this analysis. Blood PYR concentrations were natural log-transformed. Two- and three-compartment models were fitted to the data using NONMEM. The influence of covariates (age, sex, weight, height, body mass index, lean body weight (LBW), red blood cell indices, parasite count, liver function tests and geographic regions) on PK parameters was tested. Bootstrap analysis and visual predictive check (VPC) were done to evaluate the model. A two-compartment model with first order absorption and elimination best described the data. Inter-subject variability (ISV) of absorption rate constant (Ka), oral clearance (CL/F), and apparent central compartment volume (V2/F) were described using an exponential error model. The ISV of peripheral compartment volume (V3/F) and intercompartmental clearance (Q/F) could not be estimated. A log error model best described residual variability. Only LBW was found to be a significant predictor of V2/F. Typical model parameter estimates (%ISV) were Ka 29.3 1/d (109%), CL/F 1180 L/d (50%), V2/F 8540 L (82%), V3/F 13200 L and Q 1720 L/d. The estimated elimination half-life was 18 days. The final model provided estimates within the 95% confidence intervals obtained by 1000 bootstrap runs. VPC showed that the final model adequately captured the majority of the data. In conclusion, a 2-compartment model was well-fitted to pyronaridine data. LBW was an important covariate of V2/F in adult patients. The parameter estimates were plausible. The final model was robust and sufficiently captured the overall PYR PK.

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PUNICALIN AND PUNICALAGIN FAILS CEREBRAL MALARIA?

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In *AJTMH* 2003, 2004 & Multi Lateral Initiative on Malaria Yaounde-05, introduced Indo medicinal fruit called *Dalimba* (P granatum) having therapeutic & prophylactic efficacy against drug resistant malaria *Pf -Pv*, alongwith active moieties, efficacy spectrum, scavenger/anti-oxidative/anti-inflammatory, anti-viral, adjuvant, & K+ as driver candidate (as reported previously). Is a CAM, a invention on global basis termed OMARIA-Orissa Malaria Research Indigenous Attempt (BBC & Eco.Times, India, 24/25-10-2000). *In vitro* results presented from authentic source (as reported previously). Decadal mono-station continuous large scale use (Koraput-Orissa-India) indicates that >15000 individuals & whole families who all had consumed OMARIA seem to block transmission for years end, no signs of resistance, non ever developed cerebral malaria. Why? OMARIA contains 1 small, stable, (A-i) Ellagic acid rich in H+, clinically indicates hepatogen, pylori & nephron toxic symptoms at sustained/bolus doses, & 2 unstable, large, hydrolytic Ellagitanins (B-ii) Punicalagin & (B-iii) Punicalin/folin of low H+, rich in OH, wholly non toxic (C-i) K+. Which Group inflicts thus ? Or A + B + C = synergistic action ? In Indian natural sources, group B is > group-A, non confounding. K+ binds exclusively to group B, which has longer bio-availability (Sreeram-04; Soh-08). Homeopaths use diluted Acetic acid; Ellagic Acid (0.25~5%) as internal medicine to treat tertian malaria *Pf-Pv*. Initially all case report relief, then rebound with hepatic, digestive, bowl problems, long use complication = drug failure. Very same cases report feel good factor & eventual 'clinical clear status' with OMARIA. Tertian cases treated with fruits & herbs rich in group A (Chestnut bark) & non from group B is ineffective & also non gametocidal. Group A do not deliver prophylaxis nor as safe. Drug dose therapeutic response of Group-A is even not equal to Artimisinin. Group B-ii & B-iii → slow onset, long acting, potent, therapeutic, prophylactic, pregnancy safe & Gametocidal even at sub-clinical doses. K+ (C-i) thwarts neuro-cerebral morbidity.

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ACTIVITY OF 8-AMINOQUINOLINE (8AQ) ANTIMALARIAL DRUG CANDIDATES AGAINST BLOOD STAGE *PLASMODIUM FALCIPARUM*

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8-aminoquinolines (8AQ) may prove critical for malaria elimination efforts since they target hypnozoites and *Plasmodium falciparum* (Pf) gametocytes. It is unclear if 8AQs could have a role in targeting the remaining component of the transmission reservoir - asymptomatic blood stage parasitemia. The key toxicity in this class will soon be addressed with animal models predicting hemolytic toxicity in G6PD-deficiency. The Walter Reed Army Institute of Research chemical information system contains data on 1803 8AQs. Of these, 106 have been tested *in vitro* against Pf, with 33 having IC50's < 200 ng/ml. Of 1457 compounds assessed in a single dose mouse *P. bergheii* blood stage model, 195 had curative activity. Ten out of ten assessed as single agents in a 3-day Aotus monkey *P. falciparum* treatment protocol had curative activity. We plan to evaluate the *in vitro* efficacy of all available 8AQs against *P. falciparum in vitro*, to confirm their lack of cross-resistance with standard antimalarial drugs, and to determine if efficacy can be separated from hemolytic toxicity. The existing data and new data relevant to *P. falciparum* blood stage efficacy and therapeutic index will be presented.

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PHARMACOKINETICS (PK) AND QTC CHANGES AFTER COADMINISTRATION OF TAFENOQUINE (TQ) AND CHLOROQUINE (CQ) IN HEALTHY VOLUNTEERS

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Tafenoquine (TQ) is an 8-aminoquinoline in development for the treatment and radical cure of *Plasmodium vivax* malaria. TQ will be coadministered (not coformulated) with chloroquine (CQ). The PK and safety of TQ, CQ and its desethyl metabolite (DECQ) were evaluated when given concomitantly compared to TQ or CQ given alone in healthy adults. Due to the long half-lives of both TQ and CQ, a double-blind, parallel group design with 20 subjects/group was used: CQ alone (600mg on D1 and D2+300mg on D3); TQ alone (450mg on D2 and D3); and CQ plus TQ at the same doses and times. Frequent blood samples for PK were taken on D2 and D3 with additional samples taken out to D56. 12-Lead ECGs were collected in triplicate on D-1; pre-dose, 2 and 12 h post dose on D1-3; and daily on D4-7. Plasma TQ, CQ and DECQ concentrations were determined using an HPLC-MS/MS method. PK parameters were determined using non-compartmental methods. Only a short term significant effect on TQ PK was seen on D2 when taken with CQ (Cmax and AUC(0-24) increased 38% and 24%, respectively) with no significant effects seen for Cmax and AUC(0-24) on D3, AUC(0-∞) and t1/2. TQ had no significant effect on CQ and DECQ PK. No subjects had a QTcF >480msec or a change from baseline ≥ 60msec. QTcF intervals increased when treated with CQ alone but there was no trend for increased QTcF intervals in those treated

with TQ alone nor a trend for increased QTcF intervals in the CQ/TQ arm beyond those seen with CQ alone. On D2 and D3, maximum increases in mean change from baseline QTcF interval seen in the CQ/TQ arm were 5msec compared to CQ and 33msec compared to TQ. Corresponding increases from baseline QTcF intervals were seen with increases in CQ and DECQ plasma concentrations. No correlation was seen between TQ concentrations and change from baseline QTcF intervals. Mild elevations in methemoglobin occurred, maximum mean change from baseline was on D14 (6% in CQ/TQ, 4% in TQ, and <1% in CQ). Safety and tolerability for CQ/TQ were generally similar to TQ alone. Overall, no significant PK or QTcF interaction between TQ and CQ was seen in this study.

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DEVELOPMENT OF A NOVEL CHEMICAL SERIES WITH ACTIVITY AGAINST BOTH BLOOD- AND LIVER-STAGES OF *PLASMODIUM FALCIPARUM*

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Using criteria including properties disclosed in the literature, synthesizability, "drugability" and potential for toxicity, a lead with an *in vitro* IC50 of 75ng/mL against blood-stage *Plasmodium falciparum* was selected for development. By synthesizing a variety of analogs of this lead, including those bearing additional substituents in three distinct regions of the molecule, we have gained an understanding of structure-activity relationships for the compound series and have prepared analogs with increased *in vitro* efficacy against blood-stage *P. falciparum*. Additionally we have established that the series has promising activity *in vitro* against liver-stage *P. falciparum*. Since *in vivo* efficacy testing in *P. berghei*-infected mice failed to show efficacy, we have focused our recent structural modification to those expected to enhance the pharmaceutical properties of the series.

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SUSCEPTIBILITY OF NORMAL AND GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENT HUMAN ERYTHROCYTES TO PRIMAQUINE ENANTIOMERS AND POTENTIAL HEMOTOXIC METABOLITES

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Primaquine (PQ) is the drug of choice for radical cure of relapsing malaria. However, its therapeutic utility has been limited due to hemolytic toxicity, particularly in populations with glucose-6-phosphate dehydrogenase deficiency [G6PD(-)]. Reactive hydroxylated metabolites generated through cytochrome P450 (CYP)-linked metabolism are primarily responsible for hemolytic toxicity of PQ. Multiple CYP isoenzmes variably contribute to generation of hemotoxic metabolites. Previous reports have indicated enantioselective pharmacologic, pharmacokinetic and toxicologic profile of PQ. The normal and G6PD (-) human erythrocytes were exposed *in vitro* to purified enantiomers of PQ, 5-hydroxyprimaquine (5-HPQ) and 6-methoxy-8-hydroxylaminoquinoline (MAQ), the potential hemotoxic metabolites of PQ. Methemoglobin accumulation, real-time kinetic measurement of oxidative stress and depletion of intraerythrocytic reduced glutathione (GSH) were monitored as multiple biochemical end points for evaluation of hemolytic response. PQ enantiomers, which have earlier shown significantly different toxicity profile in laboratory animals, did not show significant difference in Hemotoxicity *in vitro*. 5-HPQ and MAQ produced robust increase in methemoglobin and oxidative stress both in normal and G6PD(-) erythrocytes. However, the metabolites generated concomitant depletion of GSH only in G6PD(-) erythrocytes, which may be responsible for selective susceptibility of G6PD(-) individuals to hemolytic response during treatment with PQ. Depletion of GSH may be monitored as a marker for susceptibility of individuals to PQ toxicity.

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NOVEL AMINOINDOLE INHIBITORS OF *PLASMODIUM FALCIPARUM*: *IN VIVO* EFFICACY AND PRELIMINARY SAFETY ASSESSMENT

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The aminoindole Genz-668764 is an analog of Genz-644442 which was originally identified as a hit in a high throughput live-dead screen of the Broad small molecule library against *Plasmodium falciparum*. Genz-668764 is a single enantiomer with IC50 values of 65 and 28 nM against *P. falciparum* strains Dd2 and 3D7 respectively. Similar potencies were seen against *P. knowlesi in vitro*, suggesting that the aminoindoles might be active against blood stages of *P. vivax*. Pharmacokinetic studies showed clearance values of 58, 46 and 13 ml/min/kg and half-life times of 7.30, 2.58 and 4.26 hr in mouse, rat and monkey respectively. Bioavailability was 33% in rats and 21% in monkeys. *In vivo* efficacy studies showed that when dosed 4 days twice/day, the ED50 against the *P. berghei* N-clone was 32 mg/kg/day; dosing at 200 mg/kg/day cured 3/5 mice. When tested at Swiss Tropical Institute against the ANKA strain, the ED50 was 19 mg/kg/day, and 2/5 mice were cured at 100 mg/kg/day b.i.d. The ED50 of Genz-6687864 *in vivo* against *P. falciparum* (3D7) in NOD-*scid* IL-2Rγnull mice engrafted with human erythrocytes was 40 mg/kg/day, while against ANKA strain *P. berghei* in the same model was 26 mg/kg/day. Preliminary 7-day rat safety studies showed a NOAEL of 200 mg/kg/day; primary findings were reticulocytopenia and failure to gain weight at the same rate as controls when dosed at 300 mg/kg/day; however, these findings were reversed 7 days after cessation of dosing, indicating that the effects were transient. Taken together, Genz-668764 appears to be a promising candidate for preclinical development.

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CENTRAL NERVOUS SYSTEM (CNS) EXPOSURE OF NEXT GENERATION QUINOLINE METHANOLS IS REDUCED RELATIVE TO MEFLOQUINE AFTER INTRAVENOUS (IV) DOSING IN MICE

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The clinical utility of mefloquine has been eroded through its association with adverse CNS events. These effects are dose-related. Mefloquine accumulates in the CNS. Next generation quinoline methanols (NGQMs) that do not accumulate in the CNS to the same extent may be useful antimalarials. The objective of this study was to determine whether reduction in CNS exposure relative to mefloquine was feasible amongst NGQMS with a wide range of physiochemical properties and antimalarial activity. Approximately thirty 4-position modified quinoline methanols were synthesized. The plasma and brain levels of mefloquine and these novel quinoline methanols were determined using LCMS/MS at 5 min, 1, 6 and 24 h after an IV administration (5 mg/kg) to male FVB mice. Fraction unbound in brain tissue homogenate was assessed for mefloquine and these novel quinoline methanols using equilibrium dialysis and this was then used to obtain brain unbound concentration from the measured brain total concentration. A five-fold reduction in whole and unbound brain concentrations relative to mefloquine was established as the minimum benchmark required for success. The maximum brain (whole/free) and 5 min plasma concentrations of mefloquine were 1807/4.9 ng/g and 1281 ng/ml respectively. Maximum whole brain concentrations of NGQMs ranged from 23 - 21546 ng/g. The corresponding free brain concentrations were 0.5 to 267 ng/g. Maximum brain concentrations correlated significantly with molecular weight, LogD, polar surface area, hydrogen bond donors and acceptors, albeit weakly ($r2 < 0.34$). The compound with the lowest free brain concentrations exhibited reasonable *in vitro* antimalarial activity (IC90s of 70-250 ng/ml) and is a reasonable early lead. In conclusion, reduction of CNS levels may be feasible in a next generation of antimalarial quinoline methanols. We are currently attempting to improve the potency of early lead compounds to determine if this is also feasible at projected clinically efficacious doses.

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NOVEL ACRIDONES AS BROAD-SPECTRUM ANTIMALARIALS

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to *in vitro* efficacy against the blood stage malaria. Our newly optimized derivatives were tested in the following systems: (1) Prevention of *in vitro P. berghei* sporozoite-induced development in human hepatocytes; (2) Prevention of *in vivo P. yoelii* sporozoite-induced blood stage infection in mice; (3) Inhibition of *in vitro P. falciparum* blood stage growth; (4) Efficacy in blood stage rodent malaria models; and (5) Inhibition of *in vitro P. falciparum* gametocyte growth. Details of the design, chemistry, biological activities, and preliminary studies of safety, metabolism and mechanism of action will be presented.

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A HUMANIZED MOUSE MODEL TO TEST HEMOLYTIC TOXICITY OF 8-AMINOQUINOLINES

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The goal of our research has been to develop a cost-effective, high-throughput animal model to test the hemolytic capacity of novel 8 aminoquinolines (8AQ) in the background of glucose 6-phosphate dehydrogenase(G6PD) deficiency. To achieve this goal, we first developed and validated a human (hu)RBC-SCID mouse model by giving NOD-SCID mice daily transfusions of huRBC from G6PD deficient donors for 14 days. At day 14, engraftment of huRBC was determined by flow cytometry to assess the percentage of human RBC. Levels of engraftment ranged from 55-85% with a mean engraftment of 75% huRBC. Mice were then treated with primaquine (PQ) at varying doses i.p. 2x per day for 7 days. The percentage of huRBC and mouse reticulocytes were determined by flow cytometry every day for 7 days. Treatment with PQ i.p. resulted in loss of huRBC and increase in mouse reticulocytes in a dose dependent manner. We next tested whether PQ given orally (p.o.) would also result in loss of huRBC. We tested PQ at 25 mg/kg/day and 12.5 mg/kg/day and found that the 25 mg/kg/day po induced a similar loss of huRBC compared to the 12.5 mg/kg/day PQ given i.p. As a control, we tested whether chloroquine (CQ) would induce loss of hu-RBC in this model. Chloroquine treatment was comparable to PBS control suggesting that the loss of G6PD deficient hu-RBC in this model is specific to PQ. These data suggest that we can reproducibly induce loss of G6PD deficient huRBC engrafted into NOD-SCID mice following treatment with PQ and that this effect is dose dependent. Further validation with additional hemolytic and non-hemolytic drugs should prove application of this model for screening antimalarial drugs in discovery phase. Importantly, we have developed a new experimental tool to assess G6PD hemolytic toxicity of anti-malaria drugs.

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ANTIPLASMODIAL ACTIVITY OF SOME MEDICINAL PLANTS USED IN SUDANESE FOLK-MEDICINE

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Ten plants indigenous to Sudan and of common use in Sudanese folk-medicine, were examined *in vitro* for antimalarial activity against schizonts maturation of *Plasmodium falciparum*, the major human malaria parasite. All plant samples displayed various antiplasmodial activity. Three plant extracts caused 100% inhibition of the parasite growth at concentrations of plant material 500 mg/ml. The two most active extracts that produced 100% inhibition of the parasite growth at concentration of plant material 50 µg/ml were obtained from the seeds of *Nigella sativa* and the whole plant of *Aristolochia bracteolata*. The ten plants were phytochemically screened for their active constituents. The two most active plants showed the presence of sterols, alkaloids and tannins.

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ANTI-PLASMODIAL AND IMMUNOMODULATORY ACTIVITY OF MEDICINAL PLANTS USED IN BURKINA FASO AGAINST MALARIA

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Plasmodium falciparum malaria is the most common cause of death in Burkina Faso. The persistence of drug resistance parasites makes the disease difficult to control. Moreover, the recent reports of emergence of resistance to artemisinin derivatives, which are the most effective antimalarial presently available, confirm that new drugs are greatly needed. The reliability of indigenous herbal drugs may be helpful. In Burkina Faso, the decoctions of *Canthium henriquesianum* Schum, *Gardenia sokotensis* Hutch. and *Vernonia colorata* Willd. are used to treat malaria. The study objective was to evaluate the antiplasmodial properties of these plant extracts and to check the relevance of their use. The plants aerial parts were soxhlet-extracted with water and different solvents and then screened for antiplasmodial activity through the pLDH method on *P. falciparum* sensitive (D10) and resistant (W2) strains. The aqueous extract from *C. henriquesianum* was the most active with IC50 of 80,02± 26,83 and 66.8±21.6 µg/ml on D10 and W2, respectively. The ethyl acetate extract was even more potent with IC50 24.0±7.4 µg/ml. No toxicity was observed against mammalian cells, suggesting a good therapeutic index. The decoction of *C. henriquesianum* contains hydrolysable tannins, flavonoids, saponins and no alkaloids. Extracts of *C. henriquesianum* also induced a dose-dependent inhibition of the production of IL-1β by human monocytes, thus confirming its traditional use as antipyretic. Attempts to identify the active principle for antiplasmodial and anti-inflammatory activities are ongoing.

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SEASONAL VARIATION AND CLINICAL PROTECTION OF ANTIBODIES TO A PANEL OF PRE-ERYTHROCYTICS AND ERYTHROCYTICS MALARIA VACCINE ANTIGENS IN CHILDREN BELOW FIVE YEARS LIVING IN MALARIA HYPER ENDEMIC AREA OF BURKINA FASO (WEST AFRICA)

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The sequencing of ***Plasmodium falciparum*** genome has contributed to the discovery of new antigens which can be used for the development of malaria vaccine candidates. In this study newly pre-erythrocytic (MR48a) and erythrocytic (LR181 & 1574) synthetic peptides and already well known recombinant antigen (LSA1) have been used to i) characterize the seasonal profile of total IgG response and (ii) examine the relationship between natural antibody responses and protection against clinical malaria. We performed two clinical and parasitological cross-sectional surveys, in January 2007 before the low and at the pick of malaria transmission season. From the first cross sectional survey, children were visited biweekly to record clinical malaria cases during one year period. Study includes 380 children under five years from 4 villages of Saponé health district. During cross-sectional surveys, blood films were prepared for parasites check, 5 ml of blood taken and plasma used for total IgG measurement. Mean number of malaria episodes was 1.57 (95%CI: 1.52-1.6) with an incidence of 0.7 episode per child year at risk. Geometric means of 1574 didn't show any difference during both transmission seasons (1574: P = 0.8). However LR181 show high antibodies level (P < 0.000) during low compared to high transmission season. LSA1 and MR48a increase significantly (P < 0.000) during high compared to low transmission season. Correlation to protection was seen only for IgG to LSA1 (P=0.01). In conclusion, antibodies to most of these antigens were affected by the level of malaria transmission season and only recombinant peptide (LSA1) elicited antibodies associated to protection in our study young volunteers. This finding should be taken into account when designing malaria vaccine trial in seasonal transmission settings.

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ELEVATED EFFECTOR MEMORY CD4+ T CELLS IN CHILDREN WITH SEVERE MALARIAL ANEMIA

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In malaria endemic areas, malaria-induced chronic immune activation may contribute to severe malarial anemia (SMA) pathogenesis. We, therefore, characterized CD4+ T-cell populations in children (n=54; age 12-36 months) presenting with differing severities of malarial anemia at Siaya District Hospital, western Kenya. Complete hematological measures were obtained with a Beckman Coulter Counter®, while Giemsa-stained slides were used to determine parasitemia. Participants were stratified based on hemoglobin (Hb) status as uncomplicated malaria (UM; Hb>11.0 g/dL; n=12), mild malarial anemia (M/MA; Hb>8.0<11.0 g/dL; n=22), and severe malarial anemia (SMA; Hb<6.0 g/dL; n=20)]. Venous blood was isolated and stained with anti-(CD45RA; CD62L; CCR7; CD69 and HLA-DR) antibodies. Cells were then acquired using a four-color FACSCalibur. Proportions of CD4+ T-cell were also analyzed. Children presenting with SMA had the highest proportion of effector memory T-cells (CD45RA-CCR7-CD62L-) [median (IQR) UM, 11.70% (11.00); M/MA, 10.59% (8.21); SMA, 14.80% (6.18); *P*=0.025], with no significant differences across the groups in the proportion of central memory T-cells (CD45RA-CCR7+CD62L+) [median (IQR) UM, 54.95% (17.40); M/MA, 59.92% (16.80); SMA, 56.74% (10.00) *P*=0.788)]. The expression of the early (CD69) and late (HLA-DR) activation markers on CD4 T cells was comparable across the groups, possibly due to continuous antigenic challenge from the chronicity of malaria infections in this region. Taken together, these results suggest that effector memory T-cells may play an important role in modulating the development of pediatric SMA in this holoendemic *P. falciparum* transmission area.

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ASSOCIATION BETWEEN IMMUNOGLOBULIN GM AND KM GENOTYPES AND PLACENTAL MALARIA IN HIV-1 POSITIVE AND NEGATIVE WOMEN IN WESTERN KENYA

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Immunoglobulin (Ig) GM and KM allotypes, genetic markers of γ and κ chains, respectively, are associated with humoral immune responsiveness. The clinical importance of Ig GM and KM allotypes has been evaluated for

other infectious diseases, but their role in placental malaria (PM) with HIV co-infection during pregnancy has not been investigated. We examined the relationship between Ig GM and KM allotypes and risk of PM in pregnant women with known HIV status, nested in an epidemiological study investigating the relationship between PM and perinatal mother-to-child transmission of HIV-1 in Kisumu, Kenya. DNA samples from 728 pregnant women were genotyped for major GM6 and KM alleles using a restriction-fragment length polymorphism polymerase chain reaction method. The genetic polymorphisms of Ig GM and KM were defined as (1) individual genotype for GM6 (+, +/-, -) and KM (1, 1-3, 3) respectively and (2) combined genotype of the GM6 and KM. Overall, there was no significant effect of individual GM6 and KM genotypes on the risk of PM in HIV-1 negative and positive women respectively. However, the combination of homozygosity for GM6 (+) and KM3 was associated with decreased risk of PM (adjusted OR 0.25, 95% CI, 0.08 to 0.8, $P = 0.019$) in HIV negative women while the combined heterozygosity of GM6 (+/-) and KM1-3 were associated with increased risk of PM in HIV positive women (adjusted OR 2.08, 95% CI, 1.12 to 3.89, $P = 0.021$). In addition, the combination of GM6 (+/-), KM1-3 and KM1 was associated with increased risk of PM only in the subgroup of HIV positive women with viral load <10,000 copies/ml (adjusted OR 2.99, 95% CI, 1.28 to 7.02, $P = 0.011$), suggesting that the viral load has an effect on the relationship between the combined genotypes and susceptibility to PM. These findings suggest that the combination of GM6 (+) and KM3 may protect against PM in HIV negative women, while the HIV positive women with GM6 (+/-) combined with KM1-3 or KM1 may be susceptible to PM.

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POLYMORPHIC VARIABILITY IN THE TUMOR NECROSIS FACTOR (TNF)-A PROMOTER IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA

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The pleiotropic cytokine tumor necrosis factor (TNF)- α plays a central role in the early stages of intracellular infections by activating the innate immune response. TNF- α has long been considered a candidate susceptibility gene for malaria. Although association of several TNF- α promoter polymorphisms with malaria disease outcome have been reported, their precise role, particularly in modulating severe malarial anemia (SMA) in children residing in holoendemic *Plasmodium falciparum* transmission regions, are largely undefined. The aim of the present study was to investigate the functional associations between TNF- α promoter polymorphisms [(G-238A), (C-308T), (C-376T) and (T-1031C)], and presence of parasitemia and SMA (Hb <5.0 g/dL) manifestation in children (n=736, aged 3-36 mos.). Stratification of parasitemic children (n=578) according to hemoglobin (Hb) levels revealed that the SMA (Hb <5.0g/dL; n=439) (P=0.040) group. Multivariate logistic regression analyses of genotypic variants, controlling for confounding factors, showed that heterozygosity (GA) at the -238 locus was associated with an increased risk of SMA [OR, 1.907,95% CI, 1.067-3.410, P=0.029] and non-significant elevations in circulating TNF- α (P=0.588) relative to wild-type. Additional analyses revealed that the ACCT [-238A/-308C/-376C/-1031T] haplotype was associated with protection against the acquisition of *P. falciparum* parasitemia (OR, 0.516, 95% CI, 0.277-0.961, P=0.037) relative to individuals without this haplotype, and non-significant elevations in circulating TNF- α (P=0.328). Results presented here suggest that variation in the TNF- α promoter conditions susceptibility to malaria and the development of SMA once malaria is acquired. However, the mechanism(s) through which variation in TNF- α promoter conditions susceptibility to malaria outcomes remains to be determined since circulating TNF- α levels were not significantly associated with either genotypes or haplotypes.

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ERYTHROPOIETIN (EPO) AND ANTI-EPO AUTO-ANTIBODIES IMBALANCE IS ASSOCIATED WITH PROTECTION AND CORRELATES WITH ANAEMIA IN PBANKA INFECTED SEMI-IMMUNE MICE

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Malaria anaemia is still a major public health problem and its pathogenesis still unclear. It has been observed that some individuals develop anaemia at low parasitaemia and are protected and others not due to complications from hyperparasitaemia. A study has shown that treatment of infected mice with exogenous anti-erythropoietin (EPO) auto antibodies (auAb) gives protection, suggesting an important role for anti-EPO auAb in malaria. Thus we hypothesized that elevated levels of anti-EPO auAb is associated with protection in *Plasmodium* infected semi-immune individuals. Semi-immune status was attained in four mice strains (Balb/c, B6, CBA and NZW) by repeated infections with PbANKA, and treatment with chloroquine/pyrimethamine. ELISA was used to measure EPO and anti-EPO auAb, while inflammatory cytokines measurement was done using bead-based multiplex assay kit. Measurement of transferin in the mice sera and anti-EPO auto antibodies in sera of human malaria is underway. High %Hb loss and survival (>40%) was observed in Balb/c in comparism with the other strains (<33% survival). Similar levels of anti-EPO auAb was observed in Balb/c and NZW ($p=0.61$), and were significantly higher than in other strains, $p<0.0001$. Anti-EPO auAb correlated positively with extent of Hb loss ($r2=0.41$; $p=0.0009$). However, anti-EPO auAb/log EPO ratio was significantly associated with Balb/c ($p<0.0001$) which are resistant to infection. Significant elevated levels of IL6 and IFNg ($p<0.0001$), both associated with erythropoiesis suppression were observed in the Balb/c. In conclusion, our data presented here seems to suggest that anti-EPO auAb/EPO imbalance may be an additional contributor in the pathogenesis of malaria anaemia, but of a beneficial role in playing a protective mechanism to severe malaria anaemia in some individuals and detrimental to others, hence implicating host genetic factors.

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MALARIA INFECTION AND MEASLES VACCINATION EFFICACY - CAUSE FOR CONCERN?

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Measles Virus (MV) continues to be one of the largest causes of vaccine preventable mortality, due in part to its high transmission rate, requiring greater than 95% seroconversion to induce herd immunity. Although there have been tremendous gains in global vaccination coverage, measles outbreaks continue, especially in areas where malaria is a common

childhood infection. Malaria is known to be immuno-suppressive and/or immune-modulating. Therefore the true influence of concomitant asymptomatic malaria infections on MV vaccine efficacy remains in doubt. In order to shed light on this underlying question, we compared antibody- and cell-mediated immunity to MV vaccine (Edmonton strain) in two prospective cohorts of Kenyan children (2 months until 3 years of age) residing in areas with divergent malaria transmission intensities: holoendemic versus highland, epidemic-prone malaria. Antibody levels were measured using the Luminex microsphere technology. Cell-mediated immunity was measured using IFN-γ ELISPOT assays and a panel of cytokines was measured by Luminex. We found no significant difference in the mean MV-antibody levels following vaccination associated with malaria exposure history. This remained true after controlling for age at vaccination and pre-vaccination antibody levels. There was also no significant difference in MV-specific IFN-γ response *ex vivo* in children from the holoendemic area (20%) compared to the highland children (24%). Highland children were however more likely to secrete higher levels of IL-10 in response to MV, though other cytokine levels did not differ significantly between groups. And yet, IL-10 responses did not correlate with a lack of seroconversion. These results suggest that malaria has a minimal impact on the quality of MV immunity following the first vaccination. Future studies will evaluate factors, such as second MV immunization and prolonged exposure to malaria, which may influence the quality and duration of immunologic memory to the MV vaccine.

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LONGITUDINAL ANALYSIS OF THE ANTIBODY RESPONSE AGAINST FIVE VAR2CSA DOMAINS AND MULTIPLE STRAIN VARIANTS IN CAMEROONIAN PREGNANT WOMEN LIVING IN A HIGH MALARIA TRANSMISSION AREA

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VAR2CSA, a variant surface antigen on late-stage *Plasmodium falciparum*-infected erythrocytes (IE), is responsible for IE adhesion to chondroitin sulfate A in the intervillous space of the placenta and can lead to placental malaria (PM). PM is associated with babies born with low birth weights, who are at increased risk of malaria early in life. While PM is less prevalent among multigravidae, presumably due to the presence of adhesion blocking antibodies (Ab), it remains unclear to which DBL domain(s) of VAR2CSA protective Ab are directed. The present study aimed to identify correlates of protection (absence of PM) by evaluating sera collected longitudinally from 37 pregnant women living in Ngali II who received ~0.7 infectious bite/night throughout pregnancy. Using a bead-based multiplex assay, Ab levels against recombinant VAR2CSA DBL 1, 3, 4, 5 and 6 were measured for parasite strains 7G8, IT4 and 3D7. Our results demonstrated that all five DBL domains are immunogenic, with robust and dynamic responses observed against DBL3 (7G8) and 5 (7G8, 3D7) as early as 3 months of pregnancy (MoP). Moderate responses were observed to DBL6 - 7G8 but not - IT4. While Ab responses against DBL1 (7G8, IT4, 3D7) were low or absent and generally did not appear until 6 MoP, only minimal responses were detected to DBL4 (7G8, IT4) during pregnancy. Ab levels against DBL3 and 5 increased gradually and steadily throughout pregnancy. Based on 13 women without PM (PM-) and 14 women with PM (PM+), a consistent pattern was observed in that PM- women had higher Ab levels against DBL3 (7G8) and 5 (7G8, 3D7) from 3 to 8 MoP and from 6 to 8 MoP, respectively, than PM+ women. Moreover, Ab of PM- women tended to recognize more domains than PM+ women. In summary, early production and maintenance of Ab against DBL3 and 5 at high levels throughout pregnancy appeared to correlate with the absence of PM at term. A larger repertoire of Ab to multiple domains may also contribute to enhanced protection from developing PM.

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LONGITUDINAL PATTERNS OF ANTIBODY RESPONSES AND *PLASMODIUM* SPP. INFECTION DURING PREGNANCY

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Pregnant women are more susceptible to, and more severely affected by, malaria and other infectious diseases. In malaria endemic regions pregnant women typically develop high parasite densities, placental infection and associated complications, despite substantial immunity to malaria that may have been acquired prior to pregnancy. This has largely been attributed to both the modulation of maternal immune responses and the sequestration of *Plasmodium falciparum* parasites in the placenta. The contribution of pre-existing immunity, maintenance and boosting of antibody responses throughout pregnancy, and their relation to malaria is unclear. In a nested case-control study of 467 pregnant Karen women (136 malaria cases and 331 non-infected controls), we measured antibody levels at enrolment to blood-stage antigens and pregnancy-specific antigen VAR2CSA and placental-binding isolates CS2 and HCS3. Furthermore, we also determined antibody levels at 2-weekly intervals during pregnancy until delivery in malaria cases and a subset of controls, including over 2000 samples. ELISAs were performed using novel high-throughput technology to facilitate determination of antibody levels in a large number of samples. At enrolment, the sero-prevalence of blood-stage antibodies was higher in cases than controls and the prevalence of antibodies to the pregnancy-specific binding isolates were low. Antibody levels at enrolment were associated with increased odds of parasitaemia during pregnancy. Longitudinal analysis revealed the prevalence of malaria infection (both *P. falciparum* and *P. vivax*) decreased with increasing gestation time as did antibody levels at a similar rate in cases and controls. However, antibody levels did increase with gestation time in those with concurrent *P. falciparum* parasitaemia most likely reflecting boosting of responses with each successive infection. This study provides the most comprehensive analysis, to date, of antibody maintenance, decay and boosting during pregnancy and contributes to our understanding of malaria during pregnancy and immune responses to infectious diseases during pregnancy.

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NOVEL *PLASMODIUM FALCIPARUM* BLOOD STAGE ANTIGENS: INDUCTION OF HUMORAL RESPONSE AND PROTECTION IN BURKINABE CHILDREN

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Protective human immunity against clinical malaria involves the acquisition of specific antibodies. The identification of vaccine candidate antigens that elicit the induction of protective humoral response is a valuable step for an effective malaria vaccine development. Here, we determine the

role of specific IgG antibody responses in the protection against clinical malaria in an area with stable and distinct seasonality of transmission. A total of 422 children below 5 years from the Sapone's health district (Burkina Faso) provided 844 plasma samples at low and high transmission seasons. Specific IgG antibody responses against the recombinant CSP construct (control antigen) and 3 synthetic α-helical coiled coil blood stage antigens (LR179A, AS155 and MR198) were measured. A year-round clinical malaria active case detection was conducted and malaria clinical data related to antibody titers for immunological correlates of protection. Geometric means of specific antibody levels to CSP, AS155 and LR179A significantly associated with age and season. In contrast, antibody levels induced by the antigen MR198 were influenced neither by age nor by season. During the entire follow up, the mean number of malaria episode per child was 1.57 with an incidence rate of 0.7 episode per child year at risk. The assessment of the role of the four antigens in eliciting a protective humoral response in children revealed a negative association between the geometric mean antibody level to AS155.4 and clinical malaria cases (p=0.007). In conclusion, our findings suggest that α-helical coiled coil protein motif-based vaccine candidates are immunogenic in children less than five years and AS155.4 antibodies strongly associate with protection against malaria clinical. Therefore, further clinical investigations including IgG subclasses are recommended for more insights into such a protective immunity against the malaria parasite.

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ACUTE *PLASMODIUM CHABAUDI* INFECTION LEADS TO LOSS OF TRANSITIONAL B CELLS IN THE SPLEEN

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The spleen is a critical interface between *Plasmodium* infected RBC and B cells. Because *Plasmodium* infection leads to a high antigen load and disruption of splenic architecture, we wanted to determine the effects of *Plasmodium* infection on B cell subset distribution in the spleen during the acute phase of infection. Peripheral B cell subsets in the spleen include both immature B cells (e.g. transitional T1 and T2 cells) and mature marginal zone and follicular B cells. Using a mouse model of the erythrocyte stage of *P. chabaudi* infection in C57BL/6 mice, we examined the alterations in splenic B cell subsets during the acute phase of infection. Mice were injected i.p. with 5x105 parasitized RBCs and after 6 and 12 days post infection (dpi) the spleens were removed and the B cell subsets were analyzed using flow cytometry. At 12 dpi, which is the peak of parasitemia, the spleens had an increase in overall cellularity; however, the total number of B cells was not significantly altered. Interestingly, the two immature T1 (B220+, AA4+, CD23-, IgM+) and T2 (B220+, AA4+, CD23+, IgM+) transitional B cell subsets showed a significant reduction in number at both 6 and 12 dpi. These decreases in transitional B cells was the result of apoptosis in both the T2 and to a greater extent T1 B cell subsets as determined by Annexin V staining. We also observed a decrease in the marginal zone B cell population (CD19+, AA4-, CD21hi, CD23lo) by 12 dpi. Experiments are ongoing to determine if the decline in marginal zone B cells is due to apoptosis of these cells or due to loss of the transitional B cells that differentiate to marginal zone B cells. Our results demonstrate that acute infection with *P. chabaudi* can lead to specific affects on B cell homeostasis in both the mature and immature subsets.

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FREQUENCY OF *PLASMODIUM FALCIPARUM* INFECTION AMONG UGANDAN CHILDREN AND ITS RELATIONSHIP WITH VARIANTS OF THE CYTOKINE RANTES PROMOTER

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Chemokines including Regulated on Activation, Normal T Expressed and Secreted (RANTES) control host immunity to pathogens. We have investigated two highly polymorphic nucleotide positions (-403 and -28) in the RANTES promoter of genomic DNA from Ugandan children and the relation of mutation at these positions to *Plasmodium falciparum* infection. To our knowledge, this is the first study of the relation between diversity of RANTES gene promoter and *P. falciparum* infection. In a cross-sectional study, we examined DNA from 319 Ugandan children (158 infected with *P. falciparum* and 161 healthy controls) aged between 0.5 and 9 years. *P. falciparum* infection was determined by microscopy of blood smears. Using DNA polymerase chain reaction and enzyme digestion, we determined the occurrence of mutation at nucleotides -403 and -28 of RANTES promoter. The profile of variants at nucleotide -403 was as follows: 55.8 % (178/319) heterozygous (mutation at one chromosome), 21.9 % (70/319) homozygous (mutation at both chromosomes), and 22.3% (71/319) wild type. The prevalence of *P. falciparum* infection was significantly higher in children carrying -403 mutation on one chromosome (P=0.001; odds ratio =2.6), or bearing the -403 mutation on both chromosomes (P = 0.014; OR 2.3) than in -403 wild type individuals. The prevalence of mutations at nucleotide -28 was 3.8% (12/319) heterozygous, 0 % homozygous and 96.2% (307/319) wild type. Importantly, eight out of the twelve children (66.7%, 8/12) carrying mutation at -28 also had a mutation at -403 and were 6.2 times more likely to have *P. falciparum* infection than those with wild type alleles, suggesting that dual mutations (at -403 plus -28) lead to higher risk for malaria. There was no significant relationship between the rare -28 nucleotide mutation and *P. falciparum* infection (P= 0.23). Further, there was no significant association between RANTES variants and parasitemia (P= 0.78).

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EVALUATION OF THE BINDING AND INHIBITORY PROPERTIES OF NOVEL MONOCLONAL ANTIBODIES TO *PLASMODIUM VIVAX* LIGAND DOMAIN

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The Duffy Binding protein of *Plasmodium vivax* is vital for host erythrocyte invasion and the region II (DBPII) contains critical residues for receptor recognition thereby making the molecule an attractive vaccine candidate against vivax malaria. Although an ideal target, the allelic variation within the DBPII and associated strain specific immunity may be a major challenge for development of a broadly effective vaccine for vivax malaria. To understand the specificity of protective immune responses to DBPII, we have generated a panel of monoclonal antibodies (MABs) to identify and map the various domains of the DBPII that correlate with protection to *P. vivax.* Using rDBPII from different alleles we have assayed the specificity of the MABs by ELISA and inhibition of binding by standard erythrocyte binding assay. Analysis by ELISA determined that some MABs react strongly with epitopes conserved on all rDBPII alleles tested, while other MABs react with allele-specific epitopes. Quantitative and qualitative analysis, with ELISA and *in vitro* erythrocyte-binding inhibition assays respectively, failed to demonstrate a consistent correlation between

the end point titers and functional inhibition. These data are consistent with the differential inhibitory patterns observed with human sera from individuals naturally exposed to *P. vivax*. Identification of conserved epitopes to which inhibitory antibodies bind is critical to optimizing DBP immunogenicity for protection against diverse *P. vivax* strains.

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DEFINING THE IMMUNOREACTIVE SURFACE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN REGION II

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Region II of the Duffy binding protein (*Pv*DBPII) is a high priority candidate for inclusion in a subunit vaccine designed to protect against disease caused by the malaria parasite *Plasmodium vivax*. The *Pv*DBPII coding sequence predicts an approximately 38-kDa antigen, which comprises the erythrocyte-binding domain of the Duffy binding protein (*Pv*DBP). Although the crystal structure of *Pv*DBPII is yet to be published, it is known to consist of a Duffy binding-like (DBL) domain, belonging to a family of structural homologues found in other adhesion molecules of other species of *Plasmodium*. Based on its high degree of homology with the DBL domain of *Plasmodium knowlesi* DBP, for which there is a crystal structure, *Pv*DBPII is thought to be largely alpha-helical and may be assigned into three sub-domains delineated by six disulphide bonds. Using a phage display approach to express these sub-domains individually or in combination in their correctly refolded and disulphide bonded conformations, we are currently mapping the conformation-dependent epitopes of a panel of monoclonal antibodies recognizing the antigen. Finer epitope mapping is being achieved using a random peptide library and a gene fragment library of *Pv*DBPII displayed on phage. Many of the antibodies being studied are capable of inhibiting recombinant *Pv*DBPII expressed on COS cells from binding to Duffy-positive erythrocytes. The studies performed here suggest that the epitopes of binding-inhibitory antibodies map to a different region of *Pv*DBPII compared with those of non-inhibitory antibodies. Ultimately information derived from these studies will contribute to the assessment of this antigen for inclusion in a vaccine designed to protect against disease caused by vivax malaria.

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A STAT6 SINGLE NUCLEOTIDE POLYMORPHISM IS ASSOCIATED WITH PROTECTION AGAINST CEREBRAL MALARIA IN GHANAIAN CHILDREN

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The IL-4/Stat6 signalling pathway could be crucial for Th2 mediated immunity and protection against malaria. Although we and others have previously shown associations between some IL-4 polymorphisms and severe malaria, the role of Stat6 and IL-4R-α polymorphisms in malaria pathogenesis is yet to be established. This study investigated the distinctive and interactive association of known polymorphisms of the IL-4 gene (+33C/T, 590C/T, VNTR), IL-4R gene (Arg551Gln) and STAT6 gene (1570C/T) with total IgE production and subsequently, malaria severity in Ghanaian children. PCR-RFLP was used to genotype all polymorphisms in a hospital based cross-sectional study involving 290 malaria cases and controls. Malaria cases were categorized into uncomplicated malaria (UM), severe malarial anaemia (SMA), and cerebral malaria (CM). We found that a single nucleotide polymorphism (SNP) (rs3024974) which causes a C → T change in intron 18 of the stat6 gene is associated with protection from cerebral malaria (OR = 0.361, P = 0.0107). All other polymorphisms studied did not show any association with malaria severity except the IL-4 VNTR polymorphism. Our data did not show any association between rs3024974 and levels of total IgE. Data from this study suggests that rs3024974 is associated with protection against cerebral malaria in Ghanaian children. However, this protection maybe mediated by other factors other than total serum IgE. To the best of our knowledge, this study is the first to suggest a role for the stat6 SNP (rs3024974) in malaria pathogenesis.

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DETECTION OF LATENT RESERVOIRS OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS BY SHORT AMPLICON PRIMERS AND TERTIARY NESTED PCR IN MACHA, SOUTHERN ZAMBIA

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Malaria endemic countries have scaled-up effective interventions; this has prompted the increased need to monitor asymptomatic infection reservoirs so as to avoid potential resurgences. Using short amplicon primers and an alternative tertiary amplification strategy, the present study reports the detection of widespread sub-microscopic asymptomatic *Plasmodium falciparum* infections. In a 2000 km2 area in Macha, Southern Zambia, 1500 willing residents of all ages were screened for malaria by microscopy, with simultaneous collection of dried finger-prick blood spots on Whatman 3MM filter paper. Chelex extracts from the dried blood spots (DBS) were subjected to regular nested PCR using primers targeting the *P. falciparum* 18S rRNA and DHFR genes. PfDHFR was amplified using regular nested PCR with published primers and a new tertiary PCR strategy using three sets of regular published primers. While the malarial parasite rate was 1.1 % by microscopy, nested PCR showed 47.2 % parasite rate with 18S ribosomal primers, 56.2 % with regular published primers and 86.5% with short amplicon primers. Tertiary nested PCR enhanced the detectable parasite rate to 71.9 % ($p = 0.04$, $n = 90$) compared with the same primers in two rounds of amplification. This study documents the existence of ultra-low asymptomatic *P. falciparum* parasitaemia below detection limit of microscopy and standard nested PCR in an area of apparently depleted malaria prevalence. These methods can be used to significantly enhance detection of latent reservoirs of infection to minimize risk of resurgences.

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MOLECULAR ANALYSIS OF THE *PLASMODIUM FALCIPARUM* SARCOPLASMIC AND ENDOPLASMIC RETICULUM CALCIUM ATPASE (*PF*ATPASE6 SERCA) GENE ASSOCIATED WITH ARTESUNATE RESISTANCE IN GHANAIAN PATIENTS WITH UNCOMPLICATED MALARIA

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Resistance of *Plasmodium falciparum* to first line antimalarials such as chloroquine and Fansidar® resulted in a national policy change in 2005, to artemisinin combination therapy (ACT), artesunate+amodiaquine (AS+AQ) in Ghana. Certain mutations in the *P. falciparum* ATPase6 SERCA gene (E431K, A623E and S769N) have been reported to be associated with artemisinin derivatives resistance and are known to cluster geographically. The aim of this study was to identify mutations in *Pf*ATPase6 SERCA gene that can be associated with artesunate resistance in Ghana. Archived filter

paper blood blots from patients who experienced AS+AQ clinical failures (treatment failure group) and an equal number of samples selected from responders (susceptible group) were studied. Sixty four Day 0 and 3 post-treatments samples from patients attending 7 hospitals at sentinel sites for monitoring drug resistance were used. PCR identification of *P. falciparum* was performed after which the ATPase6 SERCA gene fragments were amplified and sequenced to identify mutations. Analysis of the sequenced data did not detect any polymorphism at positions 623 and 769 in the treatment groups when compared to the reference *P. falciparum* Dd2 DNA sequenced data in the GeneBank database. However, four parasites (3 in treatment failure and 1 in susceptible group) had the E431K mutation reported in Senegal. Five novel mutations (N569K, E633K, H747Y, K776N and one synonymous at position 460) were observed in the Day 0 samples. Three of the novel mutations were in the susceptible parasites and the other in the treatment failure group. Also, two parasites in the susceptible group were found to be double mutant. Considering the absence of mutations in the post-treatment parasites' genes (n=3), the small sample size, the possibility of re-infection, no definitive conclusion could be made on their association with treatment outcome. Further studies to assess other mutations in *Pf*ATPase6 SERCA gene and other genes should be looked at to know the actual target of artemisinins and verify their usefulness in monitoring ACT resistance.

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GENETIC DIVERSITY OF THREE VACCINES CANDIDATES ANTIGENS IN *PLASMODIUM FALCIPARUM* ISOLATES FROM RURAL AREA IN SENEGAL

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Genetic variation allows the malaria parasite *Plasmodium falciparum* to overcome chemotherapeutic agents, vaccines and vector control strategies and remain a leading cause of global morbidity and mortality. Several of the intended *P. falciparum* vaccine candidate antigens are highly polymorphic and could render a vaccine ineffective if their antigenic sites were not represented in the vaccine. In this study, we characterize the genetic variability of vaccine candidate antigens as msp3, ama1 and eba-175 in isolates of *P. falciparum* from Senegal. DNA analysis was completed on 111 isolates of *P. falciparum* collected from endemic area in Keur Soce, Senegal from years 2006 to 2007. Genetic diversity was determined in immunological important in merozoite surface protein-3 (MSP-3), apical membrane antigen-1 (AMA-1) and erythrocyte binding antigen (EBA-175). Alleles identified by DNA nested PCR and RFLP were analysed by Genelex 6, Arlequin v3.1 and Epi Info v6.04. The data comparisons were made using the Chi square or Fisher's exact test, and the Student's t-test and ANOVA for normally distributed continuous data with a statistical significance threshold of P< 0.05. The allele's frequencies were estimated based on the GenAlEx AFL. The genetic diversity was calculated by determining the heterozygosity of alleles detected for each antigen in each population. From 111 samples, PCR product where obtain from 70 (63,06%), 89 (80,18%%)and 80 (72,07%) respectively for ama1, msp3 and eba-175. The results showed that the eba-175 gene presented 4 different alleles [eba175F_loop (45,9%), eba175C_loop (31,1%), eba175~400bp (12,6%), eba175~360bp (6,3%)] and the alleles found had frequencies high than 5% in the respective parasite population. Regarding the msp-3 patterns, the analysis revealed the presence of three alleles MSP3_K1 (40,5%), MSP3_3D7 (47,5%) and MSP3~350bp (11,7%). For ama1 patterns, the results showed three different alleles ama-1_K1 (37%), ama-1_HB3 (30,9%), ama-1_3D7(32,1%). In conclusion, characterization of the genetic diversity in *Plasmodium* isolates from Keur Soce (Senegal) showed that *P. falciparum* in these antigens have polymorphisms more similar to Peru than to India.

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GEOGRAPHIC STRUCTURE OF *PLASMODIUM VIVAX* IN SRI LANKA

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Understanding the genetic diversity and population structure of *Plasmodium vivax* in Sri Lanka would be helpful in differentiating indigenous from imported parasite isolates within the country. This would enable meaningful surveillance and control strategies for the elimination program currently being conducted in the country. 190 *P. vivax* isolates collected from various locations within Sri Lanka (between 1999 and 2008 were genotyped using 14 highly polymorphic microsatellite markers. All samples were PCR-amplified and the length variations of the PCR products were measured. The single or predominant allele at each locus was considered for computing allele frequencies. The presence of more than one allele at a particular locus was interpreted as a multiple-clone infection. Genetic diversity was determined by calculating heterozygosity (*HE*) and standardized index of association (*ISA*) used to test for multilocus linkage disequilibrium. STRUCTURE software was used to test for clustering of haplotypes according to geographic and temporal origins. The parasite population was highly polymorphic with 189 unique haplotypes. The number of alleles per locus varied between 13 and 47. Almost 66% (n=125) had multiple-clone infections. Mean genetic diversity (*HE*) was 0.8747. Significant multilocus linkage disequilibrium was present (*ISA*=0.0265, *P*<0.001). The population structure revealed temporal variations and partial clustering of *P. vivax* isolates according to geographic locations. Microsatellite typing would serve as an excellent tool for surveillance of *P. vivax* malaria within Sri Lanka enabling effective strategies for control depending on the origin of the parasite.

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GENETIC MARKERS AND RISK OF MALARIA INFECTIONS: GENETIC-EPIDEMIOLOGY STUDY IN A LOW MALARIA ENDEMIC AREA OF SRI LANKA

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Malaria transmission is seasonal and unstable in the dry zone of Sri Lanka and the levels have steadily declined over the past 10 years. This study is part of an immuno-epidemiological study that was conducted in residents of 8 villages in the district of Moneragala, a known malaria endemic area with transmission of *P. vivax* (predominant species >80%) and *P. falciparum*. The original study was a cohort study with active case detection of 1,951 individuals during 1992/93. In year 2006, 1133 of these individuals were traced, blood samples obtained and history of malaria attacks during the past 15 years recorded. DNA was extracted from whole blood and SNPs in selected genes including those related to

immune-response in malaria were investigated. Serum was separated for serological investigations and titers of seven antibodies, AMA1, MSP1, MSP2, NANP, IgE, Pv_AMA1 and Pv_MSP1, were determined by ELISA. Antibody levels were further classified into low and high level. SNP data were analyzed in relation to past history of malaria attacks and serum antibody levels. A total of 169 SNPs were typed in 1107 study subjects. After sample and genotype quality control, 96 SNPs in 1017 study subjects were selected for analysis. Genotype frequencies in 7 SNPs in 7 genes were found to be significantly different between those who have experienced repeated malaria attacks and those with apparent protection ($p<0.05$; Chi-square test). When classified antibody levels into low-high binary trait, we found significant association in 11 SNPs for AMA1; 5 SNPs for MSP1; 9 SNPs for MSP2; 8 SNPs for NANP; 5 SNPs for Pv-MSP1; 11 SNPs for IgE; and none for Pv-AMA1. However, there was no SNP which gave significant association in all tested antibodies. Preliminary evidence is in favour of a genetic basis for susceptibility to or protection against malaria infection in this population, which may or may not have links with the generation and/or maintenance of anti-malarial antibodies, the levels of which appear to be maintained in spite of low malaria transmission levels.

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THE APICOPLAST GENOME OF *PLASMODIUM VIVAX*

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Plasmodium vivax is responsible for causing greater than 60% of human malaria cases in Southeastern Asia and the Indian subcontinent. The World Health Organization has projected that of the 100 million total cases of malaria in the South East Asia Region, 70% occur in India with more than 50% (35 million) owing to *P. vivax*. The rising severity of the disease and the resistance shown by the parasite towards usual therapeutic regimen has put forth a demand for a novel drug target to combat this disease. Apicoplast, an organelle of prokaryotic origin, and its circular genome are being looked upon as a potential drug target. The Apicoplast genome is known to carry various genes of functional importance. Except for a few reports, this genome has not been detailed from *P. vivax*. Our group for the first time has reported any complete gene (*tufA*) from this genome of *P. vivax* (as reported previously). In the present study we have characterized major genes of the IR-A region and some genes of the IR-B regions of this genome. These include *ssu* and *lsu* ribosomal RNA and *tRNA* genes, *sufB*, *clpC*, genes, *RNA Polymerase B*, *C* and *D* subunit genes and various ribosomal protein genes. The Apicoplast genes were amplified and sequenced from *P. vivax* field samples. A comparative analysis of *P. vivax* Apicoplast genes with alleles from other *Plasmodium* species (especially *P. falciparum*) was performed along with codon usage pattern. About 8 - 13% differences were observed at both nucleotide and amino acid level. Peptides based on *P. vivax* Apicoplast Ef-TuA were used to colocalize the organelle in *P. vivax* infected blood smear slides obtained from the field..

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HIGH THROUGHPUT GENOMICS SCREENING FOR MALARIA ANTIGEN DISCOVERY

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Malaria is the most burdensome parasitic disease of man, exacting an estimated toll of 863,000 deaths and 243 million clinical cases per year. It is important to develop a vaccine that can effectively prevent the disease. Up to now, there are few identified malaria antigens, representing less than 0.3% of the 5,300 proteins encoded by the *Plasmodium* parasite, and those have limited efficacy in vaccine clinical development,. Here, we report a new approach employing adeno-array technology for high throughput discovery of pre-erythrocytic *Plasmodium falciparum* antigens using orthologues identified in the *Plasmodium yoelii* mouse model. To obtain molecular information for highly expressed *P.yoelii* pre-erythrocytic antigen genes, we performed bioinformatics data mining using publicly available genomic and proteomic databases. Based on expression abundance data from microarray analysis and protein mass spectrometry analysis by several research groups, we prioritized sporozoite stage and liver stage candidate *P. yoelii* genes with identifiable *P. falciparum* orthologues for amplification and cloning. Amplified *P. yoelii* genes were first cloned into a shuttle plasmid, tested for appropriate insert size using colony PCR and then transferred to the pAdFlex vector, which contains the full length, E1/E3-deleted adenovirus type 5 genome. pAd*Py* adenoplasmids were then purified and transfected into 293 cells seeded in 96 well plates. After several passages, the cell lysates containing high titer Ad*Py*Antigen vectors are utilized to perform the antigen discovery screen and archived for titering and identity analysis. Currently, we are building the Py adenovector array. In the antigen discovery screen, we will infect antigen presenting cells with individual adenovectors from the Py array. The infected APC will be incubated with splenocytes from mice immunized with known protective regimens of Radiation Attenuated Sporozoites (RAS), and antigen-specific CD8+ T cell responses will be measured by the FACS analysis. Novel P. yoelii antigens identified as targets of RAS vaccination induced T cells will be presented.

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CHARACTERIZATION OF MAL13P1.319, A *PLASMODIUM FALCIPARUM* SPOROZOITE GENE

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During the life cycle of *Plasmodium falciparum*, the most pathogenic of the human malaria parasites, sporozoites are infectious for both the mosquito salivary gland and vertebrate tissue. Since this dual infectivity of sporozoites is critical to the survival and development of the parasite, the sporozoite presents an effective target for control by vaccines, drug therapy, and/or novel mosquito control methods. In an effort to discover molecules that aid sporozoite invasion of host tissue, a *P. falciparum* sporozoite gene known as *MAL13P1.319* was identified by a search of the annotated *Plasmodium* genome database (PlasmoDB) using specific criteria, i.e., presence of a signal peptide sequence, expressed as a sporozoite protein as shown by mass spectrometry, and predicted to be a surface or secreted protein. The *P. falciparum* MAL13P1.319 protein demonstrates significant protein homology with other *Plasmodium spp*. and appears to be unique to *Plasmodium spp*. Transcription of *MAL13P1.319* during the sporozoite and erythrocytic stages, which was reported in PlasmoDB, was confirmed by RT-PCR and protein expression during the sporozoite and erythrocytic stages was demonstrated by immunofluorescent assays and Western blot analysis. Currently, we have transfected a MAL13P1.319-GFP construct into erythrocytic stage parasites to analyze MAL13P1.319 protein trafficking in various stages. To assess the functional role of *MAL13P1.319*, a gene disruption construct has integrated into the *MAL13P1.319* chromosomal location and a clonal population will be obtained via limiting dilution. We also have initiated a comparative study of the *P. berghei* ortholog of *MAL13P1.319* by analyzing its gene/protein expression and assessing a functional role. Overall, these studies will be used to analyze the role of *MAL13P1.319* in sporozoite biology and, more specifically, to determine if it has a role in host tissue invasion.

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CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* PROTEIN, PFE0565W

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Malaria is a resurging disease due, in part, to resistance that has developed in *Plasmodium spp.* and mosquitoes against drugs and insecticides, respectively. Because of this disease resurgence, better control methods are in great need. A key stage in the *Plasmodium* life cycle is the sporozoite because it exhibits dual infectivity in both the mosquito vector and vertebrate host and, therefore, is a promising target for discovering effective ways of controlling malaria. The *P. falciparum* gene, *PFE0565w*, was chosen as a candidate for study due to its potential role in the invasion of host tissues. This gene was selected based on data from PlasmoDB, indicating that it is expressed both at the transcriptional and protein levels in sporozoites and likely encodes a putative surface protein. Additional sequence analysis shows that the PFE0565w protein has orthologs in other *Plasmodium* species, but none outside of the genus *Plasmodium*. PFE0565w expresses transcript during both the sporozoite and erythrocytic stages of the parasite life cycle. The PFE0565w protein is expressed on the sporozoite surface, as suggested by confocal microscopy. In contrast, the protein is not expressed during the asexual stages, as demonstrated by both Western blot analysis and confocal microscopy. A GFP-trafficking construct has been made and studies are in progress to track the expression profile of the PFE0565w protein throughout the parasite's life cycle and to confirm protein expression results described above. Furthermore, both gene disruption and deletion constructs have been successfully created for PFE0565w and studies are in progress to assess the function of the protein in parasite development and to determine if it plays a role in host tissue invasion. Lastly, a comparative study between the *P. berghei* ortholog of PFE0565w, PB107985.00.0, is in progress.

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A 7TM-RECEPTOR FAMILY MEMBER IN *PLASMODIUM* PLAYS A ROLE IN PARASITE VIRULENCE AND INFECTIVITY

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The genome of *Plasmodium* encodes a number of multipass-transmembrane domain proteins that bear the structural hallmarks of integral membrane receptors mediating intercellular and environmental signal transduction. One of these, PFL0765w, is a member of a phylogenetically widespread family of putative G-Protein Coupled Receptors (GPCR) found in a range of mammals, insects, plants and protozoa, including proteins with heterotrimeric Gα-protein binding activity in *Arabidopsis thaliana*. PFL0765w is expressed in gametocytes, maturing trophozoites and in schizonts adjacent to the rhoptry bulb protein, RAP1. To investigate a role in the initiation of signal transduction pathways in *Plasmodium*, we have generated PFL0765w-orthologue gene knockout lines in the rodent malaria model, *P. berghei,* and performed phenotypic analyses throughout the parasite lifecycle in the mouse and mosquito. Knockout parasites are significantly less virulent than wildtype parasites and show a marked reduction in infectivity.

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MITOCHONDRIAL GENETIC VARIATION AND EVOLUTIONARY HISTORY IN ASIAN *PLASMODIUM VIVAX*

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The genetic diversity and population structure of *Plasmodium vivax* were investigated using mitochondrial (mt) genome sequences of parasite samples from China, Myanmar and Korea. Among 95 samples, 32 different haplotypes were defined by 29 polymorphic sites. Overall haplotype diversity and nucleotide diversity were 0.88 and 0.15, respectively. Coding sequence diversity was large with an average of one SNP every 193 bp. A total of 17 single nucleotide polymorphisms (SNPs) were detected in the coding region, resulting in 10 mutated codons. Minimum spanning network analysis of the resulting data, combined with those from previously published populations, revealed that the *P. vivax* population from Myanmar and temperate-zone *P. vivax* parasites from China branch might share the same ancestor with the population from India. This study provides further resolution of the population structure and evolution of *P. vivax*, especially in temperate-zone endemic areas.

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REDUCTION OF MALARIA TRANSMISSION BY MASS TREATMENT: A COMPARISON OF OPERATIONAL STRATEGIES

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Mass treatment as a means to reducing malaria transmission was largely discontinued after the first Global Malaria Eradication Programme but is currently being reconsidered by several regional control programmes. In the past it has shown great variation in impact in different settings. We used a dynamic transmission model to explore both the short and long term impact of possible mass treatment strategies. Our results confirm that the expected effect of a single round of mass treatment would be temporary, assuming that 100% identification and cure of cases is highly unlikely. In a scenario of high transmission with moderately seasonal vector populations and initial mean slide prevalence of 60%, slide prevalence is predicted to return to its original level within 1 year. Where slide prevalence is initially at a mean of 5% this is estimated to take ~2 years. Using gametocytocidal drugs could give some advantage, based on artemisinin and primaquine impact on infectiousness observed in clinical trials, however the reduction in transmission was limited by the reservoir of infection in those not participating in the intervention. Screening for infection and treating only test-positives would reduce numbers of treatment courses required by 70-95% in low to moderate transmission settings, but was estimated to achieve only 60-75% of the cumulative impact on transmission, mainly due to lack of prophylaxis in test-negatives. Annual mass treatment could achieve a 20-60% reduction in mean slide prevalence if sustained but was not predicted to eliminate malaria unless initial transmission levels were very low. Intense, fortnightly mass treatment for a period of 2-3 months has a limited probability of achieving local elimination in low-to-moderate transmission settings, however it would be endangered by repeated non-participation of individuals and by immigration. Increasing vector control would delay and reduce the reinvasion of the population by the parasite. The transmission reduction achieved by mass treatment needs to be carefully weighed against the enhanced risk of drug resistance.

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EFFECTS OF ANAEMIA ON THE EMERGENCE, CLEARANCE AND SEX RATIOS OF *PLASMODIUM FALCIPARUM* GAMETOCYTES IN MALARIOUS CHILDREN

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Anemia, in *falciparum* malaria, is associated with an increased risk of gametocyte carriage but its effects on transmission have been little evaluated in malarious children. The effects of anemia, defined as a haematocrit < 25%, on the emergence, clearance, population structure, inbreeding rates, and the temporal changes in *Plasmodium falciparum* gametocyte sex ratios were evaluated in 802 children with acute infections treated with artemisinin-based combination therapies (ACTs). Gametocyte sex was determined morphologically, and sex ratio was defined as the proportion of gametocytes that are male. Pre-treatment gametocyte carriage in all children was 8.5% (68/802) and was similar in children with or without anemia (9.4% v 8.3%). Following treatment, the emergence of gametocytes seven days after treatment began was significantly more frequent in anaemic children (7/106 v 10/696, P = 0.002), but gametocyte clearance was similar (2.1 d v 2.4 d). Pre-treatment sex ratio (0.36, 95%CI 0.1- 0.65 v 0.25, 95% CI 0.15-0.35, P = 0.5) was similar but estimated inbreeding rates (the proportion of a mother's daughters that is fertilized by her sons) was lower (0.28 v 0.50) in anaemic children. Pre-treatment sex ratio became more female-biased in non-anaemic children following treatment but in anaemic children, it became male-biased. Sex ratio 3 d after treatment began was significantly lower and more female-biased in non-anaemic children (P = 0.027). Anemia significantly increases gametocyte emergence and may significantly alter the sex ratio after treatment with ACTs. These findings may have implications for malaria control efforts in endemic settings.

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INCREASED AND SUSTAINED RESOURCE MOBILIZATION PREVENTION USING COMMUNITY HEALTH WORKERS (CHW'S) AS KEY INTERVENTION AGENTS IN THE DISTRIBUTION OF INSECTICIDE TREATED NETS (ITNS)

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In support of Zambia's national malaria control efforts, there was substantial national and external donor investment beginning in 2004 and increasing through 2007. However, for the past 24 months, there have been markedly reduced disbursements from Global Fund resources for malaria. We examined potential consequences of this growth followed by reduction in resources using ITN procurement and distribution systems as a marker for change. We examined national and donor financing for ITN procurement between 2005 and 2009 and examined ITN distribution and coverage and changes over time across the 72 districts in the 9 provinces of Zambia. Donor financing specifically applied to ITN procurement and distribution increased annually between 2004 through 2007 due largely to substantial investments from Global Fund, World Bank, MACEPA, and United States Agency for International Development&PMI. In 2008 and 2009, GF resources for ITNs decreased dramatically; World Bank resources for ITNs were about 5 million dollars overall, ITN distribution varied substantially on an annual basis (from 1,560 000 distributed in 2005, 1,399,000, 3,453.414 in 2007, 964553 in 2008 and 1,396,347 distributed in 2009). With an estimated 3-year life span of ITNs, if current financing for ITNs is maintained at the 2008/9 level, coverage rates ie actual use of ITNs will drop nationally by 15% from 48% to 33% by 2012. Similarly, assuming stable usage rates in households, only 4% of children under-5 years of age will be using ITNs in 2012. In conclusion, malaria control in Zambia, like many other African countries, is currently heavily reliant on stable financing and procurement and delivery to scale up ITN coverage and maintain that prevention. Recent declines in available resources may have dramatically altered the recent progress in Zambia.

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IMPACT OF ARTEMETHER-LUMEFANTRINE ON MALARIA TRANSMISSION AND UNDER FIVE MORTALITY IN TWO RURAL DISTRICTS OF TANZANIA

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Deployment of artemisinin-based combination therapy (ACT) with other malaria control measures is key to reduce malaria transmission and overall under five mortality. To date, there is scanty evidence about the contribution of ACTs such as artemether-lumefantrine (AL) in malaria endemic Africa. As part of the ALIVE [Artemether-Lumefantrine In Vulnerable patients: Exploring health Impact] project, we assessed the impact of the introduction of AL as first line treatment for uncomplicated malaria on parasite prevalence, anaemia and under five mortality. Parasite and anaemia prevalence were obtained by repeated cross-sectional surveys conducted in two rural districts (Kilombero and Ulanga) in Tanzania during the sulfadoxine-pyrimethamine (SP) era (2005 & 2006), and after AL introduction beginning in 2008 i.e. 18 months after AL introduction. Mortality rates were obtained using a Demographic Surveillance System (DSS) that covers a dynamic expanding population of about 90,000 in the same districts. Mortality rates of children under five were compared to rates obtained 2 years during and post the SP era (2007 & 2008).

A total of 5903 persons were assessed in 2005, 6324 in 2006, 4557 in 2008 and 7454 in 2009. Asymptomatic parasite prevalence in the whole population was 11.4% in 2005, 13.6% in 2006, 11.0% in 2008 and 4.6% in 2009. Gametocyte carriage rates were 0.3% in 2005, 0.2% in 2006, 1.4% in 2008 and 0.4% in 2009. Prevalence of anaemia in children under five was 17.8% in 2005, 9.7% in 2006, 10.1% in 2008 and 10.9% in 2009. Population coverage with insecticide-treated bednets was 35%, 36%, 44% and 47% respectively. Under five mortality rate per 1000 person-years was 27.0 in 2005, 23.1 in 2006, 21.3 in 2007 and 18 in 2008. After 3 years of AL implementation, there was a considerable decline in parasite prevalence but no change in anaemia prevalence. On average gametocyte carriage rate has remained < 1% throughout the period. Mortality in children <5 years decreased, but trend was consistent with pre- and post-AL introduction.

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COVERAGE OF INSECTICIDE TREATED NETS AND INTERMITTENT PREVENTIVE THERAPY FOR THE CONTROL OF MALARIA IN PREGNANCY IN SUB-SAHARAN AFRICA: MAPPING PROGRESS

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Over the past 10 years, policies for intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) and use of insecticide treated nets (ITNs) for the control of malaria in pregnant women have been almost

universally adopted in sub-Saharan African countries. Both ITNs and IPTp are delivered through antenatal clinics alongside other antenatal care packages. We assessed progress in adoption and implementation of the ITN and IPTp strategies in Africa using information obtained from national surveys (DHS, MICS, MIS, and other). This was linked with estimates of malaria transmission maps obtained through Malaria Atlas Project. ITN use among women 15-49 years was used as proxy measure for ITN use among pregnant women because of the greater availability and the excellent correlation (Pearson 0.94). Results are presented at the subnational level (admin1). ITN policies for pregnant women could be identified for 45 of 47 malarious countries in sub-Saharan Africa; the median year of adoption was 2002 (range 1998-2007). Data from 32 surveys between 2004 and 2009 showed that the median reported ITN use among women aged 15-49 years was 13.8% (interquartile range [IQR] 4.9%-26.8%, n=286). Only 7 regions had ITN coverage of >=60%; all in countries that adopted ITNs for pregnant women >5 years ago (P=0.04) and in areas with a mean *Plasmodium falciparum* (Pf) prevalence among children 2-9 yrs of age between 10-49% (2007). Thirty nine countries have adopted IPTp (median year of adoption 2004, range 1993-2007). The median IPTp coverage (any source, any number of doses) was 17.0% (IQR 4.6-74.3%, n= 282 regions) in 36 countries that had an IPTp policy in place for ≥1 year at the time of survey; 49 regions from 9 countries had a coverage of ≥60%, 42 of these (85.7%) were in areas with a mean Pf prevalence among children 2-9 yrs (2007) of 10% or more, and 25 of them were in countries which had adopted IPTp >5 years ago (P<0.001). The median use of any drug for malaria prevention was 55.6 % (IQR 37.0-73.9%, 281 admin1 from 31 countries), and the median coverage of ANC (≥1 visit) was 88.1% (IQR 66.5-95.3%, n=342 admin1, 39 countries). In conclusion, ITN coverage is still below the Abuja target for many countries in sub-Saharan Africa. Considerable progress has been made for IPTp. The high utilisation of ANC and of use of drugs for malaria prevention in pregnancy indicates there is significant potential to improve malaria prevention among pregnant women.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT IN INFANTS WITH SULFADOXINE-PYRIMETHAMINE ON MORTALITY IN THE DISTRICT OF KOLOKANI, MALI

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Intermittent Preventive Treatment in infants (IPTi) with Sulfadoxine-Pyrimethamine (SP) given during routine vaccinations is efficacious in preventing malaria disease and anemia. However the impact on mortality remained to be established. To evaluate the impact of IPTi- SP on the mortality, the 22 health sub-districts in the district of Kolokani, Mali, were randomized in a 1:1 ratio and starting in December 2006, IPTi - SP was implemented for 12 months in 11 health sub-districts (intervention zone) while the other 11 health sub-districts served as the control (non-intervention zone). A cross-sectional survey was conducted in 98 randomly selected locations in March-April 2009 to determine mortality in children in the target age group during the implementation period (Dec 2006-2007) in both intervention and non-intervention zones. Causes of death were assessed using a post-mortem questionnaire. A total of 3,122 children (1,556 in each zone) were surveyed. Preliminary results indicated that during the intervention period, there were 79 deaths in the intervention zone (mortality = 5.04 %, 95% CI of 3.89% - 6.20%) and 109 in the non intervention zone (mortality = 7.01%, 95% CI 5.62% -8.40%), giving a protective efficacy against all cause mortality of 27.5% (95% CI: 2.3%-46.4% (p= 0.035). The differences in disease specific mortalities between the two zones were not statistically significant. In conclusion, this study shows significant reduction in overall mortality in IPTi intervention zone compared to the control zone during the IPTi implementation period and supports the introduction of IPTi-SP alongside other malaria control interventions.

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SOCIAL AND ENVIRONMENTAL DETERMINANTS OF CHILDHOOD MALARIA AND THE USE OF ITN: IMPLICATIONS FOR MALARIA CONTROL STRATEGIES IN THE DEMOCRATIC REPUBLIC OF CONGO

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Various malaria control strategies have been applied overtime in countries, however, without taking into cognizance the environmental and social context of a country, these strategies may be difficult to achieve one of its intended goal; reduction of childhood malaria incidence. We therefore, want to examine social and environmental determinants associated with childhood malaria and the use of ITN in the Democratic Republic of Congo. Social and Environmental factors can negatively or positively influence individual's decisions and functions taken on behalf of children which may eventually affect the effectiveness of the various strategies to combat this scourging disease by the government. The Democratic Republic of Congo is just recovering from years of war and to combat malaria which is one of the biggest health treat in the country, the government has started with various control strategies, including appropriate case management in both community and health infrastructures, and scaling up the use of insecticide treated nets (ITNs). However, almost 47.3% of the children die annually from malaria. The analyses for this study will be based on the 2007 Demographic Health Survey of the Democratic Republic of Congo. Finding relevant literatures that look into the association of disaster/war with malaria control strategies are very sparse. Therefore, the findings of this study are expected to significantly augment knowledge on what influences malaria control strategies in a war-torn environment.

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APPRAISING EFFECTS OF THE SUPPLY CHAIN OPERATIONS OF THE LONG-LASTING INSECTICIDAL NETS (LLINS) MASS CAMPAIGNS IN NIGERIA ON OWNERSHIP AND USAGE

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Widespread use of long-lasting insecticidal nets (LLINs) within a community is a proven intervention for malaria control. Therefore, a widespread effort is being made to distribute 63 million LLINs in Nigeria, which will achieve universal coverage, before the end of 2010_a country that shoulders 25 percent of the Africa malaria burden. Between May 2009 and April 2010, the National Malaria Control Program, with support from various partners, procured and distributed 18.5 million LLINs in 11 states (representing over 30% 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% fulfil 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 qualitative appraisal of implementation strategies is underway for the supply chain operations of the LLINs universal coverage campaigns in Nigeria. Thee are preliminary findings. Although most of the households are satisfied with the distance they have to travel to the distribution points, they have major concerns

about crowd control, waiting time to collect the bed nets, and the attitude of the personnel. The recipients also question the effectiveness of the nets. Currently, there is no specific strategy to conclusively address beneficiaries' complaints about acquisition and use of the LLINs. Even though the policies and guidelines are strong enough, program managers find it difficult to determine what needs to be done and to respond quickly to unforeseen challenges, which are frequently part of campaign activities. To address these constraints, program managers and the Roll Back Malaria partners are developing the necessary framework to deal with these issues and to improve LLIN ownership and use at the household level.

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THE MALAWI NATIONAL MALARIA CONTROL PROGRAM'S "YEAR OF ACTION-2010": GAUGING PROGRESS TOWARD MALARIA CONTROL

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Malawi is receiving support from the President's Malaria Initiative and the Global Fund to implement its National Strategic Plan (2005-2010). The primary strategic areas identified for the scale-up of malaria control activities include case management, intermittent preventive treatment of pregnant women with SP (IPTp) and malaria prevention with emphasis on the use of insecticide treated nets.

UNICEF's Multiple Indicator Cluster Survey in 2006 is the most recent nationally representative evaluation that has served as a baseline of select malaria control activities before increased resources were made available. In this "year of action," NMCP is undertaking an RBM supported Malaria Program Review, a study evaluating artemether-lumefantrine efficacy and the nation's first ever Malaria Indicator Survey (MIS) to help inform the new strategic plan. A recent household survey completed in 8 of Malawi's 28 districts in 2009 has revealed interval improvement since the MICS results. Select indicators document that net usage in children under 5 increased to 61% from 23% in 2006 and coverage with 2 doses of IPTp reached 72% compared to 46% of women in 2006. Malawi's 2010 MIS is underway. Fieldwork and data collection was completed in April 2010. Children under 5 from 3500 households were interviewed. Preliminary results will be available in June 2010.

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REDUCTION IN ANEMIA IN CHILDREN UNDER TEN YEARS OF AGE AFTER DISTRIBUTION OF LONG-LASTING INSECTICIDAL NETS (LLIN) FOR CONTROL OF MALARIA AND LYMPHATIC FILARIASIS IN FOUR LOCAL GOVERNMENT AREAS (LGAS) IN SOUTHEAST NIGERIA

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In an integrated filariasis and malaria project, LLIN were distributed to all households in 4 LGAs in two states in South East Nigeria starting in April 2008. Two LGAs received LLIN for vulnerable persons (pregnant women and under fives) only while the others were targeted for full coverage.

At annual representative household surveys, household net ownership increased from a mean of 0.1 nets per household in 2007 (N=968) to 1.2 in 2008 (N=1078). However by 2009 net ownership had dropped to 0.8 per household (N=1294). Net use (all ages) showed a similar pattern, increasing from 2.0% of persons (N=5197) sleeping under nets in 2007 to 35.5% in 2008 (N=5200) but dropping to 21.4% in 2009 (N=5200). A higher proportion of children under five slept under nets in the full coverage arm (61.0%, 95% CI 50.7_70.4%) than the vulnerable arm (24.8%, 95% CI 18.2_ 32.8%) in 2008. Prevalence of malaria (any species) by blood slide declined significantly between 2007 and 2008: from 22.2% (N=1093; 95% CI 19.3-25.5%) in 2007 to 12.6% (N=1446; 9.7-16.2) in 2008. Within study arms, the decrease in malaria prevalence was significant only in the full coverage LLIN arm (22.3% (N=552) to 10.1% (N=681), $p<0.001$) but not in the vulnerable group arm (22.1% (N=541) to 14.9% (N=765), $p=0.79$). The mean hemoglobin (Hb) in children under 10 increased significantly in both study arms, from 9.5 g/dl (N=453; 95% CI 9.2 - 9.8) overall in 2007 to 10.6 g/dl(N=629; 10.4 - 10.8) in 2008; it remained higher at 10.3 g/dl (N=704; 10.1-10.5) in 2009. The proportion of children with moderate to severe anemia (defined as Hb<8 g/dl) declined significantly from 15.1% (95% CI 10.7-21.0) in 2007 to 2.3% (1.0% to 5.0%) in 2008 and 7.9% (5.8-10.6) in 2009. The difficulty of sustaining LLIN programs after initial increase is demonstrated by a drop in net ownership and use in the third year of the study. Nevertheless the results show that community-wide distribution of LLIN (as compared to only targeting vulnerable groups) is the best way to protect children in the study area from malaria and anemia.

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ZEROVECTOR® DURABLE LINING (DL) - A PROGRAM EXPENDITURE MODEL

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Community level malaria protection requires coverage and usage of an effective tool by >80% households. Durable Lining (DL) is an innovative technology for disease prevention that reduces vector densities and breaks transmission cycles. The product has residual efficacy that meets international standards for effectiveness, shown after two years of field use in Nigeria. Durable Lining (DL) may eliminate future need for IRS in rural communities. Measurements of room sizes, labor and installation activities, material and logistical requirements were collected from trials conducted in Mali, Ghana, and South Africa in 2008/09. The data were used to analyze prospective costs associated with an installation project for DL. The cost analysis was converted to a program where estimates for the various program components could be entered and an expenditure budget modeled. The extent of accuracy that the model is capable of providing is determined by the level of detail used for input. General information about project scale and timing can result in a cost estimate sufficient for project planning. When details about exact number of rooms where DL is to be installed, distances between houses and villages where installation is to occur, specific dates for the project start and completion, wage rates, and fuel costs are entered the model can produce a budget that can be used for program pricing considerations. An additional feature is that similar data for indoor residual spray programs (IRS) can be entered and a comparison between the options produced. The complexities of planning, preparing, implementing, and maintaining Durable Lining are incorporated into a model that estimates program expenditures and total budget requirements for an installation project. Expenditure components in the model include materials, delivery, training, labor, transportation, administration, monitoring, and community awareness activities (IEC). The model can be used to make direct comparisons with cost of alternative vector control options such as IRS.

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PROXIMITY TO HEALTH SERVICES AND GEOGRAPHIC FEATURES DETERMINE INSECTICIDE TREATED NET USE AMONG 5-30-MONTH OLD CHILDREN IN MALAWI

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Malaria prevention with insecticide-treated nets (ITNs) depends on both their availability and proper use. Particularly in rural areas, distance to ITN providers may impede prevention efforts. We undertook a large-scale, household-level, population-based survey to determine ITN use and malaria intensity among 5-30 month old children throughout Malawi during May 2007. In addition to questions involving demographic and environmental risk, child ITN use during the preceding 24 hours was determined. Traditional multivariable statistical methods and propensity analysis were used to evaluate how proximity to health services was associated with ITN access and use. Rates of reported ITN use were negatively associated with distances to nearest health facility, both for the shortest linear, "crow-fly" distance and for travel by road. "Hockey-stick" regression indicated that probability of ITN use declined as distance to facility increased to a breakpoint of 2.1 km ("crow-fly") and 4.7 km (road distance). Among the most proximal 20% of households, ~65% reported using ITNs in the past 24 hrs. Beyond the breakpoint, only ~40% of households reported ITN use. Access to material resources appeared to have a confounding relationship with facility distance, but ITN patterns remained similar even after controlling for material wealth. Propensity analysis was used to estimate direct effects of health facility access using 2.1 and 4.7 km as proxy measures for accessibility. After controlling for all factors that might predispose households to be located beyond these breakpoints, reported ITN use was significantly lower (OR = 0.81) compared to those living closer to health facilities. Our findings demonstrate that proximity to health services was strongly correlated with reported ITN use. This could indicate that access not only results in more available health resources, but also may reinforce health behaviors. We suggest that equitable and regular access to health services would reduce the burden of malaria, and hence should be a priority for health policy makers.

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CAUDAL CONTROLS VECTOR COMPETENCE FOR *PLASMODIUM FALCIPARUM* AS A REGULATOR OF THE TRIPARTITE INTERACTIONS BETWEEN THE INNATE IMMUNE SYSTEM, THE MICROBIOTA AND THE MALARIA PARASITE

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Anopheles gambiae, the major vector for the human malaria parasite *Plasmodium falciparum* in sub-Saharan Africa, uses its innate immune system to defend against *Plasmodium,* mainly via the Toll and Imd (Immune Deficiency) signaling pathways. Interestingly, these immune pathways are also activated by the microbiota present in the mosquito midgut, which is the primary site for *Plasmodium* invasion and development (as reported previously). *Caudal* was first identified in *Drosophila* as a developmental transcription factor as well as a negative regulator of the Imd pathway -mediated activation of the Relish transcription factor (as reported previously). We have shown through RNAi-based silencing assays that depletion of the *An. gambiae Caudal* results in a significant reduction of the midgut microbiota as well as a change of its species composition. Interestingly, *Caudal* is also a highly potent regulator of vector competence for *P. falciparum* while its implication in the defense against the rodent parasite *P. berghei* was weak. Our previous studies have also shown that the Imd pathway more efficiently defends against *P. falciparum* than *P. berghei* (as reported previously). These findings suggest that the *An. gambiae Caudal* controls the finely tuned tripartite interactions between the innate immune system, the midgut microbiota, and the *Plasmodium* parasite as a factor of the Imd pathway. We are currently conducting comprehensive whole-genome microarray studies to better understand *Caudal's* relationship to the Imd and Toll pathways and to identify potent anti-*Plasmodium* effectors that are transcriptionally controlled by this immune regulator. We also present studies on *Caudal*'s role in regulating the midgut microbial load and composition in field-derived *Anopheles arabiensis* mosquitoes, a key vector of malaria in southern Zambia.

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REACTIVE OXYGEN SPECIES-DEPENDENT CELL SIGNALING REGULATES THE MOSQUITO IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM*

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Plasmodium parasites undergo a series of complicated transformations inside the mosquito vector during which they experience significant losses. This is due in part to the mosquito innate immune response, yet the details of the cell signaling pathways that regulate this response are poorly understood. We have previously shown that provision of human insulin in a blood meal leads to increased reactive oxygen species (ROS) and decreased antioxidant production in the mosquito midgut. ROS have been implicated in direct killing of pathogens, increased tissue damage, and regulation of immune signaling pathways; all of which could affect the establishment of *Plasmodium* infection in mosquitoes. Here, we demonstrate that provision of human insulin in *P. falciparum* infected blood meals fed to *Anopheles stephensi* resulted in a trend towards increased parasite development. The addition of antioxidant significantly decreased parasite numbers in insulin-fed mosquitoes, suggesting that insulin-induced ROS are involved in the establishment of parasite infection. Our data suggest that the effect of human insulin on parasite development is not a result of ROS-induced parasite killing or tissue damage. Rather, our studies demonstrate a role for ROS in mosquito cell signaling. ROS scavenging by antioxidants resulted in decreased phosphorylation of downstream effectors of the MAPK and PI3K/Akt signaling pathways in mosquito cells. Although ROS are required for signaling downstream of insulin stimulation, scavenging of ROS had no effect on TGF-beta1-dependent MAPK activation. Furthermore, we found that ROS alone can directly activate both the MAPK and PI3K/Akt signaling pathways in mosquito cells. Taken together, these data highlight a novel and specific role for ROS as mediators of *A. stephensi* cell signaling processes that are involved in the innate immune response to *Plasmodium* parasites.

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SEMINAL FLUID PROTEIN IDENTIFICATION AND POTENTIAL FUNCTIONS IN THE DENGUE VECTOR, *AEDES AEGYPTI*

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New tools that target mosquito control may reduce vector populations and break the cycle of dengue transmission. Male mosquito seminal fluid proteins (Sfps) are one such target since these proteins, in aggregate, modulate the reproduction and feeding patterns of the dengue vector, Ae. aegypti. We identified 95 proteins of *Aedes aegypti* ejaculate that are transferred to females during mating. Using a stable isotope labeling

method, we identified sperm proteins and Sfps transferred from males to females. Sperm proteins were distinguished from Sfps by comparing the transferred proteins to sperm-enriched samples we analyzed from testes or seminal vesicles. We identified and confirmed transfer to females during mating for 56 Sfps and 39 predicted sperm proteins. The Sfp classes detected suggest roles in sperm fertility and protection from oxidative stress, semen coagulation, ecdysteroidogenesis, and protein activation/inactivation. Many of the *Ae. aegypti* predicted sperm proteins were homologous to Drosophila melanogaster sperm proteins, suggesting conservation of their sperm-related function across Diptera. This is the first study to directly demonstrate transfer of seminal fluid proteins from male *Ae. aegypti* to females.

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CAN HUMAN INSULIN AND IGF-1 SURVIVE THE MOSQUITO MIDGUT?

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Insulin and insulin-like growth factors (IGF) present in a mosquito blood meal have insect homologues, which opens the possibility of cross-talk between vertebrate hormones and insect receptors. Bovine insulin, for instance, has been demonstrated to stimulate ovaries of *Aedes aegypti* to produce ecdysteroids, as reported previously. Insulin signaling is relevant to disease transmission as activation of the insulin/insulin-like growth factor signaling pathway affects mosquito longevity and therefore the amount of time an infected mosquito can vector pathogens, as reported previously. In this study, the fate of human insulin and IGF-1 was investigated to determine their viability as signaling molecules after ingestion by *Anopheles stephensi*. Female *An. stephensi* were fed washed red blood cells spiked with physiological concentrations of radiolabeled insulin or IGF-1 through artificial feeders. Mosquitoes were dissected every 6 h for 48 h and autoradiography was used to estimate the amount of intact insulin or IGF-1 present in the midgut and hemolymph over time. Insulin and IGF-1 persisted intact in the midgut for up to 18 h. Intact insulin and IGF-1 were also detected in hemolymph samples showing that these molecules can cross the midgut and exist as viable signaling molecules within the mosquito.

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TISSUE DISTRIBUTION, BIOACTIVITY AND SIGNALING OF OVARY ECDYSTEROIDOGENIC HORMONE IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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Egg maturation in female *Aedes aegypti* is dependent on the release of neuropeptides from the medial neurosecretory cells in the female brain after blood ingestion. Ovary ecdysteroidogenic hormone (OEH) is one such peptide which shows gonadotropic activity when injected in blood-fed decapitated females and ecdysteroidogenic activity when incubated with dissected ovaries *in vitro*. A previous molecular characterization of *Ae. aegypti* OEH focused on a truncated version of the full length protein. In this study, we compare the bioactivity of short and long forms of OEH and determine the tissue distribution of OEH transcript and peptide in all life stages and during a gonadotropic cycle. In addition, we seek to identify a receptor or binding protein for OEH and determine whether there is any interaction between OEH and insulin-like peptides, also known to stimulate egg maturation. Insight into this endocrine cascade may lead to novel controls for mosquito reproduction.

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ROLE OF INSULIN SIGNALING IN BLOOD MEAL NUTRIENTS STORAGE AND VITELLOGENESIS IN *AEDES AEGYPTI*

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Reproduction in mosquitoes encompasses a highly regulated sequence of behavioral, metabolic, and synthetic processes that result in the production of eggs. As in all other animals, peptide hormones bind to cell receptors that activate signal transduction pathways to provide precise regulation of these physiological processes. In vertebrates, insulins are important growth factors and multifunctional regulators of metabolism. However, insulin like peptides (ILPs) are not restricted to vertebrates, but have also been identified in invertebrates including mosquitoes. Earlier studies from mosquitoes indicated that blood feeding stimulates egg development by triggering the release of peptide hormones from neurosecretory cells in mosquito brain. It has also been demonstrated that at least one ILP in *Aedes aegypti* regulates carbohydrate and lipid stores in the same way as insulin in vertebrates. An insulin receptor (IR) has also been identified from several mosquito species including yellow fever mosquito, *Ae. aegypti.* Knock down of IR expression by RNAi decreased metabolic stores and yolk synthesis in blood fed mosquitoes. Our results indicate that insulin signaling regulates mosquito reproduction and metabolism.

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ANOPHELES GAMBIAE: MULTIPLE IMMUNE SURVEILLANCE MECHANISMS FOR GUT COMMENSAL MICROBES

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All life stages of mosquitoes are in close association with commensal bacteria. This relationship plays a prominent role in the regulation of the mosquito's basal immunity. In an attempt to understand how mosquitoes cope with the gut commensal microbes, we monitored the gut expression of antimicrobial peptide (AMP) genes *Defensin 1, Defensin 4,* and *dual oxidase (Duox)* that mediates the reactive oxygen species (ROS) immunity as well as immune regulators *Caudal* and *PGRP-LB*. *Def 1* transcription is mediated by NF-κB (IMD and/or Toll pathways), and Caudal can reduce the NF-κB activity by occupying the NF-κB site at the promoter of an AMP gene. In the gut *Def1* and *Caudal* showed opposite transcription pattern, suggesting a balanced NF-κB mediated AMP production. *Def 4* transcription is regulated by an unknown mechanism. Gut *Def 4* and *Duox* showed a similar expression pattern throughout the life-stages of the mosquito, which suggests that they both are required to maintain the gut homeostasis. *PGRP-LB* is constitutively expressed throughout all stages. As an amidase PGRP-LB reduces the peptidoglycan load to ensure an appropriate level of IMD signaling to the commensal habitants in the gut. Our data suggest the gut homeostasis is maintained by multiple immune surveillance mechanisms.

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GENERAL PRINCIPLES AND NOVEL POSSIBILITIES FOR SINGLE-CONSTRUCT GENE DRIVE

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Mosquito-borne diseases such as malaria and dengue fever continue to pose a major health problem through much of the world. Several new approaches to disease control utilize gene drive systems to spread refractory genes into mosquito populations. Recently proposed gene drive systems include Medea, homing endonuclease genes and underdominance constructs. Through mathematical analysis of the population genetics of single-construct systems, we show that the gene

drive systems currently under consideration only represent a fraction of the full range of possibilities. We summarize the conditions that must be satisfied for a single-construct system to spread to fixation in a population or to induce a population crash. We also describe basic properties of the release threshold, above which a gene drive system is expected to spread into a population. Both autosomal and X-linked constructs are considered. Many of these hypothetical gene drive systems are prohibitively difficult to engineer; however several can be engineered with simple combinations of toxins and antidotes. We highlight a number of novel possibilities including inverse Medea constructs, which consist of a zygotic toxin linked to a maternal antidote; and Semele constructs, which encode semen-based lethality for which transgenic females possess an antidote. Implications for disease control are discussed.

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THE REGULATORY ROLE OF VITELLOGENIN PROMOTER DRIVEN *ANOPHELES GAMBIAE* NF-KB REL2 TRANSGENE IN THE DEFENSE AGAINST *PLASMODIUM* PARASITES AND OTHER MICROBES

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The innate immunity of mosquitoes is the primary line of defense against the malaria parasite *Plasmodium* and other microbes. It mainly comprises of the TOLL and IMD pathways where the two NF-kappa B-like transcription factors, Rel1 and Rel2 translocates to the nucleus and activates the transcription of several antimicrobial peptides and many other effector genes. IMD pathway is the major player in regulating resistance of several *Anopheles* species to numerous malaria parasites and is more likely appealing for the generation of genetically modified mosquitoes over-expressing Rel2 that are resistant to *Plasmodium* species. The Rel2 gene (orthologous to Drosophila Relish) of the malaria vector *Anpoheles gambiae*, has been shown to control the expression of several immune genes (LRIM1, CLIPB14, KIN1, FBN etc) and antimicrobial peptides and also regulate the bacterial and *Plasmodium* infections. The *A. gambiae* Rel2-S (Rel2 short form lacking the inhibitory ankyrin repeats and death domain) transcript has been cloned under the *A. gambiae* vitellogenin promoter to generate blood-fed inducible Rel2 transgenic mosquitoes (fat-body specific) in *Anopheles* stephensi. We have observed a decreased *Plasmodium falciparum* infection phenotype (~50% lower oocyst intensity); upon activation of Rel2 transgene (and the IMD pathway) after feeding on infectious blood-meal. Upon injection of gram positive and gram negative bacteria in blood-fed Rel2 transgene induced mosquitoes; their survival was better when compared to wild-type mosquitoes. The vitellogenin driven Rel2 transgenic mosquitoes were found to lay less number of eggs compared to the wild-type, however their longevity were very much comparable. We have furthermore explored the regulatory role of Rel2 in mosquito innate immunity in activation of other immune genes and effector molecules; Tep1, Defensin, LRIM1 were among the few which were found to be up-regulated in Rel2 transgene induced mosquitoes. Studies are ongoing to look at the *Plasmodium* infection phenotype after silencing of various immune genes in the Rel2 transgene induced mosquitoes.

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IDENTIFICATION OF AN IMMUNE-RELATED *ANOPHELES GAMBIAE* THREONINE- AND TRYPTOPHAN-RICH REPEAT (*AGTWRR*) MIDGUT PROTEIN

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Soon after ingestion by the female mosquito, the malaria parasite undergoes gametogenesis followed by fertilization and differentiation of the resulting zygote into motile ookinetes. The ookinete traverses the mosquito midgut to form an oocyst that when mature, releases thousands of sporozoites into the haemocoel. In turn, the sporozoites invade the salivary gland from where they move to the next human host when the mosquito bites again. Ookinete invasion of the mosquito midgut contributes to one of the biggest population bottlenecks of the *Plasmodium* spp. infection cycle. Here we report on a previously uncharacterized gene (AgTWRR), which contributes to this bottleneck. It encodes an unusual 515 amino acids-long protein that is rich in threonine (21.6%) and tryptophan (8.9%). Feeding mosquitoes *P. berghei*- or *P. falciparum*-infected blood or Gram-negative bacteria (*Escherichia coli*) greatly upregulated AgTWRR expression in the midgut. RNAi silencing of AgTWRR significantly increased the number of *P. berghei* parasites that developed into oocysts, indicating that this gene is part of the mosquito defense network against *Plasmodium*.

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MOSQUITO GUT-MICROBIOME-DENGUE TRIPARTITE INTERACTIONS INFLUENCES VIRUS INFECTION

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Dengue virus is one of the most important arboviral pathogens and the causative agent for dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Dengue virus is transmitted among humans by the mosquitoes *Aedes aegypti* and *Aedes albopictus* and it is estimated that at least 2.5 billion people are at daily risk of infection. During their lifecycle, mosquitoes are exposed to a range of microbes, some of which are needed for the successful growth into adulthood. Nevertheless, new evidence suggests that the microbial flora also plays an important role in influencing the mosquito's susceptibility to infections by other pathogens. Here we present an analysis of the interactions between the dengue virus, the mosquito and seven bacterial types isolated from midguts of field-collected mosquitoes. We observed a marked decrease in viral loads in mosquitoes infected with certain bacterial isolates as well as a dynamic modulation of the mosquito's immune system. Transcript abundance analysis of selected antimicrobial peptides suggests that the mosquito's microbial flora plays a critical role in the elicitation of immune activity that is in part responsible for the lower viral load. In short, this study assessed the effects of the endogenous microbial flora on mosquito dengue virus infection as well as the modulation of the mosquito's innate immune system, a tripartite interaction that likely defines viral transmission dynamics.

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CHARACTERIZATION OF ENDOGENOUS *HSP70* BASED INDUCIBLE PROMOTERS IN *AEDES AEGYPTI* EMBRYOS

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While inducible promoters capable of controlling transgene expression in specific tissues have been characterized for *Aedes aegypti*, no whole body, whole life stage inducible promoter has been described for this mosquito. Previously we characterized the gene structure and expression of heat shock 70 genes in *Ae. aegypti*. Preliminary experiments using *hsp70*-derived genomic fragments to drive transgene expression demonstrated high levels of transcription under stress conditions, as well as repression under control conditions for constructs containing the entire intergenic region between the *AaHsp70Aa/AaHsp70Ab*, and *AaHsp70Ba/AaHsp70Bb* genes. In order to further define an optimal *Aedes aegypti* Hsp70 promoter, deletion constructs of regions between *AaHsp70Aa/AaHsp70Ab* and *AaHsp70B/AaHsp70Bb* were produced and cloned into luciferase reporter constructs. Constructs were injected into *Aedes aegypti* embryos along with a Renilla luciferase control plasmid. Embryos were subsequently heat shocked at 39 C and harvested after 24 hours. Dual luciferase assays

were performed to compare the activity of each promoter construct. Significant differences in induction and repression were observed based on construct size. Isolating inducible *AaHsp70* promoter elements would be valuable for transgenesis and gene function studies, particularly when it is important to minimize the presence of exogenous sequences.

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ANOPHELES GAMBIAE SAGLIN AND SPOROZOITE INVASION: A TRANSGENIC ANALYSIS

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Sporozoite invasion of mosquito salivary glands is thought to involve specific receptor-ligand interactions. Saglin, a 100 kDa protein of *Anopheles gambiae* has been implicated as a target for *Plasmodium falciparum* sporozoite binding during salivary gland invasion. The hypothesis that saglin is directly involved in *P. falciparum* invasion of the salivary glands was tested by: 1) indirect immunofluorescence assays to determine the distribution of saglin on the surface of the salivary glands of female *An. gambiae* mosquitoes; and 2) by creating transgenic *An. stephensi* expressing *An. gambiae* saglin in the distal lateral lobes of the salivary glands and measuring *P. falciparum* abundance in transgenic salivary glands. Indirect immunofluorescence using an *An. gambiae* saglin-specific monoclonal antibody (mAb2A3) revealed the presence of saglin in the medial and proximal lateral lobes of the salivary glands of *An. gambiae*. This pattern of saglin localization was independent of the age of adult females or their gravid state. mAb2A3 did not recognize saglin in wild type *An. stephensi*. Transgenic *An. stephensi* expressing *An. gambiae* saglin under the control of an antiplatelet protein gene promoter exhibit strong constitutive transgene expression in the distal lateral lobes of the salivary glands. These transgenic mosquitoes showed no difference in their susceptibility to *P. falciparum* sporozoite invasion compared to salivary glands from non-transgenic *An. stephensi*. These observations do not support the hypothesis that saglin is directly involved in sporozoite invasion of the salivary glands.

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THE *AEDES AEGYPTI* TRANSCRIPTOME BY RNASEQ: A TOOL FOR VECTOR DISEASE CONTROL?

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Dengue is the most important arboviral disease with 50-100 million people infected annually and cases continuing to rise. The principal vector for Dengue viruses (DENV) is the mosquito, *Aedes aegypti*. The current lack of a vaccine and ineffective vector control create an urgent need for new control strategies. Recent advancements in mosquito molecular biology support the development of genetic control strategies whereby DENV-competent vector populations are replaced by mosquitoes unable to transmit viruses. Key to these strategies are the identification of promoters for expressing antiviral effector molecules, the synthesis of genes with anti-pathogen properties, the means to genetically modify mosquitoes in a stable manner with a minimum fitness load and the development of ways to introgress the antiviral-effector genes into field vectors. Illumina RNAseq technology was used to compare variation in gene expression profiles between blood- and sugar-fed mosquitoes. The results allow us to 1) refine the annotation of the *Ae. aegypti* genome, 2) analyze biochemical pathways and biological processes elicited by a blood meal, with particular attention to genes previously described as important for pathogen interactions, and 3) identify promoters and/or regulatory regions of genes highly-activated after a blood meal as candidates for driving the expression of anti-pathogen effector molecules. More than 40% of the transcripts detected were expressed differentially between blood- and sugar-fed mosquitoes. This variation in transcription corresponds with an enhancement of digestive activity and a down-regulation of genes involved in stimuli perception. Several genes previously linked to pathogen interaction also were expressed differentially. Putative cis-regulatory elements (CREs) were identified at the 5'-end flanking sequences of selected blood-meal activated genes. These CREs may be essential for accurate temporal and spatial promoter activity.

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UTILIZING TISSUE-ENRICHED EXPRESSION PROFILES TO ELUCIDATE HEMOCYTE TRANSCRIPTOME RESPONSES TO INFECTION IN *AEDES AEGYPTI*

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Hemocytes are fundamental elements of the mosquito host defense. They mediate immune mechanisms such as phagocytosis, melanization, and production of antimicrobial peptides. Previous studies investigating tissue-enriched expression of hemocyte transcriptome have not adequately addressed changes associated with infection status. Consequently, it has been difficult to identify specific transcriptional responses unique to hemocytes. Using genome-wide microarrays, we identified transcripts with enriched expression in circulating hemocytes with respect to remaining carcass (body minus hemocytes) in both naïve and immune-challenged adult female mosquitoes, and showed that infection response significantly alters the tissue enrichment ratios. Taking this effect into account, we took a combinatorial approach through integration of infection responsive expression profiles with tissue enrichment ratios, and resolved patterns of transcriptional response unique to hemocytes from those that are likely shared by other immune responsive tissues. This analysis contributes to the molecular characterization of hemocytes, reveals new insights into the distinctive features of the hemocyte transcriptome response to infection, and provides valuable resources for designing RNAi experiments specifically targeting hemocyte function.

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METABOLOMIC AND TRANSCRIPTIONAL DIFFERENCES IN HIGH AND LOW NUTRIENT *AEDES AEGYPTI*

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When raised under high population density and nutritional constraints, mosquitoes emerge smaller and with less nutrient reserves than those raised under low population density. Under field conditions large, high-nutrient mosquitoes occur less frequently than small, low-nutrient mosquitoes. Such mosquitoes do not have enough nutrient stores to successfully complete egg production from a single blood meal and therefore need a second blood meal in order to produce eggs. This can raise their vectorial capacity. This study focuses on the molecular differences between high- and low-nutrient mosquitoes and on the regulation of nutrient accumulation in the fat body. We performed transcriptome sequencing and metabolomics analysis of mosquito fat body tissue and compared the metabolic rate of individual low- and high nutrient mosquitoes. The results of this study will further our understanding of the molecular basis of mosquito nutrient metabolism and pave the way for creation of transgenic lines with altered nutrient use and increased reproductive fitness.

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TARGETED KNOCKDOWN OF SRPN6 IN TRANSGENIC *ANOPHELES STEPHENSI*

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SRPN6 is a gene encoding a serine protease inhibitor (serpin) whose expression is induced in the midgut during invasion of *Plasmodium berghei* ookinetes through the midgut wall. In *Anopheles gambiae, SRPN6* is also expressed in the salivary glands at the onset of sporozoite invasion. Functional roles in the invasions of both epithelia were proposed after injection of *SRPN6* double-stranded RNA (dsRNA) into the hemolymph of infected adult female mosquitoes resulted in increased numbers of oocysts and sporozoites. We tested the hypothesis that *SRPN6* expression in midgut and salivary gland epithelia of adult female mosquitoes has an antagonistic affect on parasite invasion. Transgenic *An. stephensi* were created that produce ds-*SRPN6*-RNA in a tissue specific manner. Expression in the midgut was under the control of the promoter from the midgut carboxypeptidase gene, while expression in the distal lateral lobes of the salivary gland was under the control of the promoter from the salivary antiplatelet protein gene. Using these novel constructs, knockdown of endogenous SRPN6 expression was confirmed in both midguts and salivary glands of *An. stephensi*. The effects of the knockdowns on transmission - i.e. oocysts in the midguts and sporozoites in the salivary glands, of the human malaria parasite *P. falciparum* and the rodent malaria parasites, *P. berghei* and *P. yoelii* will be reported.

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EVOLUTIONARY RATES OF SIRNA AND MIRNA GENES IN GEOGRAPHICALLY DIVERSE *AEDES AEGYPTI* MOSQUITO POPULATIONS

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The *Aedes aegypti* mosquito is one of the most significant transmission vectors of dengue viruses. RNA silencing has been shown to act as an effective antiviral mechanism and as an innate immune response to protect adult mosquitoes from alphavirus and flavivirus infection and unregulated dissemination. It is generally believed that a molecular co-evolutionary arms race exists between viruses and their arthropod hosts. Research has demonstrated that the rate of evolution of amino acid sequences is substantially elevated in antiviral RNA interference (siRNA) genes (Dicer2, Ago2, R2D2) in *Drosophila* compared to the genes in the microRNA (miRNA) pathway (Dicer1, Ago1, R3D1) and to the average rate in the entire genome. To quantify *Ae. aegypti* evolution in the siRNA genes, we generated sequence data for most of the exon regions of *ago2*, *dcr2*, *r2d2*, *ago1*, *dcr1* and *r3d1* in 104 *Ae. aegypti* mosquitoes from three populations from Mexico, two populations from Senegal, Africa, and one population from Thailand. Collection sites were chosen based on their diverse geographic distribution, vector competence for DENV-2 and *Ae. aegypti* subspecies. The intraspecific rates of siRNA and miRNA gene evolution were then compared. The rates of amino acid evolution and the ratio of non-synonymous to synonymous nucleotide differences were determined to be significantly elevated in siRNA genes (*ago2*: 0.306, *dcr2*: 0.230, *r2d2*: 0.221) compared to miRNA genes (*ago1*: 0.039, *dcr1*: 0.193, *r2d1*: 0.131). Phylogenetic analysis using maximum likelihood demonstrated substantial variation in all genes and distinct clade formation with one *Ae. aegypti* collection from Senegal, Africa. Likelihood ratio test for positive selection also identified a substantially higher number of positively selected sites in the siRNA genes. Even though the collection sites were selected based on *Ae. aegypti's* role in arbovirus transmission, our findings do not conclusively demonstrate a role for arbovirus infection in driving RNAi gene evolution rates.

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GENETIC STRUCTURE OF *CULEX RESTUANS* IN THE EASTERN UNITED STATES

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Studies of the population genetics of disease vectors can help explain the spread of vector-borne disease. *Culex* mosquitoes are known to be important vectors of West Nile Virus (WNV). *Cx. restuans* is a native species of mosquito and its role relative to other species in WNV transmission is not well understood. We examined the genetics of *Cx. restuans* from several populations in the eastern US using a panel of 17 microsatellite loci. Populations from distinct geographical areas were found to be highly similar, indicating a high degree of gene flow and thus the potential for arboviruses such as WNV to expand their range via movement of these mosquitoes. A similar trend has been documented in populations of *Cx. tarsalis* in the western US. Additionally, blood meal analysis (BMA) was performed on blooded specimens of *Cx. restuans* to determine feeding preferences and better understand their role in disease transmission cycles.

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THE POPULATION STRUCTURE OF THE MALARIA VECTOR *ANOPHELES MELAS* IN WEST AFRICA

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Anopheles melas, a brackish-water breeding mosquito belonging to the *An. gambiae* complex, inhabits West-African coastal marshlands from Senegal to Angola. Although it is not generally considered a major malaria vector due its limited distribution, it is frequently an important local vector, as is the case in several locations on Bioko Island, Equatorial Guinea. Given its role in malaria transmission, knowledge of *An. melas* migration patterns is important in informing vector control efforts. To this end, we are using microsatellite markers, as well as a portion of the mitochondrial ND 4 and 5 genes, to investigate the population structure of *An. melas* across its range. In addition to studying the effect of *An. melas'* patchy distribution on its population structure, we are placing a particular emphasis on estimating migration levels of *An. melas* between the African mainland and Bioko Island, where *An. melas* is the target of extensive vector control efforts under the Bioko Island Malaria Control Project II in support of the National Malaria Control Program of Equatorial Guinea. Our results thus far suggest that the *An. melas* populations on Bioko Island belong to a single larger population. In contrast, highly significant genetic differentiation was found between the mainland and Bioko Island populations, suggesting that migration between them is highly restricted. Thus far, two geographically close mainland populations did not show any significant differentiation, however analyses of samples from a wide range is underway and will provide real insight in patterns of migration across the range of this disease vector.

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THE MOLECULAR EVOLUTION OF OLFACTION GENES IN THE *ANOPHELES GAMBIAE* COMPLEX

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The main malaria vector of sub-Saharan Africa, *Anopheles gambiae s.s.,* has a strong preference for blood feeding on humans. This distinct host preference is a major reason this species is such a devastating malaria vector. This marked anthropophily is the result of the mosquito's attraction to components of human sweat and involves two classes of genes in its olfaction system; the olfaction receptors and the odorant binding proteins. It is thought that the adaptation of *An. gambiae* to human hosts has occurred after the relatively recent increase in human population size in Africa following the introduction of agriculture. We expect that this recent adaptation has left a signature of selection in the genes directly involved in this process. Therefore, we are examining patterns of genetic variation of olfaction receptors and odorant binding proteins in the species of the *An. gambiae* complex. Our goal is to identify candidate anthropophily genes by determining which genes show signatures of selection in the *An. gambiae* s.s., but not in other species of the complex.

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THE SYSTEMATICS OF THE *CULEX PIPIENS* COMPLEX, 40 YEARS LATER

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Mosquitoes in the *Culex pipiens* Complex are important vectors of human encephalitis, parasitic filaria, and avian malaria. Their geographical distribution overlaps that of humans and they are currently the most ubiquitous mosquitoes on Earth. The complex includes *Cx. pipiens pipiens* (two forms differentially domesticated)*, Cx. pipiens pallens, Cx. quinquefasciatus*, *Cx. australicus*, and *Cx. globocoxitus.* The difficulties in distinguishing the different species morphologically and the existence of hybrid zones between several of them have made this species complex "…one of the major outstanding problems in mosquito taxonomy". Hybridization between some of the members of the complex appears to have been influenced or even driven by infection with multiple *Wolbachia* strains and some hybrid zones exhibit unusual patters of generic exchange. To examine the phylogenetic relationships between the species, subspecies and forms in the Complex we used methods originally developed for population level analysis: we analyzed the microsatellite diversity at eight loci, sequenced part of the mitochondrial NADH4 gene and cloned and sequenced nuclear introns in acetylcholinesterase 2, triosephosphate isomerase and wingless. In all analyses we included specimens from several populations of each species and subspecies. As outgroups we used species morphologically similar such as *Cx. torrentium* and *Cx. pervigilans* as well as *Cx. vagans*, *Cx. restuans* and *Cx. salinarius.* We include in some of the analyses specimens of *Cx. pipiens* not infected with *Wolbachia* reported from South Africa in 2003. We present statistical and phylogenetic analyses using MicroSat 1.5b, Phylip 3.573c, and PAUP 4.0b2, of the appropriate data sets. We hypothesize on the biogeographical expansion and evolution of the Complex, as well as on the status of the above taxonomic names.

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FACTORS ASSOCIATED WITH *PLASMODIUM FALCIPARUM* INFECTIOUSNESS DETERMINED BY MEMBRANE FEEDING AND QT-NASBA

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Plasmodium falciparum gametocyte carriage is essential for mosquito infection and spread of the parasite. Human immune factors can reduce infectiousness and form the basis of transmission blocking vaccines. The occurrence and importance of this sexual stage immunity for low density infections has never been studied in natural settings. One hundred volunteers from an area of seasonal malaria transmission provided a total of 307 blood samples at the start, peak and end of the transmission season. Infectiousness was determined by membrane feeding assays and the same samples were used for gametocyte detection by Pfs25-QT-NASBA and assessment of Pfs45/45 and Pfs230 antibody responses. Natural human antibodies were purified from the same plasma samples and tested in the Standard Membrane Feeding Assay for transmission reducing activity. At least one mosquito was infected in 32.6% (100/307) of the experiments. In total, 7.5% (916/12,079) of the mosquitoes were infected with 1-97 oocysts per midgut. Human individual infectiousness and the proportion of infected mosquitoes were negatively associated with age ($p<0.001$ and $p=0.001$ respectively) after adjustment for confounding factors. Individual infectiousness also declined over time with a significant change at the peak (OR=0.56; $p<0.001$) and end of the transmission season (OR=0.20; $p<0.001$) compared to the start. Submicroscopic gametocyte carriers, as detected by Pfs25 QT-NASBA, were infectious to mosquitoes in 32.8% of the feeds. Purified antibodies appeared to completely block or reduce mosquito infection when tested in the Standard Membrane Feeding Assay. This study thus revealed age- and season-dependent patterns of gametocyte infectiousness in residents of an endemic area. Naturally acquired antibodies were found able to reduce the parasite infectiousness. These findings are relevant for the characterization of the infectious reservoir and may provide new opportunities for malaria control.

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USE OF 'ROLLING' MALARIA INDICATOR SURVEYS (RMIS) AS A MONITORING AND EVALUATION (M&E) TOOL IN MALARIA ENDEMIC SETTINGS WITH MARKED SEASONALITY

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In recent years, malaria control interventions have scaled up considerably. To guide control efforts, new M&E tools are needed to assess rapid changes in intervention coverage and malaria burden. Currently, M&E tools at the community level rely largely on national cross-sectional household surveys such as the Malaria Indicator Survey (MIS). However, these are logistically demanding, costly, and burden estimates can be susceptible to annual and seasonal variability. Growing evidence suggests that malaria seasonality also changes over time as transmission levels decrease. Monitoring these changes will be key to maximize the impact

of available control options, particularly to assess the optimal timing of seasonally targeted interventions such as Indoor Residual Spraying (IRS) or seasonal Intermittent Preventive Treatment (sIPT). To address this we developed a novel ongoing (rolling) cross-sectional survey tool: the 'rolling' MIS (rMIS). As part of a randomized trial assessing the safety and effectiveness of two different ACTs, a monthly rMIS is conducted from May 2010 onwards in 51 villages in Chikwawa district, southern Malawi (an area of perennial transmission with marked seasonality). Each month, a random sample of households will be visited covering each village at least twice a year. This rMIS will evaluate coverage of malaria control interventions and parasite and anaemia prevalence in under-fives. Most importantly, we will assess the potential role of rMIS to monitor short-term changes in the burden of malaria accounting for the potential role of malaria seasonality. Preliminary results from the rMIS will be presented based on a comparison with the standard national MIS, conducted in April-May 2010. Findings will focus on the logistical, user-friendliness and costs aspects as well as on the applicability of this tool to monitor rapid changes in the burden of malaria accounting for the effect of seasonality

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A COMPARISON OF SCHOOL- AND COMMUNITY-BASED CROSS-SECTIONAL SURVEYS FOR COLLECTION OF SEROLOGICAL MEASURES OF MALARIA TRANSMISSION

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Antibody responses to malaria antigens represent markers of exposure to infection and can be used to monitor transmission and the effect of interventions. As antibodies can persist for many years they are particularly useful at low transmission intensities. Samples are usually collected during community based cross sectional surveys; however, these surveys can be logistically demanding and expensive if conducted frequently. An alternative approach is to use school surveys, which are long established in helminth research and are being used increasingly within malaria studies. Here we compare antibody responses to the *Plasmodium falciparum* merozoite antigen MSP-119 in samples collected in community surveys with those collected during contemporaneous school surveys conducted in Rachuonyo and Kisii districts in Western Kenya. Age specific sero-prevalences and estimates of the sero-conversion rates were similar between the two survey approaches, with both discriminating areas of high and low transmission. The broader application of school-based sampling, and its potential advantages for monitoring and evaluating variations in malaria transmission, are discussed.

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EVALUATION OF DIFFERENT INDICES TO MONITOR MALARIA IN THE GAMBIA, WEST AFRICA

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For over half a century malaria research and control measures have been taking place in The Gambia. Recent analysis of malaria indices from several facilities suggests a significant decrease in disease burden in the country. There is a need to confirm this finding as it has implications on the current control measures and design of new interventions. Although important, the available indices are constrained in determining the characteristics of the emerging trends, the impact of the expanding control measures and assessing novel tools that may appear in the future. Robust indices of the current malaria epidemiology will help prioritize malaria control measures and research activities. This was a countrywide study that evaluated clinical, parasitological and serological indices in varied transmission settings for monitoring malaria over time and space. It also examined the usefulness of health facility surveys as a method for collecting data to describe area specific malaria epidemiology. The presentation will focus on the utility of data from different sources and indices for monitoring malaria. Data analysis is ongoing however provisional results shows significant shift in demographic and gender patterns of malaria infections. Young teens compared to young children [19.9% vs.10.0%, 95% 5.8, 13.9; p<0.001] currently have the highest burden of infection. Again there is preponderance of more infections in adolescent males (23.9% 95%CI 19.3, 28.8) compared to females[15.2% 95% CI 11.8, 18.9] due to behavioural and differential ITN use. Additional information will be presented on how malaria indices from health facility surveys correlate and explain current infection burden in their catchment communities and further issues on the utility of filter paper for collecting serum to monitor transmission intensity will also be considered. Malaria is changing hence the continuous use of existing malaria related indices in children as a proxy for defining malaria burden in endemic regions may need review. Moreover, integrated approach to data collection including health facility surveys and filter paper based specimen antibody assays present potential tools for monitoring area specific changes in malaria.

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MALARIA SURVEILLANCE IN HAITI, POST-EARTHQUAKE, 2010

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On January 12, 2010, a 7.0 magnitude earthquake struck Haiti. The earthquake's epicenter was 10 miles west of the Haiti capital city of Port-au-Prince. According to the Haitian government, approximately 200,000 persons were killed, and over 2 million were displaced. *Plasmodium falciparum* malaria is endemic in Haiti where the principal vector is the *Anopheles albimanus* mosquito, which frequently bites outdoors. Thus, displaced persons living outdoors or in temporary shelters in Haiti are at substantial risk for malaria. We conducted a survey of 1,629 consecutive suspected malaria patients presenting to medical clinics managed by Save the Children in the earthquake affected areas of Leogane and Jacmel from March 4 to April 9, 2010. Suspected malaria accounted for 3.0% of all consultations. Females accounted for 59% of suspected malaria consultations. A malaria rapid diagnostic test (RDT) was performed on 96% (1,564/1,629) of these patients with an overall positivity rate of 20.3% (317/1,564). Among 341 children less than five years of age, 7.6% were RDT positive, 87.7% were RDT negative, and 4.7% had no RDT result recorded. Among 1288 individuals five year of age and older, 22.6% were RDT positive, 73.6% were RDT negative, and 3.8% had no RDT result recorded. Among 463 women aged 15-49 years, 21.0% were RDT positive, 75.6% were RDT negative, and 3.5% had no RDT result recorded. This included 40 pregnant women among whom 27.5% were RDT positive, 65.0% were RDT negative, and 7.5% had no RDT result recorded. Of the 317 patients with a positive RDT, 87.7% received chloroquine, 2.5% received quinine, and 9.8% had no anti-malarial documented. Malaria is an important public health problem in Haiti post-earthquake with the potential for an increase in cases given the large number of displaced individuals and the onset of the rainy season. Continued malaria surveillance is essential to monitor prevalence, identify areas of potential increased transmission, detect epidemics should they occur, and help direct and monitor interventions and response.

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RECURRENT AND SUB-PATENT INFECTIONS ARE A COMMON OCCURRENCE IN *PLASMODIUM VIVAX* PATIENTS TREATED WITH CHLOROQUINE AND PRIMAQUINE: A ONE-YEAR COHORT STUDY IN PERU

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Worldwide, *Plasmodium vivax* has re-emerged and developed in a major problem in areas where it had been eradicated, and has become increasingly prevalent in areas where it is sympatric with *P. falciparum*. It has the ability of producing relapses originating from dormant liver forms. Fifty one *P. vivax* patients living in communities around Iquitos, in the Peruvian Amazon region, where treated with chloroquine and primaquine and then followed up monthly for 1 year. Passive detection of malaria cases was also carried out throughout the study period. At each visit a blood sample was systematically collected and screened with species-specific PCR. Positive samples were then genotyped using 16 polymorphic microsatellites. Eighty four recurrent infections were identified, 61% within 6 months after treatment (median time 203 days), 22 of them positive also by microscopy. The majority (71%) of recurrences was asymptomatic and in 13 patients the infection persisted for several months at sub-patent level. The genotype of most (75%) recurrent infections was different from that at day 0; 41% of recurrent infections carried different alleles as compared to any previous episode. Only 8 infections were polyclonal. The average expected heterozygosity was 0.55. There was strong linkage disequilibrium (*ISA* = 0.29, $p < 1.10\text{-}4$) which remained also when analyzing only the unique haplotypes, suggesting common inbreeding. In Peru, similarly to Brazil and Vietnam, *P. vivax* recurrent infections, despite the low transmission intensity, were common and displayed a high turnover of parasite genotypes. Most infections were asymptomatic, persisting for several months and detectable only by PCR. *Plasmodium vivax* patients, even when appropriately treated, may still represent an important parasite reservoir from which transmission can be maintained. Therefore, any elimination effort should consider approaches able to identify and treat this hidden reservoir.

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THE FEASIBILITY OF MALARIA ELIMINATION: A FRAMEWORK FOR ANALYSIS

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The realization that the Global Malaria Eradication Program was unable to interrupt transmission in every region due to a variety of technical, operational, administrative, or socioeconomic factors led to understanding of the importance of carefully assessing the feasibility of such a campaign - with all its significant programmatic, financial, and technical implications - before embarking upon it. However, specific criteria for such an evaluation were never clearly proposed. Today, as many countries again contemplate the potential for malaria elimination within their borders, advice on how to conduct such a complex assessment remains scant. To establish a rigorous, quantitative framework for evaluating the feasibility of malaria elimination, a comprehensive evaluation was undertaken to provide explicit guidance for those grappling with this decision. Following a thorough historical review, the feasibility of malaria elimination was defined along three dimensions: technical, operational, and financial. Technical feasibility was defined in relation to two key concepts: the rate of importation of infections from neighboring regions and the probability of these infections leading to onward transmission. These parameters are quantified for a specific context using observational evidence and mathematical models that can estimate reductions in transmission achievable with available tools as well as the potential for maintaining elimination. The operational feasibility component can then evaluate whether the interventions needed to achieve and sustain elimination according to the technical models can be implemented given the capacity of the national malaria program and the health system. Finally, the financial feasibility component should evaluate whether the costs of these required elimination interventions can be sustained over time and compare these costs to those required to control endemic transmission without eliminating it altogether. Together, these analyses will allow malaria programs to make informed decisions and set appropriately evidence-based strategies.

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CD23-BOUND IGE AUGMENTS AND DOMINATES RECALL RESPONSES THROUGH HUMAN NAÏVE B CELLS

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Peripheral blood human naïve B cells express high levels of CD23 and circulate pre-loaded with IgE. The antigen specificity of CD23-bound IgE presumably differs from the B cell receptor (BCR) and may reflect the antigen specific mix of free serum IgE. CD23-bound IgE is thought to enhance B cell antigen presentation raising the question of how a B cell might respond when presented with a variety of antigens and CD23-bound IgE specificities. We recently reported that an increase in CD23+ B cells is associated with resistance to schistosomiasis highlighting the potential importance of CD23-bound IgE in immunity. We sought to determine the relationship between BCR and CD23-bound IgE mediated B cell activation in schistosomiasis. Crude schistosome antigens downregulated basal B cell activation levels in individuals hyper-exposed to infectious worms. However, schistosome-specific IgE from resistant, occupationally exposed Kenyans recovered responses of naïve B cells to schistosome antigen. Furthermore, cross-linking of CD23 overrode intracellular signals mediated via the BCR illustrating its dominating role in B cell activation. Notably, the nature of the cognate antigen appeared to dictate the threshold of antigen-specific CD23-bound IgE required for B cell activation suggesting that CD23-bound IgE functions as a rheostat for B cell responses to antigenic stimuli. These results suggest that CD23-bound IgE augments and dominates host recall responses through naïve B cells.

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A SOMATICALLY DIVERSIFIED LECTIN (FREP3) IS INVOLVED WITH RESISTANCE OF SNAILS TO DIGENETIC TREMATODE INFECTION

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Transcriptional analysis of *Biomphalaria glabrata* snails following challenge with the digenetic trematodes *Schistosoma mansoni* and Echinostoma paraensei revealed a number of transcripts associated with snail resistance to infection. Comparison of the transcriptional profiles expressed by snails resistant to infection because of size, strain (BS-90 resistant/M-line susceptible) or prior exposure to homologous parasites (acquired resistance) yielded a small group of resistance-associated transcripts that were commonly up regulated in all three resistance models. Fibrinogen related protein 3 (FREP3) was identified as one such transcript, demonstrating an increased expression beginning as early as 12 hours

post exposure (dpe), and continuing until 8 dpe. The common recurrence of FREP3 in all of our transcriptional studies of snail resistance and the sequence heterogeneity that arises in FREP3 molecules due to a high rate of point mutation and gene conversion events made FREP3 a high priority for further functional analysis. In situ hybridization studies co-labeling for newly produced hemocytes (BrdU) and FREP3 suggested that newly developed hemocytes are major producers of FREP3. Using an anti-FREP3 antibody we purified native FREP3 from *B. glabrata* plasma and used both FREP3 and the antibody to it, to analyze FREP3 function. We have identified that FREP3 is involved in binding and recognition of galactose sugars, and that it can act as an opsonin to enhance phagocytosis of bound targets. Injection of FREP3-specific small interfering RNA into size resistant snails and subsequent challenge of these normally resistant snails with *E. paraensei* results in a partial loss of the resistance phenotype, suggesting FREP3 is important for immune-associated resistance to trematode infection.

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

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Blood flukes of the genus *Schistosoma* infect 200 million people. As a result of host parasite co-evolution, *S. mansoni* has evolved to exploit host immune factors as signals to coordinate its own development within the host. Worms fail to develop normally in RAG-/- mice that lack all T and B cells, while development is restored when CD4+ T cells are transferred into RAG-/- mice, suggesting that CD4+ T cells play a central role in regulating parasite development. Recent findings suggest the role of CD4+ T cells in this process is indirect, limited to provision of non-cognate T cell help for innate responses which, in turn, facilitate parasite development. In support of this hypothesis, we have found that administration of LPS to RAG-/- mice, in the absence of CD4+ T cells, also restores worm development, indicating that innate immune signals are sufficient for parasite development to proceed normally. LPS, a pathogen-associated molecular pattern (PAMP), activates toll-like receptor 4 (TLR-4), resulting in signaling through both MyD88-dependent and TRIF-dependent pathways. Interestingly, specific stimulation of TRIF-dependent signaling failed to restore worm development in RAG-/- mice suggesting that worm development is not dependent on TRIF-mediated induction of type I interferon expression. However, high levels of PAMPS are not present during the normal course of a *S. mansoni* infection. We therefore hypothesize that, during schistosome infection, endogenous danger-associated molecular patterns (DAMPs) induce the innate responses required for parasite development, following their release by damaged host cells such as hepatocytes. In support of this, we show that stimulation of the NALP3 inflammasome, a MyD88-dependent sensor of endogenous DAMPs, restores worm development in RAG-/- mice. Current research efforts are focused on dissecting the MyD88-linked signaling events that influence schistosome development. Elucidation of the innate immune signals that control schistosome development may help in the development of new drug targets and vaccine strategies.

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GLYCOPROTEIN CHARACTERIZATION OF THE *TAENIA SOLIUM* ONCOSPHERE AND ITS ROLE IN ADHERENCE MECHANISMS

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The molecules by which oncospheres of *Taenia solium* attach to the intestine prior to invasion have not been studied. Knowledge of these molecules could greatly aid in the design of novel preventive and interventional strategies against this parasite. We characterized the carbohydrate/lectin surface membrane of the *T. solium* oncosphere and used an *in vitro* adhesion CHO cell system and polysaccharide or glycoconjugates, laminin and fibronectin substrate assay to investigate the molecules responsible for *T. solium* oncosphere adherence to host cells. This study demonstrated that the activated oncosphere had a lectin with a strong affinity for sialic acid, and that the predominant carbohydrates on the *T. solium* oncosphere are α-D-galactose, α-galNAc and α-D-Mannose. We found that the oncospheres bound to CHO cells and that this binding was augmented by laminin and to a lesser extent by fibronectin and fetal bovine serum. Laminin even at low doses increased oncosphere adherence but exhibited decreased adherence at higher concentrations. Adherence by laminin and fibronectin was specific since antibodies to both inhibited oncosphere adherence. Also adherence of oncospheres to CHO cells even in the presence of laminin was significantly inhibited by heparin. Furthermore, as demonstrated by immunofluorescense heparin bound strongly to activate oncospheres. This study provides us with significant insight into the mechanism of activated *T. solium* oncosphere binding, and will be useful for the development of vaccines that will prevent attachment of oncospheres to the intestinal wall.

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A RECOMBINANT ATTENUATED *SALMONELLA* VACCINE SYSTEM FOR *TAENIA SOLIUM* CYSTICERCOSIS INFECTION IN PIGS

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Cysticercosis is a parasitic infection produced by the larval stage of *Taenia solium*. The clinical importance of the disease appears when the larvae develop in the central nervous system of humans producing neurocysticercosis. Although a number of intervention trials have demonstrated that transmission of *T. solium* can be inhibited temporarily; recrudescence occurs as a consequence of tapeworm reintroduction through infection of a susceptible intermediate host. Vaccination of the intermediate host will remove the source of human infection and interrupt the *T. solium* life cycle. As reported previously, the oncosphere antigen Tsol18 can confer protection when it is administrated as a recombinant GST fusion protein. However, production of a recombinant protein is

expensive and therefore impractical for large-scale use. Consequently, the potential of a live vector vaccine system to deliver *Taenia solium* Tsol18 was investigated. An attenuated strain of *Salmonella enterica* serovar Typhimurium χ9402 was used to develop an oral delivery system for Tsol18 antigen. Tsol18 gene was cloned downstream from the β-lactamase signal sequence in a multicopy Asd+ plasmid vector pYA3620 to yield plasmid pYA3620/Tsol18 and then transformed into the vaccine strain. The recombinant attenuated *Salmonella* vaccine harboring Tsol18 was stable and expressed rTsol18. Immunization of mice with either one or two doses of 109 CFU of the recombinant vaccine strain carrying plasmid pYA3620/Tsol18 elicited specific IgG responses to *Salmonella* self antigens (LPS and SOMPs) and to rTsol18. Moreover, oral immunization of piglets with a single dose of 1012 CFU reduced the numbers of viable cysts after challenge. The use of a recombinant attenuated *Salmonella* vaccine will not only reduce the cost of vaccine production but also the number of vaccine doses needed per animal. The data we present provides the basis for an affordable and easy vaccine delivery system that can be used as an adjunct in cysticercosis/taeniasis control programs.

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INVESTIGATION OF POST-TREATMENT INFLAMMATORY RESPONSES IN A RAT MODEL OF NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) resulting from infection by the cestode *Taenia solium* is the leading cause of epilepsy in endemic regions. Anthelmintic treatment leads to inflammation around the dying cysts, often resulting in a worsening of symptoms. In an intracerebral (IC) infection model using metacestodes of *T. crassiceps* (Tc) in rats, we studied the effects of anthelmintics on parasite-host interactions. Tc metacestodes were surgically implanted in the brain parenchyma, and growth of IC cysts were tracked and quantified by magnetic resonance imaging (MRI). IC infection resulted in 60% mortality by day 80 post-infection (PI). Six to eight weeks PI the rats received 18 days of treatment with praziquantel, albendazole, or a combination thereof. Treatment with PZQ and PZQ plus ABZ resulted in growth arrest of the cysts by MRI, suggesting damage or death of the parasite. Histopathlological examination of pericystic brain revaled patchy areas of mononuclear inflammatory cell infiltrates. A majority of rats had detectable serum IgG responses to cyst antigens (Ags), and high titers of Ag-specific IgG1, IgG2a, and IgG2b consistent with a mixed Th1/Th2-type response to the parasite. Preliminary studies of whole brain samples for gene expression of pro-inflammatory and regulatory genes by RT-PCR did not reveal significant differences between the treated and untreated rats. Focused gene expression analysis of the pericystic region using laser dissection microscopy and characteriation of cellular immune responses to the IC cysts, including cell phenotyping by immunohistochemistry and flow cytometry are ongoing, aiming to determine the mechanisms underlying parasite-associated immune regulation and post-treatment neurological infalmmation. This model reflects cerebral infection in humans and may have utility in the investigation of novel anti-inflammatory therapies or the control of post-treatment inflammation associated with anthelmintics.

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IN VITRO EFFECTS OF ANTHELMINTICS ON *TAENIA CRASSICEPS* REVEAL LIMITATIONS OF ITS USE AS A MODEL FOR *T. SOLIUM* CYSTICERCOSIS

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Taenia crassiceps (Tc) cysts have been commonly used as a substitute for cysts of T. solium (Ts) *in vitro* and *in vivo* in a rodent model of cysticercosis. Although there are common features and characteristics of both organisms, some studies suggest that the two parasites differ in their biology, and that not all experimental conclusions from one can be applied to the other. One area of investigation for which Tc is particularly attractive is in the evaluation of anthelmintic drug efficacy. We investigated the utility of secreted/released parasite proteins for the evaluation of drug effects on Tc. Using changes in AP secretion as a measure of drug activity, as previously applied to the study of Echinococcus spp., we investigated the effects of the anthelmintics albendazole sulfoxide (ABZ) and praziquantel (PZQ) on Tc cysts. In contrast to the results using Ts cysts, we observed an initial increase of AP secretion followed by a significant decrease in secretion of AP with both ABZ and PZQ. Higher doses of both drugs caused earlier maxima of AP secretion; for example, peak secretion changed from day 4 to day 2 with PZQ concentrations of 10 ng/nml and 100 ng/ml, respectively. Overall, the release of AP on exposure of Tc to PZQ and ABZ resembled the patterns reported for *Echinococus* spp., but was observed at significantly lower concentration of both drugs. The secretion/release of a parasite specific antigen found in the serum and cerebrospinal fluid of Ts-infected patients. was evaluated using immunofluorescence microscopy with Tc cysts and monoclonal antibodies. These monoclonal antibodies were observed to cross-react with Tc, and studies aimed at characterizing the effects of drugs on the localization of the target antigen on Tc before and after exposure to anthelmintics are under way. These data suggest that secreted/released enzymes and immunoreactive antigens may provide sensitive measures of drug effects on Tc and also suggest that results of studies with Tc differ significantly from those reported with Ts.

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43KDA EXCRETORY-SECRETORY ANTIGENIC PEPTIDE OF *TAENIA SOLIUM* METACESTODE AS A POTENTIAL DIAGNOSTIC MARKER IN HUMAN NEUROCYSTICERCOSIS

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Taenia solium taeniasis/cysticercosis is being reported increasingly from India and that it is an important constraint for regional agricultural and health development. Diagnosis of neurocysticercosis (NCC) is complicated because of the variability in clinical presentations and course of the disease where viability of parasite is a major determinant. The living parasite continues releasing metabolic byproducts or excretory-secretory (ES) substances. Hence detection of ES substances in body fluids or an antibody response to the ES substances might help in diagnosing an early stage of infection before to the onset of parasite degeneration in typical symptomatic NCC in human. The objective of the present study was to characterize the ES substances collected from *in vitro* culture of *T. solium* metacestode larvae, and to identify specific ES peptides as diagnostic markers. Three ES peptides viz., 67kDa, 43kDa and 32kDa, were found to be diagnostic for NCC based on high sensitivity and specificity of their detection in either serum or cerebrospinal fluid (CSF) specimens. More remarkably, the 43kDa ES peptide was found reactive with CSF and serum specimens from confirmed NCC patients with absolute specificity and a high sensitivity (88.23% in serum and 89.28% in CSF). This peptide was also detected by sera and CSF from clinically suspected NCC patients but with a decreased sensitivity correlating with the decreasing order of the certainty of diagnosis. The 43kDa ES peptide is suggested to be an important peptide of diagnostic utility in NCC with an application in either clinical laboratory practice or mass screening studies in endemic areas.

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FIELD-APPLICABLE COPROANTIGEN ASSAY TO DETECT *TAENIA SOLIUM* CARRIERS

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Taeniasis by *Taenia solium* occurs only in humans and is the unique source of cysticercosis infection to humans and pigs. Cysticercosis causes economic losses to pig farmers and morbidity and mortality in humans, being the most important infectious factor increasing epilepsy burden in endemic areas. Diagnosis by microscopy or molecular techniques requires the presence of eggs or progglotids, which is infrequent. Serum antibody detection doesn't indicate active infection. Coproantigen detection is the best alternative, with 96.5% sensitivity and 98% specificity. A major limitation of coproantigen ELISA detection is its limited availability and technical requirements: ultralow temperature (-70ºC) storage, expensive blocking agents, ultra pure water and the use of a spectrophotometer to interpretation of the results. We initially measured the stability of polyclonal antibodies kept at -20ºC for 1 month, with no decrease in activity. Then we compared use of bottle commercial water and powder commercial milk instead of ultrapure water and fetal bovine serum respectively, again without major changes in the ratios (19.4 vs 18.9, OD pool positive / Mean OD + SD of 6 negative samples). Finally, applying both assay versions to 308 stool samples from patients with *T. solium* taeniasis and 78 from negative controls we found a Pearson's r of 0.97 comparing the OD results of a field-applicable version versus the standard coproantigen test. Additionally, using a visual color gradient chart we compared the classification of cases and controls as positive (taeniasis by *T. solium*), indeterminate, and negative cases with the results obtain from the spectrophotometer. Categorization was the same in all cases. Development of an ELISA test with minimal technical requirements and maintaining the sturdiness of the original assay will provide a simple, cheap, available tool to detect and follow up *T. solium* carriers. This assay will greatly facilitate surveys, epidemiological studies, control/eradication programs and individual management of patients in endemic areas for taeniasis/cysticercosis.

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A RETROSPECTIVE REVIEW OF CALCIFIED NEUROCYSTICERCOSIS IN A NEW YORK CITY MUNICIPAL HOSPITAL

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Neurocysticercosis (NCC) is the most common cause of acquired adult onset seizures and epilepsy in endemic countries. Calcified lesions are the most common cerebral findings and previously had been classified as "inactive disease", but over the past decade there have been increasing reports demonstrating this is a frequent finding and associated with episodic seizures. The objective of this study was to document the frequency of perilesional edema associated with calcifications and to define clinical symptoms associated with the finding in an immigrant population seen in a nonendemic region. A retrospective chart review of patients with calcified lesions with NCC since 1995. MRIs and CT scans were reviewed by a neuroradiologist to identify the presence of calcified lesions and perilesional edema. Charts were reviewed for demographic data and the presence of edema related symptoms. 44 patients had calcified lesions consistent with NCC, of which 77% had positive W. blots. 22 (50%) patients were female and the mean age was 35 $\pm$13 years. All but one patient was an immigrant from an endemic region. The mean time from immigration was 6.6 $\pm$8 years. In descending order of frequency, patient's geographic locations of origin were Mexico, South America, Caribbean islands, and Central America. Additionally, 2 patients were from India, 1 was from Korea and there was one patient from the US who was a short term traveler to India. Of the 44 patients, 15 (34%) had perilesional edema, all of whom were symptomatic at the time of the scan. Symptoms in decreasing order of frequency were headache, seizure, hemiparesis and altered mental status. Recurrent perilesional edema was evident in 7 patients, with two patients having 2 or more recurrences. In conclusion, this study found that 34% of patients with parenchymal NCC and calcifications were found to have perilesional edema on retrospective review and is congruent with findings at other institutions. Calcified perilesional edema seems to be a frequent phenomenon in NCC, often associated with headaches and seizures.

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GEOSTATISTICAL MAPPING OF MALARIA AND LYMPHATIC FILARIASIS CO-ENDEMICITY IN AFRICA

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Malaria and lymphatic filariasis (LF) cause the largest public health burden of all diseases worldwide. Some 350-500 million clinical episodes and 1 million deaths each year are caused by malaria, of which approximately 60% and 80% respectively occur in Africa. More than 50 million people are also thought to be infected with lymphatic filariasis in 39 endemic countries in sub-Saharan Africa, with approximately 14.6 million individuals living in these endemic countries estimated to suffer from the two major filarial debilitating conditions, lymphodema or hydrocoele. Both diseases are vector-borne and in many parts of sub-Saharan Africa are transmitted by the same vector, namely the *Anopheles* mosquito. Recently there have been calls for an integrated approach to disease control, however, to successfully achieve this, accurate maps of the geographic distributions of the infections, and maps highlighting co-endemic regions are crucial for 1) guiding the planning of control programmes and 2) assessing whether an integrated control strategy is cost-effective. We attempt to map the prevalence of LF and malaria infection across Africa using a Bayesian generalised spatial linear model in conjunction with community-level infection data obtained from the published literature. We use the package 'geoRglm' implemented in 'R' to create a disease prevalence distribution model with spatially correlated random effects. The model parameters are estimated using Markov chain Monte Carlo techniques. We use the model to assess how different environmental risk factors influence the distribution of LF and malaria, and how this could be affected by the expected changes in climate using future predictions from the global climate models. We apply simple overlay map functions to these maps to identify areas of co-infection, estimate the number of co-infected people and assess the implications for an integrated control strategy, specifically, whether this approach is more cost-effective and it is more suited to some regions than others based on their environmental conditions and current levels of endemicity.

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COST SAVINGS OF SCALING UP TO INTEGRATED, CO-ADMINISTRATION OF IVERMECTIN, ALBENDAZOLE, AND PRAZIQUANTEL: TRIPLE DRUG ADMINISTRATION IN NIGERIA

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Nigeria is one of the first countries to implement co-administration of the drugs ivermectin (IVR), albendazole, (ALB) for lymphatic filariasis and praziquantel (PZQ) for schistosomiasis at scale. For safety reasons, one year of separate, stand-alone treatment was needed, after which all three drugs were given concomitantly in what has come to be known as Triple Drug Administration (TDA). In 2008, 9 Local Government Areas (LGAs) in north central Nigeria received a stand-alone treatment of IVR with ALB given to 1,367,927 adults and children, followed a week later by treatment with PZQ to 295,641 children. The next year IVR+ALB was administered to 1,355,455 persons, 303,346 of whom were children also receiving PZQ. Costing of the program was done through the use of work and travel logs, retrospective surveys and finance records for both MoH and partner organizations. Data collected included capital costs, salaries, transport, supplies, per diems, intervention materials, overheads, and time. Operational data were also collected for specific activities. These included advocacy, data management and reporting, drug delivery and distribution, field supervision, health education and community mobilization, M & E, morbidity control, planning and budgeting, procurement, and training. Total costs and costs per treatment were compared and cost curves examined for economies of scale and scope. Efficiency was measured using DEAP v2.1. From year 1 to year 2, total costs had reduced by nearly 60%. The greatest cost savings were seen in recurrent costs such as transportion and administration. Some diseconomies of scale were witnessed in districts with large populations due to distribution requirements in those areas; however costs were still lower than during the previous year and the cost curve had flattened from year 1 to year 2. Preliminary analysis of efficiency showed minor improvements in 2009 when compared to 2008, however consideration should be given to making intervention "packages" too complex. The up-front cost of a stand-alone distribution requires a higher, initial financial commitment before any cost savings can be realized.

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THE CHALLENGES OF THE 'END-GAME' OF AFRICAN LF MDA PROGRAMS WITH RESPECT TO COENDEMICITY OF ONCHOCERCIASIS: MUST IVERMECTIN TREATMENT FOR ONCHOCERCIASIS CONTINUE WHEN LF TRANSMISSION HAS BEEN INTERRUPTED?

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A strategy for the elimination of lymphatic filariasis (LF) in Africa is to provide combined mass drug administration (MDA) with ivermectin (IV) and albendazole (ALB). If MDA is administered with good treatment coverage, it is believed that LF transmission will be interrupted after 5-7 years and MDA can cease. LF occurs in all 30 local government areas (LGA) of Plateau and Nasarawa states of central Nigeria, while onchocerciasis occurs in just 12 of them. MDA with ivermectin (IV) has been administered in these 12 LGAs beginning in 1993, and together with ALB (for coendemic LF) since 2000. Assessments of LF in 2008 indicated that MDA with IV/ALB could be halted for LF in five of these coendemic LGAs. However, that led to the question of whether IV MDA as monotherapy needed to continue to be administered for onchocerciasis. Accordingly, in 2009 we conducted assessments to determine if onchocerciasis transmission has been interrupted and if IV could likewise be halted. Our skin snip study for microfilaridermia consisted of two elements 1) sampling of school-aged children resident in the five coendemic LGAs where LF transmission had been interrupted to determine if any recent Onchocerca infections had taken place, and 2) community-wide surveys conducted in six sentinel villages located in four other coendemic LGAs where 1992 baseline surveys showed a mean skin snip prevalence of 72%.

In the school surveys in five LGAs, we found only 1 skin snip positive among 2779 children (0.04%). In the six sentinel villages in four additional LGAs, we found 8 (0.4%) infections among 1919 persons. This represents a 98% decrease compared to the 1992 baseline. We believe that interruption of transmission of onchocerciasis throughout all or most of the two state area has likely been achieved, and that both IV and ALB can be stopped in the five LF/oncho coendemic LGAs. The next step should be the design and implementation of integrated post MDA surveillance for recrudescence of one or both filarial diseases.

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DATA-CAPTURE AND DATA-MANAGEMENT FOR OPERATIONAL RESEARCH TO SUPPORT LARGE-SCALE NTD IMPLEMENTATION PROGRAMS

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To define the best diagnostic test for use in assessing end points in the elimination of Lymphatic Filariasis, an 8 country trial evaluating 8 different diagnostic tests was conducted. This study gathered data on more than 63,000 tests, from over 14,000 specimens, collected from nearly 10,000 individuals. To support the activities of this study, the Electronic Data Gathering and Evaluation (EDGE) system, a PDA based informatics solution, was developed. The EDGE system was designed to facilitate the collection of survey and gps data, to enable differential synchronization between in country and US based central servers, to utilize a bar code label tracking system, and to employ a specimen inventory management database. Additionally, the EDGE system produces on demand analysis datasets and provides secure online access to real-time reporting. Building on diagnostic knowledge gained from this initial study, 10 additional countries were designated as sites to evaluate a protocol for MDA stopping and post-MDA surveillance. The EDGE system was used to collect, manage, and report on these data. To date, activities from these studies have yielded an additional 15,000 test results. In anticipation of the need to expand collection of epidemiological data for monitoring and evaluation efforts to other NTD programs, a project to modify the EDGE system to utilize Wi-Fi and 3G communication technology is currently underway. This platform is designed to be cost effective, rapidly deployable, and flexible enough to support the operational needs of Lymphatic Filariasis, Schistosomiasis, STH, and Trachoma research projects.

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EXPANSION OF THE UNITED STATES AGENCY FOR INTERNATIONAL DEVELOPMENT NTD CONTROL PROGRAM: 2010 AND BEYOND

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In the 3½ years since the start of the U.S. Congressional and Presidential initiatives to support integrated efforts to control or eliminate 5 Neglected Tropical Diseases (NTDs), national programs in 12 countries have been enabled to progressively expand and provide more than 250 million (largely donated) drug treatments to 60 million individuals - most, multiple times - in yearly MDAs (mass drug administration) targeting lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminthiasis and trachoma. The success of this United States Agency for International Development NTD Control Program (managed by RTI International and its NGO partners) has resulted from a strategic design that demands full compliance with WHO guidelines and strict country selection criteria that include, most importantly, national government commitment to NTD control. With time a 'best practice' strategy for program implementation has evolved the following recommended, step-wise progression: 1) Country situation analysis - a standardized compilation of all existing NTD information; 2) Disease mapping - not prevalence maps, but action-maps defining where and what type of MDAs will be required; 3) Financial gap analysis - a standardized tool to identify the financial costs and needs for effective program implementation; 4) National strategy and plan of action (POA) - developed to meet WHO recommendations and guidelines, including integration of existing vertical NTD programs; 5) National stakeholders meeting - to agree to support the national POA and identify agreed roles and responsibilities; 6) Implementation of POA - with simple, but rigorous monitoring and evaluation; and 7) Advocacy within country - to ensure sustained support based on program success. Though challenges remain (e.g., policies for disease-specific mapping, treatment and surveillance; human capacity and training), this successfully developed model for effective program roll-out will serve as the foundation for the significantly expanded, new United States Agency for International Development support targeting the NTDs; details of this new program and its funding mechanisms will be available for presentation.

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EFFECTS OF INTEGRATION ON FINANCING AND COVERAGE OF NEGLECTED TROPICAL DISEASE PROGRAMS

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Neglected Tropical Disease (NTD) program activities are increasingly being integrated with the goal of improving resource availability and enhancing program reach. To evaluate the true impact of the United States Agency for International Development program for NTD control on program financing and coverage, data was collected by NTD program managers and non-governmental organization partners in Burkina Faso, Mali, and Uganda from 2 years prior to integration (2005, 2006) and 2 years post-integration (2007, 2008). Program coverage indicators included the number of people treated by district, geographic and therapeutic coverage, and program scale-up, by disease. Program financing indicators included financial support only and analyzed trends in governmental, internal non-governmental and external sources of funds, by disease. With United States Agency for International Development funding, overall geographical coverage and the number of persons treated for NTDs has increased, and high rates of therapeutic coverage of preexisting programs have been maintained. United States Agency for International Development funding has also enabled some countries to start treating persons for diseases not previously targeted (e.g., trachoma in Burkina Faso and Uganda) as well as to rapidly scale up existing programs (e.g., LF in Mali). When United States Agency for International Development funding began, NTD support from other sources in each country experienced post-integration changes that varied by disease; funding for those more established programs (i.e., LF and onchocerciasis) remained generally unchanged across study countries while for the more marginally supported programs (schistosomiasis, soil-transmitted helminths and trachoma) funding generally decreased. However, when the new United States Agency for International Development support is considered, every country experienced a significant increase in overall funding available for the integrated NTD programs. These findings suggest that significant decreases in external support might threaten program sustainability, so that seeking increased government commitment to establish NTD budget lines should be a top priority of these programs.

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OVERCOMING THE CHALLENGES OF TARGETING THE NTDS IN THE URBAN SETTINGS OF AFRICA

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Neglected tropical diseases (NTDs) can be eliminated or controlled through effective Mass Drug Administration (MDA) to at risk populations. Urban settings, however, have proven to be a barrier to programmatic success, particularly because of challenges in mapping disease distribution and achieving satisfactory treatment coverage and compliance. In African NTD programs, the major challenges confronting *mapping* in urban settings are: (1) the large size of cities, (2) the lack of social and cultural homogeneity and (3) the rapid rural to urban migration contributing to slum development. The major challenges confronting the implementation of *MDA* in urban settings are related primarily to: (1) a complex urban social structure, (2) focal transmission in areas of low socioeconomic status resulting in an inappropriately low perception among decision-makers of the true disease threat and (3) inadequate advocacy and social mobilization leading to insufficient program resources and low population compliance. In-depth analysis of experiences of ongoing programs reported through detailed surveys and expert panels, has led to two sets of potential solutions to addressing NTD program success in urban settings. For the short term, recommendations include: (1) re-defining smaller implementation units and sampling frames that represent distinctly different levels of socioeconomic development and vector transmission foci; (2) implementing extensive social mobilization; (3) proper selection and in-depth training of drug distributors; and (4) strengthening pharmacovigilance. Longer term solutions will require operational research focused on (1) understanding the dynamics of LF transmission by different vectors in urban settings (which may impact both mapping and MDA costs); (2) use of hospital or clinic records to rapidly and effectively determine the endemicity status of NTDs; and (3) understanding the impact of imported cases on urban transmission.

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ALLELE-SPECIFIC EFFICACY OF THE MONOVALENT APICAL MEMBRANE ANTIGEN 1 (AMA1) MALARIA VACCINE FMP2.1/AS02A

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The limited efficacy to date of blood stage malaria vaccines may be explained by extreme genetic diversity in vaccine antigens resulting in allele-specific efficacy. Apical membrane antigen 1 (AMA1) gene sequences from clinical malaria episodes experienced by 400 children randomized 1:1 to receive either the FMP2.1/AS02A AMA1 malaria vaccine or rabies vaccine in a Phase 2 safety and efficacy trial conducted in Bandiagara, Mali were used to assess whether FMP2.1/AS02A vaccine is cross-protective against allelic variants of AMA1. Blood samples were collected at baseline, at days 90, 120, 150, 180, 210, 240, and whenever ill participants presented with malaria symptoms. For the primary analysis we defined homology to the vaccine strain 3D7 based on polymorphic codons in the cluster 1 loop (c1L) of Domain I of AMA1, a region previously shown to be immunologically important. For secondary analyses the definition of homology was extended to include polymorphic codons in Domain II and III. Time to first malaria episode with AMA1 c1L sequence identical to the five most frequent strains (3D7, Fab9, DD2, M5 and FVO) was assessed, the hazard of a clinical episode with parasites carrying AMA1 c1L homologous to any of these strains was measured, and FMP2.1/AS02A protective effect against malaria infection was evaluated. Vaccine efficacy against first malaria clinical episode with a 3D7-type c1L allele was 64% (p=0.03). The vaccine also showed significant efficacy against two strains that have c1L sequences identical to 3D7 but have differing amino acids in Domains II and III, but no significant efficacy against four strains with c1L different from 3D7. In conclusion, this first trial of the FMP2.1/AS02A malaria vaccine showed efficacy against strains with AMA1 c1L sequence identical to the vaccine strain 3D7, supporting the idea that polymorphism in this region of the molecule is an important determinant of allele-specific vaccine-induced immunity. A multi-allelic AMA1 vaccine is likely to be needed for broad efficacy against diverse parasites.

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SAFETY, IMMUNOGENICITY AND IMPACT ON PARASITE MULTIPLICATION RATES OF THE CANDIDATE BLOOD-STAGE VACCINE AMA1-C1/ALHYDROGEL WITH THE NOVEL ADJUVANT CPG 7909 AGAINST BLOOD-STAGE MALARIA CHALLENGE IN HEALTHY MALARIA-NAIVE VOLUNTEERS

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Human malaria challenges provide valuable evidence of candidate vaccine efficacy in non-immune populations prior to large-scale field trials. Growth-inhibitory assays (GIA) are widely used as a marker for biologic activity of the induced antibody, although there is no direct evidence that inhibitory activity in vitro relates to impact on parasite growth in humans. Inoculation of Plasmodium *falciparum* infected erythrocytes allows standardisation of the challenge dose, and may be more sensitive than sporozoite challenge for detecting subtle effects of blood-stage vaccines. We sought to compare in vivo and in vitro growth inhibitory activity of the bi-allelic AMA1 vaccine formulated on Alhydrogel, co-adjuvanted with the TLR agonist CPG 7909. We enrolled ten healthy malaria-naive volunteers aged 18-50 in the UK. Seven volunteers were immunised with two doses of AMA1-C1/Alhydrogel mixed with CPG 7909. Immunisations were safe, well tolerated and immunogenic, stimulating AMA1-specific antibodies and apparently higher levels of AMA1-specific T cells than have previously been reported with other protein-in-adjuvant blood-stage vaccines. Immune responses were boosted by the second dose of vaccine. Two vaccinated volunteers withdrew consent prior to challenge for non-clinical reasons. We inoculated the remaining five vaccinated volunteers and three unvaccinated controls with parasitised erythrocytes.

All challenged volunteers developed blood film patency at a similar level of parasitaemia to sporozoite challenged volunteers, but with a significant reduction in the frequency of symptoms. We observed no significant differences between the vaccinees and controls in time to first detectable PCR, time to positive blood film, magnitude of parasitaemia at blood film patency, or parasite growth rates. GIA is in progress to allow comparison with in vivo growth rates. We observed no evidence of an impact on in vivo parasite multiplication in this model.

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PHASE 1 STUDY OF THE SAFETY AND IMMUNOGENICITY OF BSAM-2/ALHYDROGEL®+CPG 7909, AN ASEXUAL BLOOD STAGE VACCINE FOR *PLASMODIUM FALCIPARUM* MALARIA IN ADULTS IN MALI

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A single blind, randomized, controlled Phase 1 clinical trial is being conducted to assess the safety and immunogenicity in malaria exposed adults of the *Plasmodium falciparum* blood stage vaccine BSAM-2, containing a four recombinant protein mixture of AMA1 (AMA1-FVO+AMA1-3D7) and MSP142 (MSP142-FVO+MSP142-3D7) / Alhydrogel® with the novel adjuvant CPG 7909. Participants are healthy adults 18-45 years old living in the village of Bancoumana, Mali. A total of 30 participants will receive up to 3 doses (Days 0, 56, and 120) of either BSAM-2 or Euvax® B/Hepatitis B vaccine. Enrollment and first vaccinations occurred in March and April of 2010. Initial vaccinations were well tolerated, with related adverse events being mostly mild or moderate injection site reactions. Adverse events and antibody responses up to two weeks after the third vaccination will be presented.

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ANALYSIS OF CELL-MEDIATED IMMUNE RESPONSES IN VOLUNTEERS STERILELY PROTECTED AGAINST *PLASMODIUM FALCIPARUM* SPOROZOITE CHALLENGE FOLLOWING IMMUNIZATION WITH A GENE-BASED VACCINE

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We tested safety, immunogenicity and protective efficacy of a DNA prime / serotype 5 adenovirus boost *Plasmodium falciparum* (Pf) malaria vaccine in 15 malaria naïve adults. Both DNA and Ad5 constructs encoded the Pf circumsporozoite protein (CSP) and Pf apical membrane antigen-1 (AMA1). Three immunizations with DNA at four week intervals followed by a single immunization with Ad5 16 weeks later completely protected 4 of 15 volunteers against malaria sporozoite challenge. Antibody responses as measured by IFA, ELISA and growth inhibition assay were poor to moderate, and showed no relationship with protection. In contrast, *ex vivo* IFNg ELISpot assays performed at time of challenge using pools of synthetic 15mer peptides overlapping by 11 amino acids demonstrated that the two volunteers showing the strongest ELISpot responses to CSP and the three volunteers showing the strongest ELISpot responses to AMA1 were protected. Each of these individuals strongly recognized a single CSP and/or AMA1 peptide pool. In contrast, ELISpot responses of non-protected volunteers were more widely distributed among peptide pools and were not as robust. Significant recall responses were not observed in the fourth protected volunteer, to either antigen; we are currently synthesizing minimal HLA-restricted CD8+ T cell epitopes to see if the 15 mers used to stimulate the PBMC's from this volunteer may have been suboptimal for recalling responses. We are also conducting ELISpot assays following CD4+ and CD8+ T cell depletions as well as flow cytometry with ICS to delineate the role of different T-cell subsets in the protective responses observed in this trial. These encouraging results indicate the feasibility of protecting volunteers against Pf sporozoite challenge using a gene-based heterologous prime-boost approach designed to elicit strong cell-mediated immunity targeting the sporozoite and hepatic stages of the parasite.

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THE FIRST PHASE 1/2A TRIAL OF THE METABOLICALLY-ACTIVE, WHOLE ORGANISM *PLASMODIUM FALCIPARUM* SPOROZOITE (PFSPZ) VACCINE

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It was shown in the 1970's that volunteers immunized by exposure to mosquitoes infected with radiation attenuated Pf sporozoites were protected when challenged by the bites of mosquitoes harboring fully infectious sporozoites, establishing a gold standard for the induction of sterile immunity to malaria. However, the technical difficulties of mass-producing a live sporozoite-based vaccine have, until recently, been considered insurmountable. Manufacturing obstacles have now been overcome and a metabolically-active (live), non-replicating (attenuated), cryopreserved Pf sporozoite (PfSPZ) vaccine suitable for parenteral injection has been produced (SanariaTM PfSPZ Vaccine). To evaluate the safety, tolerability, immunogenicity and protective efficacy of the SanariaTM PfSPZ Vaccine, we conducted a Phase 1/2a open-label, dose-escalation study in malaria-naïve, healthy adults aged 18 to 50 years. Eighty volunteers were randomized to receive four doses of intradermal or subcutaneous injection of the vaccine at 7,500, 30,000 or 135,000 sporozoites/ immunization. One group receiving high dose also received a fifth and sixth immunization. There were no vaccine-related severe or serious adverse events and reactogenicity to the study vaccine was mild in all dose groups, with approximately 85% of the reported adverse events (AEs) at an intensity level of Grade 1. There were no breakthrough infections, a finding supported by the absence of detectable antibodies to asexual stage parasites (IFAT) or to the blood stage antigens MSP-1 or EBA-175 (ELISA). In contrast, the vaccine induced antibody and T cell responses to whole sporozoites (IFAT, ELISpot) and antibody responses to the sporozoite

antigen PfCSP (ELISA) with a dose response observed. Protective efficacy was evaluated by challenging volunteers by the bites of 5 malaria-infected *Anopheles stephensi* mosquitoes. The full results of the safety, tolerability, immunogenicity, and protective efficacy of the vaccine and plans for subsequent clinical trials will be presented.

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DEVELOPMENT OF A SAFE AND REPRODUCIBLE HUMAN SPOROZOITE CHALLENGE MODEL FOR *PLASMODIUM VIVAX* IN HEALTHY ADULTS IN THE UNITED STATES

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With ~70 to 80 million cases per year, *Plasmodium vivax* is the most widespread malarial infection of man, causing a highly debilitating disease characterized by multiple relapses. Increasing resistance to anti-malarials and to insecticides highlights the requirement for a *P vivax* vaccine and the urgency to develop a safe mosquito challenge model for vaccine and drug evaluation. This study is the first experimental challenge for *P vivax* conducted in the US under regulatory oversight. Objective: To conduct a proof-of-concept study to develop a safe and reproducible sporozoite challenge model for *P. vivax* in humans with a goal of 100% infectivity rate. Methods: Because *P. vivax* has proved difficult to culture *ex vivo*, *P. vivax*-infected blood from patients diagnosed with malaria in Mae Sot, Thailand was fed through membrane to laboratory-colonized *Anopheles dirus* mosquitoes from AFRIMS insectary. The blood was screened for potential confounding pathogens including other malaria species, filariasis, Japanese encephalitis, chikungunya, HIV, and hepatitis B & C viruses. Mosquito batches with highest oocyst infection and fed on pathogen negative blood were selected for an initial challenge of six healthy US volunteers, who subsequently received five infectious bites and were monitored for development of parasitemia by daily blood smears from day 5 until volunteers have 3 consecutive negative smears post treatment. All six developed parasitemia between days 12 to 14 post challenge and were treated with chloroquine @ 1500 mg base for 3 days and primaquine @ 30 mg/day for 14 days by direct observation therapy, with six month follow-up to document resolution of all symptoms and lack of recurrences. A second cohort will be conducted in summer 2010 to demonstrate reproducibility of the challenge procedure. Safety data, prepatent period, relapse rate, and parasite genotype data for both challenges will be presented.

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ESTABLISHING THE MALARIA HUMAN CHALLENGE MODEL IN A NEW REGULATORY ENVIRONMENT

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Malaria human challenge studies are a critical assessment tool to refine leading vaccine candidates and ensure that only the most viable candidates advance to large-scale trials. However, the existing worldwide infrastructure for conducting these unique studies is inadequate to meet the projected global need over the next decade. In partnership with the Walter Reed Army Institute of Research (Walter Reed Army Institute of Research) and the PATH Malaria Vaccine Initiative (MVI), the malaria human challenge model was recently established at Seattle Biomedical Research Institute (Seattle BioMed). Activities at both centers were aligned to adapt to a new regulatory environment for conduct of the malaria human challenge model. Production of the challenge material was described in a Biologics Master File (BB-MF) and the challenge conducted under an Investigational New Drug Application (IND), both on file with the FDA. Likewise, efforts to harmonize the challenge model procedures with other centers worldwide are ongoing. In order to demonstrate establishment of the quality systems and procedures required to conduct the model, a demonstration challenge trial was conducted in six healthy malaria-naïve volunteers. Volunteers were challenged with wild-type NF54 strain *Plasmodium falciparum* by the bite of five infected *Anopheles stephensi* mosquitoes under controlled conditions. All participants developed a patent parasitemia and were treated upon first positive blood smear with standard doses of chloroquine by directly observed therapy. Immunologic assessments (IFN-γ EliSPOT and antibody ELISA) and qRT-PCR detection of subpatent parasitemia were performed. Prepatent and incubation periods and adverse event profile were consistent with published reviews of challenge cohorts at other centers. No serious adverse events occurred. Standardization of the human challenge model will ensure consistency in early-phase malaria vaccine efficacy assessment across centers and uniformly stringent safety monitoring of volunteers. Expanded infrastructure provides critical mutual backup for infected mosquito production between centers to reduce disruptions to product development and clinical testing.

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TRENDS IN HOSPITALIZATION FOR CYSTICERCOSIS-UNITED STATES, 1981-2000

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Recent studies suggest that the occurrence of cysticercosis may be increasing in the United States. The purpose of this study was to examine secular trends in the diagnosis of cysticercosis among persons admitted to U.S. hospitals since 1980. Using data from the National Hospital Discharge Survey for 1981 through 2000, we estimated annual numbers and rates of cysticercosis-related hospitalization for the U.S. population. During this interval, an estimated 23,000 patients were hospitalized with this diagnosis; when averaged over 5-year periods, rates increased consecutively. The mean annual rate during 1996-2000 (6.6 per million population) was nearly five times the mean annual rate during 1981-1985

(1.3 per million population). Rates were highest in the western United States. Enhanced public health surveillance of this condition appears warranted to focus prevention programs with greater efficiency and reverse trends identified in this study.

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CYSTIC ECHINOCOCCOSIS IN SOUTHERN AFRICA: MANY OPEN QUESTIONS

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Cystic Echinococcosis (CE) is a zoonotic infection caused by the cestode Echinococcus granulosus. The life cycle of the parasite involves herbivores like sheep, goats, cattle and others as intermediate hosts and carnivores as the definite host. CE is highly endemic in Northern and Eastern Africa, but few epidemiological data on hydatid disease from Sub-Saharan Africa exist. Transhumant pastoralist populations such as in the Turkana region of Kenya carry the highest burden of disease. To date no published epidemiological data of note on hydatid disease exist from Southern Africa; however, there are a number of case reports and the disease is considered to be common, particularly in rural communities. In addition, little is known about co-infections with CE and either HIV or Tuberculosis (TB), or viral hepatitides; whilst it has been observed that the clinical course and treatment outcome can be significantly altered, as there is evidence that the host immune response to the larval cyst greatly influences the clinical course of CE. Unusual cases of disseminated CE have been documented in patients co-infected with HIV and also the Tb-specific response in patients co-infected with echinococcosis and TB is diminished, but improves with successful treatment of echinococcal infection. Apart from more severe clinical courses of CE in the presence of HIV and/or TB, treatment of all three conditions poses a great problem due to significant drug interactions and the possibility of Immune Reconstitution Inflammatory Syndrome (IRIS). We present a retrospective analysis of patients with CE treated between 1995 and 2009 at two large urban hospitals in Johannesburg with a large population of migrant workers from rural areas. The analysis is conducted with particular reference to geographical distribution of cases, risk factors for acquiring CE and presentation and course of disease in the presence of co-infections with HIV and/or TB.

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HUMAN CYSTIC ECHINOCOCCOSIS IN PERUVIAN ENDEMIC AREAS

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Cystic Echinococcosis (CE) is a zoonosis widely distributed around the world; CE affects principally to livestock and accidentally to humans; CE is acquired by the ingestion of *Echinococcus granulosus* eggs that are transmitted by infected dogs. Clinical presentation is characterized by the formation of cystic lesion in the infected host; liver is the principal organ involved (52 to 77%), followed by the lungs (9 to 44%); signs and symptoms of CE depend on the organ involved, the size of the cyst and the presence of any complication, it is asymptomatic during the first years of the infection. Diagnoses is based on imaging techniques (ultrasound-US, chest X Ray, CT scan), serological evaluation is used to support the presumptive diagnose. Peru is considered an endemic country, previous studies in endemic regions showed a CE prevalence of ~7%; nevertheless there is not any current control program. The aim of the present study is to determine the current human CE prevalence in 3 endemic regions. We performed an imagenological (abdominal US, and Chest X-Ray) and serological evaluation (Electro immune transfer blotting) in the population of 9 districts located in 3 Peruvian endemic regions (Junín, Huancavelica y Puno); A total of 1456 participants (Junin), 1675 participants (Puno) and 953 (Huancavelica) were evaluated. In Junin, we found a liver compromise in 4.7% (52 of 1106), lung involvement in 1.96 % (20 of 1018), and a serological positive result in 4.8% (57 of 1188); In Huancavelica, we found a liver involvement in 1.47% (12 of 690), lung involvement in 0.72 % (5 of 691), and a serological positive result in 3.1% (15 of 498); In Puno, we found a liver involvement in 2.33% (26 of 1114), lung involvement in 0.93% (11 of 1185), and a serological positive result in 2.86% (29 of 1014). Human CE is still a public health problem in Peruvian endemic areas, furthermore despite there is not any control program in endemic regions, these results showed a decrease in the last 10 years, (from ~7% to ~5%); nevertheless it is important to recognize the necessity of a local control program to reduce the prevalence found in our study.

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THE IMPACT OF CYSTIC ECHINOCOCCOSIS ON HUMAN HEALTH AND QUALITY OF LIFE IN RIO NEGRO PROVINCE, ARGENTINA

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Cystic echinococcosis (CE), a zoonosis which results in substantial morbidity and economic losses for affected individuals, is highly endemic in Rio Negro Province, Argentina (RNP). A cross-sectional study, which incorporated abdominal ultrasound screening and a questionnaire, was conducted in a rural community in RNP in order to evaluate potential risk factors for CE as well as the health and financial impacts of the disease. A total of 341 adult participants were examined, 33 (9.7%) of whom were identified as being CE positive. The participants, 78% of whom were female, ranged in age from 18 to 89 years, with a mean age of 42 years. Neither age, nor gender, was significantly associated with being CE positive. However, CE positive participants had a significantly ($p = .02$) higher unemployment rate compared to CE negative participants, with rates of 75% and 56%, respectively. In order to examine the impact of CE on the physical and mental health and quality of life of previously undiagnosed individuals, the short form 12 v2 (SF-12v2) health survey was administered to all participants prior to abdominal ultrasound examination. This study found that individuals with a positive diagnosis for CE had a significantly lower mean score for five (physical functioning, role physical, bodily pain, general health, and role-emotional) of the eight domains measured by the health survey. The CE positive participants' mean scores for vitality, social functioning, and mental health, did not differ significantly from those of CE negative individuals. These results suggest that CE is associated with a decrease in the overall health and quality of life of patients prior to formal diagnosis, which is likely to be associated with decreased productivity and a diminished ability to earn income. The results of the questionnaire and the SF-12 v2 health survey will be used in the evaluation of indirect costs associated with human CE among previously undiagnosed individuals. These data will then be incorporated into a larger project examining the total economic impact of CE in this region.

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G1 STRAIN OF *ECHINOCOCCUS GRANULOSUS* INFECTS SOUTH AMERICAN CAMELIDS IN PERU

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Cystic echinococcosis (CE) is a zoonosis widely distributed throughout the world. CE is caused by the taenia *Echinococcus granulosus* that lodges in the intestines of dogs. Herbivores and, accidentally, humans could be infected by the ingestion of *E. granulosus* eggs, resulting in cystic larvae in their liver, lungs, or other tissues. Molecular identification of Echinococcus strains were described as important information to be considered for implementing local control measures in endemic countries. Previous studies performed in Peru and other South American countries described the presence of G6 strain (camel strain) in human and animal population, in addition to the knowledge of CE infection in South American camelids. In order to assess if South American camelids behave as a natural reservoir of the G6 strain (present in goats in the region), we determined the strains of *E. granulosus* in samples from infected South American camelids. First we carried out a survey using PCR and CO1 sequencing of *E. granulosus* isolates collected from llamas located in Peruvian Andes; macroscopic information on the appearance, size, and status of the larvae was collected; the nature and fertility of the sample were confirmed by microscopic observation; total *E. granulosus* DNA was extracted using the DNeasy Tissue kit (QIAGEN, Hilden, Germany); using PCR reactions, and *E. granulosus* genotype was determined by mitochondrial cytochrome c oxidase subunit 1 (CO1) sequencing; sequences were compared using Macrogen kit (Korea). DNA was amplified from 12 isolates; 3 isolates (25%) were identified as G1 and 9 (75%) did not correlate with known strains but had 81% identity to CO1 of Taenia hydatigena, leaving their origin unclear. In this small series, G1 was present and G6 was absent in South American camelids.

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IN VITRO SCREENING FOR NEW COMPOUNDS AGAINST *ECHINOCOCCUS MULTILOCULARIS* METACESTODES IDENTIFIES ANTI-ECHINOCOCCAL ACTIVITY OF MEFLOQUINE

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Alveolar echinococcosis is caused by the metacestode stage of the fox tapeworm *Echinococcus multilocularis* and causes severe disease in the human liver and occasionally other organs, which is fatal if treatment is unsuccessful. The present chemotherapy of AE is based on mebendazole and albendazole, which has been found to be ineffective in some instances, parasitostatic rather than parasiticidal, and usually involves the lifelong uptake of massive doses of drugs. Thus, new treatment options are urgently needed. Within this study, a recently validated parasite viability assay was applied, based on the release of phosphoglucose isomerase (PGI) by dying parasites. A range of 30 thiazolides, 19 pentamidine- and 12 artemisinin-derivatives, and of mefloquine and its (+) and (-) erythro-enantiomers, were tested for their efficacy against E. multilocularis metacestodes *in vitro*. Initial screening of compounds was performed at 40 µM, and those compounds exhibiting considerable anti-parasitic activity were assessed also at lower concentrations. Mefloquine was chosen for subsequent studies. *In vitro* mefloquine treatment at 24 µM resulted in rapid and complete detachment of large parts of the germinal layer from the inner surface of the laminated layer within a few hours, and prolonged treatment for a period of 10 days was parasiticidal as determined by bioassay in mice. Interestingly, as determined by the PGI-assay, the (-) erythro-enantiomer of mefloquine was more active than the (+) enantiomer or a mixture of both erythro-enantiomers. Affinity chromatography employing epoxy-agarose-coupled mefloquine and E. multilocularis extracts identified fructose-bi-phosphate-aldolase and malate-dehydrogenase as mefloquin-binding proteins. In conclusion, mefloquine represents an interesting drug candidate, and is currently followed in appropriate *in vivo* studies on alveolar echinococcosis in the mouse model.

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LONG-LASTING EFFECT OF OXFENDAZOLE AGAINST CYSTIC ECHINOCOCCOSIS IN NATURALLY INFECTED SHEEP

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Cystic Echinococosis (CE) is a zoonotic disease caused by larval stage *Echinococcus granulosus*. It is a public health problem with considerable economical losses in both humans and animals. Treatment options in animal intermediate hosts might have two objectives: animal model for drug use in humans, or eliminate / reduce the parasite burden in animals as potential control strategy. We determined the long-lasting effect of high dose of Oxfendazole (OXF) against CE in naturally infected sheep. A randomized placebo-controlled trial was carried out on 10 randomly selected ewes. Liver ultrasound was performed in order to select those infected sheep. They were assigned to one of the following groups: 1) placebo (n=3); 2) OXF 60mg/Kg of body weight (BW) weekly for four weeks (n=7). Necropsies were performed 7 month after finishing the treatment. Treated animals had significantly smaller cysts in both lung and liver than the controls (approximately 22mm smaller) ($p<0.05$). Percent protoscolex (PSC) viability, evaluated using a 0.1% aqueous eosin vital stain for each cyst, demonstrated a significant decrease in the treatment group as compared to the control ones for both organs lungs and livers (more than 50% reduction) ($p<0.05$). Follow-up ultrasound examination also exhibited a progressive degeneration stages in treated sheep. We demonstrate that Oxfendazole at 60mg weekly is a successful schema that can be added to control measures in animals and merits further study for the treatment of animal CE. Apparently, Oxfendazole has a long-lasting effect on cyst degeneration at least for 7 months after stopping the treatment. Further investigations on different schedules of monotherapy or combined chemotherapy are needed, as well as studies to evaluate the safety and efficacy of Oxfendazole in humans.

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BABESIA SPP. IN WHITE-TAILED DEER AND EASTERN COTTONTAIL RABBITS IN TENNESSEE

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Babesiosis is an emerging zoonotic disease in the United States caused by protozoan parasites of the genus *Babesia*. *Babesia spp.* parasites are transmitted by hard ticks (Ixodidae). Endemic human disease in the United States occurs in the northeast and upper Midwest. Most human cases in the United States are caused by *B. microti*. Recently there have been human cases in Missouri, Washington and Kentucky attributed to a *B. divergens*-like protozoan termed "MO-1". The first confirmed human case of babesiosis in Tennessee occurred in 2008. To investigate the potential public health risks of babesiosis in Tennessee we conducted an environmental survey in the area where the Tennessee babesiosis case reported exposure to wildlife and ticks. To assess potential reservoirs for common North American *Babesia spp.* we collected blood samples from 5 white tailed deer (*Odocoileus virginianus*) and from 7 eastern cottontail rabbits (*Sylvilagus floridanus*). Spleens from 8 rabbits were collected and stored. Additionally, 167 *Ixodes scapularis* and 1 *Amblyomma americanum* were collected from wildlife and flannel tick drags and stored for *Babesia* parasite screening using conventional PCR. Serum was separated and detection of antibodies to *B. odocoilei*, *B. microti*, and *B. divergens*-like MO-1 was determined by indirect immunofluorescence. Serum samples were considered reactive with an IgG titer ≥1:64. Evidence of exposure to a specific *Babesia spp.* was determined by comparing the titers of the 3 *Babesia spp.* tested. A four-fold higher titer was considered evidence of exposure to a specific *Babesia spp.* Four deer serum samples were reactive to *B. odocoilei* antigen, with the last deer serum sample being non-reactive. Two of the rabbit serum samples were non-reactive, 2 were reactive to *B. odocoilei* antigen, and 3 were reactive to MO-1 antigen. We described wildlife with serologic evidence of exposure to *B. odocoilei* and MO-1 parasites in the environment most likely associated with transmission of *Babesia* in Tennessee. Since *B. MO-1* has been attributed to human cases in the United States, including nearby Kentucky, babesiosis should be considered in the differential diagnosis of patients in Tennessee with febrile illness and hemolytic anemia.

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IDENTIFICATION OF *BARTONELLA* SPECIES IN SMALL MAMMALS FROM AN URBAN AND A RURAL LOCATION IN KENYA

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Bacteriological and ecological investigations of small mammals from rural homesteads within Nyanza Province in western Kenya, and from Kibera, an urban slum in Nairobi were conducted in order to characterize the distribution and diversity of *Bartonella* spp. in East Africa. Small mammals were trapped using Sherman live traps placed in and around human dwellings. Whole blood from mammals was placed on blood-enriched agar to culture *Bartonella* bacteria. Additionally, blood samples were screened by PCR using citrate synthase gene (*gltA*) primers for identification of non-cultivable bacteria. *Bartonella* was identified in 13/53 (24.5%) small mammals of 4 species from Nyanza Province: *Rattus rattus* (3), *Mastomys natalensis* (6), *Crocidura olivieri* (3), and *Lemniscomys striatus* (1). In Kibera, 20/33 (60.6%) peri-domestic rats but 0/146 house mice were *Bartonella* positive. Sequence analyses of the *gltA* revealed that Kenyan rodents harbored 4 *Bartonella* spp.: *B. tribocorum* (16), *B. elizabethae* (12), *B. queenslandensis* (5), and a novel strain, which is different from all known *Bartonella* species with the highest percent of identity to *B. birtlesii*. The most prevalent *Bartonella* spp. (*B. elizabethae* and *B. tribocorum*) found in Kenyan rats are highly specific for *Rattus* and *Bandicota* rats in Asian countries. In America and Europe, both *Bartonella* spp. were detected only in urban settings from two domesticated rats, which were introduced within historic times to these continents from Asia. By contrast, in Kenya, these *Bartonella* species were observed not only among peri-domestic rats in Kibera, but also in wild rodents (*M. natalensis* and *L. striatus*) from the rural area. Surprisingly, domestic rats in Kibera also carried *B. queenslandensis*, the bacterium previously identified in Queensland, Australia. The genetic diversity of *Bartonella* spp. found in Kenya is significantly lower than in Asia. The novel strain was detected in the African giant shrew (*C. olivieri*) and additional genetic and phenotypical characterizations are required for description of the new bacterial species. The isolation of the human pathogenic *B. elizabethae* from rodents trapped in close proximity to humans, in an area of high HIV prevalence, raises the possibility of spillover of this pathogen into humans.

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MOLECULAR CHARACTERIZATION OF *BARTONELLA* SPECIES ASSOCIATED WITH DOGS AND THEIR FLEAS AND TICKS IN THE COASTAL LOWLANDS OF MANABI, ECUADOR

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Bartonella cause both zoonotic and vector-borne diseases and infect a wide variety of vertebrate hosts. Thirteen species of *Bartonella* have been associated with human disease while eight species are known to be pathogenic for dogs. In Ecuador, we investigated *Bartonella* species in the blood of domestic dogs and in ectoparasites found on those dogs. Fleas, ticks, and blood samples were collected from 22 dogs residing at twenty-one households in the rural communities of El Bejuco and San Francisco, Manabi Province, Ecuador during June-July 2009. Ectoparasites were collected in 70% ethanol, washed in water, processed by freezing in liquid nitrogen and crushing, and their DNA was extracted. Genetic analysis included PCR amplification of *Bartonella* RibC or GltA gene fragments and sequencing. Fleas and ticks were identified using taxonomic keys along with molecular analyses of mitochondrial 12S and 16S rDNA gene sequences. Matched blood samples to ectoparasite collections were obtained from 17 dogs by venipuncture using EDTA-vacutainers, and DNA was extracted and analyzed as above. The number of fleas collected from single dogs ranged from 1-24, with multiple flea genera being found on single dogs in many cases. Fleas were identified as *Ctenocephalides felis*, *C. canis*, *Pulex irritans*, and *Xenopus cheopis*. In addition, ticks (engorged and flat) were collected from 12/17 dogs, with 1-7 ticks collected per dog. Ticks were identified as *Amblyomma triste*, *A. ovale*, one *Rhipicephalus* spp, and one *Dermacentor* spp. In blood samples taken directly from dogs, only *B. henselae* was detected by PCR in 5/17 dogs. This is in contrast to *Bartonella* spp detected by PCR in both dog-associated fleas and ticks that included *B. vinsonii* subsp. *berkhoffii*, *B. quintana*, *B. henselae*, *B. tribocorum, and B. elizabethae*. Excluding *B. tribocorum*, these *Bartonella* spp are all pathogenic for dogs. Our findings suggest that further investigations of the ecology and epidemiology of *Bartonella* in Ecuador are warranted.

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SOCIOECONOMIC AND ENVIRONMENTAL FACTORS: INCREASING THE POTENTIAL RISK OF TICK-BORNE DISEASES IN THE REPUBLIC OF KOREA

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Man-made and natural ecological events have changed the landscape of the Republic of Korea (ROK) since the 1900's. During the Japanese occupation (1910-1945), forested hillsides were cleared, leaving hills and mountains largely covered by grasses and shrub vegetation. Following WWII (>1945), local populations scavenged for wood for heating during the cold winters and cooking. Just as the ROK was recovering, North Korea attacked, beginning a long drawn-out conflict (1950-1953), which ended in an uneasy cease fire between the two countries. In the 1960's, a tree planting policy was instituted that reestablished the long-ago lavish forested mountains and hillsides that make up >70% of the ROKs landscape. Today, mountains and hillsides are completely forested, which has resulted in increased protection and habitat for large and small mammals, birds, and associated tick populations, all of which increase the establishment and maintenance of zoonotic tick-borne pathogens in these populations and potential transmission to man. A tick-borne disease surveillance program established by the Eighth US Army (2001) has resulted in an increased knowledge of the prevalence of known tick-borne pathogens and identification of new pathogens in small and large mammals, their ectoparasites, and man. The increased use of permethrin-treated uniforms and repellents, reduce the risk of potential for transmission of tick-borne pathogens to US military, but not US civilians and family members while conducting outdoor activities. In addition, training environments of unmanaged forests and grasslands with abundant small and large mammal populations at some field training sites remain largely unchanged, with personnel training in tall grass/forested areas and sleeping in tents that abut forested areas where animals and their ectoparasites are present. Increased surveillance by military personnel and civilian agencies has expanded our knowledge of the prevalence tick populations and tick-borne pathogens, including several spotted fever group rickettsial pathogens previously unreported in the ROK.

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DETECTION OF *RICKETTSIA* SPECIES IN ECTOPARASITES FROM AREAS OF COSTA RICA ENDEMIC FOR SPOTTED FEVERS

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The ecology of spotted fevers in Costa Rica is not well known: even though outbreaks have been reported since the 1950s, the most recent studies date back to the 1980s and the vectors responsible for transmission to humans have not been directly identified. Moreover, rickettsial diseases in several regions of the world have been associated recently with species of *Rickettsia* that had not been described or were considered non-pathogenic to humans. In this study, species of *Rickettsia* were detected in ectoparasites from five areas of Costa Rica where cases of spotted fevers have been reported. In each area, ectoparasites were collected using drag cloths or directly from domestic and wild animals, identified, and organized into lots according to species, host, and location. One pooled sample of 1 to 10 specimens was prepared from each lot for PCR analysis. Specific *Rickettsia* DNA fragments of the *gltA* (citrate synthase), *htrA* (17 kDa protein), and *ompA* (190 kDa protein) genes were detected by PCR in each of the pools, and pools were considered positive when fragments of at least two of the genes were evidenced. Several of the *gltA* gene fragments were sequenced to confirm the presence of *Rickettsia* and identify species. A total 205 pools of ectoparasites were analyzed, and 29% were considered positive for *Rickettsia* DNA by evidence of at least two of the gene fragments. These included 62% of *Amblyomma cajennense,* 60% of *Ctenocephalides felis,* 44% of *Amblyomma ovale*, 18% of *Boophilus microplus*, 10% of *Dermacentor nitens*, and 4% of *Pulex simulans* pools analyzed. No *Rickettsia* DNA was detected in *Rhipicephalus sanguineus* (39 pools). Positivity also varied between the areas evaluated. Sequencing of gene fragments confirmed the presence *Rickettsia felis* as well as species from the typical spotted fever group (SFG). Results show the presence of rickettsiae in vectors that may be responsible for transmission to humans in Costa Rica, and evidence suggests rickettsial infection in the human environment may be common. This is the first study to report different species of *Rickettsia* in various species of ticks and fleas in Costa Rica.

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A COMPARATIVE META-ANALYSIS OF TICK PARALYSIS IN NORTH AMERICA AND AUSTRALIA: EPIDEMIOLOGY, CLINICAL AND ELECTRODIAGNOSTIC MANIFESTATIONS AND OUTCOMES

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Tick paralysis (TP) is a neurotoxic poisoning that mimics polio and primarily afflicts young girls worldwide, especially in hyperendemic regions of the North American Pacific Northwest and Eastern Australia. A comparative meta-analysis of the scientific literature was conducted using Internet search engines to assess the epidemiology, clinical and electrodiagnostic manifestations, and outcomes of TP in North America versus Australia. Well-documented cases of TP were collected in North America and Australia. Continuous data including age, time to tick removal, and duration of paralysis were compared for statistically significant differences by unpaired t-tests; categorical demographic data including sex, geographic distribution, tick vector, and misdiagnosis were compared for statistically significant differences by chi-squares. TP following gravid female ixodid tick bites occurred seasonally and sporadically in individuals and in more clusters of children than in adults of both sexes in urban and rural locations in North America and Australia. The case fatality rate for TP was low, and the proportion of misdiagnoses of TP as the Guillain-Barré syndrome (GBS) was greater in North American than in Australia. Although electrodiagnostic manifestations were similar in North America and Australia, neurotoxidromes differed significantly with prolonged weakness and even recurrent neuromuscular paralysis following tick removal in Australian cases compared to North American cases. TP was a potentially lethal poisoning that occurred in children and adults in a seasonally and regionally predictable fashion. TP was increasingly misdiagnosed as GBS during more recent reporting periods in North America. Such misdiagnoses often directed unnecessary therapies such as central venous plasmapheresis with intravenous immunoglobulin G, and delayed correct diagnosis and tick removal. TP should be added to and quickly excluded from the differential diagnoses of acute ataxia with ascending flaccid paralysis, especially in children living in TP-endemic regions worldwide.

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MEADOW VOLES SERVE AS COMMON BLOODMEAL SOURCES FOR SUBADULT DOG TICKS IN SITES WITH ACTIVE TULAREMIA TRANSMISSION

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The enzootic cycle of *Francisella tularensis tularensis*, the agent of Type A tularemia, remains poorly described. Dogma suggests that the cottontail rabbit, *Sylvilagus floridanus*, is the main reservoir for Type A infection, but the evidence for this remains largely circumstantial. Martha's Vineyard, Massachusetts has continuously reported human tularemia cases each year for the last 10 years. We have implicated the American dog tick, *Dermacentor variabilis*, as critical to perpetuation but have yet been unable to define the role of rabbits in Type A tularemia ecology there. To determine the identity of the animals that serve as the main hosts for feeding subadult dog ticks and thereby serve as the likely Type A reservoir, we identified bloodmeal residues in host seeking adult dog ticks by the use of a reverse line blot (RLB) assay. Host seeking dog ticks were collected from well characterized tularemia natural foci on Martha's Vineyard. DNA was extracted from individual ticks, and mammalian DNA was amplified by PCR using primers targeting a conserved region of the 12S rRNA gene. The amplicons were then assayed by RLB using species-specific probes, and probe binding was detected using chemiluminescence. The probes included the major reservoir host candidates such as meadow voles, white footed mice, and cottontail rabbits, as well as other animals found on the island. Of 106 ticks that have been tested to date, 58 (55%) did not have amplifiable mammalian DNA. Of the ticks that yielded an amplicon, the vast majority were from voles (46%). Only 6% of the ticks appeared to have fed as a nymph on rabbits, with a similar proportion from white footed mice. Interestingly, skunk and raccoon also appeared to serve as hosts for subadult dog ticks, an unexpected finding inasmuch as carnivores are only known to feed adult dog ticks. A third of the amplicons are as yet unidentified. We conclude that voles, not rabbits, are the main hosts for subadult dog ticks and are more likely to serve as reservoir for *F. tularensis tularensis* on Martha's Vineyard.

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WOLBACHIA ENDOSYMBIONTS INVADE THE GERMLINE OF YOUNG ADULT *BRUGIA MALAYI* FROM THE LATERAL CHORDS

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The lymphatic filarial parasite *Brugia malayi* contains *Wolbachia,* α proteobacteria,that are crucial for filarial development and reproduction. In filarial larvae, *Wolbachia* mainly reside in the hypodermis and in hypodermal precursor cells. Developing reproductive tissue is free of endobacteria. In mature adult female worms *Wolbachia* can be observed in the ovaries, oocytes and developing microfilariae. We used immunohistology, in situ hybridization and transmission electron microscopy to study the germline invasion process of *Wolbachia.* We found massive multiplication of *Wolbachia* in the lateral chords of immature adult stage female worms (5 weeks pi) in cells that border the ovaries. *Wolbachia* enter the pseudocoelomic cavity in the median region of the developing ovaries and invade germ cells. In inseminated females (8 weeks pi) *Wolbachia* were detected in embryos and in decreasing numbers in the lateral chords. In young adult stage males (5 weeks pi) *Wolbachia* were found in distinct zones of the developing testis and in large numbers in the lateral chords in the vicinity of testicular tissue. *Wolbachia* were never detected in mature spermatids or spermatozoa in older male worms. The invasion of *Wolbachia* into the reproductive tissue in young adult worms is essential for transovarial propagation of *Wolbachia.* This may be an Achilles heel in the filarial life cycle that could be explored for further intervention.

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THE INVOLVEMENT OF THE *WOLBACHIA* WSP-LIKE PROTEINS IN THE ENDOSYMBIOTIC RELATIONSHIP WITH *BRUGIA MALAYI*

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The parasite *Brugia malayi* is a lymphatic dwelling filarial nematode that infects over 138 million individuals worldwide, primarily in the tropics, and causes Lymphatic Filariasis. *B. malayi* harbors endosymbiotic intracellular bacteria, *Wolbachia* (*w*Bm), that are required for the development and reproduction of this worm. The crucial role of the endosymbiont for *B. malayi* survival suggested novel anti-*Wolbachia* chemotherapeutic approaches for the treatment of human filarial infections. Our study aims to identify proteins that have potentially an essential function in this endosymbiotic relationship. The major *Wolbachia* surface protein (WSP) contains transmembrane domains and a standard signal peptide for secretion. In previous studies, this protein was shown to act as an inducer of the innate immune system and was also implicated in the pathogenesis of the filarial infections in the host. The *Wolbachia* genome contains 6 WSP-like proteins (Wbm0100, Wbm0152, Wbm0284, Wbm0432, Wbm0506 and Wbm0575). Immunoelectron microscopy analyses using anti-WSP (Wbm0284) antibodies confirmed that the protein is not only present on the surface of *w*Bm within the hypodermal region of the worm and inside the oocytes, embryos and microfilaria, but that it is also found in the body cavity within the uterine wall of the adult worms, eggshells surrounding the developing microfilaria and in the hypodermal region of the cuticle. This points to the possibility that WSP plays a role in the symbiotic relationship. To further explore whether the WSP-like proteins interact with proteins produced by the filarial host, we expressed them as GST or HIS tagged recombinant proteins. Each recombinant protein was found to bind specifically to *B. malayi* crude extracts using a modified ELISA assay. The putative *B. malayi* target protein for WSP (Wbm0284) was identified by the panning of a *B. malayi* cDNA phage library, and was named WSP/G2. The *B. malayi* WSP/G2 protein contains a BTB/POZ-like domain proven experimentally in other systems to be involved in Protein:Protein interactions. The specific interactions between the *B. malayi* WSP/G2 protein, WSP and the other WSP-like *Wolbachia* proteins will be presented. Our studies further improve our understanding of this particular symbiotic relationship.

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FUNCTIONAL ANALYSIS OF POLYMORPHISMS IN THE SL CORE PROMOTER DOMAINS OF RIBOSOMAL PROTEIN GENES OF *BRUGIA MALAYI*

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Previous studies have indicated that the promoters of *Brugia malayi* are unusual in that they do not exhibit the CAAT or TATAA sequences usually found in the core domains of promoters of most eukaryotic organisms. Analysis of the promoters of the ribosomal proteins (RPs) showed that the 22nt region flanking the splice leader (SL) addition site plays an important role in transcription and may function as the core promoter domain in *B. malayi*. Interestingly, the promoters of the *B. malayi* RPs demonstrated a wide variation in activity when evaluated using a homologous transfection assay. To evaluate the relative contribution that polymorphisms in the SL core domains made to observed variation seen among the RP promoters, the SL addition site of the BmRPL13 gene was replaced with the SL

addition domains derived from six other RP genes. The promoter activity of the replacement constructs was found to be intermediate between that of BmRPL13 and the corresponding wild type RPs. Comparison of the activity of the replacement constructs to the wild type promoters indicated that on average 85% of the variation in activity observed among the RP promoters could be ascribed to variation in the SL core domain. The activity of replacement mutants containing the 10nt upstream of each RP promoter produced activity levels that were similar to those produced with the substitution mutants containing the entire 22nt SL domain. These data suggest that the majority of the variation in promoter activity seen among the *B. malayi* RP promoters is a result of polymorphisms in the SL core domain, and that the 10nt upstream of the SL addition site represents the primary determinant of promoter activity.

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THE TRANSCRIPTOME OF THE *WOLBACHIA*-FREE FILARIAL NEMATODE *ONCHOCERCA FLEXUOSA*

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We have recently reported the presence of *Wolbachia* -like sequences in DNA from filarial nematode species that do not contain *Wolbachia* endosymbionts. These results suggest that ancestors of these parasites contained *Wolbachia,* acquired *Wolbachia* DNA by horizontal gene transfer, and later eliminated the bacteria. RT-PCR studies showed that some of the *Wolbachia* -like sequences were transcribed. We now report studies of the transcriptome of *Onchocerca* flexuosa. One objective of this work was to identify the most abundantly transcribed *Wolbachia* sequences in this *Wolbachia* -free parasite. The transcriptome was sequenced using GS-FLX 454 Titanium technology. 575,491 reads were assembled with 2,124 EST sequences derived from a conventional cDNA library to produce 13,129 isogroups and 30,861 singletons. 48,372 peptides were predicted based on transcript sequences, and deduced amino acid sequences were analyzed by blastp. 49% of predicted proteins had significant homology to known filarial nematode proteins, 3% had homology to proteins in other nematodes, and 4% had homology to proteins in other organisms. KEGG analysis showed that the most highly represented metabolic pathways were purine metabolism, oxidative phosphorylation, pyrimidine metabolism, and glycolysis. At the nucleotide level, 144 isogroups and 175 singletons showed homology to *Wolbachia* sequences. 42 of these isogroups and 19 of these singletons also contained sequences with homology to filarial genes on other regions of the transcript. 66 of the transcribed *Wolbachia* -like sequences contained putative open reading frames. These results confirm the presence of *Wolbachia* -like sequences in O. flexuosa transcripts and show that these sequences are often found on transcripts that also contain typical filarial sequences. Studies in progress will determine whether any of these *Wolbachia* -like sequences are expressed at the protein level. This may explain the ability of a minority of filarial species to live and reproduce without an endobacterial partner.

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A RELIABLE *IN VIVO* APPROACH TO RNA INTERFERENCE IN MOSQUITO-BORNE FILARIAL WORMS

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Diseases caused by parasitic nematodes perpetuate socioeconomic instability in developing countries by inflicting crippling morbidity and significant mortality. One reason for the persistence of these diseases is the limited portfolio of effective drugs available to combat parasitic nematodes. A major obstacle to the rational development of new anthelmintics is the experimental intractability of parasitic nematodes; they simply are not amenable to many of the techniques commonly used to develop drugs for other diseases. Illustrative of this is RNA interference (RNAi) - a reverse genetic tool that allows researchers to rapidly and specifically 'turn off' genes of interest in an organism or cell line. RNAi has become a standard tool in rational drug development and validation of potential new drug targets for many diseases, but a reliable and reproducible protocol has yet to be established for animal parasitic nematodes. We describe an innovative strategy for the application of RNAi to study gene function and validate drug targets in animal parasitic nematodes. Our approach uses the filarial nematode *Brugia malayi* as a model and targets developing parasites in the mosquito host. We can profoundly suppress expression of a cathepsin-L-like gene using and RNAi trigger injected directly into infected mosquitoes. RT-qPCR confirms that suppression is specific and results in an 83% decrease in transcript abundance. Most importantly, cathepsin L-like suppression stunts parasite growth and development and elicits profound motility defects that effectively abolish transmission potential. Finally we present evidence that other genes are susceptible to this approach; we have successfully now used this method to suppress genes encoding a beta-tubulin (Bm-tub-1), G-protein linked acetylcholine receptor 2 (Bm-gar-2), prohomone convertase (Bm-pc-2), and a transcript with homology to a nicotinic acetylcholine receptor alpha subunit.

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BEYOND FILARIAL GENOMICS: PROTEOMIC ANALYSES PROVIDE INSIGHTS INTO BOTH THE FILARIAL HOST AND ITS *WOLBACHIA* ENDOSYMBIONT

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Global proteomic analyses of pathogens have thus far been limited to unicellular organisms (e.g. protozoa and bacteria). Proteomics of most eukaryotic pathogens (e.g. helminths) have been restricted to specific organs, specific stages or secretomes. We report here a large-scale proteomic characterization of almost all the major mammalian stages of *Brugia malayi* (Bm), a causative agent of lymphatic filariasis and its endosymbiont *Wolbachia* (wBm). Proteomes of both the *B. malayi* and *Wolbachia* of multiple stages were analysed by nanobore reverse-phase liquid chromatography-tandem-MS (nanoRPLC-MS/MS). The obtained spectra were searched against *B. malayi* and *Wolbachia* databases using SEQUEST. Methionine oxidation and phosphorylations on serine, threonine and tyrosine were included as dynamic modifications in the database search. A total of 7267 proteins (~62% of the 11610 genes predicted from the *Brugia* genome) from adult male, adult female, microfilariae, L3 larvae and uterine immature microfilariae (UTMF) were identified. Genomic analysis predicted that 4956 (42.4%) of the total number of genes as being hypothetical proteins; the present study was able to confirm 2336 (47.1%) as *bonafide* proteins. Among the identified proteins in each stage studied (except UTMF [13%]), 4-5% were determined to have 'stage-specific' protein expression. Gene set enrichment analysis demonstrated that extracellular matrix related and immunologically related proteins are enriched in the microfilarial and L3 stages compared to the other stages. Proteomic analysis of *Wolbachia* resulted in the identification of 557 of the 805 predicted wBm proteins, some of which appeared to be expressed stage-specifically. Parallel analysis of Bm and wBm protein families and domains in concert with each of their stage-specific expression highlight important pathways (both parasite and endosymbiont) that benefit the parasite during its development in the host.

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IVERMECTIN DISRUPTS THE FUNCTION OF THE EXCRETORY-SECRETORY APPARATUS IN MICROFILARIAE OF *BRUGIA MALAYI*

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In vivo, ivermectin (IVM) treatment of filarial infections is characterized by a rapid drop in the levels of circulating microfilariae (Mf) followed by the long term suppression of their production. Nevertheless, the direct effect of this drug on the Mf of most filarial nematodes is still debatable as there is no clear *in vitro* evidence of its microfilaricidal action. IVM acts upon binding to nematode Glutamate-gated Chloride Channels (GluCl), resulting in disruption of the neurotransmission processes that are regulated by the activity of these channels. To identify the physiological effects of IVM on Mf, we cloned and localized two AVR-14 subunits from *Brugia malayi*, which constitute their only putative IVM-sensitive GluCl subunits. Bma-AVR-14 subunits co-localized with a muscle structure surrounding the Mf- Excretory-Secretory (ES) vesicle. It suggests that under the control of GluCl, protein release in the ES apparatus is driven by the contraction of this vesicle. Consistently, *in vitro* IVM treatment led to a decrease in total protein released from Mf. Protein release decreased in 0.1 µM IVM up to 58, 68 and 42 % the amount released by the control at 24, 48 and 72 h, respectively. To understand how IVM can affect secretion of proteins released by the parasite, we identified 3 different localization patterns among a group of 5 known Mf- ES products; suggesting that either the parasite surface or the ES apparatus are probable anatomical pathways for physiological protein release. Nevertheless, the presence of muscle association with the ES-vesicle and the low permeability characteristic of the Mf sheath points to the ES-apparatus as the main source of Mf protein delivery to the mammalian host. Mf treatment with IVM targets the ES apparatus, which constitutes the main source of parasite protein release to the mammalian host. This leads to the inference that rapid Mf clearance related to IVM treatment *in vivo* reduces the secretion of immunomodulatory parasite proteins and thus induces a parasiticidal effect of the host immune system.

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AN ALPHAVIRUS REPLICON BASED DENGUE VACCINE IS IMMUNOGENIC AND PROTECTIVE IN RHESUS MACAQUES AND INDUCES PREDOMINANTLY DOMAIN III REACTIVE NEUTRALIZING ANTIBODIES

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Domain III of flavivirus E protein (EDIII) has been identified as a major target of strongly neutralizing monoclonal antibodies. However, natural infection of humans with DENV and WNV results in only a small proportion of antibodies directed to EDIII, representing a minor contribution to the neutralization potency of the DEN and WNV immune sera. Therefore, it is likely that tetravalent live attenuated dengue vaccines, which mimic natural infection and are currently in clinical trials, will induce a small proportion of EDIII neutralizing Abs. We have developed a vaccine modality based on an alphavirus replicon vector (VRP) expressing soluble DENV E protein (Es) that induces neutralizing antibodies directed predominantly to EDIII and complete protection in macaques. Interestingly, we found that neutralizing antibodies induced in macaques infected with live DENV, mostly recognize epitopes outside EDIII, as seen in humans. Rhesus macaques (n=6) were immunized by s.c. inoculation with 10e8 IU DEN3 Es-VRP at weeks 0, 7 and 18. All animals produced neutralizing antibodies to DEN3 after the first immunization, and maintained them throughout the study. To determine protective efficacy, vaccinated macaques (at 15 wks-post 3rd dose) along with 4 unvaccinated controls, were challenged with 10e5 pfu of DEN3 live virus. All VRP-immunized animals showed complete protection from viremia, as measured by focus assay on Vero cells. The contribution of EDIII reactive antibodies to the neutralizing response was determined by depleting DEN3 EDIII binding antibodies from the sera of DEN3 Es-VRP vaccinated and DEN3 live virus infected monkeys. EDIII adsorbed sera from VRP immunized macaques lost most of its neutralizing activity; while EDIII adsorbed sera from live virus infected animals retained most of its neutralizing activity. We propose that VRP delivery of soluble DENV E protein allows EDIII to become visible to the immune system, in the presence of EDI and EDII. This may represent an advantage over other vaccine platforms. A tetravalent Es-VRP has been constructed and will be tested in macaques.

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TOWARDS DEVELOPING A NON-INVASIVE INTRADERMAL VACCINATION STRATEGY WITH A TETRAVALENT DENGUE VACCINE (DENVAX): IMMUNOGENICITY AND EFFICACY STUDIES IN MICE AND NON-HUMAN PRIMATES

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Among the mosquito-borne viruses, dengue (DEN) viruses are notable for their global distribution and the frequency of large scale epidemics that they cause. To date, there is no effective vaccine to prevent against DEN infections. DENVax is a tetravalent DEN vaccine based on using the DEN-2 vaccine, strain PDK-53, as a vector. Three chimeric recombinant viruses were constructed, each bearing the vector`s capsid and non-structural gene backbone while expressing DEN-1,-3 or -4 prM and E structural genes.

In a series of experiments we examined the immunogenicity and protective efficacy of DENVax administered via the ID route in mice and non-human primates. Comparison of the immunogenicity of DENVax-4 vaccine via the ID vs. subcutaneous (SC) route in AG129 mice clearly indicated the superiority of the ID route over the SC. The neutralizing anti-DEN-4 antibody responses were 5-fold higher. Additional groups of AG129 mice were immunized ID or SC with a tetravalent DENVax formulation bearing the ratio of 105: 104: 105:105 PFU of DENVax-1, DENVax-2, DENVax-3 and DENVax-4, respectively. The neutralizing titers against DEN-1, -3 and -4 were higher when DENVax was given ID. However, responses to DEN-2 were unaffected by the route of immunization. Non-human primates (Cynomologous macaques) were immunized with a tetravalent DENVax formulation containing 105 PFU of each vaccine virus by SC administration with needle and syringe or ID administration using a needle-free device. Needle-free ID administration induced neutralizing antibody titers against all four DEN serotypes greater than those elicited after SC injection. All ID vaccinated animals challenged SC with 105 PFU of wild-type DEN-1 West Pacific (WP) or DEN-2 New Guinea C (NGC) viruses were shown to be free of viremia. Needle-free ID DENVax delivery has the advantage of making vaccine administration simple and eliminating concerns about needle reuse and disposal. These preclinical studies set the stage for human clinical testing of DENVax formulations in dengue naïve adult volunteers.

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CLINICAL DEVELOPMENT OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics with an estimated 50-100 million cases of dengue annually. To date no specific vaccine or therapy has been licensed to combat this important disease. To address this unmet need, Hawaii Biotech, Inc. is developing a tetravalent recombinant subunit vaccine to protect individuals against dengue virus induced disease. GMP manufacture of the four truncated dengue envelope proteins to support human clinical trials has been completed. Preclinical studies conducted in mice and rabbits have demonstrated the immunogenicity of both monovalent and tetravalent formulations adjuvanted with alum, and formal toxicology studies have demonstrated acceptable safety. An alum-based monovalent DEN1 formulation was shown to be immunogenic and protective in non-human primates. A Phase 1 clinical study of monovalent DEN1 has been conducted in healthy volunteers. Currently, final preparations are ongoing for a Phase 1 clinical study of the tetravalent subunit vaccine. The current status of preclinical and clinical development will be presented.

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EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF TETRAVAX-DV, A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE

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Dengue virus (DEN) has become the most important arbovirus worldwide with a 30-fold increase in DEN infections and hundreds-fold increase in the more severe form, dengue hemorrhagic fever/shock syndrome. Because a secondary DEN infection with a serotype different from that which caused the primary infection is a significant risk factor for DHF/DSS, a DEN vaccine must induce a long-lived immune response to all four DENV serotypes. The goal of the National Institutes of Health (NIH) DEN vaccine program is to produce a minimally reactogenic, highly immunogenic, genetically stable, live attenuated DEN vaccine that is cost-effective and safe for the community. Over the past ten years, the NIH has developed numerous live attenuated candidate vaccines against the four individual DEN serotypes. We have tested 8 monovalent vaccines in 15 Phase I clinical trials to identify DEN1, DEN2, DEN3, and DEN4 candidate vaccine viruses that are safe and maintain the optimal infectivity and immunogenicity profiles for inclusion in a tetravalent formulation. Each monovalent candidate was well tolerated by volunteers with no volunteer experiencing a dengue-like illness. Following a single subcutaneous injection of 1,000 PFU, each of the candidate vaccines induced seroconversion rates of 80 - 100% to its parent wild-type virus. Factors such as infectivity, immunogenicity, and reactogenicity were used to determine which vaccine candidates to include in the tetravalent formulation, and this selection process will be discussed. A Phase I Clinical trial evaluating 3 different tetravalent admixtures in flavivirus naïve subjects was initiated in 2010. Preliminary safety and immunogenicity results from this Phase I clinical trial will be discussed.

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DIFFERENT INNATE SIGNATURES INDUCED IN HUMAN MDCS BY WILD-TYPE DENGUE 3 VIRUS, ATTENUATED BUT REACTOGENIC DENGUE 3 VIRUS, OR NON-REACTOGENIC ATTENUATED DENGUE 1-4 VACCINE VIRUSES

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Dengue infection is a major and growing public health issue worldwide. Different vaccine candidates are being developed, including YFV 17D vaccine-based chimeric dengue virus vaccines (CYDs). Dendritic cells (DCs) play a key role in initiating immune responses and are primary targets of dengue infection. The consequence of human monocyte-derived DCs (mDCs) infection by various wild type (wt) dengue strains has been investigated by several authors. We addressed the innate profile induced in mDCs upon infection by each of the 4 CYDs and their tetravalent combination. In a first study, a limited set of activation markers, cytokines and chemokines was assessed by ELISA, flow cytometry and qRT-PCR. This first study showed that CYDs induced DC maturation, a controlled immune response, limited inflammatory cytokine production, and consistent expression of anti-viral interferons, confirming clinical observations of safety and immunogenicity. A second study used 22K and 44K Agilent DNA microarrays to assess mDCs infected by the 4 CYDs alone or in combination, or by a wt serotype 3 virus, or a classically attenuated serotype 3 virus (VDV3) shown to be reactogenic in a clinical trial. The results of this second study confirmed and expanded upon the first: we observed a very reproducible signature for each of the 4 CYDs, involving stimulation of Type I IFN genes and associated ISGs, together with genes encoding chemokines and other mediators involved in the initiation of adaptive responses. In contrast, the wt virus induced a predominantly inflammatory profile, while VDV3 appeared to induce a blunted response, which may have been insufficient to trigger early immune responses and prevent initial viral replication. This could have contributed to VDV3 symptomatic outcome in clinical trials. These studies contributed to documenting the safety and immunogenicity of the 4 CYD candidate vaccine viruses, which are currently in evaluation in large scale efficacy trials.

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PRIMING EFFECT OF PREVIOUS JAPANESE ENCEPHALITIS VACCINATION ON HUMORAL IMMUNE RESPONSE TO TETRAVALENT DENGUE VACCINE

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The potential priming effect of prior Japanese encephalitis vaccination (JEV) on antibody responses to tetravalent live-attenuated chimeric dengue virus vaccine (TDV) was assessed. The TDV vaccine viruses were constructed by replacing genes for non-structural pre-membrane (PrM) and envelope (E) proteins of the attenuated YF 17D vaccine with wild-type dengue serotypes sequences. In a randomized, controlled, multicenter, Phase II study in Mexico City, 18-45 year-old participants received two injections of TDV at days 0 and 105 (Group 1) or 3 injections of JE-VAX® at days -14, -7, and 0 followed by one dose of TDV at day 105 (Group 2). Dengue antibody levels were determined by microneutralization assay before, 28 and 60 days after each TDV vaccination and at day 365. Viremia was assessed by PCR methods 7-14 and 21 days after each TDV vaccination. Safety was documented after vaccination through day 28. Seropositivity rates (antibody level ≥10 1/dil) 28 days after 2 doses of TDV (Group 1, n=31) were 61%, 36%, 71% and 75% respectively against dengue serotypes 1-4. After 1 dose of TDV among JEV primed (Group 2,

n=30), these rates were 85.2%, 85.2%, 85.2%, 92.6%. GMTs against dengue serotypes 1-4 for Group 1 / Group 2 were: 18.4/37.4, 12.6/27.4, 16.7/42.4, and 56.5/219. The percentage of seropositive subjects for at least 3 serotypes was 46% for Group 1 and 78% for Group 2. JEV priming did not increase viremia, which remained low and infrequent in both groups. Serotype 4 was the most frequently detected followed by serotype 3. Both groups had acceptable and similar safety profiles. Injection site pain and headache were the most common solicited reactions. In conclusion, seropositivity rates and GMTs of subjects primed with JEV vaccine receiving 1 dose of TDV were higher than those who received 2 doses of TDV, suggesting that pre-existing JE immunity may boost antibody responses to the TDV without modifying the safety profile.

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PRE-EXISTING IMMUNITY TO JAPANESE ENCEPHALITIS VIRUS IS ASSOCIATED WITH AN INCREASED RISK OF SYMPTOMATIC ILLNESS FOLLOWING A DENGUE VIRUS INFECTION

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Dengue viruses (DENVs) and Japanese Encephalitis virus (JEV) co-circulate in Southeast Asia, where they are both important causes of human morbidity and mortality. They have significant cross-reactivity in serological assays, but the possible clinical implications of this remain poorly defined. Previous studies have suggested a protective effect of JEV immunization against dengue hemorrhagic fever (DHF). An improved understanding of whether and how JEV immunity modulates the clinical outcome of DENV infection is important as large-scale DENV vaccine trials will commence in areas where JEV is co-endemic and/or JEV immunization is routine. The association between pre-existing JEV neutralizing antibodies (NAbs) and the clinical severity of subsequent DENV infection was evaluated in a cohort of school children in Northern Thailand. Covariates considered included age, baseline DENV antibody status, school of attendance, epidemic year, and infecting DENV serotype. The presence of JEV NAbs in serum collected prior to DENV infection was associated with an increased probability of symptomatic versus subclinical infection (OR=2.02, p<0.01). This association was strongest in children with negative DENV serology (DENV-naive) (OR=3.13, p<0.01); the OR declined with seropositivity to an increasing number of DENV serotypes. Significant differences in the association were observed by infecting DENV serotype in multivariate analysis. There was no significant effect of JEV NAb on the probability of DHF (OR=1.40, p=0.30). The prior existence of JEV NAbs was associated with an increased probability of symptomatic as compared to subclinical DENV illness. JEV seropositivity was not associated with the occurrence of DHF, but the small number of DHF cases limited the power of this comparison. These findings are in contrast to previous studies suggesting a protective effect of heterologous flavivirus immunity on DENV disease severity. Further evaluations of possible clinical and immunological interactions between flaviviruses are warranted.

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ROLE OF RAPID DIAGNOSTIC TESTING (RDT) IN THE CONTEXT OF HOME MANAGEMENT OF CHILDHOOD FEVER (HMCF) WITH DISPERSIBLE ARTEMETHER-LUMEFANTRINE: AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL IN A RURAL AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

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Delivery of prompt and adequate treatment (currently ACTs) for uncomplicated malaria at community level remains a key strategy to reduce the burden of malaria in sub-Saharan Africa. Rapid Diagnosis Tests (RDT) could help improve diagnosis at home level by trained community health workers (CHW) and therefore rationalize the use of ACTs. This study aimed to assess fever management based on RDT or presumptive ACT treatment and RDT use by CHW. In an open label, cluster randomized study, 12 villages were randomized (1:1) ratio into an intervention and a control arm. Children aged 6-59 months (mo) with a history of fever (last 24 hours) or axillary T°> 37.5°C received presumptive treatment with antimalarial drug (dispersible artemether-lumefantrine, DAL) in the control arm. In the intervention arm, RDT and Respiratory rate counting was performed so that febrile children received DAL and/or antibiotic based on an algorithm followed by the CHW. In both arms, a blood smear was obtained at baseline (Day 0). Participants were assessed at Day 3 and Day 7 and febrile children were referred to a community clinic. A total of 732 children (354 in the control arm; mean age 29.4±15.4 mo vs. 378 in the intervention arm; mean age of 27.6±15.1 mo) were enrolled during the malaria high transmission period. Prevalence of malaria infection was comparable at baseline across arms (76.2% in the control vs. 76.4% in the intervention arm, P= 0.97). Specificity and sensitivity of RDT as compared to microscopy were respectively 28.7% and 98.6%. On day 3, fever clearance rate (FCR) was 96.3% in the control vs. 93.3% in the intervention arm (P = 0.08), and on Day 7, 99.0% in the control vs. 97.3% in the intervention arm (P=0.1). Data for the dry season are being collected, and an analysis by season (Low vs. High transmission season) will be presented. A high fever clearance rate was achieved with DAL in both arms. The study has shown that the use of RDT by community Health Workers is feasible in our context. However a more specific test is needed for use at community level to be recommended.

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MANAGEMENT OF FEBRILE YOUNG CHILDREN IN MALARIA MIX-ENDEMIC AREAS (*PLASMODIUM FALCIPARUM* AND *P. VIVAX*). QUICK ATTENDANCE, RAPID TESTING AND EFFECTIVE TREATMENT: A SAFE ATTITUDE IN PAPUA NEW GUINEA

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The management of febrile children in malaria endemic areas has evolved and the following approach has been proposed: febrile children should attend quickly to get tested [rapid diagnostic test (RDT) or microscopy] and receive efficient malaria treatment if positive. There are safety concerns about withholding antimalaria drugs from children with negative test. More generally, we have no data on the accuracy of this strategy, especially in areas with mixed endemicity (Pf and Pv). The present study explores the feasibility of this approach in Papua New Guinea (PNG), a highly endemic country for both Pf and Pv. Alongside a malaria preventive drug trial (IPTi), a morbidity surveillance was set up to record all illness

episodes. When presenting fever, study participants were screened by health workers for malaria using RDT's (ICT combo®) and treated with Coartem® when tested positive. Blood slides (BS) were also collected. From 2006 to 2009, 1605 infants 3 months old were enrolled and followed-up for 2 years. A total of 7004 febrile episodes were recorded. The median symptoms' duration was 2 days. 3807 (54%) had a negative RDT. Among them, 146 (3.8%) re-attended the clinic within 7 days for fever, 1 died (negative RDT & BS) and 24 (0.6%) presented a serious adverse event: 13 had a negative RDT, 3 had a positive RDT or quick read (but negative BS) and 8 had no RDT's results (2 had negative BS, 1 positive BS, 4 without BS were treated without antimalarial drugs for alternative diagnostics, 1 without BS received Coartem®).There were 1677 positive RDT's. All treated with Coartem®, 39 (2.3%) re-attended within 7 days for fever, none died and 5 (0.3%) presented a serious adverse event (2 possible severe malaria, 1 possible meningitis, 1 severe pneumonia and 1 gastroenteritis). This study provides good evidence that the approach "quick attendance, rapid testing and effective treatment" is safe and feasible in infants in countries with limited resources and a high level of Pv infections.

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HOW CAN MALARIA RAPID DIAGNOSTIC TESTS ACHIEVE THEIR POTENTIAL? A QUALITATIVE STUDY OF A TRIAL AT HEALTH FACILITIES IN GHANA

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Rapid diagnostic tests (RDTs) for malaria are at the early stages of introduction across malaria endemic countries in an effort to improve case management. Evidence of the effect of introducing RDTs on the prescription of antimalarials is mixed. A recent trial of RDTs in rural health facilities in Ghana reduced overprescription of antimalarials, but found that 45.5% patients who tested negative were still prescribed an antimalarial. We conducted a qualitative study of the implementation of RDTs in this trial. We interviewed health workers who had been shown to either continue to prescribe anti-malarials to most patients with negative RDT results or who largely restricted anti-malarials to patients with positive RDT results. Interviews explored the experiences of using RDTs and their results amongst trial participants.

Meanings of RDTs were constructed by health workers through participation with the tests themselves as well as through interactions with colleagues, patients and the research team. These different modes of participation with the tests and their results led to a change in practice for some health workers, and reinforced existing practice for others. Many of the characteristics of RDTs were found to be inherently conducive to change, but limited support from purveyors, lack of system antecedents for change and limited system readiness for change were apparent in the analysis. When introduced with a limited supporting package, health workers had learnt to use RDTs differently. To build confidence of health workers in the face of negative RDT results, a supporting package should include local preparation for the innovation; unambiguous guidelines; training in alternative causes of disease; regular support for health workers to meet as communities of practice; interventions that address negotiation of health worker-patient relationships and encourage self-reflection of practice; feedback systems for results of quality control of RDTs and of prescribing practices; and RDT augmentation such as a technical and/or clinical troubleshooting resource.

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A LARGE PROPORTION OF ASYMPTOMATIC MALARIA INFECTIONS WITH LOW PARASITE DENSITIES IN TEMOTU PROVINCE, SOLOMON ISLANDS: CHALLENGES FOR MALARIA DIAGNOSTICS IN AN ELIMINATION SETTING

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Many countries are scaling up malaria interventions towards elimination. This transition changes demands on malaria diagnostics from detecting parasites in ill patients to detecting asymptomatic reservoirs. The selection of diagnostic methods must be solved prior to transitioning a malaria control program to elimination. A baseline malaria parasitological survey was conducted in Temotu Province, Solomon Islands, in late 2008, as the first step in a provincial malaria elimination program. The survey provided opportunities to obtain point prevalence and epidemiological characteristics of malaria infections on the island, as well as to assess how well different diagnostic methods performed in this particular setting.

During the survey, 9491 blood samples (~50% population) were collected and examined by microscopy with a subset also examined by PCR and RDTs. A total of 256 samples were determined positive by microscopy, of which 17.5% and 82.4% had Plasmodium *falciparum* and P. vivax, respectively. Interestingly in this low transmission setting, only 17.8% of the P. *falciparum* and 2.9% of P. vivax infected subjects were febrile (≥38oC) at the time of survey with 50% and 66.7% of the P. *falciparum* and P. vivax fever observed in the 5-14 age group. Overall, 40% of the P. *falciparum* and 65.6% of the P. vivax infected subjects had parasite density below 100/µL. There was increase in the proportion of parasite density below 100/µL with age for P. vivax infections, but no such a correlation was observed for P. *falciparum* infections. The observed large proportion of infections with densities below 100/µL presents a major challenge to microscopy, RDT and PCR. In general, there is a reasonable agreement between microscopy and PCR in detecting P. vivax, but poor agreement in detecting P. *falciparum* and mixed infections, particularly in samples below 100/µL where 85.7% of the discrepancies occur. The results suggest a combination of methods, or new diagnostics, may be required to detect infections in asymptomatic parasite reservoirs, the prevalence of which is high even in low transmission settings.

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DOES THIS PATIENT HAVE MALARIA? A META-ANALYSIS OF THE DIAGNOSTIC UTILITY OF CLINICAL FACTORS FOR ENDEMIC AND IMPORTED MALARIA

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Malaria commonly infects residents of and travelers to tropical regions, and the clinical features, if any, are notoriously nonspecific. We endeavored to systematically review and synthesize data regarding the predictive value of clinical findings for the diagnosis of malaria in endemic areas and in returning travelers. We searched MEDLINE (1950-July 2009) to identify English-language studies of endemic malaria and "imported malaria," and additional studies were identified from reference lists. We included studies that compared the presence or absence of pre-defined

clinical findings with blood smear confirmation of parasitemia in patients suspected of acute malaria. Two authors independently identified studies, appraised study quality, and extracted data. We identified over 500 studies for endemic malaria, but only 15 met review criteria. Individual symptoms are of limited diagnostic utility, but splenomegaly (summary positive likelihood ratio [LR] 3.3; 95% confidence interval [CI] 2.0-4.7) and hepatomegaly (summary positive LR 2.7; 95% CI 1.9-3.5) make malaria infection more likely. Combinations of findings can impact the likelihood of malaria, but their performance varies by setting. We identified over 900 studies for imported malaria, but only 28 met criteria. The presence of fever (positive LR 5.1; 95% CI 4.9-5.3), splenomegaly (summary positive LR 6.5; 95% CI 3.9-11), hyperbilirubinemia (positive LR 7.3; 95% CI 5.5-9.6), or thrombocytopenia (summary positive LR 4.9; 95% CI 2.4-10) make malaria much more likely. In endemic areas, the likelihood of finding parasitemia is increased by the presence of splenomegaly and hepatomegaly, but individual findings are overall of limited utility; clinical algorithms may be useful to risk-stratify patients but their performance is variable between settings. In returning travelers, the clinical assessment can provide substantial diagnostic benefit, but all patients still require laboratory testing.

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PHRP-2 PLASMA CONCENTRATIONS DISTINGUISH BETWEEN MALAWIAN CHILDREN WITH RETINOPATHY-POSITIVE AND RETINOPATHY-NEGATIVE CEREBRAL MALARIA

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As malaria control and eradication efforts expand, it will be increasingly important to identify cases of severe malaria. The standard clinical case definition of cerebral malaria incorrectly identifies 25% of children as having cerebral malaria when compared with post-mortem histology. The best clinical predictor, malarial retinopathy, is >95% sensitive and specific, but well-trained personnel and expensive equipment are required. We investigated plasma concentrations of pHRP-2, a *Plasmodium*-specific protein released mainly on schizont rupture, as an indicator of "true" cerebral malaria in patients fulfilling the standard clinical case definition. In a group of 64 patients with clinically defined cerebral malaria who died and underwent autopsy, 47 patients had histological evidence of sequestration in the cerebral microvasculature. The sensitivity and specificity of pHRP-2 concentrations for distinguishing the patients with true CM were 98% and 94%, respectively (area under the ROC, AUROC, was 0.98). In a larger group of children with clinically defined cerebral malaria (n=260), a cut-off concentration of pHRP-2 distinguished retinopathy-positive children from those without retinopathy with a sensitivity and specificity of 0.90 (AUROC = 0.90). In contrast, the AUROC associated with peripheral parasitemia was 0.53. pHRP-2 is a measure of both circulating and sequestered parasites, and the association of higher concentrations with histologically- and fundoscopically-confirmed cerebral malaria suggests that parasite burden is important in disease pathogenesis. Plasma concentrations of pHRP-2 may be a more field friendly approach to identifying patients with retinopathy-positive (ie, "true") cerebral malaria, and if so, this would facilitate appropriate treatment and simplify disease surveillance.

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REAL-TIME QUANTITATIVE RT-PCR FOR MONITORING PARASITEMIA IN MALARIA HUMAN CHALLENGE TRIALS

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Malaria vaccine clinical trials using the malaria human challenge model require accurate and sensitive methods for detection of parasitemia. Currently the gold standard is peripheral blood smear, although development of highly sensitive and reproducible quantitative assays that detect pre-patent parasitemia is desirable. We developed a real-time quantitative reverse transcription PCR (qRT-PCR) for the A-type 18S rRNAs of *Plasmodium falciparum*. Methods: Total nucleic acids were extracted from frozen whole blood samples spiked with an exogenous competitive control RNA. Quantitative RT-PCR with dual hybridization probes for the malaria 18S rRNA was performed. The standard curve generated from *in vitro*-transcribed RNA standards was correlated against a parasite-containing whole blood standard to determine the number of 18S rRNA molecules per parasite. Results: The analytical sensitivity of the assay is 20 parasites/mL. The assay correctly identified all parasite-containing samples (ranging from 40 parasites/mL to 4x107 parasites/mL) and all negative samples (clinical sensitivity and specificity 100%). The reportable range is ≥20 parasites/mL. The observed values for >98.7% of samples tested were within 0.5 log10 units of the nominal values. At 80 parasites/mL, the within-run coefficient of variation was 1.8% and between-runs 4.0%; at 4x107 parasites/mL, the within- and between-run variation was 0.6% and 1.6%, respectively. This method was used to assess A-type 18S rRNA expression throughout the blood-stage lifecycle of highly synchronized *P. falciparum* parasites. We also used the assay to monitor parasitemia in a *P. falciparum* human challenge trial. Conclusions: This method offers advantages over comparably-sensitive DNA-based PCR assays because specimen handling requirements are reduced (filtering of leukocytes is not required) and a small blood volume can be used. This qRT-PCR format can be adapted for other *Plasmodium* species. The assay will be an important tool for monitoring malaria clinical trials and also may be adapted for diagnostic purposes in the clinical laboratory.

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COMMON RED BLOOD CELL POLYMORPHISMS CONFER DIFFERENT LEVELS OF PROTECTION AGAINST *PLASMODIUM FALCIPARUM* MALARIA: RESULTS FROM A PEDIATRIC COHORT IN MALI, WEST AFRICA

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In Mali, 5 red blood cell (RBC) polymorphisms are extremely common, are frequently co-inherited, and are associated with reduced risk of severe *falciparum* malaria. These include sickle hemoglobin S (HbS), HbC, alpha-thalassemia, G6PD deficiency, and type O blood group antigen. To determine whether and to what degree each of these RBC polymorphisms protect against uncomplicated *falciparum* malaria in a single study population, we initiated a 5-year longitudinal cohort study in three villages

in rural Mali. From June 2008 to December 2009, we enrolled 1419 children aged 6 months to 17 years. We found that the vast majority of children carry at least 1 RBC polymorphism: 15% HbS, 8% HbC, 28% alpha-thalassemia, 15% G6PD deficiency, and 40% type O blood group antigen. During the 2008 and 2009 transmission seasons (June-December), we diagnosed 1277 children with 1980 episodes of *falciparum* malaria (92% uncomplicated, 8% severe - mostly prostration, repetitive vomiting, and cessation of eating and drinking). To estimate the relative risk (RR) of malaria by RBC polymorphism, we compared malaria incidence rate ratios using a Poisson regression model that took into account age (a surrogate of acquired immunity), sex, ethnicity, and village. Since only 11 episodes of cerebral malaria or severe malarial anemia were diagnosed, we combined all cases of malaria for ease of analysis. Compared to HbA, HbS reduced the incidence of malaria by 39% (RR 0.61, 95%CI 0.51-0.73, p <0.0001). This reduction was comparable to that associated with age 6-10 years (RR 0.77, 95%CI 0.68-0.88, p <0.0001) and 11-17 years (RR 0.35, 95%CI 0.29-0.42, p <0.0001), when compared to age 0.5-5 years. Other RBC polymorphisms were not associated with statistically significant reductions in malaria incidence. The malaria protective effect of HbS was not associated with reduced parasite density. In contrast, the protective effect of age was strongly associated with reduced parasite density. Relative to children aged 0.5-5 years, children aged 6-10 years and 11-17 years showed 63% and 83% reductions in mean parasite density. Contrasting the protective effects of HbS and age on the development of malaria may yield important clues about the mechanism of protection by the sickle-cell trait.

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IRON DEFICIENCY DECREASES THE RISK OF *PLASMODIUM FALCIPARUM* MALARIA AND DEATH IN CHILDREN

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Iron supplementation in malaria endemic areas may increase malaria morbidity and mortality. We explored whether iron status alters malaria risk in Tanzanian children (N=785) living in an area of intense malaria transmission. Children were enrolled at birth, and monitored for malaria and iron status for up to 3 yrs with an average of 47 blood smears and 3 iron status determinations/child. We evaluated the impact of iron deficiency (ID) on malaria outcomes and mortality using multivariate models accounting for repeated measures and potential confounders. Compared to iron-replete children, children with ID had reduced prevalence of concurrent parasitemia (6.6-fold lower), hyperparasitemia (24.0-fold lower) and severe malaria (4.0-fold lower), and, if infected, 3.9 fold lower parasite density (all P < 0.001). ID predicted significant decreases in the odds of subsequent parasitemia (23% decrease, P<0.001) and subsequent severe malaria (38% decrease, P=0.04). Children with ID on half or more of their iron status measurements (N=407) had 63 % lower all cause mortality (P = 0.04) and 73% lower malaria-associated mortality (P = 0.07) compared to children with ID on fewer than half of their iron status measurements (N=378). Together with published data, our results indicate that malaria risk is influenced by physiologic iron status as well as iron supplementation. Future interventional studies should assess whether ID and malaria control measures can mitigate the risks of iron supplementation for children in areas of malaria transmission.

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THE ROLE OF VARIANT ANTIGEN SWITCHING IN THE DEVELOPMENT OF SYMPTOMATIC MALARIA

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Var genes encode proteins that mediate adherence of erythrocytes infected by the malaria parasite *Plasmodium falciparum* to human tissue and comprise 50 to 60 genes within each parasite's genome. The parasite's ability to switch *var* gene expression may permit it to sequester in different sites in the body and potentially avoid the immune system, thereby contributing to virulence. Previous work in Bandiagara, Mali has shown that cerebral malaria is associated with the expression of a group of structurally distinct *var* genes. To date, there have been no longitudinal studies evaluating how *var* gene expression changes in a population. We aimed to compare *var* gene expression changes in individuals who progress from asymptomatic to symptomatic malaria infection compared to individuals who remain asymptomatic over time. We enrolled 300 children in an ongoing longitudinal cohort study of malaria incidence in Bandiagara, Mali. Blood samples obtained quarterly and during episodes of clinical malaria were evaluated for parasite RNA expression of *var* genes with reverse transcription PCR using degenerate primers specific to this gene family. During the first year of follow-up, study subjects experienced on average at least one clinical malaria episode each, and six subjects were diagnosed with cerebral malaria. The parasite RNA collected during symptomatic and asymptomatic time points is being evaluated for *var* gene expression, and results will be presented describing association of expression of particular *var* gene groupings with clinical illness and the extent to which a change in *var* gene expression increases an individual's risk of developing symptomatic malaria.

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VARIATION WITHIN THE TOLL-LIKE RECEPTOR-9 (TLR-9) GENE PROMOTER (-1237C/T) IS ASSOCIATED WITH PROTECTION AGAINST PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IFN-Γ

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Plasmodium falciparum malaria still remains one of the leading global causes of infectious disease burden. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia (SMA) results in high rates of pediatric morbidity and mortality. Since Toll-like receptors (TLRs) affect innate and adaptive immune responses, the roles of polymorphic variants within TLR-9 in conditioning susceptibility to SMA were investigated. The relationship between the TLR-9 variant (-1237C/T, rs5743836) and susceptibility to SMA (Hb<6.0 g/dL, any density parasitemia) was investigated in children (n=277) with *falciparum* malaria from a holoendemic *P. falciparum* transmission region in western Kenya. Hematological and parasitological profiles were determined in all study participants. TLR-9 -1237C/T genotypes were determined using a bi-directional allele-specific PCR amplification. Circulating interferon (IFN)-γ levels were determined using BiosourceTM hu multiplex inflammatory profile. Frequencies of the -1237CC, CT and TT were 6.8%, 44.4%, and

48.7%, respectively. Multivariate logistic regression analyses controlling for potential confounders demonstrated that homozygous C individuals (OR; 0.31, 95% CI, 0.10-0.95; *P*=0.040) and heterozygous (TC) individuals (OR; 0.57, 95%CI, 0.34-0.95; *P*=0.030) were protected against SMA relative to TT carriers. In addition, carriers of the CC genotype had significantly lower circulating IFN-γ levels relative to TC (*P*=0.040) and TT individuals (*P*=0.098). Results presented here demonstrate that variation in TLR-9 at -1237 is associated with protection against SMA and functional changes in circulating IFN-γ levels.

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C5A IS ELEVATED IN AFRICAN CHILDREN WITH CEREBRAL MALARIA (CM) AND C5A RECEPTOR DEFICIENCY IMPROVES SURVIVAL IN EXPERIMENTAL CEREBRAL MALARIA (ECM)

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Cerebral malaria (CM) in humans and in animal models (ECM) is associated with a dysregulated host innate immune response to infection. For example, high levels of inflammatory cytokines contribute to endothelial cell activation and increased expression of adhesion molecules resulting in the sequestration of parasitized erythrocytes in the cerebral microvasculature. The complement system is an essential component of host innate immunity, and its activation culminates in the generation of pro-inflammatory anaphylatoxins, C3a and C5a. Using a panel of inbred and congenic mice, our laboratory has recently demonstrated that susceptibility to ECM is associated with the generation of C5a, which contributes to dysregulated inflammatory and angiogenic responses to parasite products. C5a can bind two receptors, C5aR and C5L2. The pro-inflammatory effects of C5a are thought to occur via its interaction with C5aR. Based on the hypothesis that excessive complement activation contributes to severe malaria, we have further investigated the role of C5a in the pathogenesis of CM by: i.) examining the levels of C5a in Ugandan children with CM versus those with uncomplicated malaria (UM); and ii.) determining if mice deficient in C5aR or C5L2 are protected from ECM. In a case-control study of Ugandan children, we show that children with CM had significantly elevated plasma levels of C5a compared to children with UM (median(range); CM, 45.4(10.0, 126.2) vs. UM, 22.4(3.3, 226.7); p =0.0003, Mann-Whitney). In ECM studies we show that C57BL/6 mice deficient in C5aR (C5aR KO), had significantly improved survival following challenge with *Plasmodium berghei* ANKA (PbA) compared to wild-type controls. Survival was associated with increased levels of angiopoietin-1, an angiogenic factor linked to endothelial cell quiescence. These results are consistent with a role for C5a in the pathogenesis of ECM and in CM in human infection. Additional studies examining the role of the alternate receptor, C5L2, in ECM are underway.

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ELEVATED LEVELS OF CSF TETRAHYDROBIOPTERIN AND NEOPTERIN DISTINGUISH NON-MALARIAL COMA FROM CEREBRAL MALARIA WITH HIGH SPECIFICITY AND SENSITIVITY

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Based on finding abnormal aromatic amino acid (AAA) metabolism in children with cerebral malaria (CM), we conducted a prospective observational cohort study to test the hypothesis that CNS products of AAA, biogenic amine neurotransmitters (BAN), i.e. catecholamines and serotonin, were deficient. BAN metabolites were quantified in CSF of CM cases and compared to: a) children with coma from non-malarial conditions (NMC) and b) a reference database of normals (Biodef). AAA metabolism requires a pterin cofactor, tetrahydrobiopterin (BH4), for BAN synthesis. We hypothesized that BH4 was deficient in CM. We measured BH4 levels in CSF of children with CM and NMC and compared the results to Biodef. CSF was collected per protocol and analyzed by Medical Neurogenetics, Atlanta, GA, for BAN metabolites and pterins. Study groups were defined by WHO criteria. Excluded were children in whom LP was contraindicated and those with severe anemia. All participants (6 months to 6 years) were entered with IRB-approved guardian consent. We enrolled 33 with CM and 43 with NMC. CSF levels of BAN metabolites were normal and indistinguishable between CM and NMC participants. CSF BH4 was elevated in 70% of children with CM but it was normal in all children with NMC (p = 0.002; 100% specificity for CM). Neopterin (another CSF pterin) was elevated in all with CM but in only 28% of NMC subjects (p = 0.003; 100% sensitivity for CM). Elevated CSF neopterin was reported in CM, encephalitis and hemophagocytic syndrome. Elevated CSF BH4 is very unusual; it was found in a rare genetic disorder, Aicardi-Goutières syndrome, caused by mutations of *RNASEH2B* or *TREX1* or *SAMHD1* genes. The common feature of this inflammatory brain disease is uninhibited signaling through TLR-9 by non-degraded nucleic acid polymers. The recent finding of TLR-9 signaling by *Plasmodium falciparum* DNA bound to hemazoin (as reported previously) may relate to increased CNS pterin synthesis as a manifestation of a brain-specific inflammatory pathway leading to malarial coma.

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IN VIVO EVIDENCE THAT MICROCIRCULATORY OBSTRUCTION IS A CENTRAL PATHOLOGICAL PROCESS IN SEVERE *FALCIPARUM* MALARIA

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First identified more than a century ago by Marchiafava and Bignami, the quantitative contribution of microcirculatory obstruction to the pathogenesis of severe *falciparum* malaria is still disputed. We have studied the different factors compromising the microcirculation and their relationships with disease severity. Direct microscopic observation of the rectal microcirculation in living patients reveals obstructed capillaries in

patients with severe malaria. Red cell deformability (RCD) is decreased in relation to disease severity, and is likely to contribute to reduced perfusion in capillaries already partly obstructed by sequestered erythrocytes. The sequestered parasite biomass, estimated from parasite derived plasma PfHRP2 concentrations, correlates strongly with disease severity. Marked sequestration is evident in the brain microvasculature of patients who died from cerebral malaria (CM) and cytoadhesion of mature parasitized red blood cells to the venular and capillary endothelium is readily seen on electron microscopy. In living patients evidence of microcirculatory obstruction in the brain in CM is difficult to obtain. Observation of the retinal vasculature has the advantage that it is the only central nervous system vascular bed easily accessible for visualisation and provides a unique opportunity to observe vascular pathology and its effect on neurological tissue. A specific retinopathy has been described in African children with CM and its severity correlates with outcome. Detailed fluorescein angiography suggests this results from microcirculatory obstruction. Recent studies have found the same retinopathy in adults. This is most common (around 80%) in cerebral and fatal malaria and its severity correlates with disease severity. Markers and causes of microvascular obstruction (RCD, rectal microvascular blood flow, blood lactate and PfHRP2) correlate strongly with the severity of retinopathy. These data all point to obstruction of microcirculatory flow as a central pathophysiological mechanism causing coma and death in CM.

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CHARACTERISTICS AND ETIOLOGY OF MODERATE-TO-SEVERE DIARRHEA OF PROLONGED OR PERSISTENT DURATION AMONG CHILDREN LESS THAN FIVE YEARS OLD IN RURAL WESTERN KENYA, 2008-2009

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Diarrheal disease is a leading cause of illness and death among children <5 years old in sub-Saharan Africa. Data on diarrhea of extended duration is limited. We examined diarrhea duration in Kenyan children <5 years old participating in the Global Enterics Multicenter Study. Children presenting at a clinic were enrolled if they met the case definition for acute moderate-to-severe diarrhea defined as ≥3 loose stools in the last 24 hrs, within 7 days of illness onset, with ≥1 of the following: sunken eyes, skin tenting, dysentery, IV rehydration, or hospitalization. To determine diarrhea duration, the child's caretaker was asked to recall the number of days the child had diarrhea in the 7 days pre-enrollment, and to record each day of diarrhea post-enrollment on a form for 14 days. Stool specimens were collected at enrollment, and the post-enrollment form was collected during a home visit. We defined acute diarrhea (AD) as ≤6 days duration, prolonged diarrhea (ProD) as 7-13 days, and persistent diarrhea (PD) as ≥14 days. From January 31, 2008 to January 30, 2009, 485 children with acute moderate-to-severe diarrhea were enrolled. Of these, 47% (n=226) had AD, 46% (n=221) had ProD, and 8% (n=38) had PD. For males (n=271) and females (n=214) respectively, 42% and 52% had AD, 49% and 41% had ProD, and 8% and 7% had PD. Infants (n=219), toddlers (n=138), and older children (n=128) had AD (40%, 46%, and 59%); ProD (50%, 45%, and 38%); and PD (10%, 9%, and 3%), respectively. Children with ProD or PD respectively, had enteroaggregative *E. coli* (EAEC) identified in 21% and 32% of their stool specimens, *Giardia* in 19% and 11%, enterotoxigenic *E. coli* (ETEC) *in* 14% and 11%, rotavirus in 13% and 11%, enteropathogenic *E. coli* in 13% and 5%, *Campylobacter* in 12% and 16%, *Cryptosporidium* in 10% and 26%, norovirus in 9% and 8%, *Shigella* in 7% and 3%, and *Salmonella* 5% and 5%. A high proportion of moderate-to-severe diarrheal illness was of prolonged duration, and the most common etiologies of ProD were EAEC, *Giardia* and ETEC, and of PD were EAEC, *Cryptosporidium* and *Campylobacter*.

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A PROSPECTIVE AETIOLOGICAL, EPIDEMIOLOGICAL AND CLINICAL STUDY ON DIARRHOEAL DISEASE IN CHILDREN UNDER FIVE YEARS OF AGE IN HO CHI MINH CITY, VIETNAM

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Childhood diarrhoea remains an important public health challenge in developing countries, where the disease represents the second leading cause of mortality in children under the age of five. Routine identification of the aetiological agents causing diarrhoea is seldom performed in resource limited countries. Vietnam is typical of a country undergoing economic transition where the spectrum of infectious diseases is changing rapidly. We conducted a prospective study to investigate the aetiology, epidemiology and clinical features of acute diarrhoea in children under the age of five in Ho Chi Minh City, in southern Vietnam. The study was designed to enroll 1,500 patients admitted to three referral hospitals over a period of one year. Our preliminary data demonstrated that viral pathogens were responsible for more than 60% of diarrhoeal infections with rotavirus being the most prevalent cause followed by norovirus. Rotavirus G1P[8] and norovirus genogroup II were the two most common viral genotypes isolated. The results also demonstrated that more than 80% of these diarrhoea episodes were treated with antimicrobials. This unnecessary use of antimicrobials may contribute to the dramatic level of antimicrobial resistance seen in the bacterial pathogens isolated, including common resistance to fluoroquinolones and 3rd generation cephalosporins. Bacterial pathogens accounted for approximately 20% of all cases, of which *Shigella spp*, *Salmonella spp*, *Campylobacter jejuni* and *Campylobacter coli* were the major agents identified. We were also able to identify specific symptoms, which may help in developing clinical algorithms, hence aiding diagnosis and guide appropriate treatment regimens. Our findings highlight a change in the aetiology and epidemiology of childhood diarrhoea in Ho Chi Minh City. This change is concurrent with socio-economic development and will help inform public health policy for the prevention and treatment of childhood diarrhoea.

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SPATIAL AND TEMPORAL RELATIONSHIP OF CIRCULATING *SALMONELLA* TYPHI IN KATHMANDU, NEPAL

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Enteric fever, the disease caused by invasive Salmonella serovars Typhi and Paratyphi A is still a considerable public health problem in some setting. Enteric fever is mainly isolated to parts of Asia, South America and Africa, and is limited to densely populated areas with poor sanitation, which facilitates ongoing transmission of the organisms. The disease is restricted to humans, therefore, understanding the structure and

dynamics of Salmonella Typhi is essential to study ongoing interactions between the pathogen and host. The amalgamation of genetic, phenotypic, spatial and temporal data can provide an extensive view of the epidemiology and evolution of this bacterial pathogen. However, the lack of a suitable technique has made it difficult to identify changes in Salmonella Typhi populations through time and space. New generation sequencing technologies have been applied to identify ~2,000 SNPs within the Salmonella Typhi population, providing loci for refined single nucleotide polymorphism (SNP) typing of clinical isolates. Four hundred and fifty Salmonella Typhi strains isolated over a four year period from a an urban area in Kathmandu were genotyped and analyzed along with GPS, epidemiological and geospatial data. We find the circulation of a dominant genotype, which we describe as the epidemic strain, which co-localizes with the areas surrounding the municipal water spouts. We additionally find evidence of microevolution and specific localities in which less common genotypes circulate. Our findings demonstrate that, contrary to popular belief, acute transmission within the household is highly limited and that specific genotypes show integral spatial relationships. These data add insight into the dissemination of these organisms in this community and will provide the framework for molecular epidemiological studies of other bacterial pathogens.

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CHARACTERIZATION OF ANTI-*SALMONELLA ENTERICA* SEROTYPE TYPHI ANTIBODY RESPONSES IN BACTEREMIC BANGLADESHI PATIENTS USING IMMUNO-AFFINITY PROTEOMIC-BASED TECHNOLOGY (IPT)

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Salmonella enterica serotype Typhi (S. Typhi) is the cause of typhoid fever and a human-restricted pathogen. Currently available typhoid vaccines provide only 50-75% protection for 2-5 years, and available diagnostic assays to identify individuals with typhoid fever lack both sensitivity and specificity. Identifying immunogenic S. Typhi antigens expressed during human infection could lead to improved diagnostic assays and vaccines. Here we describe a platform Immuno-affinity Proteomic-based Technology (IPT) that involves the use of columns charged with IgG, IgM or IgA antibody fractions recovered from humans bacteremic with S. Typhi to capture S. Typhi proteins subsequently identified by mass spectrometry. This screening tool identifies immunogenic proteins recognized by antibodies from infected hosts. Using this technology and the plasma of patients with S. Typhi bacteremia in Bangladesh, we identified 57 proteins of S. Typhi, including proteins known to be immunogenic (PagC, HlyE, OmpA, and GroEL), and a number of proteins present in the human-restricted serotypes S. Typhi and S. Paratyphi A but rarely found in broader host-range Salmonella spp. (HlyE, CdtB, PltA, and STY1364). We categorized identified proteins into a number of major groupings, including those involved in energy metabolism, protein synthesis, iron homeostasis, biosynthetic and metabolic functions, and those predicted to localize to the outer membrane. We assessed systemic and mucosal anti-HlyE responses in S. Typhi infected patients, and detected anti-HlyE responses at the time of clinical presentation in patients but not in controls. These findings could assist in the development of improved diagnostic assays.

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LEPTOSPIROSIS AMONG HOSPITALIZED PATIENTS WITH FEBRILE ILLNESS IN NORTHERN TANZANIA

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The epidemiology of human leptospirosis in Tanzania is not well described. We examined leptospirosis as a cause of febrile illness among inpatients in northern Tanzania to determine its importance and inform control measures. We identified febrile patients among consecutive admissions to two hospitals in Moshi, Tanzania, from September 2007 through August 2008, recorded standardized demographic and clinical information, and collected acute and convalescent sera. Confirmed leptospirosis was defined as a ≥4-fold increase in MAT titer; probable leptospirosis as any reciprocal MAT titer ≥ 800; and exposure to leptospirosis as any titer ≥100. Among 870 patients enrolled in the study, 453 (52.1%) had paired sera available, and 40 (8.8%) of these met the definition for confirmed leptospirosis. Of 831 patients with ≥1 serum sample available, 30 (3.6%) had probable leptospirosis and an additional 277 (33.3%) had evidence of leptospirosis exposure. Of 70 persons with confirmed or probable leptospirosis 39 (55.7%) were male, the median age was 23 (range <1-78) years, and none were diagnosed clinically. Among those subsequently found to have confirmed or probable leptospirosis the most common clinical diagnoses were pneumonia in 18 (25.7%) and malaria in 31 (44.3%); 14 (20.0%) were treated with antimalarials alone. Among adults and adolescents, leptospirosis was associated with thrombocytopenia (OR 2.1, p=0.019). Leptospirosis was associated with living in a rural area (OR 3.4, p<0.001). Among 40 patients with confirmed leptospirosis, the predominant reactive serogroups were Mini and Australis, and 16 (40.0%) had evidence of co-infection with ≥1 additional pathogen. Leptospirosis is underdiagnosed yet accounts for a substantial proportion of febrile illness in northern Tanzania where it appears to be endemic. Clinicians should suspect leptospirosis in febrile patients, particularly those with thrombocytopenia or rural residence. Based on the pattern of serogroup reactivity, livestock are likely reservoirs. Further research to inform control measures in Tanzania is warranted.

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ALTERNATIVE COMMUNITY-BASED SCREENING FOR RAT INFESTATION TO IDENTIFY HIGH-RISK HOUSEHOLDS FOR LEPTOSIROSIS IN URBAN SLUM SETTLEMENTS

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The domestic rat is believed to be the principal reservoir for urban leptospirosis. However, few if any studies have identified markers for rodent infestation in slum environments and evaluated their use in predicting the risk for leptospirosis. We performed a case-control study,

which enrolled households of leptospirosis cases identified between 2007 and 2009 in Salvador, Brazil and neighboring control households in the same slum communities. Households were surveyed for signs of rodent infestation and environmental characteristics. We used conditional logistic regression modeling to identify risk factors and develop a predictive score for leptospirosis with data collected from 2007 to 2008. We used receiver operating characteristic (ROC) curve analysis to evaluate the performance of the prediction score with an independent data set collected in 2009. We identified signs of rodent infestation in 63% (60/95) and 35% (64/184) of the cases and control households, respectively. Independent risk factors for acquiring leptospirosis in a household were rodent burrows (OR, 3.30; 95% CI, 1.50-7.26), *Rattus norvegicus* feces (2.86; 1.24-6.59), rodent runs (2.57; 1.06-6.22), household bordering an abandoned house (2.48; 1.04-6.02), and unplastered walls (2.22; 1.02-6.02). A prediction score was developed by assigning points (3, 3, 2, 2 and 2 respectively) to each risk factor. The area under the ROC curve for the scoring system was 0.70 (95% CI, 0.64-0.76) and 0.71 (0.65-0.79) for the development and validation datasets. In conclusion, our study indicates that high proportions (>44%) of urban slum households are infested with *R. norvegicus*. A simple prediction score demonstrated good performance in identifying high-risk households for leptospirosis within slum communities. These findings need to be confirmed in other urban centers. Yet, they suggest that community-based screening for rodent infestation may be a feasible strategy to target rodent and environmental control measures to populations at highest risk for leptospirosis.

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Q FEVER AMONG HOSPITALIZED FEBRILE PATIENTS IN NORTHERN TANZANIA, 2007-2008

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Little is known about *Coxiella burnetii* as a cause of febrile illness in sub-Saharan Africa, and its putative role as an HIV coinfection is unclear. We identified febrile patients among consecutive admissions to two hospitals in Moshi, Tanzania, from September 2007 to August 2008, recorded standardized clinical data, and collected acute and convalescent sera. After *C. burnetii* phase II antigen ELISA screening, positive samples with paired sera were tested by IgG immunfluorescence assay. A >=4-fold increase in titer to *C. burnetii* phase II antigen defined acute Q fever; a titer >=1/1,000 to *C. burnetii* phase I antigen defined probable chronic Q fever. Predictors of Q fever and patient management were examined. Among 870 febrile patients, 483 (55.5%) had sera screened; results suggested acute and chronic Q fever among 24 (5.0%) and 7 (1.4%) patients, respectively. The median (range) age for acute and chronic cases was 26 (1, 73) years and 29 (1, 49) years, respectively. Clinical features of acute Q fever included headache (82.4%), cough (75.0%), rigors (58.8%), and anemia (60.9%). Acute Q fever was associated with hepato- or splenomegaly (OR 3.1, p=0.026), anemia (OR 3.5, p=0.003), leukopenia (OR 4.0, p=0.012), jaundice (OR 7.0, p=0.008), and livestock parturient season (OR 3.2, p=0.004). HIV infection was not associated with acute Q fever (OR 1.7, p=0.217). Q fever was never clinically diagnosed; the most common diagnoses among those subsequently found to have acute Q fever were malaria in 8 (33.3%) and pneumonia in 5 (20.8%); 2 (8.3%) patients received antimicrobials active against *C. burnetii*. No chronic cases had clinical endocarditis and no patients with Q fever died. Despite being an important cause of febrile illness in northern Tanzania, Q fever is not considered in the differential diagnosis and patients are seldom treated with an agent active against *C. burnetii*. Increased awareness, access to Q fever diagnostic tests and more frequent use of tetracyclines may improve patient outcomes. *C. burnetii* does not appear to be an HIV-related coinfection.

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DISEASE ASSOCIATION MAPPING IN *ANOPHELES GAMBIAE*: WHAT IS THE EFFECT OF SINGLE NUCLEOTIDE POLYMORPHISMS ON MALARIA INFECTION?

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There are over one million deaths and 500 million cases annually of malaria, a disease caused by parasites of the genus *Plasmodium* that are vectored by anopheline mosquitoes. We have adopted a "disease association mapping" approach, commonly used in humans, in a novel way to determine whether single nucleotide polymorphisms (SNPs) in immune signaling genes of *Anopheles gambiae* are associated with *Plasmodium falciparum* infection. We examined the encoded conserved domains of more than 30 mosquito immune signaling genes to determine SNP presence and predicted effects on protein function. For preliminary functional analyses, we have developed a "designer SNP" approach to analyze SNPs that are predicted to inhibit the interaction of the *A. gambiae* signaling proteins MEK and ERK. These proteins are components of a MAPK signaling cascade that has been shown to regulate *P. falciparum* development in the mosquito host. Our data can be used not only to answer important basic science questions about susceptibility under natural conditions, but also to discover genetic markers that can be used for surveillance and to develop genetically engineered mosquitoes that are refractory to malaria parasite transmission.

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SMALL RNA PROFILE ANALYSIS PROVIDES NEW INSIGHT INTO MOSQUITO-DENGUE INTERACTIONS

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In vector mosquitoes, such as Aedes aegypti, arboviruses hijack host cell processes to establish a successful infection, evade the immune response, and support transmission to mammalian hosts. RNA interference (RNAi) is one way that mosquitoes defend themselves against Dengue virus (DENV). This pathway is an important regulator of host cell gene expression and also cleaves viral genomes. Small RNA (sRNA) regulatory pathways (SRRPs) control host gene expression in a variety of ways. A major product of this control mechanism is the production of small RNAs in different size classes. RNAi and PIWI pathway-produced sRNAs are 20-23 nts and 24-30 nts, respectively. We show that one component of the Ae. aegypti RNAi pathway, Argonaute-2, binds to small RNAs in the expected size range and is associated with a large molecular weight complex (RNA-induced Silencing Complex). These complexes are present in mosquitoes prior to a bloodmeal and are depleted by 1 day after bloodfeeding, suggesting that available RISC complexes may be rate-limiting in anti-viral defense. We used deep sequencing technology to analyze changes to small RNA profiles during DENV infection. Enrichment of sRNAs for a given target mRNA indicates depletion of the target by an SRRP. This method allows us to assess biological pathways that are altered during arbovirus infection in an RNAi-dependent manner. We found that transcript levels in the following categories are altered during DENV infection at 2 and 4 dpi:

immunity, transport, transcription factors, and energy metabolism. Each of these functional categories is important to establishing a successful DENV infection. In addition, viral sRNA profiles change over the course of infection. The data presented will provide insight into the cellular processes that are affected during DENV infection.

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A PEROXIDASE/DUAL OXIDASE SYSTEM MODULATES MIDGUT EPITHELIAL IMMUNITY IN *ANOPHELES GAMBIAE*

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A large number of commensal bacteria reside in the gut of insects, like in most metazoa. Gut epithelial cells need to protect the host from pathogenic organisms but must do so without mounting immune responses against the normal microbiota. This is especially challenging in blood-feeding insects because commensal bacteria proliferate extensively during blood digestion. We have found that a heme peroxidase (Immuno modulatory peroxidase, IMPer), secreted by the mosquito *Anopheles gambiae* midgut, and dual oxidase (DUOX) form a dityrosine network that decreases gut permeability to immune elicitors. This network protects the microbiota by preventing activation of epithelial immunity. IMPer midgut expression is upregulated around 12h after a blood meal and its activity is localized to the ectoperitrophic space. IMPer dsRNA mediated knock down leads to a decrease in gut bacteria and to an up regulation of mosquito immune related genes, suggesting that during normal digestion IMPer activity prevents a midgut immune response against bacteria. IMPEr or DUOX dsRNA mediated knock down decrease infection of the midgut by *Plasmodium berghei* or *Plasmodium falciparum* by up regulating nitric oxide synthetase (NOS) expression. This indicates that IMPer and DUOX normal activity prevent the induction of NOS expression in response to *Plasmodium*. Peroxidases are known to form protein netwoks by formation of dityrosine covalent bonds, for which DUOX can generate a required substrate (H2O2). Immunostaining of the mosquito midgut detected dityrosine bonds in the luminal side of the midgut epithelia, which decreased upon dsRNA mediated knock down of IMPer or DUOX. Midgut permeability to fluorescent dextran increases upon dsRNA mediated knock down of IMPer or DUOX. The results indicated that IMPer and DUOX prevent immune activation of the midgut by forming a dityrosine protein network that decreases permeability to immune elicitors, a novel function for a Peroxidase/oxidase system.

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THE ROLE OF AUTOPHAGY IN FOLLICULAR ATRESIA DURING OOGENESIS IN THE MOSQUITO *AEDES AEGYPTI*

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Autophagy is a process for degrading and recycling cytoplasmic contents via formation of an autophagosome that fuses with a lysosome. Autophagy is triggered by diverse physiological stressors including starvation and infection; indeed recently published and preliminary data indicate that autophagy is an innate immune response in arbovirus-infected flies. To explore the importance of autophagy in oogenesis in the mosquito, autophagy-related genes (Atg) were identified in the *Ae. aegypti* genome and profiled for spatial and temporal transcript production by RT-PCR. Autophagy-related gene 1 (Atg1) was of particular interest because it is a protein kinase that regulates induction of autophagy directly downstream of the target of rapamycin (TOR), and we observed an increase in Atg1 transcript in *Ae. aegypti* ovaries post-blood feeding. Adult *Ae. aegypti* were subjected to Atg1 suppression by injection with double-stranded RNA. At 20 and 48 hours post blood meal (hpbm), ovaries were dissected and subjected to neutral red staining, and gene suppression was confirmed by RT-PCR. In Atg1 suppressed mosquitoes, significantly more resorbing, atretic follicles are observed at 48 hpbm as compared to those in normally progressing ovaries in control mosquitoes. This distinct phenotype is consistent with Atg1 Drosophila mutants that show decreased apoptotic cell death in mid-stage oogenesis when follicles are undergoing atresia, suggesting that autophagy functions upstream of apoptosis in clearing atretic follicles from the ovariole. These data reveal similar and significant interplay between apoptosis and autophagy in the process of follicular atresia in a mosquito, and highlight the importance of autophagy in mosquito oogenesis. Given that autophagy is detrimental to arbovirus persistence, but critical for oogenesis, vertically transmitted mosquito-borne viruses must strike a careful balance in the ovary to avoid and not suppress autophagic events.

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MOLECULAR CLONING OF SLIMFAST FROM THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI* REVEALS A HISTIDINE-SPECIFIC TRANSCEPTOR

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Anautogenous mosquitoes are vectors of numerous diseases because they require blood feeding for their egg maturation and as a consequence they transmit pathogens. Following blood feeding, the surge of amino acids in the hemolymph of female mosquitoes induces multiple events including yolk protein precursor (YPP) gene expression in the fat bodies via direct activation of the TOR (target of rapamycin) signal transduction pathway controlling the activation of ovarian development and egg maturation in conjunction with the ecdysone-coupled signaling. Principle mediators and plasma membrane molecular components controlling those nutritional signaling changes remain to be identified in vectors insects. Two members of the cationic amino acid transporter (CAT) subfamily of the solute carrier family 7 (SLC7) named slimfast (slif) and iCAT2 were previously identified as essential nutrient signaling mechanisms in the fruit fly and the mosquito *Aedes aegypti*. The cloned mammalian CAT-SLC7 members are y+ system exchangers for cationic L-amino acids; however, functions of CAT-SLC7 in other organisms were enigmatic. We determine the expression and transport function of slif from Ae. aegypti. AeSlif gene transcription increases during postembryonic development and peaks in the 3rd and 4th larval stages, but the corresponding protein was detected only in pupae and emerging adult mosquitoes. Functional expression of AeSlif in Xenopus oocytes reveals a sodium-independent cationic amino acid transporter with a preference for transporting L-histidine. Therefore, AeSlif represents the first CAT-SLC7 member acting as a L-His transceptor. This protein can execute two functions: absorption of L-histidine with K 0.5 L-His ~0.4 mM, and transport of L-histidine messengers across cell membranes of fat bodies' trophocytes for signaling nutrient availability in the female mosquitoes. This study has contributed to our understanding of the role of nutritional blood meal mediated signaling of egg development in vector mosquitoes.

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A NEWLY DESCRIBED NATURAL POPULATION SUBGROUP OF THE MOSQUITO *ANOPHELES GAMBIAE* IS EXCEPTIONALLY SUSCEPTIBLE TO HUMAN MALARIA

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Capture of indoor-resting mosquitoes via aspirator or pyrethroid spray is a commonly used approach for *Anopheles* population sampling. However, indoor collections do not efficiently recover behaviorally distinct compartments of the population, and larval sampling followed by genotyping and genetic analysis may represent a comprehensive and less biased alternative. When this approach was used in the Sudan Savannah region of Burkina Faso, a novel group of outdoor-resting *A. gambiae* was found at high frequency in larval captured *A. gambiae*. The newly described exophilic subgroup freely segregates for both molecular form markers (>35% M/S hybrids) and the 2La chromosome inversion (>50% 2L+ chromosomes). In contrast, contemporaneous site-matched indoor collections confirmed the canonical description of the *A. gambiae* population in this region of West Africa: namely M and S molecular forms speciating and near fixation of the inverted form of the 2La inversion. Most striking is the larger population abundance and the greater susceptibility to *Plasmodium falciparum* infections of the exophilic subgroup of *A. gam*biae. The existence of a group of *A. gambiae* that is both highly susceptible to *P. falciparum* infections and not indoor resting could have significance for the efficiency of malaria control efforts, particular those targeting indoor resting mosquitoes.

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CYTOGENETIC MAP FOR *ANOPHELES NILI*: APPLICATION FOR POPULATION GENETICS AND COMPARATIVE PHYSICAL MAPPING

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Anopheles nili is one of the major malaria vectors in Africa with wide geographic distribution. However, the taxonomic and population genetic studies on this species are scarce. New research tools are urgently needed to genetically characterize this important malaria vector. In this study, a high-resolution cytogenetic map was developed for *An. nili* polytene chromosomes. Chromosomes were straightened and subdivided into 46 numbered divisions according to the banding pattern. Population analysis of *An. nili* females collected in Burkina Faso revealed the presence of two highly polymorphic inversions on the 2R chromosomal arm. To determine chromosome homologies and gene order conservation between *An. nili* and other major malaria vectors, PCR probes based on the *An. gambiae* coding sequences were mapped to An. nili chromosomes. Comparative mapping demonstrated that An. nili chromosomes have an An. stephensi-like arm association and that whole arm translocations and paracentric inversions were the major types of rearrangement in evolution of these mosquitoes. The minimum number of fixed inversions among An. nili, An. gambiae, and An. stephensi was calculated using the Multiple Genome Rearrangements (MGR), Genome Rearrangements In Man and Mouse (GRIMM), and Sorting Permutation by Reversals and block-INterchanGes (SPRING) programs. The data suggest that the An. nili is, at least, as diverged from An. gambiae as An. stephensi. We provided evidence that 2La/a arrangement of An. gambiae is present in outgroup species An. nili and An. stephensi confirming the ancestral status of the 2La inversion in the An. gambiae complex. Availability of the new polytene chromosome map, polymorphic inversions, and physically mapped DNA markers for An. nili will further stimulate population genetic, taxonomic, and genomic studies of this neglected malaria vector.

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TRANSDUCTION OF *SCHISTOSOMA MANSONI* WITH VESICULAR STOMATITIS VIRUS GLYCOPROTEIN PSEUDOTYPED LENTIVIRUS

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Retrovirus-mediated transduction offers a means to insert reporter transgenes into the schistosome genome, to elucidate schistosome gene function and expression through vector-based RNA interference, and to establish transgenic lines of schistosomes. Previously we have reported that murine leukemia virus (MLV) pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) can transduce developmental stages of Schistosoma mansoni. In addition, we have been investigating whether human immunodeficiency virus (HIV-1) lentivirus (a complex retrovirus) might likewise be utilized for transgenesis of schistosomes. We constructed lentiviral vectors using the ViraPower Gateway (Invitrogen) system; we modified pLenti6/R4R2/V5-DEST by insertion of an endogenous schistosome gene promoter; from the spliced leader (SL) RNA gene, upstream of the reporter gene encoding jellyfish green fluorescent protein (GFP). 293 FT producer cells were transformed with this construct and viral packaging plasmids to produce replication incompetent lentivirus virions pseudotyped with VSVG. We investigated early steps of lentivirus infection of schistosomes including attachment of virions to the schistosome tegument, reverse transcription to synthesize proviral DNA, and integration of the provirus into the schistosome genome. Schistosomes were incubated with HIV virions in the presence of the cationic polymer polybrene. At several times from 0 minutes to four hours thereafter, schistosomes were washed and the surface cross-linked with formalin. Using a VSVG specific antibody as the probe, time course dependent immunolocalization was evident to both schistosomules and adult worms, with increasing fluorescence signals from 0 to 180 min after exposure. Downstream events were investigated at one day post infection. Genomic DNAs (gDNA) were extracted from infected worms and used as the template for quantitative real time PCR (qPCR). qPCR targeting linear viral cDNA and integrated viral genome were performed with single step PCR and two step anchored PCR approaches, respectively, which revealed the presence of linear viral cDNA and integrated proviruses. We are now investigating integration junctions and reporter gene activity, with the aim of establishing the potential of VSVG-HIV-1 lentivirus as a vector for genetic manipulation of schistosomes.

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MOLECULAR MIMICRY BETWEEN ALLERGENS AND HELMINTH PROTEINS UNDERLIES RESPONSES AT THE HELMINTH-ALLERGY INTERFACE

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Although chronic helminth infection can diminish allergic skin test reactivity, many helminth infections are associated with allergic symptoms, possibly mediated by cross-reactivity between allergens and helminth proteins. To investigate the extent of this cross-reactivity, we performed in silico comparisons of 410 common (and molecularly defined) allergens

against the predicted proteins of the entire *Brugia malayi* genome. Among the 410 allergens, we found 170 that had at least one filarial orthologue (identities ranging from 22% to 92%). To investigate the implications of this finding, we assessed IgE levels specific to common allergens in 132 filarial-infected subjects and in 165 uninfected controls. When compared to uninfected individuals, filarial infection was associated with increased IgE prevalence to extracts of both house dust mite (HDM, Der p) (71% x 51%, P=0.02) and cockroach (Bla g) (60% x 16%, P<0.001) both of which contain several orthologues in filariae. In contrast, the IgE prevalence to timothy grass extract (Phl p), containing virtually no orthologues, was no different between the filarial-infected and -uninfected individuals. To extend these findings *in vivo*, mice were infected twice with H. polygyrus and the IgE response to helminth homologous and non-homologous allergens of cockroach and HDM were analyzed. Infected animals developed cross-reactive IgE only to allergens with helminth homologues (Der p10 and Bla g5), but not to those without homologues (Der p7 and Phl p6). Furthermore, cross-reactive IgE was functional as infected animals developed immediate hypersentivity skin test reactions to recombinant allergens (Der p1, Der p10 and Bla g5) structurally related to parasite antigens but not to structurally unrelated allergens (Der p7). These data suggest that molecular mimicry between helminth proteins and aeroallergens may cause the development of cross-reactive IgE and allergic sensitization and provide new insights into the allergy-helminth interface.

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TRANSFECTION OF DEVELOPMENTALLY COMPETENT *BRUGIA MALAYI*

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Transient transfection of isolated *Brugia malayi* embryos by biolistic bombardment has proven to be useful in defining promoter structure and function in this human filarial parasite. However, isolated transfected embryos are developmentally incompetent. A method of producing developmentally competent transfected parasites is therefore needed. We have found that L3 parasites can be chemically transfected in situ in the peritoneal cavity of a gerbil. The in situ chemically transfected parasites are developmentally competent, producing adult parasites with an efficiency similar to that obtained from implanted untreated L3. To refine this system, a reporter plasmid was constructed consisting of the a secreted gaussia luciferase reporter gene (gLUC) sequence flanked by the 5' and 3' untranslated domain of the *B. malayi* HSP70 gene, and containing the first intron derived from the *B. malayi* HSP70 gene inserted into the gLUC ORF. Cultured adult parasites and progeny mf derived from L3 transfected with this construct secreted gLUC into the culture medium. When the transfected mf were mixed with blood, fed to mosquitoes and the resulting L3 collected, the L3 also secreted gLUC into the culture medium. Transfected adults and progeny mf contained transgenic DNA, and the transgenic mRNA produced in these parasites was found to be correctly cis- and trans-spliced. These data suggest that it is possible to produce developmentally competent transfected *B. malayi* and that the transgenic sequences are inherited remain transcriptionally active in all lifecycle stages. Furthermore, the data demonstrate that gLUC may be employed as a selectable marker to identify transfected parasites. These studies open the way to using transgenesis to study all lifecycle stages of *B. malayi*.

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TBEIF4E-3 IS A PUTATIVE AND DIVERGENT TRANSLATION INITIATION FACTOR EIF4E ORTHOLOGUE IN *TRYPANOSOMA BRUCEI* THAT IS ESSENTIAL FOR CELL CYCLE PROGRESSION AND A POTENTIAL CANDIDATE FOR DRUG TARGETING

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Trypanosoma brucei is a unicellular, flagellated protozoan of the kinetoplastid group of eukaryotes causing 'African sleeping sickness' in humans. Current drugs have limited efficacy and high toxicity. Pathogenesis of *T. brucei* and related parasites rely on a complex life cycle involving proliferation, differentiation and transmission between polymorphic insect and human stages. Remarkably, mRNA transcription and processing are generally constitutive in trypanosomes, and thus, gene expression control driving their life cycle may instead rely predominantly upon regulated mRNA turn-over and translation. In most eukaryotes, the mRNA 5' cap binding protein eIF4E plays a central role in both the process and regulation of mRNA translation and turn-over. To explore translational regulation in *T. brucei*, we studied unique and essential features of TbeIF4E-3, one of several putative, divergent orthologues of eIF4E. First, unlike canonical eIF4E, it does not appear to bind well with 5' cap analogue 7mGTP, consistent with *ab initio* X-ray structure modeling of its cap-binding pocket suggesting only partially conserved, cap-interacting amino acid residues. Second, down-regulation of its expression by inducible RNAi caused multiple defects in cell cycle progression in both procyclic and bloodform stages of the parasite, inevitably leading to cell death. Finally, down-regulation of TbeIF4E-3 in bloodforms caused remarkable hypersensitivity to Rapamycin, a drug also causing cell cycle defects in *T. brucei*, and inhibiting TOR complexes central to cytoskeletal remodeling and/or translational regulation. These findings indicate that TbeIF4E-3 is involved in crucial cellular pathways regulating normal cell cycle and survival in *T. brucei*. The potential presence of a divergent cap-binding pocket in TbeIF4E-3 according both to its predicted structure and biochemical behavior, as well as its synergistic interaction with a known growth inhibitor drug, provides strong rationale for exploring TbeIF4E-3 and the pathway it controls as potential target for drug therapy against this pathogen.

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BACTERIA-MEDIATED RESISTANCE TO *PLASMODIUM* INFECTION IN *ANOPHELES* MOSQUITOES

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Before transmission to a vertebrate host occurs, *Plasmodium* parasites must transition through discrete developmental stages in the mosquito. During the earliest stages of development in the midgut lumen, the parasite encounters a diverse environment of mosquito-derived factors, host blood-derived factors, and the resident microbiota. Our lab and others have shown that bacteria have a profound effect on the ability of *Plasmodium* to infect the mosquito. Removal of resident bacteria increases mosquito susceptibility to infection more than two-fold. However, data generated on bacteria-mediated resistance to infection is in large part limited to lab isolates of bacteria and no mechanism has been resolved. To further examine the interaction between bacteria and *Plasmodium*, we first assessed microbial exposure of *Anopheles* mosquitoes collected in Zambia and then performed molecular and phenotypic analyses in regards to *Plasmodium* infection. Similar to previous reports, Gram-negative bacteria inhibited oocyst development. However, we identified a Gram-negative bacterium that potently inhibits infection during early stages of parasite development. Characterization of the bacteria-parasite-mosquito interaction shows that the antimicrobial immune response does not play

a major role in the observed microbe-mediated *Plasmodium* refractoriness but large populations of replicating bacteria are required. Physical interaction between bacteria and parasite were not observed following oral co-introduction in mosquitoes and supplementing nutrients required for parasite development do not rescue infection. We are currently using biochemical and continued phenotypic analyses to elucidate the mechanism of inhibition, which could lead to the identification of novel anti-*Plasmodium* molecules.

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ANTIBODIES PRODUCED AGAINST RECOMBINANT SIX-CYSTEINE GAMETE SURFACE HOMOLOGY FRAGMENTS FROM *PLASMODIUM FALCIPARUM* PFS48/45 AND PFS230 RECOGNIZE SEXUAL STAGES AND MAY BLOCK TRANSMISSION

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The development of a *falciparum* malaria transmission blocking vaccine is being evaluated as a complement to a pre-erythrocytic vaccine. Antibodies against the sexual stage protein, Pfs25, significantly inhibit oocyst development when assessed by a membrane feeding assay, though it requires higher antibody titers in humans to achieve complete blocking in transmission. In order to evaluate whether the transmission blocking activity of Pfs25 may be enhanced by the inclusion of additional sexual stage antigens, we aimed to produce two recombinant forms of the cysteine-rich *Plasmodium* gamete surface homology fragments derived from Pfs48/45 and Pfs230. Using a modified *Pichia pastoris* host that overexpresses protein disulfide isomerase, multiple forms of Pfs48/45 have been expressed and purified that contain 8 to 10 cysteines, which form a "double domain". An amino-terminal region of Pfs230 containing a single "double domain" was expressed and refolded from inclusion bodies derived from *Escherichia coli*. Antibodies generated in rabbits against both of these recombinant forms of Pfs48/45 and Pfs230 recognized unfixed gametes by indirect immunofluorescence. In contrast, only rabbit antisera against Pfs230 inhibited oocyst development by greater than 97% (reduction in oocyst prevelance was 50 to 90%) using neat sera in the presence of complement. The failure of the Pfs48/45 antisera to inhibit oocyst development may be the result of a poorly folded immunogen or that the Pfs48/45 "double domain" is not a significant biological target. Now that a recombinant form of Pfs230 has been produced using a scalable system that induces inhibitory antibodies, pre-clinical studies evaluating Pfs25 and Pfs230 may be performed.

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HUMAN ANTIBODY RESPONSE TO *ANOPHELES* SALIVARY GSG6-P1 PEPTIDE: NEW IMMUNO-EPIDEMIOLOGICAL TOOL FOR EVALUATING THE EFFICACY OF INSECTICIDES TREATED NETS (ITNS) IN MALARIA VECTOR CONTROL

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To optimize malaria control, WHO has emphasized the need for new indicators to evaluate the efficacy of vector control strategies. Previous studies have shown that the quantification of human antibody (Ab) response to *Anopheles* salivary proteins represent an epidemiological biomarker of exposure to *Anopheles* bites and malaria risk. In particular, only one salivary peptide, the gSG6-P1, is one clear candidate to evaluate the level of exposure to *An. gambiae* and *An. funestus* bites. The aim of the study was then to validate this peptide as a new tool to evaluate the efficacy of ITNs use. One longitudinal study, concerning individuals (n=108) living in malaria endemic area was performed from March 2005 to January 2007 (Angola). The cohort was followed for parasitological, entomological and immunological data, before and after the well-controlled use of ITNs (installation in Feb. 2006). Significant decrease of the percentage of immune responders and of anti-gSG6-P1 IgG Ab level was observed just after the ITNs use and was correlated with the decrease of malaria parasitemia, the current and referent criteria of ITNs efficacy. Interestingly, the decrease of specific IgG level was observed in all age groups (0-6; 7-14 and >14 years-old) and for the majority of ITNs-protected individuals, suggesting its potentiality as an individual biomarker. However, in concordance with the considerable loss of ITNs and lack of ITNs use, specific IgG response increased only four months after ITNs introduction in this studied population. It suggests that this salivary tool could be also an indicator to the time-dependent incorrect use of ITNs. This study shows that the assessment of IgG response to gSG6-P1 salivary peptide could be a pertinent tool to evaluate the ITNs efficacy, whatever age, and potentially a biomarker of efficacy at an individual level. This study represents a first approach to elaborate new tools of evaluation of malaria vector control and future studies are needed to confirm this hypothesis in other areas and using different vector control strategies.

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TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* MALARIA WITH ARTEMETHER-LUMEFANTRINE TO REDUCE DISEASE TRANSMISSION: A MODELING AND SIMULATION STUDY

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Asymptomatic carriers (AC) of *Plasmodium falciparum* serve as a reservoir for malaria transmission. Identification and treatment of AC within a region should reduce the reservoir and thus transmission intensity in that area. Using computer simulation, the factors that influence the impact of this intervention, i.e. community screening campaigns (CSC) followed by artemether-lumefantrine (AL) treatment on disease transmission were explored. The model of Okell et al (2008) was modified with malaria vector seasonality added and components modified to represent screening and treatment of AC with AL. The age grouping, relative distribution of age in a region, and degree of heterogeneity in disease transmission were maintained. The impact of the number of CSC and their timing on malaria transmission throughout a period of 1 year was explored. A sensitivity analysis to determine factors with the greatest impact was done. The simulation showed the intervention reduces transmission in a region with marked seasonal transmission (6 months) of moderate intensity (EIR<100). Three CSCs scheduled in close succession (monthly intervals) at the start of the dry season had the greatest impact. Adding an extra CSC did not bring improvement. In areas with low transmission intensity (EIR<10) the reduction was sustained for years after a single intervention while gradually tapering off with return to initial setting. Repeated intervention at least every other year allowed to sustain the effect. The simulation results show that screening and treatment of asymptomatic carriers with AL in a region reduces malaria transmission significantly. Transmission intensity has the greatest impact on the magnitude and duration of malaria reduction. When combined with other strategies (LLINs, RDT, Prompt diagnosis & treatment, IRS), the effect of this intervention can persist for many years, and it may become a tool to accelerate the reduction transmission intensity to pre-elimination level. The modeling supports the evaluation of this approach in a prospective clinical trial in an area with marked seasonality.

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PROTECTIVE EFFECTS OF WHO-RECOMMENDED LLINS AGAINST *ANOPHELES DARLINGI* IN THE FIELD

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The Andean Malaria Program has adopted Long Lasting Insecticide Net (LLIN) distribution as an important strategy to prevent and control malaria in high risk areas of malaria transmission in the Amazon Region. The nets that have been distributed to date include Interceptor®, PermaNet 2.0®, and Olyset®. Respectively, these exploit the pyrethroids alpha-cypermethrin, deltamethrin and permethrin. All published studies on the field efficacy of these compounds when incorporated into LLINs refer only to African (An. gambiae s.l.) and Asian (An. culicifacies and An. fluviatilis) malaria vectors. Only a single comparative study between these compounds applied to bed nets has been completed (also in Africa). It is therefore essential to characterize the relative efficacy of these LLINs against An. darlingi, the most common and efficient malaria vector in the Amazon basin. This was done using a set of experimental huts sited close to the Amazon town of Iquitos, Peru. Using huts with open eaves and exit traps in the windows we examined 1) the lethal effects of the three LLINs on mosquitoes, 2) their impacts on mosquito exit and entry behavior, and 3) the protection that holed nets afforded their human occupants. We also examined the protection that nets gave to humans that were in the same house as the nets, but not under the nets.

Highly significant differences between the LLINs were noted, with some nets exerting far greater lethal effects, and some far greater repellent and irritant effects than others. An. darlingi is an early biting mosquito, which exhibits its peak in biting behavior before people have retired under their bed nets. These differences between the three LLINs therefore have profound implications for the patterns of protection that they give to humans against An darlingi.

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INNOVATIVE TOOL TO EVALUATE MALARIA RISK: TOWARD THE DEVELOPMENT OF A BIOMARKER OF INFECTING BITE?

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Malaria causative agent, i.e. Plasmodium parasite, is transmitted to human during the blood meal of the Anopheles mosquito. During blood feeding, the vector injects parasite and saliva into the vertebrate host skin. This saliva contains bioactive components which induced an immune response in the vertebrate host. Our team has previously developed a serological biomarker to assess the human exposure to mosquito bite. This tool is based on the evaluation of human antibody response against mosquito salivary proteins. Here we investigate whether a salivary antigen could be specific of the infecting bite and constitute a biomarker of the risk of disease. To assess this question, we have compared the human antibody response against salivary extracts infected or not by P. *falciparum*. Experimental infections of An. gambiae by P. *falciparum* were carried out and salivary glands were dissected 14 days post-infection. The infective status of each salivary gland was confirmed by PCR. Then two-dimensional western-blots were realized with different pools of infected vs non infected sera. These pools were constituted with sera from Senegalese 1-2 y.o. children leaving in deeply exposed village to Anopheles and presenting or not a high parasitemia.

The results of 2D-blots showed that immunogenic proteins around 70kDa are detected in both infected and non infected vector. Mass spectrometry analyses identified these proteins as the 5'nucleotidase and Apyrase, proteins which inhibiting platelet aggregation. Furthermore one immunogenic protein from infected salivary glands extracts was detected only with sera from infected children by P. *falciparum*. Mass spectrometry analysis on this protein is underway. These results indicated that human immune system could discriminate between an infective bite and a non infective bite. This work opens the way to design epidemiological tools to evaluate the risk of malaria in area of (re) emergence, but also have strong implications for the vector control and monitoring.

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STRATEGIES TOWARDS *PLASMODIUM FALCIPARUM* MALARIA ELIMINATION IN AFRICA USING CURRENTLY AVAILABLE TOOLS

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In the past decade malaria intervention programs have been scaled up across Africa. However, it remains unclear what levels of decrease in transmission are achievable using currently available tools. We developed an individual-based simulation model for *Plasmodium falciparum* transmission in an African context incorporating the 3 major vector species (*Anopheles gambiae* s.s., *An. arabiensis* and *An. funestus*) with parameters fitted to parasite prevalence data from 34 sites across Africa. We explored the impact on transmission of increased roll-out of of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), mass screening and treatment (MSAT) and a future RTS'S/AS01 vaccine in six settings with varying entomological inoculation rate (EIR), vector species combinations and patterns of seasonality under a target of 80% coverage of interventions. In the low transmission setting (EIR approx. 3 infectious bites per person per year (ibppy)), LLINs alone can reduce malaria transmission to low levels if high usage levels are sustained. In two moderate transmission settings (EIR approx. 43, 81 ibppy) additional rounds of IRS with DDT coupled with MSAT could drive parasite prevalence below the 1% level. However, in the third (EIR=46) with *An. arabiensis* prevailing, these interventions are insufficient to reach this threshold. In both high transmission settings (EIR approx. 586, 675 ibppy) either unrealistic coverage levels (>90%) or novel tools and/or substantial social improvements will be required, although existing tools with realistic coverage levels greatly reduce prevalence. In conclusion, interventions using current tools can result in major reductions in *P. falciparum* transmission and associated disease burden in Africa. Reduction to below the 1% parasite prevalence level is possible in low to moderate transmission settings when vectors are primarily endophilic, provided a comprehensive and sustained intervention program is achieved. In high transmission settings and those in which vectors are mainly exophilic, additional new tools are likely to be required to achieve this level of control.

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QUANTIFICATION OF EASTERN EQUINE ENCEPHALITIS VIRUS IN FIELD-COLLECTED MOSQUITOES TO EVALUATE THEIR ROLE AS BRIDGE VECTORS

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Eastern equine encephalitis virus (EEEV) is maintained in an enzootic cycle involving *Culiseta melanura* mosquitoes and avian hosts, whereas other mosquito species that feed opportunistically on mammals have been incriminated as bridge vectors to humans and horses. To evaluate the capacity of these mosquitoes to transmit EEEV, we estimated the infection prevalence and virus titers in mosquitoes collected in Connecticut by cell culture, plaque titration, and quantitative RT-PCR. *Cs. melanura* yielded the greatest number of EEEV isolations (n=83) followed by *Ochlerotatus canadensis* (10) and *Aedes cinereus* (6). Relatively few (≤4) or no EEEV isolates were obtained from the remaining mosquito species collected. *Cs. melanura* contained significantly higher titers of virus (mean= 6.53 log10 PFU/mosquito pool) than all other mosquito species combined (mean=2.26 log10 PFU/mosquito pool). None of the remaining mosquito species had high enough titers to transmit virus. Our findings suggest that *Cs. melanura* is the primary if not sole vector of EEEV in this region, which may explain the paucity of human cases. More generally, this study emphasizes the importance of evaluating virus titers from field-collected mosquitoes to assess their vectorial capacity.

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WESTERN EQUINE ENCEPHALITIS VIRUS PATHOGENESIS IN OUTBRED MICE

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Western equine encephalitis virus (WEEV) is a naturally occurring recombinant virus derived from ancestral Sindbis and Eastern equine encephalitis viruses. Relatively little is known about virulence determinants of WEEV. We previously showed that infection by WEEV isolates McMillan (McM) and IMP-181 (IMP) results in high and low mortality, respectively, in outbred CD1 mice when virus is delivered by either subcutaneous or aerosol routes. McM and IMP infectious clones were used to generate chimeric constructs. By characterizing the pathogenic phenotypes of these chimeras, the pathogenic determinants were mapped to a region within the E2 glycoprotein from McM nt 8950 through McM nt 9658. This region of 709 nt contains 18 nt and 7 aa differences. Additional mapping is underway to further narrow the pathogenic determinants. We have also conducted studies with cationic lipid DNA complexes (CLDC), a potent immunomodulatory compound, in mice before and after WEEV-McMillan challenge. Subcutaneous CLDC administration up to 12 hours after challenge can protect mice from WEEV-induced disease and mortality. In virus-infected animals, large increases in production of IFNγ, TNF-α, MCP-1, IL-12, and IL-10 in the brain were observed by 72 hours after infection, consistent with neuroinvasion and viral replication in the CNS. Mice receiving CLDC treatment show early increased serum IFNγ, TNF-α, and IL-12, suggestive of a T_H1-biased activation of the innate immune system, but no increase in brain cytokine levels. These studies are lending insight into the mechanisms of WEEV pathogenesis and protection from WEEV encephalitis.

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INFIRMATUS VIRUS: A NEWLY DESCRIBED *ORTHOBUNYAVIRUS* (CALIFORNIA SEROGROUP) ISOLATED IN FLORIDA

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A novel virus, tentatively designated Infirmatus virus, was isolated as part of an ongoing study on the ecology of arbovirus transmission in Florida. The virus was isolated by Vero cell culture from a pool of *Aedes infirmatus* mosquitoes collected in 2008. The virus was not detected using previously reported flavi-, alpha- and bunya-broad RT-PCR methods. To identify the virus, sequence-independent single-primer amplification and a sequence-specific genome walking technique was used to generate the nearly complete sequence of the virus. BLASTn analysis of the sequence identified 86% nt homology to Trivittatus virus in the small (S) segment, 77% homology to Trivittatus virus in the medium (M) segment, and 75% homology to La Crosse virus in the large (L) segment. Infirmatus virus was also detected in 6 other mosquito pools (3 *Ae. infirmatus* pools, 2 *Cx. quinquefasciatus* pools, 1 *Cx. nigripalpus* pool) at the site. Host species identification on blood-fed mosquitoes found that *Ae. infirmatus* and *Cx. nigripalpus* frequently fed on eastern cottontail rabbits in this area, suggesting rabbits as its natural reservoir host, similar to California encephalitis virus. Earlier surveillance studies in the area

isolated a "Trivittatus-like virus" in the 1960s that was not classified or further characterized by molecular techniques. An isolate of this virus collected in 1965 was obtained for comparison; 98% nucleotide sequence homology (~900 bp) was found to the Infirmatus strain collected in 2008. Intracerebral inoculation of suckling mice with the novel virus resulted in neurovirulence and mortality three days post inoculation. This virus thus represents a novel California serogroup *bunyavirus* first isolated in the 1960s that may pose a risk to public health in Florida.

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IGM PERSISTENCE IN PROBABLE CASES OF CALIFORNIA SEROGROUP INFECTION

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Snowshoe hare (SSH) and Jamestown Canyon (JC) viruses are mosquito-borne zoonotic agents belonging to the California serogroup (CS) of viruses (Genus *Orthobunyavirus*). These viruses have a wide geographical range throughout North America and are associated with febrile and neurological disease. Serological procedures to identify probable cases of CS-associated illness usually consist of IgM detection in patient sera. Persistent IgM can cause diagnostic issues with case identification, therefore, a study was performed to evaluate lingering SSH and JC specific IgM in exposed individuals. Fifteen patients / individuals positive by SSH and JC IgM ELISA screening and neutralization assays were tested for CS virus IgM antibody at yearly intervals. In addition, 90 sera collected from patients during the winter months in Canada were screened for CS IgM to provide additional evidence for lingering IgM antibody during times when mosquitoes were not present. Evidence for California serogroup virus IgM persistence was identified among patients screened for arbovirus antibody over several years. Fifteen individuals had significant JC or SSH IgM titres that persisted for over a year with several exhibiting positive IgM levels for 2-5 years. Of 90 sera collected from randomly picked febrile patients during the months of January to March approximately 9% (8/90) were positive for California serogroup virus specific IgM. The high number of IgM positive sera collected during the late spring and winter months is consistent with JC and SSH exposures occurring the previous mosquito season, a time frame of 6 months or longer. Our studies indicate that JC and SSH IgM titres may be maintained for at least a year, a finding previously observed with West Nile virus infections. Persistent IgM may pose a diagnostic dilemma when identification of California serogroup infections is based only on presence of IgM in patient sera. Confirmation of current JC and SSH- associated illness should be demonstrated by diagnostic rises in antibody titre or the presence of virus in clinical samples.

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EXPANDED FRAMEWORK OF HANTAVIRUS EVOLUTION FROM NEWLY IDENTIFIED MYOSORICINE SHREW HOSTS IN TANZANIA

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Sub-Saharan Africa has long been considered the birthplace of numerous vector-borne and zoonotic infectious diseases affecting humans and domestic animals. For example, numerous viruses, including prototype viruses that define families and genera (such as Bunyamwera, Lassa and Ebola), were first discovered in Africa many decades ago. By contrast, hantaviruses have only recently been detected, in the African wood mouse and Therese's shrew from Guinea. Recent observations that multiple species of shrews (Order Soricomorpha, Family Soricidae) harbor genetically distinct hantaviruses in widely separated geographic regions throughout Eurasia and North America suggest the existence of additional shrew-borne hantaviruses in Sub-Saharan Africa, where numerous unique shrew lineages have evolved and diversified. To investigate this issue, archival tissues from the geata mouse shrew (*Myosorex geata*) and Kilimanjaro mouse shrew (*M. zinki*), two myosoricine shrew species restricted to Tanzania, were analyzed for hantavirus RNA by RT-PCR. Pairwise alignment and comparison of nearly full-length S- and L-genomic sequences of Uluguru virus (ULUV) and Kilimanjaro virus (KMJV) indicated moderately low nucleotide and amino acid sequence similarities with representative rodent- and soricid-borne hantaviruses. Phylogenetic analyses, using the maximum-likelihood and Bayesian methods under the best-fit GTR+I+Γ model of evolution, showed that ULUV and KMJV shared a common ancestry and were most closely related to Thottapalayam and Imjin viruses, two hantaviruses harbored by crocidurine shrew species in Asia, in keeping with the evolutionary relationship between crocidurine and myosoricine shrews. However, the newfound hantaviruses were distantly related to Tanganya virus in *Crocidura theresae* from Guinea. Discovery of genetically divergent hantaviruses in myosoricine shrews in eastern Africa further expands the host range and distribution of soricid-borne hantaviruses across four continents, lending additional support to the emerging paradigm-altering concept that ancestral soricomorphs, rather than rodents, may have served as the original mammalian hosts of primordial hantaviruses. Studies, now underway, of other African shrew genera, such as *Sylvisorex*, may provide greater insights into the evolutionary history of hantaviruses.

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FACTORS ASSOCIATED WITH PERSON TO PERSON NIPAH VIRUS INFECTION

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Since 2001, we have identified 9 Nipah virus (NiV) outbreaks in Bangladesh with a high case fatality (106/146; 73%). Family members or friends care for patients in the home or hospital. Since more than half of all cases followed close contact Nipah infected patients we attempted to identify factors responsible for NiV transmission to close contacts from 2007. Close contact was defined as sharing a room, veranda, or vehicle with a Nipah case for at least 15 minutes. We defined secondary infection as one developed evidence of NiV infection 5-20 days after close contact with a Nipah patient. All contacts except five deaths were serologically tested for NiV IgM antibody. Confirmed NiV infection was defined as a positive NiV IgM antibody test or features of encephalitis without laboratory confirmation. We identified 38 Nipah cases and 612 contacts; mean16 contacts per case. Only three (8%) cases transmitted NiV. The overall proportion of secondary cases was 2.5% (15/612) of which 25% (4/15) had mild febrile illness, and none were asymptomatic. The mean age (36 years vs. 33 years) and sex (53% male vs. 47% male) of secondary cases and contacts were similar. Compared to seronegative contacts, secondary cases were more likely to have spent time in a vehicle with a case (OR 2.9; 95% CI 0.9-9.5), had close contact with a case within the first 5 days of illness (OR undefined; 95% CI 1.0-undefined), fed a case with their hands (OR 3.2; 95% CI 1.0-10.4), helped patient to use the toilet (OR 4.7; 95% CI 1.3-15.3), cleaned vomit from the patient's body (OR 5.3; 95% CI 0.9-21.0) and received a cough in the face (OR 4.8; 95% CI 1.1-17.0). In conclusion, person-to-person transmission of NiV was uncommon among persons who cared for Nipah patients, but hand contamination with body fluids, receive cough in the face and close contact within five days of onset of illness increased risk. Hand washing with soap and water and avoiding to receive cough in the face while caring for a Nipah patient could interrupt transmission of NiV to caregivers.

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HOSPITAL-BASED SURVEILLANCE FOR INFLUENZA VIRUSES IN GHANA, WEST AFRICA

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Ghana began sentinel investigation of influenza-like-illness (ILI) in selected health institutions in 2007. This is in line with global systems to identify circulating/novel influenza virus strains, inform annual vaccine composition and provide early warning for an influenza pandemic. Initial sites were located in 3 regions where Avian Influenza outbreaks in poultry had occurred. For the past 32 months, health facilities across Ghana have investigated patients presenting with respiratory disease signified by fever >38ºC and/or cough, sore throat, headache, body-aches and coryza. At the national influenza center, Influenza (flu) virus presence was determined by virus isolation in cell culture and real time reverse transcriptase polymerase chain reaction. Antigenic, phylogenetic and anti-viral susceptibility analyses were also performed. Five thousand, eight hundred and ninety-two cases of ILI have produced 1284 flu viruses (22% of samples). The 6-15 years age group was most affected (38%). Initially, in 2007, flu A H3N2 subtype was predominant but this was replaced in 2008 by flu A H1N1 with flu B Yamagata strains also in circulation. Flu A H3N2 then dominated in 2009 alongside with flu B Victoria strains. By early 2010, pandemic flu A H1N1 2009 (pH1N1) became the prevailing flu virus with A H3N2 and B Victoria strains also present. Fever, cough, headache and coryza were the most frequent clinical presentations. Molecular markers of resistance to Oseltamivir in flu A H1N1 isolates was first observed in 2008 and has persisted, but has not been found in other subtypes. The pH1N1 isolates have anti-viral resistance to Adamantanes with genomic comparability of 99% with A/California/7/2009 (pH1N1) strain. Four cases of flu AH3N2 and pH1N1 co-infections have been recorded. Amongst hospital admissions for severe illness, seasonal flu viruses have now been replaced by pH1N1. In conclusion, scrutiny for flu virus is now part of Ghana's public health system with full regional coverage by mid-2010. Soon, the profile of flu virus activity over the tropical climate will be discerned that would be applicable to West Africa as well. Due to the data obtained, the contribution of influenza virus to respiratory disease burden in Ghana is now better understood. As a result, control and response strategies for pandemic influenza have been established.

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FEMALE UROGENITAL SCHISTOSOMIASIS IN TANZANIA'S LAKE ZONE REGION: A HIGHLY-SPORADIC DISTRIBUTION AMONG WOMEN IN EIGHT RURAL VILLAGES

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Female urogenital schistosomiasis (FUS) is a parasitic infection caused by *Schistosoma haematobium* that leads to genital tract inflammation and ulceration and may predispose to incident HIV infection. FUS affects an estimated 45 million girls and young women in sub-Saharan Africa. The prevalence among women of reproductive age has been reported to be as high as 30-40% in areas where the parasite is endemic, including regions of northern Tanzania. We conducted a community-based prevalence study among women aged 18-50 living in northwest Tanzania's Lake Zone region, where *Schistosoma haematobium* infection had been diagnosed in 30-90% of schoolchildren in 2006. Women received screening for urogenital schistosomiasis, HIV, sexually-transmitted infections, intestinal schistosomiasis, and cervical cancer in collaboration with local cancer screening programs. In 472 women living in eight villages, the prevalence of FUS varied by village and ranged from 0 to 14%. Living in a village in which at least 3% of adult women had FUS and age less than 25 years old were highly-significant risk factors for FUS in this population ($p<0.0001$ for each risk factor). The overall prevalence of HIV in these women was 5.9% (28 of 472), but the prevalence of HIV in women with FUS was more than 50% greater (3 of 33, 9.1%). In conclusion, FUS is sporadically distributed among women in northwest Tanzania, with some villages having rates of *Schistosoma haematobium* infection as high as 14% in women of reproductive age, while other nearby villages have none. Women younger than 25 years old were almost four times more likely to have FUS than older women. As these young women are also at increased for HIV and FUS has been associated with HIV, this may have implications for schistosomiasis and HIV control programs in the region.

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ASSESSMENT OF QUALITY OF LIFE AS A TOOL FOR MEASURING MORBIDITY DUE TO SCHISTOSOMIASIS AND THE IMPACT OF TREATMENT

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Mass treatment programs targeting schistosomiasis in a number of African countries are designed to reduce morbidity associated with the infection. In order to better monitor and evaluate treatment programs, tools for assessing morbidity are needed. Because schistosomiasis can persist for years as a chronic infectious disease, one approach is to utilize questionnaires that capture measures of quality of life. To evaluate whether the WHO quality of life assessment (WHOQOL-BREF) is a useful measure of the health impact of *Schistosoma mansoni* infections, we enrolled nonpregnant adult members of the community of Usoma, Kenya who had no recollection of previous treatment with praziquantel. Based on WHO recommendations and previous studies showing high prevalence of schistosomiasis in children, the entire community was eligible for mass drug distribution. Prior to treatment, the WHOQOL-BREF was administered to consenting participants. These individuals were

evaluated for schistosomiasis by both stool examination and presence of urine circulating cathodic antigen (CCA). Additionally, participants were tested for infection with soil transmitted helminths, malaria, and HIV. Two days after taking praziquantel, individuals were asked about tolerance of treatment and tested again for CCA. Preliminary results suggest no association between schistosome infection status (positive or negative) or intensity of infection and quality of life at baseline. However, following praziquantel treatment, persons with higher intensity infections demonstrated more pronounced side effects than individuals with light or no infections ($p < 0.0001$). Urine CCA levels were reduced within 2 days of treatment ($p < 0.0001$) and those with light infections were more likely to become negative. Ongoing work will reassess WHOQOL-BREF scores at 6 months after treatment to help determine how useful this tool will be to evaluate and compare the impact of mass drug administration programs.

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PREVALENCE AND INTENSITY OF *SCHISTOSOMA* SPP TWO YEARS AFTER A PRAZIQUANTEL TREATMENT AMONG SCHOOL-AGE CHILDREN FROM A RURAL VILLAGE IN MALI

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Schistosomiasis remains a major neglected and important health problem in developing countries. In regions where praziquantel (PQZ) has been used over prolonged periods for schistosomiasis morbidity control one central question builds around the optimal interval between PQZ treatment rounds to achieve significant long term decrease in worm loads. Our aim was to study the impact of a single dose of PQZ treatment on the prevalence and infection intensity of *Schistosoma mansoni* and *S. haematobium* in a rice irrigated village in Mali. Two cross-sectional parasitological surveys among children (6-14 years old) were carried out in 2005 and 2007 within a single village in Mali. Stool and urine samples were examined for *S. mansoni* and *S. haematobium* eggs, respectively. Difference in prevalence and infection intensity between the two years was tested using age and sex adjusted logistic and negative binominal regression models, respectively. At 2 years post-treatment, the overall prevalence of *S. mansoni* and *S. haematobium* infection fell from 93% to 88% [OR 0.55, CI95 0.26-1.10] and 74.5% to 28.0% [OR 0.12, CI95 0.07-0.20], respectively. Geometric means of *S. mansoni* and *S. haematobium* infections decreased significantly from 179 to 83 eggs/gram of faeces [egg count ratio (ECR) 0.58; CI95 0.42-0.78] and 12.3 to 1.8 eggs/10 ml urine [ECR 0.074, CI95 0.044-0.127]. The proportion of children with heavy infections decreased significantly from 42% to 26% for *S. mansoni* and 26% to 0.9% for S. haematobium. The validity of these results needs to be put into the context of the epidemiological setting, drawing attention to the issue of scale and specific control measures. In conclusion, praziquantel appeared to have a long term effect on *S. haematobium* but not on *S. mansoni* thought this might also suggest species-specific differences in praziquantel treatment. Current control efforts do not attain sufficient reduction of schistosomiasis infection in this particular setting which points us to the need for additional control measures specific to the 'Office du Niger' irrigation scheme.

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IMPACT OF TWO ROUNDS OF PRAZIQUANTEL TREATMENT ON *SCHISTOSOMIASIS HAEMATOBIUM* IN SELECTED SENTINEL COMMUNITIES OF DELTA STATE, NIGERIA

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Urinary schistosomiasis is a parasitic disease caused by the trematode *Schistosoma haematobium* (SH). Nigeria is one of the most endemic countries for SH in Africa. Following an initial prevalence survey in 2003, annual mass drug administration (MDA) for SH was initiated in Delta State in 2004. Following 1993 WHO guidelines, single dose praziquantel (PZQ) was provided to all school-aged children in 57 communities where reagent strip (dipstick) testing of urine show hematuria prevalence of ≥20% in a sample of 30 school aged children (aged 6-14 yrs), and to the entire village in 7 communities where the prevalence in children was ≥50%. Children had a physical examination and were asked about passing blood in urine ('red urine'). A visual diagnosis of bloody urine was recorded by nurses (NVD) before results of dipstick testing was known. Cross-sectional surveys of school children were conducted in 8 sentinel villages (3 mass, 5 school-aged) at baseline (n=240) and after two annual doses (n=402) to determine the impact of PZQ. Following 2 rounds of PZQ MDA hematuria measured by dipstick decreased by 88.5% and only 1 sentinel village (SV) still qualified for MDA (≥20% prevalence). Mid upper arm circumference (MUAC) increased by 6.8% ($p<0.001$). Although specific, history of hematuria and NVD had a low sensitivity compared to dipstick results (31% and 44%, respectively). Prior to treatment, history and NVD identified only 1 (31%) and 3 (44%), respectively, of the 8 SVs as needing treatment. Neither history nor NVD identified the remaining SV still in need of treatment after two MDA rounds. PZQ mass treatment was associated with a decrease in the prevalence of hematuria and an increase in MUAC. Questioning children or using NVD failed to identify the majority of the communities in need of treatment. In this part of Nigeria, history of hematuria or gross hematuria are not sufficiently sensitive for either initial mapping or impact assessment.

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DYNAMICS OF TRANSMISSION AND REINFECTION PATTERNS OF SCHISTOSOMIASIS AFTER TREATMENT

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The high burden of schistosomiasis in Africa is due to *Schistosoma haematobium*, which causes urinary schistosomiasis, and *S. mansoni*, which causes intestinal schistosomiasis. Evidence has accumulated of the dynamics of schistosomiasis epidemiology and increasing co-endemicity of these two species. In order to assess the dynamics of transmission, the efficacy of treatment and the re-infection patterns in mixed infection foci, a multicentre study was conducted in several countries including Cameroon, Senegal and Niger, where suitable villages with *S. haematobium* and *S. mansoni* co-infections were selected. The studies were conducted following a designed standard protocol, including parasitological baseline survey, treatments and follow up surveys at several time intervals up to 12 months after treatment. The results showed an overall good efficacy of praziquantel against *S. haematobium* and *S. mansoni*. However, there were significant differences in transmission dynamics, cure rates and re-infection patterns. The efficacy of praziquantel

against *S. mansoni* was lower in Senegal and Niger compared to Cameroon. In the mixed infection foci in Senegal, the re-infection rates were higher for *S. mansoni*, contrary to Cameroon where *S. haematobium* exhibited higher re-infection patterns. These studies provided an important insight into the understanding of praziquantel efficacy and post-treatment re-infection dynamics of schistosome in co-infections with *S. mansoni* and *S. haematobium*. The findings and a better understanding of schistosome interactions are important for recommendations to optimize control strategies in mixed infection foci.

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GEOSTATISTICAL MODEL-BASED ESTIMATES OF SCHISTOSOMIASIS RISK IN WEST AFRICA

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Schistosomiasis is a water-based disease caused by trematodes of the genus Schistosoma. It is believed that the disease affects some 200 million people worldwide with more than 95% of the infections concentrated in Africa. However, the current figures are largely based on population re-adjusted estimates originally published by Utroska and colleagues in 1989. These estimates might be outdated due to, for example, water-resource development and management, improved sanitary facilities and large-scale preventive chemotherapy. For planning, coordination and evaluation of control activities, it is essential to have reliable schistosomiasis risk maps and burden estimates. We analyzed survey data compiled on a newly established open-access global neglected tropical disease (GNTD) database (www.globalntddatabase.org) to (i) create smooth empirical risk maps for Schistosoma mansoni and S. haematobium for children aged below 20 years in West Africa, including Cameroon, and (ii) to calculate country prevalence estimates. We used Bayesian geostatistical models based on environmental and climatic predictors to take into account potential spatial clustering due to common spatially structured exposures. Our estimates suggest that a total of nearly 70 million West African children are currently infected with either S. mansoni or S. haematobium. The country prevalence estimates range between 0.7% (Gambia) and 35.5% (Liberia) for S. mansoni and between 17.7% (Gambia) and 50.8% (Sierra Leone) for S. haematobium. We observed that the combined schistosomiasis risk for both species is two-fold lower in Gambia than previously reported, while the existing estimates for Liberia seem to be seriously underreported (24.0% compared to our estimates of 61.7%). However, our predictions might overestimate overall country prevalence, since modelling was based on children aged below 20 years who are known to carry the highest infection risk.

To our knowledge, these are the first empirical estimates of S. mansoni and S. haematobium risk at high spatial resolution throughout West Africa.

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OPTIONS FOR THE TREATMENT AND CONTROL OF SCHISTOSOMIASIS *HAEMATOBIA* AND *MANSONI*: EVIDENCE FROM TWO SYSTEMATIC REVIEWS AND META-ANALYSES

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Treatment and control of schistosomiasis relies on praziquantel with virtually no alternatives. Uncertainties remain on praziquantel doses. Systematic reviews and meta-analysis can help inform both policy and research. A systematic search for randomized controlled trials of drugs used alone or combined for treating *Schistosoma haematobium* and *S. mansoni*. We used risk ratio (RR) or weighted mean difference (WMD) to analyze combined dichotomous or continuous outcomes, including confidence intervals. Overall, 24 trials (6,315 participants) met the inclusion criteria for *S. haematobium* and 49 trials (9,608 participants) for *S. mansoni*. Praziquantel, metrifonate (against *S. haematobium*) and oxamniquine (against *S. mansoni*) were better than placebo in producing parasitological clearance and egg reduction. Praziquantel shows no dose-effect for *S. haematobium* between 20 and 40 mg/kg, while there is a dose-effect on *S. mansoni* (40 mg/kg > 20-30 mg/kg, no increment with 60 mg/kg). For *S. haematobium*, metrifonate is effective, but requires multiple dosing (10 mg/kg fortnightly x 3); no study compared directly the standard doses of praziquantel and metrifonate. With oxamniquine, there is a dose-effect for parasitological efficacy against *S.mansoni* (40 mg/kg > 10-30 mg/kg; no increment with 50-60 mg/kg). Oxamniquine (20-60 mg/kg) was not different to praziquantel (40 mg/kg). No dose-effect was demonstrable on clinical improvement with oxamniquine or praziquantel. Data on artemisinins for both *S. haematobium* and *S. mansoni* are inconclusive. In conclusion, praziquantel is effective and well-tolerated at the WHO-recommended dose of 40 mg/kg for both *S. haematobium* and *S. mansoni* in all endemic areas. There is no advantage with higher doses. Alternatives are metrifonate for *S. haematobium* and oxamniquine for *S. mansoni* but both have limitations and neither is available today for use in programmes. There is a pressing need for studies of combination therapy (e.g. praziquantel plus metrifonate, oxamniquine and artemisinins).

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INCIDENCE AND ETIOLOGY OF ACUTE DIARRHEA IN A FRENCH MILITARY COHORT IN CHAD

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The objective of this study was to assess the incidence, etiology and behaviors associated with acute diarrhea among French Forces deployed to N'Djamena, Chad Sub-Saharan Africa. Prospective study based on physician consultation for diarrhea during a 5-month French forces mandate. Diarrhea was defined as >=3 stools per 24h or >=2 stools per 8 h. For each diarrheic episode an anonymous clinical-administered questionnaire and stools sample were collected. The global incidence rate was calculated using the mean number of soldiers based in N'Djamena during the study period (N=1024) as denominator. As the overall number of military personnel staying in N'Djamena slightly varied during the study period, diarrhoea incidence rates were also estimated for the 11 two-week-periods of stay. A case-crossover analysis estimated behaviors associated to diarrhea. A total of 240 acute diarrheas were notified by military physicians, resulting to an overall incidence rate of 49 diarrheas per 1000 person-months and a 23% risk to develop diarrhea. The incidence rate raised from 8.8/1000 person-two-weeks at the beginning of stay to 54.4/1000 person-two-weeks after one month and decreased after two months to stabilize between the end of November 2007 to early January 2008. Pathogens were identified in 40% stool samples, enteric viruses were predominant (28.6%, 14.8% for noroviruses). Three behaviors were significantly associated to acute diarrhea in the case-crossover multivariate analysis: diarrhoea in the close circle in the previous days increased the risk (OR:3.8 [2.0-7.0]); always eating at Mess (OR: 0.2 [0.1-0.5]) or staying in temporary encampment (OR:0.3 [0.1-0.8]) were protective. In conclusion, identification of viruses lead to high risk of man to man transmission. Independently of the risk of traveler's diarrhea due to poor sanitation environment confirmed by the study, our results underline the importance of relevant hygienic measures and primary care during military deployment.

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POOR TOLERANCE OF NIFURTIMOX IN TREATMENT OF CHAGAS DISEASE IN UNITED STATES-BASED POPULATION

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Chagas disease (CD), caused by the protozoan *Trypanosoma cruzi*, causes the most important parasitic disease burden in Latin America, where an estimated 8 million persons are infected. Chronic CD results in symptomatic cardiac and/or gastrointestinal disease in 10-30% of infected persons with 20,000 deaths annually. Approximately 17 million persons born in the endemic countries currently reside in the U.S. and roughly 300,000 of these immigrants are thought to have chronic CD. There are only two drugs available for the treatment of CD: nifurtimox and benznidazole. It has been over 40 years since the development of both drugs, yet neither is approved by the FDA. Currently, The CDC has obtained special approval for the use of nifurtimox. Though numerous clinical studies have evaluated efficacy and tolerance for nifurtimox in acute CD, little data exists on the tolerance of nifurtimox in older patients in the chronic or indeterminate stage of CD. Twenty nine consecutive Latin American immigrant patients with CD were treated with nifurtimox at the Center of Excellence for CD at Olive View-UCLA Medical Center for a planned treatment course of 90 days. The majority of patients were symptomatic with either chest pain or palpitations and 48.3% of patients had evidence of early cardiac involvement on electrocardiogram. None of the patients had clinical congestive heart failure. The median age of patients was 45 with ages ranging between 15 and 58. The most observed side effects of nifurtimox were: anorexia (79.3%), psychiatric manifestations including depression, anxiety, insomnia and memory loss (75.9%), nausea & vomiting (69.0%), headache (65.5%), myalgia & arthralgias (48.3%), abdominal pain (34.5%), fatigue (24.1%), parasthesias & neuropathy (20.7%), and rash (6.9%). Eleven (37.9%) patients required dose adjustment because of side effects. Nine patients (31%) stopped treatment temporarily, then resumed at a lower dose. Three of these patients (10.3%) were unable to tolerate the lower dose and stopped treatment prematurely. Most patients who required dose adjustment cited the psychiatric manifestations as the primary reason. The poor side effect profile of nifurtimox is the primary reason behind treatment intolerance and significantly hinder treatment efforts. Given the significant limitations of the current drugs, new drugs with better efficacy and lower side effect profiles need to be developed for CD.

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QUALITY SYSTEMS IMPROVEMENT FOR COLLEGE OF AMERICAN PATHOLOGISTS ACCREDITATION OF A CLINICAL LABORATORY TO SUPPORT BIOMEDICAL RESEARCH IN BAMAKO, MALI

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High-quality clinical laboratory capacity is oftentimes lacking in resource-poor settings and areas of high disease endemicity, impacting the ability to support biomedical research where it is needed most. As the regulatory climate changes globally, human research studies will require higher quality laboratory support for protecting study volunteers in assessing biological parameters in natural history studies and in assessing safety and immunogenicity which guide the clinical development of products. The University of Bamako in Mali and its NIAID partners have undertaken a comprehensive Quality Management Systems (QMS) improvement plan to scale-up productivity, technical ability and quality by utilizing Clinical and Laboratory Standards Institute (CLSI) internationally accepted consensus standards and guidelines. This QMS improvement and accreditation is part of the NIAID program for promoting best practices in clinical research in developing countries. The laboratory passed inspection by the College of American Pathologists (CAP) in April 2010. We report on the QMS implementation process, pitfalls in capacity building, staff training initiatives, and CAP-accreditation timeline as it may apply to other laboratories in resource-poor settings that support clinical research.

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MILD AND SEVERE CLINICAL FORMS OF URBAN LEPTOSPIROSIS: ACTIVE OUTPATIENT-BASED SURVEILLANCE IN A SLUM COMMUNITY IN BRAZIL

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Outbreaks of leptospirosis occur each year in slum communities in Brazil. The burden due to these outbreaks is underestimated since routine surveillance relies on identifying hospitalized cases. We performed active outpatient-based surveillance to measure the leptospirosis incidence in a slum community in Salvador, Brazil and determine the proportion of cases which develop severe disease forms. We identified all patients with acute fever and >5 years of age at an urgent care facility which serves a community of 62,952 inhabitants. The study team recruited a sample of patients five days a week to obtain information on clinical outcome and paired serum samples. The microscopic agglutination test was performed to confirm cases of leptospirosis. We recruited a sample of 1,119 patients among 4,509 identified with acute fever during a nine-month period

(04/09-12/09). Of these, 15 (1.4%) were confirmed cases of leptospirosis. The annual incidence of leptospirosis was 143 (95%CI, 120-166) cases per 100,000 population. Cases were mostly males (67%), had a mean age of 27 years (SD± 16.8 years) and were not suspected as having leptospirosis during their outpatient evaluation. The majority (11) of cases had a self-limiting illness while 4 developed Weil's disease requiring hospitalization. Risk factors for leptospirosis among subjects with acute fever were residence <10 meters from open sewage (OR 4.5; 95%CI 1.5-13.7) and sighting of rats (OR 7.0; 2.0-25-0), contact with trash (OR 5.7; 2.0-16.0), sewage (OR 5.9; 2.1-16.1), and mud (OR 3.4; 1.2-9.4) in the household environment. In conclusion, our study found that severe disease accounted for a small proportion (23%) of leptospirosis cases, indicating that the burden of urban leptospirosis is greater than previously believed. Outpatient physicians were unable to diagnose leptospirosis during the initial phase of illness. However, we identified environmental risk exposures which can be evaluated during outpatient evaluations and used to identify cases who would benefit from antimicrobial therapy.

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CHARACTERISTICS OF MALNOURISHED CHILDREN WITH DIARRHEA IN A RURAL AREA IN EGYPT

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Diarrhea and its association with malnutrition are major causes of morbidity and mortality in children in developing countries. Our aim was to compare the epidemiologic and clinical characteristics associated with better-nourished and malnourished children seeking diarrhea medical care. A hospital-based surveillance study was conducted during 2000-2007 in rural Egypt enrolling children less than 5 years of age seeking medical care for diarrhea. Data was collected, including height and weight to categorize a malnourished child as wasted (weight for age z-scores ≤ -2 SD) and/or stunted (height for age z-scores ≤ -2 SD). Better-nourished (not wasted and/or stunted) children served as a comparison group. Stool samples were collected for routine microbiological diagnostics. Of 3813 children enrolled, 610 (16%) were malnourished (6% wasted, 6% stunted, and 4% both wasted and stunted). For malnourished children, the mean age was 16 months and 59% were male, compared to 13 months and 54%, respectively, for better-nourished children. Compared to better-nourished children, stunted and both [wasted and stunted] children were less likely to be breastfed [(OR=0.6, p=0.003), (OR=0.6, p-value=0.02) respectively]; they were also more likely to be dehydrated [(OR=2.4, p=<0.0001), (OR=2.1, p=<0.0001) respectively]. Malnourished children were more likely to be hospitalized due to diarrhea than better-nourished children (OR=2.4, 2.4 and 3.5) for wasted, stunted and both [wasted and stunted] compared to better nourished children, respectively (p≤0.0001). *Cryptosporidium spp.* was the only pathogen more commonly found among wasted and stunted children (10%, 9.5%) compared to better-nourished children (6%), p=0.006 and 0.01. In conclusion, a significant percentage of children in rural Egypt seeking diarrhea medical care are malnourished and experience greater severity of illness than better-nourished children with diarrhea. Steps to improve sanitation and nutrition (including breastfeeding) may help to decrease diarrhea-associated morbidity in children.

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RELATEDNESS OF *VIBRIO CHOLERAE* O1/O139 FROM PATIENTS AND THEIR HOUSEHOLD CONTACTS, DETERMINED BY MULTILOCUS VARIABLE NUMBER TANDEM REPEAT ANALYSIS (MLVA)

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The genetic relatedness of *Vibrio cholerae* O1/O139 isolates obtained from 100 patients and 146 of their household contacts in Dhaka, Bangladesh, between 2002 and 2005 was assessed by multilocus variable number tandem repeat analysis (MLVA). Isolate genotypes were analyzed at five loci containing tandem repeats. Across the population as well as within households, isolates with identical genotypes were clustered in time. Isolates from individuals within the same household were more likely to have similar or identical genotypes than isolates from different households, but even within a household, isolates from different individuals often had different genotypes. Isolates with genotypes related to the index case appeared in household contacts on average ~3 days after the household index case, while isolates with unrelated genotypes appeared in contacts ~6 days later. Limited data revealed that multiple isolates from the same individual may have identical, similar, or unrelated genotypes as well. Our results demonstrate that genetically-related *V. cholerae* cluster in local outbreaks, but also suggest that multiple distinct strains of *V. cholerae* O1 may circulate simultaneously within a household.

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LUCIO'S PHENOMENON IN LEPROMATOUS LEPROSY: A VASCULITIS "MIMIC" IN TWO IMMIGRANTS FROM MEXICO

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First described by Lucio in 1852, Lucio's phenomenon is a necrotizing skin reaction associated with non-nodular diffuse lepromatous leprosy (Latapi's leprosy). Although rare in the United States, we have seen two cases of Lucio's mimicking cutaneous vasculitis in patients from endemic regions of Mexico (Jalisco; Sinaloa). Case #1: A 36 year old male presented with a one month history of painful, erythematous lesions on the lower extremities; within two weeks, similar lesions appeared on the hands, arms and subsequently ulcerated. The patient also had a history of eyebrow loss x 1 year and evidence of lepromatous leprosy (+FITE stain) on skin biopsy with full-thickness epidermal/dermal necrosis. The patient responded to a multidrug regimen including rifampin/dapsone/clofazamine. Case #2: A 49 year old male with cirrhosis/ESLD presented with peripheral neuropathy and bilateral necrotic lower extremity ulcers. The patient also had coarse facial features, partial loss of eyebrows/eyebrows, hepatosplenomegaly and thickening of peripheral nerves. Biopsy of skin lesions demonstrated mononuclear cell infiltrate with numerous AFB on Fite stain. The patient was started on multi-drug therapy for leprosy (rifampin/dapsone/clofazamine); however, he died two weeks later after developing aspiration pneumonitis and associated respiratory failure. Patients with Lucio's phenomenon present with painful erythematous macules that evolve into discrete purpuric or necrotic lesions suggesting cutaneous vasculitis. Diagnosis is often delayed since patients may have positive serologies (RPR; ANCA; anti-cardiolipin) suggesting other infectious or rheumatologic conditions (anti-phospholipid syndrome; cryoglobulinemia). Associated findings of lepromatous leprosy are commonly present but definitive diagnosis depends on pathological confirmation of lepromatous

leprosy (+ Fite stain) and characteristic findings of vasculitis and/or tissue necrosis on skin biopsy. Patients usually respond to multi-drug therapy for lepromatous leprosy; however, in severe disease, the outcome may be fatal despite appropriate therapy. The role of adjunctive therapy (corticosteroids; thalidomide) remains controversial but specific agents may be helpful in selected cases. Consider the possibility of lepromatous leprosy with associated Lucio's phenomenon in immigrants from endemic regions with cutaneous vasculitis.

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THE EFFECT OF VISCERAL LEISHMANIASIS ON GENE EXPRESSION IN HUMAN MONONUCLEAR CELLS: GLOBAL PROFILING OF CELLS FROM PATIENTS DURING DISEASE AND AFTER SUCCESSFUL TREATMENT

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Visceral leishmaniasis (VL) is a potentially fatal disease caused by Leishmania infantum chagasi (Lic) in South America. Lic infection can be symptomatic or lead to progressive disease. The factors that determine whether an individual will develop asymptomatic or symptomatic infection are not fully defined. Leishmania infection suppresses macrophage microbicidal responses and IFN-γ pathway signaling *in vitro*. In order to identify genes critical to the immune response to Lic *in vivo*, we evaluated gene expression in peripheral blood mononuclear cells (PBMCs) from Brazilians with acute VL and compared this to PBMCs three months after successful treatment. PBMCs were stimulated with soluble leishmania antigen for 4 hours before RNA extraction. RNA was hybridized to Affymetrix arrays. Comparisons showed 362 genes were differentially expressed during disease versus after resolution. Transcripts that were up-regulated after resolution of VL included CXCL5 (p=0.032, fold change 1.92), CXCL10 (p=0.051 fold change 2.63), CCL22 (p= 0.046 fold change 2.00), FAM177B (p=0.006), BCL11A (p=0.011), AIDA (p=0.0007), ADAM28 (p=0.022), THBS1 (p=0.040). Transcripts that were up-regulated during acute VL included APOC1 (lipid metabolism; p=0.055 fold change -1.68), ARG1 (arginine metabolism; p=0.05 fold change -2.89) and DHFR (nitrogenous base biosynthesis; p=0.01 fold change -2.15). The comparison of gene expression between disease and post-recovery from VL are consistent with suppression of chemokines as CXCL5 and CXCL10 and induction of lipid biosynthesis APOC1 and non-classical macrophage microbicidal response as Arginase 1 during acute VL. Data indicate previously unreported pathways are likely involved in pathogenesis of VL due to Lic.

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SUBUNIT LEPTOSPIRAL IMMUNOGLOBULIN-LIKE (LIG) PROTEIN VACCINE PROTECTS AGAINST LETHAL CHALLENGE IN THE HAMSTER MODEL OF LEPTOSPIROSIS AND PROTECTION IS ANTIBODY MEDIATED

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Subunit vaccines are a potential intervention strategy to prevent leptospirosis, an important neglected disease in developing countries. Lig proteins are a putative virulence factor which has bacterial Ig-like repeat domains and is expressed on the surface of *Leptospira*. We previously reported that immunization of recombinant Lig protein fragments in Freund's adjuvant conferred protection against lethal challenge in the hamster model of leptospirosis. This work aimed at evaluating Lig immunoprotection using aluminum hydroxide as an adjuvant, which is acceptable for human use. The terminal portion of LigA, the recombinant peptide LigANI, corresponding to nucleotides 1873-3675 of *ligA* gene from *Leptospira interrogans* Copenhageni strain Fiocruz L1-130, was produced as a soluble peptide and adsorbed to aluminum hydroxide. Golden Syrian hamsters (n = 8 or 10) were immunized with two doses (20-80 µg) of purified LigANI in aluminum hydroxide adjuvant, by either subcutaneous or intramuscular routes, and challenged two weeks afterwards with a lethal dose (2.5× LD50%) of *L. interrogans* strain Fiocruz L1-130. Immunization with LigANI conferred 100% protection against mortality (five experiments, P≤0.0005) by either route used. By Real Time PCR, leptospiral charge was statistically different in LigANI-immunized hamsters when compared to adjuvant-immunized hamsters in the lungs, spleen and eyes, but not in the liver and kidneys. Immunofluorescence studies of pre-challenge sera found that immunized hamsters produced surface-binding antibodies. Specific hyperimmune serum anti-LigANI was raised in New Zealand White rabbits immunized with LigANI. Passive transfer of rabbit hyperimmune sera conferred protection against lethality of 50 to 85% in hamsters (two experiments, P≤0.02). Albeit LigANI-based vaccine protected hamsters against lethal infection and serum anti-LigANI partially protected hamsters against mortality, no sterilizing immunity was observed. Together these findings indicate that immunization with recombinant Lig proteins in aluminum hydroxide confers robust immunoprotection in the standard animal model of leptospirosis and that the mechanism of immunity is antibody-dependent. Lig proteins may therefore serve as a sub-unit vaccine candidate for human and animal leptospirosis. Improvement efforts should focus on sterilizing immunity and heterologous protection.

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ETIOLOGY OF NONSPECIFIC ACUTE FEBRILE SYNDROME IN A DENGUE ENDEMIC URBAN AREA IN COLOMBIA

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Although it is assumed that the nonspecific acute febrile syndrome (SAFI) is cause by this arbovirus, the etiology of this syndrome is still unknown in Colombian cities where dengue is endemic. Methods: We conducted a prospective cohort study (2003-2008) in subjects ≥ 5 years with SAFI in Bucaramanga. The etiologies were defined as follows: Dengue Negative: Negative test for specific IgM (ELISA) in convalescent serum. Influenza: Seroconversion or fourfold increase in antibody titers anti Influenza A or B of an acute and convalescent serum or titers> 1:40 (HIA test) in the latter. Leptospira: titers ≥ 1:50 by microagglutination technique. Rickettsiae: fourfold increase in the titers from the acute to the convalescent serum (IFA test). Rubella and Measles: Seroconversion of specific IgM (ELISA). Unusual Virus: We used cell culture in order to rule out Flavivirus (Yellow Fever, Encephalitis, St. Louis), Arenaviruses (Tacaribe Group) Bunyavirus (Oropuche) and Alphavirus (Venezuelan Equine Encephalitis) Results: Between March 2003 and August 2008, 2063 subjects with SAFI were followed and 1124 (54%) from those subjects were negative for dengue. In the latter group 228 (20.2%) had influenza and 52 (4.6%) had Leptospira. 11 suspected cases of Rubella and 7 of Measles were negative on confirmatory testing. None was positive for Rickettsiae or other virus. The majority 454 (40.3%) of total had no defined etiology..The acute sera of 27 cases initially considered to be dengue-negative, were positive for dengue in the cell culture. In conclusion, in a dengue endemic city in Colombia, other agents different from this virus are prevalent as the etiology of SAFI.

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EFFECTS OF VARIOUS METHODS OF INACTIVATION OF HEMORRHAGIC FEVER VIRUSES ON CLINICAL LABORATORY PARAMETERS MEASURED IN HUMAN BLOOD

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Viral hemorrhagic fever (VHF) is caused by infection with one of over 25 different lipid-enveloped viruses, including Ebola, Marburg, and Lassa. These viruses pose a threat not only to infected persons, but also to healthcare workers, laboratorians, and researchers in potential contact with blood and other bodily fluids. Although specialized Biosafety Level 4 laboratories have been developed for researchers to safely manipulate these viruses and diagnose VHF, few standard clinical laboratories have these means of protection, potentially putting workers in these laboratories at risk. However, various means of inactivation of lipid-enveloped viruses have been reported that may help protect workers in clinical laboratories, including solvent/detergent combinations (Triton X-100/TnBP/Tween 80), heat, gamma-irradiation, formalin, psoralens, and UV light. However, since the point of testing in clinical laboratories is to measure parameters important in guiding clinical management, it is important to understand the effect of the various inactivation techniques on each parameter in question. We performed a study to evaluate the effects of proven or assumed virus inactivation techniques on clinical laboratory parameters commonly measured in the blood and useful in the treatment of patients with VHF, including complete blood cell counts, electrolytes, and chemistries, including coagulation parameters. Each parameter is measured before and after the inactivation step on the Piccolo Xpress blood analyzer (Abaxis Co.), a point-of-care instrument, and the percent change noted. Triton X-100/TnBP/Tween 80 has minimal effect on tested clinical parameters. In contrast, there were significant changes after heat and formalin inactivation. Results from gamma irradiation, UV light, and psoralen/UV light are pending but will be discussed, as well as proposed guidelines for safe handling and testing of blood from patients with VHF in the clinical laboratory.

902

CLINICAL STUDY OF SAFETY AND APPARENT EFFICACY OF ANTIVIPMYN® AFRICA FOR THE TREATMENT OF SNAKEBITE IN KINDIA, A FOREST REGION OF GUINEA

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An open phase IV pragmatic clinical study was conducted to measure the safety and assess the apparent efficacy under field conditions of Antivipmyn® Africa (AA), an equine lyophilized $F(ab')_2$-based antivenom. The study was conducted at the Insitut Pasteur de Guinée (IPG) from August 2009 until February 2010. All people reporting snakebite, presenting clinical symptoms of envenomation (edema, necrosis or neurological signs) and who formally accepted to participate in the study were included. Antivenom was administered by slow direct intravenous push. In 6 months 228 snakebite victims arrived for consultation at the IPG. The mean delay from bite to consultation was greater than 24 hours. Of these, 150 (65.8%) were included, mostly young men; of these, 124 (82.7%) exhibited signs of viper envenomation (inflammation and/or bleeding and/or necrosis) and 26 (17.3%) exhibited manifest neurological signs compatible with Elapid envenomation (local-regional paresthesias, cranial nerve paralyses, dyspnea, severe problems of awareness.) All patients received treatment, a mean of 1.41 vials per patient (± 0.99), and more for those with signs of neurotoxicity ($P < 10^{-5}$). Four patients (2.7%), apparently bitten by Viperidae, had a necrosis of variable extension which healed without sequels. Four others, in all likelihood bitten by Elapidae, died with hours (range: 1-7) of arrival to the IPG in spite of antivenom administration. For 2 of them, the delay between bite and arrival might explain in part the absence of a therapeutic response; for the other 2, the evolution of neurotoxicity continued in spite of treatment. Ongoing analysis of blood samples collected during the trial should permit an identification of the offending species and inform on the evolution of blood venom levels. Ten patients exhibited mild adverse events (pruritus or eruption), 5 of which are likely to be attributable to treatment.

903

FALSE-POSITIVE RAPID PLASMA REAGIN TESTING IN PATIENTS WITH ACUTE *PLASMODIUM VIVAX* MALARIA

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Prior to the introduction of penicillin, a common therapy for patients with neurosyphilis was the induction of fever with deliberate *Plasmodium* infection. Investigators at that time described positive serologic tests for syphilis in malaria-infected control patients without evidence of syphilis infection. Currently, the diagnosis of syphilis consists of screening with non-treponemal tests such as the rapid plasma reagin (RPR), followed by confirmation with a specific test such as the *Treponema pallidum* hemagglutination assay (TPHA). In this study, we compare the rate of false-positive RPR tests in patients with vivax malaria compared with patients with other febrile illnesses in Peru. Patients ≥ 5 years of age were offered enrollment into an ongoing febrile surveillance protocol in Peru if they had a temperature of ≥ 38.0 degrees C for ≤ 7 days without distinct localizing symptoms, such as a purulent cough or meningismus. Malaria was diagnosed by microscopy and PCR. RPR and TPHA were performed on all acute serum specimens. Groups were compared by two-tailed Fisher's exact test. 73 patients with vivax malaria and 76 control patients with other febrile illnesses were identified. 54.9% of patients with malaria were male with a mean age of 31.5 years, compared with 40.8% and 28.9 years in patients without malaria. In patients with malaria, positive RPRs were detected in 8/73 (11.0%), of whom 2/8 (25%) had positive TPHA tests. RPR titers ranged from 1:1 to 1:16 among the false-positive tests. In patients without malaria, a positive RPR and TPHA were detected in 1/76 (1.3%) with no false-positives. Overall, false-positive RPRs were detected in 6/73 patients with malaria (8.2%) versus 0/76 patients with non-malarious fever (0%) (p=0.0124). The positive predictive value of the RPR in patients with malaria was 25% (95%CI: 4.4-64%). In conclusion, false-positive RPRs are common in patients with vivax malaria. The RPR is a widely-used screening test for syphilis in pregnant women, persons with HIV, and other groups at risk for malaria. As such, the RPR should be interpreted with caution in malarious settings.

904

RISK FACTORS FOR METABOLIC SYNDROME IN CHILDREN AND ADOLESCENTS FROM URBAN AND RURAL AREAS OF NORTHEASTERN VENEZUELA

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Obesity represents one of the major problems associated to public health for its association to increased risk to type 2 diabetes, cardiovascular disease and stroke. Recent studies have showed that obesity is a world

wide epidemic, with the age of onset for diabetes, metabolic syndrome and cardiovascular disease decreasing every year. In order to study the prevalence of children and adolescents with increased risk of obesity in urban areas compared to rural areas, we studied 1,764 individuals (3 to 17 years old) from the cities of Barcelona (n=972), Cumana (n=325), and four rural communities (n=467). We have calculated body mass index (BMI), fasting glucose, total and HDL cholesterol, triglycerides, uric acid, creatinine, and total serum proteins. The prevalence for the risk of obesity (BMI>85 percentile) was 30.2% in the cities and 9.9% in the rural areas, without statistical differences between sexes. There were differences among age groups of the prevalence for risk of obesity. High fasting glucose (BMI>100 mg/dL) was found in 2.2 and 4.9% of the individuals from urban and rural areas, respectively. High cholesterol (above the 90 percentile, according to age) was found in 9.0 and 13.5% of individuals, respectively, and high triglycerides (above the 90 percentile) were found in 8.3 and 5.7% of the subjects. We found a relationship between age and fasting glucose, creatinine and total serum protein in both sexes, and total cholesterol but only in males. Individuals with high risk for obesity showed higher values of triglycerides and total cholesterol. These results show that there is a high prevalence of overweight among children and adolescents in urban areas but not in rural areas, most likely related to physical activity and nutrition. The risk factors found in the studied individuals can contribute to the progression of diseases such as type 2 diabetes, cardiovascular disease and metabolic syndrome. Prevention programs have to initiate in very early stages, thus more studies in children and adolescents have to be carried out.

905

ACCEPTABILITY OF A PRE-REFERRAL LIFE SAVING DRUG ADMINISTRATION FOR THE PREVENTION OF SEVERE MALARIA AND UPPER RESPIRATORY INFECTION RELATED DEATHS IN CHILDREN LIVING IN RURAL AREAS

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Malaria and acute respiratory infections (ARI) worldwide account together for 25% of deaths among less than 5 years old children. Clinical manifestations of those diseases make differential diagnosis difficult in the absence of medical doctors and proper diagnosis. Co-infections between malaria and ARI are generally treated as a malaria case, and result in a number of treatment failures leading to late antibiotic administration, enhanced morbidity and death. In rural areas, difficulty in reaching health facilities is responsible for many deaths during transportation of a child to the nearest hospital. Therefore, implementation of a home based treatment directed against these two major infectious diseases is of great interest in reducing infant mortality. In this context, we are developing a pre-referral intra rectal antibiotic - antimalarial combination to initiate the treatment of aggravated febrile illness in children. Beyond the rational for the R&D related to the development of a proper drug formulation, one of the current objective is to evaluate whether a pre-referal drug combination administered before any clinical or biological diagnosis is done, will be acceptable by the health authorities, health professionals, and the community. A qualitative study was conducted from May to July 2009 in Guinea Bissau. Health officials, health professionals, and community members leaving in rural areas were interviewed. At the institutional level, participants were asked about the acceptability of the combination and how to integrate the drug within the health system. The overall intervention strategy was well accepted by all health representatives in Guinea Bissau, but should be based on proper trainings aiming to optimize the use of the drug at the community level and to ensure proper treatment after admission to a referred health structure. Such therapy should be proposed in agreement with other health programmes related to malaria control such as the TDR/WHO artersunate rectal project, and the introduction of rapid diagnostic tests for malaria at the community level. Severity of illness was evaluated differently depending on mothers at the community level, but fever associated to at least one symptom of severity was mostly associated to the need for an emergency intervention. However, drugs for the treatment of non-severe malaria should also be implemented to prevent misuse of the emergency treatment at the community level.

906

ACCEPTABILITY TO PARTICIPATE IN A DENGUE VACCINE TRIAL AMONG RESIDENTS IN PUERTO RICO

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Dengue is endemic in Puerto Rico with the most recent outbreak occurring in 2007. Vector control has shown limited impact. Dengue vaccines may be effective for disease control. Knowledge of dengue prevention and the acceptability to participate in a Phase III dengue efficacy trial in Puerto Rico was assessed. Two structured questionnaires with open-ended questions were developed; 1) key informant interview questionnaire for university researchers, mayors, school principals/teachers, community leaders, and parents; 2) focus group questionnaire for children and parents. Sixty-four interviews with key informants and 45 focus groups with 96 parents and 111children were conducted in 12 municipalities with the highest dengue rates. Most participants knew about dengue prevention and perceived children as the most affected population; all felt at risk for dengue. Participants knew that vaccines prevent illness and indicated that a dengue vaccine would be "fabulous"; 99% of parents would not allow their child to take part in a clinical trial. Barriers to child participation included: lack of trust and information on vaccine development and trial procedures; fear of infection due to vaccination; side effects (SE) and sequelae; and lack of transportation. Lack of trust and fear was associated with news reports on Influenza A-H1N1vaccine SE. Motivators for participation were: altruism, information on vaccine development and trial procedures, gaining protection against dengue, and getting free medical care and stipends for transportation or participation. Researchers stated that community and captive populations were the best settings for a clinical trial. Face to face interventions may be the best strategy to motivate parents to allow their children to participate in a dengue vaccine trial. Therefore, the following information should be included: study purpose, procedures and duration; results of prior studies; vaccine development, risks of SE and sequelae; differences between Influenza A H1N1and dengue vaccines; benefits; safety regulations for subjects; and transportation.

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UPDATE ON TETRAVALENT DENVAX: PREPARATION FOR PHASE I CLINICAL TRIALS IN THE US AND COLOMBIA

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Inviragen's DENVax tetravalent dengue vaccine consists of a mixture of the live, attenuated DEN-2 PDK-53 virus and three chimeric recombinant viruses, each bearing the DEN-2 PDK-53 non-structural gene backbone while expressing DEN-1, DEN-3 or DEN-4 structural genes. The four live, attenuated dengue virus strains (DENVax 1, DENVax-2, DENVax-3 and

DENVax-4) express the antigenic prM and E proteins from each of the four dengue serotypes and share the common dengue 2 (strain PDK-53) genetic background. Inviragen is currently preparing for two phase I clinical trials of DENVax formulations. The first trial will be conducted in the US at St. Louis University. In the study, two formulations (high and low dose) will be tested and administered by the subcutaneous and intradermal routes. A similar study is being planned for Colombia in the town of Rionegro (Ant), a high altitude area with low incidence of dengue. Required documents for both trials have been submitted to the US (FDA) and Colombia (INVIMA) regulatory authorities.

908

INCREASED REPLICATION OF DENGUE-2 VIRUS SEROTYPE IN MIXED CO-INFECTION STUDIES

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A double mosquito infection with two Dengue viruses (DENV) serotypes under field conditions has been documented recently. To our knowledge, no studies have been conducted characterizing experimental infection in mosquitoes exposed to two distinct DENV serotypes and the impacts of coinfection on mosquito biology and transmission dynamics remain unknown. We have conducted preliminary studies *in vitro* and *in vivo* of mosquito mixed co-infection using strains of DENV serotypes 2 and 3 isolated from the Medellin area. To test this, C6/36 HT cells were co-infected with 2x108 genome copies/ml of DENV-2 and -3 serotypes and the replication capacity was evaluated at 96 hours post-infection. Additionally, A. aegypti mosquitoes were artificially fed with a mixture of DENV -2 and -3 at 2x108 genome copies/ml of each serotype. Mosquitoes were also infected with single viruses; negative controls consisted of blood without virus. At different days post-feeding viral genome was quantified by RT-qPCR and viral antigen was detected by immunofluorescence. Our results *in vitro* show that there are significant differences in the replication capacity between the DENV-2 and -3 different serotypes, but not between strains of the same serotype isolated from the same geographic area 5-10 years apart. Similar results showing significant differences between serotypes, but not between DENV strains, were obtained from co-infection studies in A. aegypti mosquitoes. The amount of antigen and viral genome were significantly ($P<0.05$) greater to DENV-2. These results are of great importance considering the epidemiology of DENV infection, since it is known that the frequency of infection with DENV-2 was higher than others serotypes.

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ESTIMATING THE MAGNITUDE AND DIRECTION OF ALTERED ARBOVIRUS TRANSMISSION DUE TO VIRAL PHENOTYPE

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Vectorial capacity (C) is used as a measure of the transmission potential of a vector borne pathogen within a susceptible population. Vector competence (b), a component of the vectorial capacity equation, is the ability of an arthropod to biologically transmit an infectious agent following exposure to that agent. Comparisons of arbovirus strain-specific vector competence estimates have been used to support observed or hypothesized differences in transmission capability. Typically, such comparisons are made at a single (optimal) time point during the extrinsic incubation period, the time in days it takes for the virus to replicate and disseminate to the salivary glands. Instead of evaluating vector competence at discrete time points, utilization of the rate of change gives a more accurate measurement of the transmission potential of an arbovirus within its vector. Accordingly, we investigated the rate of change in vector competence of dengue virus in *Ae. aegypti* mosquitoes and combined it with the survival function of the vector to produce a vectorial capacity curve. The areas under the resulting curves represent the cumulative transmission potentials of the arboviruses within a population of mosquitoes. We used the calculated area under the curve for 5 dengue strains and the corresponding variance estimates to test for differences in cumulative transmission potentials between strains of dengue based on our dynamic model. To further characterize differences between dengue strains, we devised a displacement index (DI) which we define as the capability of a newly introduced strain to overtake and displace the established, dominant circulating strain. The computation of a displacement index can be used to better understand the transmission dynamics in systems where multiple strains/serotypes/arboviruses circulate. We postulate that this displacement index will lead to a better measurement of the true differences in transmission potential of pathogens, as well as inform models of the serotype/strain switching phenomena such as that seen in dengue transmission systems.

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USING HUMAN MOVEMENT DATA TO DERIVE DENGUE VIRUS TRANSMISSION NETWORKS

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Movement patterns and social structure play an important role in modulating human-vector contact rates, affecting transmission dynamics, and the spread and persistence of vector-borne pathogens. For dengue virus (DENV), limited dispersal range of its day-biting vector, *Aedes aegypti*, points to movement of viremic humans as a plausible explanation for the rapid spread of infection across urban environments. We used field data from spatially-explicit semi-structured interviews (SSI) and GPS data-loggers to derive contact networks of individual humans for DENV transmission in Iquitos, Peru. We obtained movement data for 300 participants and expressed their contact network as an undirected bipartite graph representing the locations participants had in common as a consequence of their routine movements. Different measures of network topology were estimated for the full contact network and "key sites" network containing only those locations where exposure to *Aedes aegypti* is most likely (houses and schools). Places where participant's spent the most time outside their home were other residential locations (71% of total time); markets and stores (18%); parks, cemeteries, and recreational areas (3%); and hospitals and health posts (2%). Average degree of a participant (number of locations visited) increased with age from an average (SD) of 2.8 (1.1) for 3-8 yr-olds to 7.1 (4.3) for 45-69 yr-olds. The derived key-sites network had a main component with 69% of all the participants, indicating a high degree of connectivity at residential locations. Current targeted vector control programs focus on neighboring homes within 100 m of a diagnosed dengue case's house. Our quantitative empiric contact networks indicate that residential exposure can occur beyond 100 m of a person's home and are consistent with the notion that movement of viremic people is a prime driver of rapid DENV propagation in urban environments.

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INTERACTION BETWEEN PRECURSOR MEMBRANE AND ENVELOPE PROTEINS OF DENGUE VIRUS

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The envelope (E) protein of dengue virus (DENV) is the major immunogen for vaccine development. The C-terminus of E protein contains two α -helices (EH1 and EH2) in the stem region and two transmembrane domains (ET1 and ET2) in the anchor region. After synthesis, E protein forms a heterodimer with the precursor membrane (PrM) protein; such interaction is critical for the assembly of virus particles in the ER and maturation along the secretary pathway. At the C-terminus of PrM protein, there is an α-helical domain (MH) and two transmembrane domains (MT1 and MT2). Previous studies of the tick-borne encephalitis virus reported the domains of E protein important for PrM-E interaction and production of virus-like particles (VLPs). In addition, PrM protein was reported as a chaperone for proper folding of E protein. However, the domains of PrM protein interacting with E protein remain largely unclear. A series of constructs containing C-terminal truncation of E protein in the DENV4 PrM/E, E or PrM expression vector were generated. After transfection to 293T cells, cell lysates and cultural supernatants were collected and subjected to Western blot analysis, radioimmunoprecipitation, ultracentrifugation and co-sedimentation assays. In the absence of E protein, the expression of PrM protein was reduced and completed abolished after truncation of MT1. In the presence of E protein, the expression of PrM protein was greatly reduced after truncation of ET1 and EH2, suggesting that they are important for expression of PrM protein.Radioimmuno-precipitation by using anti-E mAb revealed greatly reduced PrM protein after truncation of ET1 and EH2, suggesting they are required for PrM-E interaction. Pulse-chase experiment suggested that EH2 is important for the stability of PrM protein. The production VLPs was reduced for ET2 truncation mutant, suggesting that ET2 is involved in the efficient production of VLPs. In conclusion, our results suggest that PrM protein with C-terminal truncation is not stable. EH2 and ET1 domains are involved in PrM-E heterodimerization, and EH2 domain is important for maintaining the stability of PrM protein. Moreover, ET2 domain is involved in the production of VLPs. These information adds to our understanding of the biology of the major immunogen of dengue vaccine development.

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CLIMATE AND MAJOR DENGUE EPIDEMICS IN THE AMERICAS

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Dengue is a major public health threat, with at least an estimated one third of the world population at risk of infection. This study explored the relationship between several climate indicators and the likelihood of major dengue epidemics. The analysis was based on annual dengue incidence data from 46 countries from North, Central and South America, as well as the Caribbean, as reported by PAHO from 2000-2008. The climatic factors examined were annual average of daily mean, maximum and minimum temperature, annual average of monthly total precipitation and the El Niño Southern Oscillation (ENSO) phases. Multivariate logistic regression models were fitted to the proportion of major dengue epidemics. Annual incidence rates of 200 per 100,000 population or larger were considered as a major dengue epidemic. Precipitation alone explained 20.90% of the total variation in the occurrence of major dengue epidemic. The final multivariate model contained precipitation, the cold ENSO phase, mean annual temperature, minimum annual temperature as well as the interaction of mean and minimum temperature. This model explained 39.14% of the total variation. Our ecological analysis suggests that global indicators of precipitation and temperature predict the probability of large dengue epidemics. Understanding the climate's role in the probability of large dengue epidemics may aid in the prediction of such events.

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DENGUECON: AN ECONOMIC TOOL FOR INFORMED VACCINE DECISION-MAKING

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Several dengue vaccines are in phase 2 clinical trials. Preparing for introducing with well-thought out introduction plan could accelerate introduction of an effective vaccine; however health economic data for dengue is often not available. The objective of this study was to design a model that introduces basic cost-effectiveness concepts, that is sufficiently simple so that practicing public health officials can readily use it and interpret input and output values and users can use the model without specialized software, programming skills, or advanced knowledge of statistics to identify data needs and stimulate country-specific economic studies for more precise evidence-based decision making. We designed interactive, Excel-based models that require four types of data: Disease burden, vaccine effectiveness, cost of treatment and cost of vaccination. The models were made self-contained, have low RAM requirements, are easily down loaded or emailed, and can be operated on any computer using the commly available Microsoft Office software. To limit the complexity of the model we assumed a healthcare perspective for calculating medical costs and cost-effectiveness ration. We developed two primary models: DenguEcon and DenguEconDALY. These models will allow for the analysis of Dengue Vaccine introduction for a single cohort over 10 years that examine the cost-per-case averted, cost-per-death averted or cost-per-disability-adjusted life year averted, respectively. In addition, we developed a third model, DenguEconCompare, which will allow for head-to-head comparison of two vaccine introduction strategies (e.g. addition to EPI vs. catch-up campaigns) examining up to 10 cohorts over a 10 year period. In conclusion, this analysis will allow the user to project the economic and health consequences of vaccination in a specific country setting. However, users should cautiously interpret their findings. Estimated cost-effectiveness ratios should serve as just one barometer for policy-makers when evaluating the appropriateness new vaccine introduction.

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THE TEMPORAL AND SPATIAL ANALYSIS ON THE EFFECTIVENESS OF DENGUE INTERVENTION ACTIVITIES IN TAINAN, 2007

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Dengue hemorrhagic fever has become one of the major causes of pediatric deaths, particularly in endemic/hyperendemic countries. In many tropical and subtropical regions, climate conditions facilitate the viral transmission through local mosquitoes. The specific aims of this study are to analyze epidemiological data and to evaluate the two most important disease control strategies - source reduction and insecticide spraying. We used 1403 total laboratory-confirmed dengue cases occurred in Tainan from the 1st week of July 2007 to the 2nd week of January 2008 to construct models illustrating both the temporal and spatial effects of dengue interventions on the epidemic. To consider mosquito's life cycle, accumulated 3-week total numbers of dengue cases in the smallest administrative unit - Li in Taiwan were compiled. Data of intervention measures, including information of spraying insecticides, and source reduction, obtained from the work logs of Tainan City government, were served as the two independent variables. Finally, we used Li-specific case differences as the outcome measures (subtracting case numbers in the prior three weeks from the current three weeks) and spatial lag models

with k-nearest neighbors set as 4 to analyze the effects of the two control measures. The results showed source reduction significantly decreased dengue cases ($p<0.05$), particularly at early stage of the epidemic. When the case numbers increased strikingly, neither insecticide nor source reduction efficiently controlled the epidemic. Only until all the manpower and resources were allocated properly with more integrated efforts during the peak period of the epidemic, both spraying insecticide and source reduction demonstrated significant effectiveness in reducing number of dengue cases. In conclusion, our data revealed that timely and thoroughly city-wide intervention by source reduction of mosquito breeding sites once dengue cases were identified early through surveillance system was the most effective strategy at the initial stage of dengue season and/or epidemic period when case numbers were low. Prevention and control of the mosquitoes has to be implemented at the right place, time and most importantly based on the data analysis of spatial epidemiology. We believe our experiences can shed more light on to set up standard operation procedures and evidence-based dengue prevention/control-related public health policies.

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ADVANTAGES OF A FIELD SITE CONSORTIUM

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Dengue is a major public health issue in developing countries. Several dengue vaccines are in currently in development. The Pediatric Dengue Vaccine Initiative (PDVI) provided funding and/or technical support to researchers to establish field sites in anticipation of future trials. Non-funded sites also expressed interest to form what has been called the PDVI Field Site Consortium. The goal of the Consortium was to open a forum for dengue researchers to allow sharing of experience and research, in preparation for the sites to be used in clinical vaccine trials. There are currently 9 field sites in the Consortium in 8 countries: Managua (Nicaragua, 2004-9), Medellin (Colombia, 2008-9), Patillas (Puerto Rico, 2005-7), Colombo (Sri Lanka, 2008-9), Kolkata (India, 2008-10), Kampong Cham (Cambodia, 2006-8), Kamphaeng Phet (Thailand, 2004-7) Ratchaburi (Thailand, 2006-8), Long Xuyen (Vietnam, 2004-8) and Cebu (Philippines, 2009-10). We review the development of the Consortium, the meetings proceedings, and PDVI work plans to assess the advantages of Consortium participation. The first Consortium meeting was March 2007 in Bangkok, Thailand, where terms of reference were developed and agreed upon. Since that time, the Consortium assisted field sites by [1] developing standardized case report forms [2] sharing programs and databases for data collection in the field (e.g. using hand held computers); [3] development and sharing of epidemiologic databases; [4] sharing of a sophisticated laboratory database; [5] implementation of a Good Clinical Practice program; [6] negotiating with industry for lower unit prices on dengue test kits; [7] provision laboratory training to and sharing of testing protocols; and [8] development of mult-country protocols for additional dengue research. In conclusion, the Consortium has accelerated the development of field sites ideal for phase 3 clinical trials. Sharing of methods and skills has led to accelerated infrastructure, staff development and over-all reduced costs of field site development.

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ECONOMIC IMPACT OF DENGUE OUTBREAK ON TOURISM

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Dengue fever is endemic in most tropical and subtropical tourist locations around the world. Dengue infection has been documented in returning traveler. We compared the volume of hotel room reservations collected routinely and prospectively by the World Tourism Organization to national surveillance data for four countries: Thailand, Vietnam, Brazil, and Puerto Rico. We categorized data from National Surveillance as either outbreak or non outbreak years based on National Public Health alerts for dengue outbreaks. The average cost of a room was multiplied by the total volume of rooms reserved, to determine the total lost revenues.

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GLOBAL RISK AND BURDEN OF TRAVEL-ASSOCIATED DENGUE

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Dengue fever is endemic in most tropical and subtropical tourist locations around the world. Dengue infection has been documented in returning traveler. We conducted a systematic review of literature to determine the risk of dengue travels. We then multiple the volume of hotel room reservations collected routinely and prospectively by the World Tourism Organization to national surveillance by that average risk to estimate the total number of dengue infections occurring in travelers.

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SEROEPIDEMIOLOGICAL EVALUATION AFTER THE OUTBREAKS OF DENGUE IN SOUTHERN TAIWAN AREAS WITH AND WITHOUT FREQUENT PAST EPIDEMICS

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Residents lived below N 23 latitude in Taiwan have suffered dengue epidemics in past years. From 2007 to 2008, Tainan experienced the largest outbreak in 60 years caused by dengue virus serotype 1 (DENV-1) with total 1,498 confirmed cases. Recently, Kaohsiung had also faced several epidemics of dengue/dengue hemorrhagic fever (DHF) caused by multiple serotypes of dengue viruses (DENV-1, -2, and -3), with total 675 confirmed DF cases and 9 DHF cases from May 2009 to May 2010. To understand the magnitude of dengue virus infection and the correlations between infection and cases, we conducted two community-based seroepidemiological studies at one year post-epidemic period in Tainan (Aug. and Sept., 2008), and at mid-epidemic period in Kaohsiung, (Nov., 2009). Moreover, two school-based cohort studies were implemented to evaluate possible asymptomatic infection and the seroprevalence and seroincidence of dengue infection in children. Laboratory tests included dengue-IgM, dengue-IgG, and dengue-specific NS1 antibody. The preliminary results showed that seroprevalence of dengue-IgG in the communities of Kaohsiung with high dengue incidence in 2009 reached 33%, which was similar to the seroprevalence evaluated in the communities of Tainan with high dengue incidence in 2007. Further spatial analysis showed that the magnitude of dengue infection was higher and broader than the officially identified 2007 cases. On the other hand, the seroincidence in the communities in Kaohsiung during the intermediate phase of the 2009 epidemic was quite high (16%, 14/87). Interestingly, three dengue-seroincident persons were located in the same family about two months after the 2009 outbreak, and most dengue-IgM positive cases distributed quite locally in HisaoKang District. Furthermore, the seroprevalence of dengue-IgG in Tainan's schoolchildren was 4%, significantly lower than in adults indicated that Tainan had not become a dengue-endemic area yet. Since Kaohsiung has multiple-serotypes of DENV versus occasional outbreaks by a single serotype of DENV in Tainan are quite different, future efforts in serotyping by dengue-specific NS1 antibody, prospective epidemiological analysis, and quality research of risk communication will provide better clues on the roles of asymptomatic

persons and isolated dengue viruses in the series of chains of transmission and on establishing a community-based effective health education program as well.

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ADULT DENGUE HEMORRHAGIC FEVER CASES WITH SECONDARY INFECTION FACILITATED VIRAL TRANSMISSION IN KAOHSIUNG, TAIWAN

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Dengue viruses (DENV, genus Flavivirus, family Flaviviridae), one of the most significant emerging threats to global public health, have increased in geographic range, prevalence, and disease severity in recent years. DENV is mosquito-borne human pathogen which causes diseases varying from asymptomatic infection, dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). The clinical manifestations have been examined from genetic, immunologic, and virological aspects. To further elucidate the disease progress, 63 serum samples (paired or triple samples) of the 24 DENV-2 laboratory-confirmed dengue patients at different stages after the onset of illness, including 5 primary DF cases, 1 primary DHF case, 7 secondary DF cases, and 11 secondary DHF cases were collected and analyzed. The preliminary findings show that the mean of viral load at defervescence stage for secondary DF cases was higher than primary DF cases, and such trends lasted until the convalescent stage. In addition, the mean of viral load for secondary DHF patients was significantly higher than secondary DF cases at convalescent stage ($p = 0.022$), although DHF cases reached the peaking viral load at a later time point than DF cases. These findings on the longer duration and higher levels of viral load in DHF cases than in DF cases imply that more efficient prevention and control measures are required once DHF cases occur. Future research will focus on integrating temporal and spatial information with viral load and cytokines/chemokines analysis for better understanding the interplays between viral pathogenesis and immunopathogenesis at different stages of disease progress.

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SPATIAL EPIDEMIOLOGY OPENS A NEW DIRECTION FOR GLOBAL CONTROL OF DENGUE HEMORRHAGIC FEVER - TAIWAN'S EXPERIENCE

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Dengue epidemics involving the more severe dengue hemorrhagic fever (DHF) have been expanding and increasing worldwide. In Taiwan, where dengue is not endemic, we have had an unprecedented opportunity to develop spatial-temporal indices in Kaohsiung, southern Taiwan that may help identify risk patterns for predicting large-scale epidemics and the emergence of DHF. To understand the epidemiological pre-conditions for severe DHF epidemics, we initiated spatial epidemiological analysis using geographical information system (GIS) to analyze the relationship between dengue fever (DF) cases and DHF cases. Through past large-scale epidemics in southern Taiwan, we found the following important spatially epidemiological characteristics. First, the distribution of DHF cases were more focalized than DF cases. Second, DHF cases occurred more likely in those areas with more cluster dengue cases. Third, epidemiological linkage to identify sources of the infection will provide better clues than residential areas to be targeted for minimizing DHF cases. Fourth, spatial-temporal diffusion of dengue cases clearly showed the correlation between large clusters and temporal increases of dengue case numbers. Fifth, DHF cases were associated with a longer lasting waves and/or waves with more intense transmission, despite low annual incidence in these areas. Finally, dynamic transmission of dengue cases, environment factors and abundance of mosquitoes plus effectiveness of control measures can be clearly analyzed and evaluated through an integrated manner. Virologic/serologic surveillance monitoring high risk populations in high risk areas is necessary after the occurrence of DHF or a large-scale dengue epidemic. We believe that the world can prevent or minimize severe epidemics of DHF by improving surveillance, implementing more integrated community-based prevention and control programs, using GIS to closely monitor tempo-spatial trends of dengue clusters and developing dengue vaccine.

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FIRST RECORDED OUTBREAK OF DENGUE 3 IN SENEGAL

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In October 2009, a 44 years old patient living in Turin (Italy) for 20 years and who stayed in the region of Louga in Senegal from the 3rd of July to the 3rd of October 2009 was suspected then diagnosed with Dengue in the hospital of Lazzaro Spallanzani in Rome. During this period, dengue RNA were identified in the National Reference Centre for Arboviruses in Paris in a second patient living in Marseille, France who also visited families in the region of Thiès, Senegal. Faced with these two cases, the arboviruses and viral haemorrhagic fever unit of Pasteur institute in Dakar took hold of the health authorities in Senegal for increased surveillance. Thus, between October 2009 and January 2010, a total of 696 samples were received in the laboratory. The suspected cases lived mostly in Dakar but also in other regions of the country. All samples received were tested by two techniques (RT-PCR in real time using specific primers for dengue and by ELISA for detection of immunoglobulin M. Of the samples tested, dengue was identified by at least one of the techniques in 196 patients. Sequencing of the E, NS5 and NS5/3'UTR of the DENV-3 strains isolated in Dakar at the last trimester of 2009 in order to analyze the phylogenetic relationship among them. Our sequences were aligned with homologous DENV-3 retrieved from Genbank. All the trees showed that DENV-3 strains circulating in Senegal were more closely related to strains isolated in Cambodgia in Asia. One death was recorded among the positive cases. Entomological surveys were conducted in and around homes of patients. These investigations allowed the identification of the vector *Aedes aegypti* in almost all the visited site, the identification of the RNA of dengue virus by RT-PCR real time and the isolation of the virus in a cellular system in three batches of mosquitoes. These results indicate the first time dengue virus type 3 appears in Senegal following the outbreak of dengue 3 recorded in Ivory Coast.

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REDUCTION IN THE USE OF UNNECESSARY INJECTIONS IN MALARIA TREATMENT AMONG TERTIARY HEALTH INSTITUTIONS IN NIGERIA

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Following an intervention to reduce unnecessary injections at health care institutions in Nigeria, United States Agency for International Development's Making Medical Injections Safer (MMIS) project conducted

a follow-up study in five selected tertiary health institutions to collect information before and after the interventions. The goal was to reduce the unnecessary use of injectable medications. The main objective was to measure whether training and policy changes have had any effect on the way these medications are used. After the MMIS health care workers were trained for the study, they assessed prescription patterns, collecting baseline information from August 2004 to January 2005 and follow-up data from August 2007 to January 2008. A secondary analysis of the data from this study shed light on the treatment of malaria in tertiary facilities. Between the two periods of study, standard first-line treatment in Nigeria shifted from injectable to non-injectable medication. When baseline and follow-up study results were compared, a significant reduction was noted in the use of unnecessary injections to treat malaria cases, which reflects the change in treatment policy. However, it should be noted that, in the outpatient department, 7% of malaria cases sampled at the follow-up were prescribed injectable medication for treatment. This is one of the highest injection rates of all the sampled diagnoses that do not usually require an injection. Given that the new malaria treatment protocol in Nigeria calls for non-injectable treatment_unless there are clear signs of *falciparum* resistance or treatment failure with artemisinin-based combination therapy (ACTs)_malaria cases are probably still receiving more injectable medications than necessary. This study indicates that, in Nigeria, there is still a long way to go in the fight against malaria. A surprisingly large number of malaria cases in that country are still being treated at tertiary health care facilities instead of the primary health care level.

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THREE-LEVEL APPROACH FOR ENSURING THE QUALITY OF MEDICINES IN RESOURCE-LIMITED COUNTRIES

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The availability and use of medicines for the treatment of diseases are basic components of any health care system. Poor quality medicines may result in impaired therapies and jeopardize patients' safety, posing a serious threat to consumers and wasting significant financial resources. Assessing a product's compliance with the appropriate quality standards requires performing quality control (QC) analysis by the Official Medicine Control Laboratory (OMCL). However, OMCLs in resource-limited countries may be understaffed and not have appropriate financial support, lacking the necessary infrastructure, equipment, and personnel to perform QC analysis according to product specifications. Geographical barriers to accessing OMCLs, which tend to be located in large cities, impose an additional constraint. Because of the above limitations, a three-level approach for QC is proposed that could help resource-limited countries improve quality control within their regulatory framework. This approach encompasses the following: Level 1 analyses that include visual inspection of the package and label and physical inspection of the product; Level 2 analyses that utilize easy-to-use, simple, rapid, and cost-effective basic analytical methodology that can be implemented in the field to assess medicines quality; and Level 3 analyses that require the assessment of all critical quality attributes of a medicine via complete validated or compendial methodologies performed at the OMCL. The level to employ for a product at a given stage in the supply chain is based on risk-benefit analysis. By strategically implementing this approach throughout the supply chain, from procurement to patients' use, health authorities may increase the frequency and number of medicines tested within their limited financial and human resources, resulting in more effective control of the national pharmaceutical market and the quality of medicines.

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MARKET ACCESS HURDLES FOR INNOVATIVE DISEASE CONTROL TOOLS

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Recent innovations in vector control tools include combination long lasting insecticidal nets (LNs), and insecticide incorporated Durable Lining which is installed on the walls of houses as a long lasting alternative to Indoor Residual Spraying (IRS). There is no specific category for either of these tools, which creates confusion amongst decision and policy makers regarding their utility. There are currently no appropriate testing guidelines for independent evaluation of these novel tools. As such, clear recommendations on efficacy - which are required by most vector control programs and donors as an external 'stamp of approval' - cannot be given, thereby delaying the deployment of these tools. Another example of a novel health product is an instant microbiological water purifier designed to reduce or eliminate the need for repeat interventions for effective use. Similar market access hurdles have been encountered with this tool due to the absence of a separate category, and hence most donors cannot even request this tool because policy is linked with products that require repeat intervention (e.g., chlorine). Despite continuous calls from the international community to expand the existing disease control toolbox, there remains a need to develop internationally accepted and clearly defined categories in order to maintain the motivation for innovation. Analysis of market access hurdles for innovative disease control tools needs to be addressed on both an international and local level. This is exemplified by the development of the LN category within the WHO Pesticide Evaluation Scheme (WHOPES) that established a platform for increased competition thereby enabling the significant and rapid scale-up in net coverage necessary to achieve the Millennium Development Goals.

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DOGS AND THE SOCIAL EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS: KNOWLEDGE, PERCEPTION AND PUBLIC HEALTH POLICY

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Zoonotic visceral leishmaniasis (VL), caused by *Leishmania chagasi infantum,* was responsible for over 51,000 new cases of human VL between 1980 and 2003 in Brazil. Infected dogs are a major risk factor for human infection. Culling of seropositive dogs has been utilized in endemic areas in northeastern Brazil as a result. Despite these efforts, the seroprevalence of *Leishmania* infection in the region remains stable, suggesting this method of control is not effective. We developed a survey which was administered in interview format to adults in 270 randomly selected households in peri-urban areas of Natal, Brazil; an area endemic for VL with a canine seroprevalence rate greater than 25%. Sampling was based on Global Positioning System (GPS) random sampling selection. The purpose of this cross-sectional survey was to enhance the current understanding of environmental, social, and cultural factors which predispose to VL. This study quantitatively described demographics related to the risk of canine and human infection in this location and identified behavioral, cultural, and disease perceptions associated with a higher risk for *L. chagasi* exposure. We also assessed the perception of dogs as a risk for disease and the role of knowledge and perception in the social epidemiology of visceral leishmaniasis in Brazil. Lastly, the study evaluated the role of canines in household life and its effect on how the current culling program is perceived. This study identified multiple gaps in the knowledge of the study population regarding VL, and the current system of addressing canine disease in high risk populations. This information is being used to develop educational materials to address these areas of

need to prevent further spread of disease. Understanding the beliefs, priorities, and capabilities of the population at highest risk for VL will help develop disease prevention policies which will be more effective in this area of endemicity.

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IMPACT OF ESTUARINE WETLAND DEGRADATION ON ZOONOTIC PATHOGEN TRANSMISSION

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The loss of estuarine wetlands is a global phenomenon; 67% of wetland habitats along estuaries and coastal seas have been lost worldide. Rising sea levels resulting from global climate change are expected to lead to increased inundation and subsequent loss of estuarine wetlands in locations where the marshland cannot retreat inland due to urbanization or agricultural practices. Increased intensity of rainfall events predicted to occur as a result of climate change in some African countries could also contribute to greater nonpoint source fecal pathogen pollution into waterways and estuaries. The goal of our research was to evaluate the effect of coastal wetland degradation on contamination of estuarine and coastal waters with terrestrially derived zoonotic pathogens. *Toxoplasma gondii*, whose oocyst stages are shed in feline feces and infective to a diverse range of warm-blooded animals, including marine mammals and humans, was our model system. Experiments were conducted with surrogate microspheres and a specially designed flume that was deployed in vegetated and mudflat (non-vegetated) estuarine wetland habitats. The flume-in-field study design allowed for replication of experiments with specific hydrological parameters, while conducting the study within a natural estuarine environment using in-situ vegetation, substrate, and water. Compared to vegetated sites, significantly more surrogates were recovered from unvegetated mudflat habitats that represent degraded wetlands. Specifically, in Elkhorn Slough where a large proportion of otters are infected with *T. gondii*, erosion of 36% of vegetated wetlands to mudflats was calculated to increase the flux of oocysts by greater than two orders of magnitude. Total degradation of wetlands may result in increased *Toxoplasma* oocyst transport of six orders of magnitude or more. Our results provide novel insights into the consequences of changes in wetland habitats on the ecology of zoonotic infectious disease organisms in coastal marine ecosystems.

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ADDRESSING HEALTH WORKFORCE CRISIS IN RURAL HEALTH FACILITIES THROUGH INTEGRATED INFECTIOUS DISEASE CAPACITY BUILDING OF MID- AND LOWER-LEVEL HEALTH PRACTITIONERS IN UGANDA

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The Integrated Infectious Disease Capacity Building Evaluation Program (IDCAP) is providing On-Site Support (OSS) to 36 rural health facilities spread out in all regions of the country. IDCAP conducted a training needs assessment (TNA) in the rural facilities of Uganda and noted that the already constrained workforce mainly comprising of mid level health Practitioners (Clinical officers and Registered Nurses) is challenged with inadequate and insufficient skills. They feel incompetent in offering care and management of infectious diseases among others. The package for OSS consists of Multi Disciplinary Training (MDT) of all the health workers involved in the patient care pathway (Medical officers to nursing assistants), Continuous Quality Improvement training and one on one coaching and mentoring of key staff who are involved in making daily clinical decisions on site. In the first IDCAP OSS field work conducted in April 2010 in 18 study sites, on average 8 clinical staff were mapped out for one on coaching and mentoring. Of these, 2 were Medical Clinical Officers, 2 Nursing Officers and 4 enrolled Nurses per site. On average, 30 health staff participated in the MDT training per site. This number appears to be big because most of the staff involved in patients' care are of lower cadre which often times can be attracted and retained in these rural health facilities i.e Enrolled Nurses, Nursing assistants, Health Educators and Inspectors, Records officers and counselors. The scope of work for non-professional personnel is tailored operationally to enable them perform less technical tasks such as counseling, adherence monitoring, home visiting, patient registration and maintaining flow client. All these aspects are addressed in the MDT sessions. In conclusion, participation of health facility staff in on-site activities i.e. mentoring, coaching, multi-disciplinary and Continuous Quality Improvement training could be one of the strategies towards improving individual and site performance given the staff shortages in rural health settings in delivering health services.

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THE EFFECT OF IDCAP IMPLEMENTATION ON IMPROVEMENT OF HEALTH MANAGEMENT INFORMATION SYSTEMS IN RURAL UGANDA

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Integrated Infectious Diseases Capacity Building Evaluation (IDCAP) is providing classroom and on-site training and continuous quality improvement of mid-level practitioners in 36 rural health centers in Uganda. IDCAP seeks to measure incremental impact and cost-effectiveness of integrated infectious diseases management. In order to measure the impact of site-level performance, IDCAP has had to strengthen the Ministry of Health (MOH) Management Information System (HMIS). This paper discusses the HMIS improvements that have been achieved due to IDCAP efforts. A Data surveillance system was set up to collect data on management of infectious diseases in Outpatients, Maternity, TB, HIV, Antenatal and Postnatal clinics. For Outpatients, the MOH Medical Form (MF5) was modified to capture in a coded format patients' demographics, history, examinations, diagnosis, treatment, referral and dispensed drugs. Health centers received a computer, printer, power supply and/or backup systems and modems for electronic reporting. Records personnel were trained in electronic data entry, analysis, reporting, quality assurance, facilitation and receive technical support. The rate of data completeness has improved to over 95%. Over 90% of clinicians using the new MF5 have found it timesaving and easy-to-use. Data losses and reporting timeliness have improved. Over 300,000 records have been collected during the four months of baseline. Using a computer, data entry, analysis and reporting is timely reducing from over seven days to less than two days. In conclusion, efforts to collect quality data to measure IDCAP site performance and impact has had a profound effect on improvement and strengthening of the HMIS in the rural health centers implementing IDCAP. The new MF5 and data surveillance system have improved quality of collected data, timely electronic entry, data analysis and reporting. It is also strengthening treatment and drug supply systems.

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CARETAKERS KNOWLEDGE, ATTITUDES AND PRACTICES ABOUT ANTIBIOTIC USE IN CHILDREN IN A SETTING WHERE ANTIBIOTICS ARE AVAILABLE WITHOUT MEDICAL PRESCRIPTION

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The misuse of antibiotics is associated with the emergence of resistant pathogens. It has been assumed that the availability of antibiotics without prescription has lead to higher self-medication rates. We surveyed caretakers in settings where antibiotics are available without prescription in order to determine their knowledge, attitudes and practices about antibiotic use. In a house-to-house survey performed in three peri-urban districts of Lima, 1201 caregivers were asked in a semi-structured questionnaire regarding antibiotic use in children less than five years of age. An educational leaflet about antibiotics and resistance concepts was explained after the interview. Only 3% of the caregivers identified correctly all antibiotics. Amoxicillin and cotrimoxazole were the best known drugs. Anti-inflammatory drugs like ibuprofen and naproxen were missed indentified as antibiotics in 45%. Only 11% of the caregivers knew the concept of antibiotic resistance. 83% of the children had used antibiotics before one year of age, 40% before 6 months of age. In 86% of the cases antibiotics were prescribed by a physician. Given the hypothetical case of common cold caretakers would seek medical advice in 76% of cases and 14% would use the drug of last prescription; 51 believed necessary to use antibiotics in this case. In the hypothetical case of non-dysenteric acute diarrhea, 87% would ask for medical advice and 4% would use the drug of last prescription; 65% of caregivers believed that an antibiotic was necessary in this case. In general, 84% of caregivers respected medical decision even if an antibiotic was not prescribed. In conclusion, knowledge about antibiotics and resistance is poor in this setting. However caregivers usually ask for medical advice when their children get ill and respect the medical recommendations. Despite this, infants are often and early exposed to antibiotics. Interventions to improve use of antibiotics should emphasize on physicians recommendations.

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CULTURAL EFFECTS IN EPIDEMIOLOGY: FACTORS AFFECTING POLYPARASITISM RESEARCH IN COASTAL KENYA

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Epidemiological studies are impacted by low participation and high attrition rates. One frequently cited explanation is the lack of participant comprehension of the study's aims and objectives. In an evaluation of informed consent practices for an epidemiology study in Coastal Kenya, our findings provide support to this explanation; participants scored well on two standard measures of comprehension. However, semi-structured interviews revealed that these scores may not be a direct reflection of participant comprehension. Participant and fieldworker interviews revealed key issues impacting participation and comprehension. Specifically, these issues address participant perceptions of research processes and how these perceptions affect participant behavior. Factoring these issues in epidemiological studies may lead to greater participation and less attrition among research participants.

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A SYSTEMATIC REVIEW OF THE SAFETY OF LICENSED LIVE ATTENUATED VACCINES

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The concept of vaccination with live organisms has been around for centuries, and was initially inspired by the practice of variolation in China, so named because infectious matter was taken from dried scabs or pustules from people with a milder form of the disease (Latin varius=speckled) and used deliberately to infect healthy recipients. Live attenuated vaccines today are derived mainly from cultures of living microorganisms that have been modified such that their virulent properties are diminished or eliminated, or which use closely related but less dangerous organisms to produce a broad immune response. Modern advances have facilitated the development of subunit vaccines, which rely on technology including the utilization of recombinant proteins. Nevertheless, immunization via live attenuated vaccines continues to be a highly effective means of generating protective immunity. Because our group is conducting a Phase 1 trial of a live metabolically active, non-replicating, radiation atttenuated *Plasmodium falciparum* sporozoite vaccine we reviewed the world's literature on the safety of live vaccines. There are currently US licensed vaccines against 25 pathogens with live attenuated vaccines available for 16 of these pathogens. The risks associated with receipt of a live vaccine include 1) reversion of virulence 2) risk of disease due to immunocompromised state 3) risk of transmission to others via shedding of the vaccine strain and 4) risk of autoimmune response to the vaccine. For each live vaccine, we review the method of attenuation, the associated safety issues, and the populations at highest risk for complications after exposure to the vaccine.

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EVALUATING THE EFFECT OF CULTURAL COMPETENCE TRAINING ON PHYSICIAN-PATIENT INTERACTION AND OUTCOMES

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In the context of global health and migration, the need for cultural competence in health care is urgent. There have been some such initiatives in the sexual health field. However, the claims regarding outcomes have not been substantiated by formal research and evaluation. The current study hypothesized that the education and training of doctors in culturally appropriate practice has a positive effect on assessment, diagnoses, and treatment of their patients with sexual health concerns. Using data from a recent 5 year project (RISHTA) conducted in 3 low income communities in India, this study examined the effect of cultural competence training on the effectiveness of 44 trained (intervention) and control doctors. Doctors were trained in a holistic approach to treatment of men's sexual health concerns called Narrative Prevention Counseling (NPC). NPC incorporated biological, psychological, relational, and social-cultural factors. Coding methodology was developed for analysis of qualitative data. Coding was done independently by two researchers to control for inter-rater variability. The physicians who had received formal NPC scored significantly higher on total cultural competence as well as separately in assessment, diagnosis, and treatment regardless of case type. The independent samples t-test for Total Cultural Competence was significant ($t(42)=6.143$, $p<.001$, 95% CI:12.06-23.71). Separate t-tests for subcomponent scores revealed significantly increased scores for the individual subcomponents Construction 7.51 ($p<.001$, 95% CI:5.55-9.48), Deconstruction 4.91 ($p<.001$, 95% CI:2.71-7.11), and Reconstruction 5.56 ($p<.001$, 95% CI:3.03-8.08). Exploratory analysis suggested that practitioners tended to have higher cultural competence when treating non-STI cases in

comparison to STI cases. This study shows that a culturally competent curriculum has positive effects on medical practice. Further studies that explore the effects of such training on individual fields of healthcare as well as individual types of medicine are recommended.

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BUILDING COMMUNITY PARTNERSHIPS FOR RESEARCH IN LIMPOPO PROVINCE, SOUTH AFRICA

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Rural populations are under-represented in biomedical research, particularly in sub-Saharan Africa. The Mal-ED project is a multi-country effort to evaluate the interactions of malnutrition and enteric disease in eight communities. Two of the sites for the project are in Africa, and one site, in rural South Africa, had not previously participated in international biomedical research. We describe the development of relationships with the rural communities which have agreed to participate in this important research. The University of Venda is a comprehensive university located in Thohoyandou, South Africa. Its mission includes a commitment to engagement with the surrounding rural communities. The University of Virginia's Center for Global Health has partnered with the University of Venda since 2003 to develop joint educational experiences for students and to pursue collaborative research. Since 2008, the partners have developed a close relationship with two communities through connections bolstered by projects led by teams of students from the two universities. These projects have been completed with significant input from community members and, in many cases, in response to their concerns. The projects have included the construction and revision of a slow sand filter to aid in purification of surface water for home use; a series of PhotoVoice projects to elicit the communities' perceived strengths and challenges relative to the provision of water and to health; geographic information system (GIS) mapping of the communities' water supplies and sanitation facilities; and a census. Participants in the PhotoVoice project were queried about their impressions. Many noted how "empowered" they felt by participation in the research process. One noted, "I felt so proud when I talked to the University people... when I saw that they were interested in our photos." In addition to the ties fostered by the student projects, community meetings of the research team with the traditional and civic leadership were essential and defined the communities' expectations which included: efforts to hire staff from the community, the formation of a community advisory board, and the development of a health education program. Informed community consent is not institutionalized in the way that individual consent is. Documenting strategies to promote and achieve community consent, particularly in rural areas, must be a priority.

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CHALLENGES IN THE DEVELOPMENT, REVIEW AND APPROVAL OF MULTI-CENTER STUDIES OF MALARIA CONTROL AND ELIMINATION STRATEGIES

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Studies of tropical diseases at single sites face challenges similar to the studies of other diseases. However, the challenges of study development, review and approval are substantially greater for multi-center studies of malaria control and elimination strategies. The first challenge will be to develop consensus protocols because the results of these studies will be interpretable only if the protocols used are the same at each site (so differences in the results reflect differences among sites, not differences in study protocols). The second challenge is that the review and approval of protocols by multiple Institutional Review Boards (each of which is an independent entity with its own FWA Number) must be sufficiently independent to guarantee the integrity of the process, and sufficiently expeditious to ensure that the time for the initial review of individual protocols is ≤ 2-3 weeks, not months to years. Preliminary discussions with IRBs suggest the possibility of a two-stage strategy. The first stage would be simultaneous submission of the initial protocol to endemic area IRBs which would be responsible for recruitment strategies, supportive care and confidentiality because of their closeness to these culturally-related issues. The second stage (after the addition of those revisions to the protocol) would be the simultaneous submission of the revised protocol to all IRBs to examine the entire range of issues being studied. This approach, if successful has the potential to yield consensus protocols across substantial numbers of IRBs and to do so within a reasonable time frame.

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INTERNET ACCESS AND BAND WIDTH AS FACTORS THAT LIMIT THE IMPROVEMENT OF MALARIA CONTROL AND PUBLIC HEALTH IN WEST AFRICA

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In contrast to East and South Africa, which have benefited from an array of Internet cables in the Indian Ocean, Internet access and band width are less available and more expensive in West Africa. For example, a digital subscriber line (DSL) with a band width of 250 kb is typically $300-500 per month with a $500-1000 installation charge and a 2-3 month wait for installation. As a result, the Internet resources readily available elsewhere (on-line clinical trial and study software, molecular data bases, sample size and other statistical programs) are either less available or unavailable - e.g., on-line clinical study software cannot be used because it requires consistent Internet access and band widths ≥ 250 kb. The current situation in West Africa has resulted from a number of factors, which include: government monopolies, limited competition among other Internet service providers and years of delay in the provision of long-promised high-speed (fiber-optic) cable by international aid agencies. Limited Internet access

and insufficient band width are two of the most important and least recognized obstacles that will need to be addressed in order to improve malaria control and public health in West Africa.

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EVIDENCE FOR NEGATIVE SELECTION ACTING ON THE GENE ENCODING THE MEROZOITE SURFACE PROTEIN-9 (MSP-9) IN *PLASMODIUM* SPP

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Merozoite Surface Protein 9 (MSP-9) is a vaccine candidate found in all malarial parasites species. In order to investigate the extent and maintenance of the genetic diversity found in MSP-9, we analyzed a total of 52 sequences of the following species: *Plasmodium falciparum, P. vivax, P. cynomolgi, P. knowlesi, P. coatney, P. fieldi* and *P. simiovale* and evaluated the signature of natural selection by estimating the difference between synonymous (dS) and non synonymous (dN) substitutions. The Z- test was used to determine the significance of such differences and, the null hypothesis was that the polymorphism was strictly neutral (dN=dS) at the 5% level. We found that in all orthologs of MSP-9 there was an excess of dS over dN, which suggest that this gene is under negative or purifying selection. We further explore how selection affected different regions of MSP-9 for *P. falciparum, P. vivax, P. knowlesi* and *P. cynomolgi*. We found that the C-terminal repetitive region of *P. cynomolgi* and *P. vivax* and the N-terminal region of *P. knowlesi* and *P. cynomolgi* were under negative selection. In contrast, evidence for positive selection was found in the N-terminal region of *P. falciparum* which may suggest that such polymorphism was important for the parasite in terms of avoiding recognition by the host immune response. In conclusion, we found evidence of negative selection acting on the MSP-9 protein in several *Plasmodium* species, this result may be indicative of the importance of this protein in the invasion of the red blood cell. However, the N-terminal region in *P. falciparum* shows a different pattern consistent with positive selection. Overall the effect of natural selection on the N-terminal region of the gene is different in *P. vivax* and *P. falciparum*. This observation implies that the genetic polymorphism observed in these human parasites may have a different interaction with the host immune response. Thus, the findings in one species cannot be directly translated into others.

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PREGNANCY-ASSOCIATED MALARIA IN RELATION TO FREE FETAL HEMOGLOBIN IN MATERNAL BLOOD AND SUSCEPTIBILITY TO DEVELOP PREECLAMPSIA

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Pregnancy-associated malaria (PAM) and preeclampsia (PE) are major causes of maternal and perinatal mortality in developing countries. PAM results in sequestration of parasites in the placenta, causing placental inflammation and impaired placental function. Placental malaria (PM) has been associated with an increased risk of pregnancy-induced hypertension (PIH), but the mechanisms linking PAM and PE are not known. PE is believed to progress in two stages. In stage 1 placental hypoxia causes oxidative stress and local inflammation. Stage 2 is a systemic syndrome characterized by endothelial dysfunction, multiorgan damage, and clinical symptoms. PE is associated with placental over-expression of hemoglobin (Hb) and increased concentrations of free fetal Hb (HbF), adult Hb (HbA), and oxidation markers in maternal plasma from early gestation. Free HbF may have a role in the etiology of PE by induction of oxidative stress in the placenta and leakage over the fetomaternal barrier to induce a maternal systemic oxidative stress. The objective of this study was to investigate the association of PAM with free HbF in maternal plasma and development of PE in a cohort of pregnant women in an area endemic of malaria. In a longitudinal prospective study in Korogwe, northeast Tanzania, 1000 pregnant women are followed throughout their pregnancy with clinical and parasitological examination and collection of blood samples at 4 antenatal visits, emergency visits, and at delivery. Plasma from women with positive rapid diagnostic test and women developing PIH, PE, or eclampsia will be analyzed for free HbF and compared to a healthy control group. Follow-up is expected to be complete in September 2009. Preliminary results will be presented. The interrelation between PAM, levels of free HbF in maternal plasma, and development of hypertensive disorders in pregnancy will be investigated. Measurements at multiple time-points will be used to investigate the longitudinal changes in plasma HbF throughout the pregnancy in relation to subsequent development of PIH, PE and eclampsia.

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COPPER FLUORESCEIN COMPOUND CONFIRMS THE PRESENCE OF NITRIC OXIDE IN *PLASMODIUM FALCIPARUM* TROPHOZOITES

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The presence of nitric oxide (NO)-derived reactive nitrogen species (RNS) in the food vacuole (FV) of *Plasmodium falciparum* parasites suggests that this free radical might play a role in the biochemical processes that take place in this organelle. Previously, we showed the presence of NO-derived RNS in the FV of 3D7 *P. falciparum* trophozoites using the fluorescence NO indicator DAR-4M AM. This fluorophore provided topographical information on the presence of NO-derived radicals, which was typically in close proximity with hemozoin deposits in trophozoites as well as in other blood stages of the malaria parasite. Although the function of NO in this organelle is not known, recent work suggests that NO can modulate heme speciation in isolated FVs exposed to NO generated *in situ.* There is, however, some uncertainty about the nature of the nitrated species present in the FV *in vivo*. Using anti-nitrotyrosine antibodies we observed nitrosation activity specifically localized in the FV envelope of trophozoite and gametocyte stage parasites, suggesting that nitrated radicals with strong oxidative potential, such as peroxynitrite (ONOO-), are present in this environment. The fluorophore we used previously, DAR-4M AM, does not react with NO directly but with N2O3, an oxidation product of NO in aqueous media. Consequenlty, we wanted to verify the actual presence of the NO radical in FVs. In this study we used a Cu(II)-complexed fluorescein compound, a new generation NO indicator that specifically reacts with NO over other RNS (N2O3, NO2, NO2-) present in biological systems. The Cu(II)-fluorescein compound has Ex/Em: 503-530 nm respectively, it is not toxic and safe to use in live cell imaging. *P. falciparum* 3D7 trophozoites treated with the Cu(II)-fluorescein compound displayed fluorescent signals in the food vacuole region of trophozoite stage parasites, lacking any noticeable fluorescence from the erythrocyte cytoplasm. These results confirm that NO radical is present in trophozoite food vacuoles and strongly suggests its involvement in FV biochemistry.

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THE NEUROLOGICAL IMPACT OF *IN UTERO* EXPOSURE TO EXPERIMENTAL PLACENTAL MALARIA

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Placental malaria (PM) is associated with poor fetal outcomes including pre-term delivery, intrauterine growth restriction (IUGR) and low birth weight (LBW). LBW is correlated with abnormal cognitive and neurological development. Severe malaria infection in children has been associated with long-term cognitive impairments, including deficits in language, pragmatics and non-verbal functioning. Taken together, these studies suggest that PM may also impact neurological and cognitive development in the fetus. We hypothesize that offspring of mothers with PM will show behavioural impairment in tests of learning and memory, as well as abnormal neuroanatomical development, compared with control offspring from uninfected mothers. We will also examine a possible role of complement in PM-induced neurological impairments. Excessive activation of complement has previously been associated with adverse clinical outcomes in severe malaria and placental malaria. We hypothesize that mice lacking C5a-C5a receptor signaling will be protected from the neurological impairments associated with *in utero* exposure to PM. Using a mouse model of PM that replicates the pregnancy outcomes and placental pathology of human malaria, we examined the impact of PM on the neurological development of offspring. BALB/c wild type and C5aR-/- dams were infected at gestational day 13 with the rodent malaria parasite, *Plasmodium berghei* ANKA. Control animals were offspring brought to term by uninfected BALB/c and C5aR-/- dams. All offspring were reared by uninfected, healthy surrogate dams. Control and PM offspring were tested in a battery of behavioural tests including the open field test as a control to assess normal behaviour and the novel object recognition test to assess learning and memory. Following cognitive testing, neurological changes were examined using neural imaging data collected from magnetic resonance imaging. Volumetric analysis of imaging data was used to compare neurological development between offspring of PM infected mothers as well as control animals.

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IDENTIFICATION OF PROTEINS ON THE SURFACE OF RETICULOCYTES INFECTED WITH THE RODENT MALARIA PARASITE *PLASMODIUM YOELII*

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Malaria parasites replicate inside host red blood cells and export parasite proteins out of the parasitophorous vacuole to the erythrocyte plasma membrane. Previous studies have shown that multigene families of *Plasmodium* species encode antigenically variable proteins on the membrane of infected RBCs. However, non-variant parasite-encoded erythrocyte membrane proteins have also been suggested to be involved in import/export pathways, adherence to vascular endothelium and localization to reticulocyte-rich tissues. Such surface exposed parasite proteins represent potential vaccine targets as antibodies generated against them could block essential functions, enhance phagocytosis and/or promote complement mediated lysis of infected RBCs. The long-term goal of this project is to identify and characterize the subset of non-variant parasite-encoded proteins expressed on the surface of infected erythrocytes. Our initial approach was to identify parasite-encoded proteins associated with the infected RBC membrane that possessed an extra-cellular domain. *P. yoelii* 17X infected RBC membranes were isolated by surface biotinylation, neutravidin pull down and analyzed by immunoblotting. In this analysis, we identified a subset of putative surface exposed parasite-encoded membrane proteins including unusually high molecular weight proteins (>300 kDa). We also showed that membrane proteins around 30-35 kDa bind to recombinant mouse CD36, an endothelial cell receptor known to be critical in adherence of infected RBCs to vascular endothelium. In order to identify CD36-binding parasite proteins, we performed two dimensional electrophoresis of infected membrane proteins and mass spectrometry (LC-MS/MS) analysis which revealed four candidate *P. yoelii* proteins, such as PY06644(Enolase), PY00631(putative adhesin), PY01841(14-3-3 protein) and PY07648. These candidate antigens are currently being studied for their role in binding to mouse CD36 and in tissue specific sequestration of parasitized RBCs.

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EXAMINING THE FINE SCALE STRUCTURE OF THE CROSSOVER LANDSCAPE IN *PLASMODIUM FALCIPARUM* BY HIGH-THROUGHPUT ALLELE SCANNING

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Malaria is one of the most persistent and devastating parasitic diseases in humans. Understanding the mechanisms of genetic variation is fundamental to understanding the origin and spread of drug resistance and the influence of selection pressures on parasite virulence. Recombination generates new variants and regulates linkage disequilibrium (LD). Crossovers (CO) break up linkage, while non-crossover gene conversions (GC) weaken LD between nearby loci. Existing marker systems, e.g. microsatellites, lack the resolution necessary to capture the subtle, localized allele changes that occur as a result of COs and GCs which may have important phenotypic relevance. The detection of COs and precise measurement of their breakpoints and distribution is challenging - ideally relying on the observation of all four products derived from a single meiosis. This is not possible in malaria parasites; therefore an alternative is to directly scan the genomes of independently recombinant progeny at fine-scale resolution. We describe a method for SNP allele detection using massively parallel shotgun sequencing of two *Plasmodium falciparum* progeny clones resulting from a cross between a multi drug sensitive (HB3) and multi drug resistant line (Dd2), using the 454/Roche FLX sequencing platform. Of 24,599 high quality SNP markers identified, approximately 8000 allelic positions were used to differentiate between the progeny clones. A sliding window approach was used for the prediction of recombination break points (BPs). We reclassify eight previously misidentified COs as GCs, as well as discover previously unknown COs and GCs. Of 63 putative GCs, 36% span genes from polymorphic gene families associated with pathogenesis, cell adherence and rosetting. This global, high resolution genome view begins to clarify the types and locations of genetic exchange in the context of local sequence. This is a necessary step to reveal mechanisms, hotspots and gene functions that comprise the repertoire of genetic variation contributing to drug resistance, gene diversification and genome organization.

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RHOPH COMPLEX FROM MOUSE MALARIA PARASITE INTERACTS WITH ERYTHROCYTE CALMYRIN

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It is essential for blood parasitism of plasmodial merozoites to recognize hosts' erythrocytes and to establish parasitophorous vacuoles. The proteins from three apical microorganelles, i.e. micronemes, dense granules, and rhoptries, should be involved in this critical process. Here we focused on the high molecular weight rhoptry protein (RhopH) complex and searched the recombinant mouse erythrocyte proteome for interaction partners of *Plasmodium yoelii* (Py) RhopH. The PyRhopH extracts was prepared by freeze-thawing schizont-rich pellet and labeling with monoclonal antibody (mAb) #32 specific to RhopH3 component of the complex. We then

referred to the study of human erythrocyte proteome (Blood 2006: v108: p791) to select mouse orthologs. A total of 441 biotinylated recombinant mouse proteins were prepared by wheat germ cell-free synthesis to conduct the PyRhopH interaction partner(s) analysis. Among positives the strongest signal was detected with calcium binding protein calmyrin (also called CIB-1). The specificity of this interaction was confirmed by dose-dependence test, mAb species selection, and divalent cation-chelator (EDTA) inhibition. Finally, the detection of calmyrin in mouse erythrocytes suggested that the *in vitro* results above reflect *in vivo* interaction between PyRhopH complex and mouse calmyrin.

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FREQUENT SEVERE THROMBOCYTOPENIA IN CASES OF *PLASMODIUM VIVAX* MALARIA FROM THE PERUVIAN AMAZON

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Thrombocytopenia has been described as a complication related to *Plasmodium falciparum* malaria and less frequently to vivax malaria, but recent reports in South America have described a frequent association between thrombocytopenia and vivax malaria. To determine the prevalence of low platelet counts in the Peruvian Amazon we studied 224 vivax malaria cases enrolled in a study on the efficacy of antimalarial drugs and 179 afebrile, thick smear-negative subjects from the same area. A platelet count per µl of blood was performed in each participant using a Giemsa-stained thin blood smear obtained by finger stick. Follow-up platelet counts were made in a small, convenience sample of malaria cases who were receiving the prescribed antimalarial treatment, chloroquine plus primaquine. Counts of <100,000 platelets/µl were defined as low. There was no statistical difference in the average age of malaria positive and negative groups (26.0±16.3 vs. 25.2±16.7, p=0.637), but malaria cases were more frequently male (122/224=54% vs 77/179=43%, p=0.022). The geometric mean of platelet counts was 74,919/µl (23,000 - 198,000) and 138,071/µl (66,000 - 293,000) in the malaria and non-malaria groups, respectively. Low platelet counts were clearly more frequent in malaria cases than in the group without malaria (176/224=79% versus 17/179=9.5%, p<0.001), and 12.2% (28) of malaria cases had a severely low platelet counts (<50,000/µl) versus zero in malaria-negative subjects (p<0.001). Among malaria cases, higher parasitic density (parasites per µl of blood) correlated significantly with lower platelet counts (Spearman Rho=-0.342, p<0.001). After commencement of malaria treatment, platelet recovery was rapid and occurred in all 10 studied cases by 48 or 72 hours. In conclusion, thrombocytopenia is very frequent in cases of *P. vivax* malaria in Peru, but it resolves within a few days after antimalarial treatment.

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FY^A AND FY^B DUFFY ANTIGEN POLYMORPHISM AFFECTS BINDING OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN (PVDBP) AND BLOCKING BY SPECIFIC ANTIBODIES AND CHEMOKINES

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The Duffy antigen has two known polymorphisms, Fya and Fyb, which differ by only one amino acid in the N-terminal region. This region, which is known for its critical role in *Plasmodium vivax* invasion of human red blood cells (RBC) by use of the malarial parasite protein *P.vivax* Duffy binding protein (PvDBP), is also important for chemokine binding, notably for interleukin-8 (IL-8) and RANTES. IL-8 has been shown to inhibit invasion of *P.knowlesi*, which also uses the Duffy antigen to invade human RBC. Previous results suggest that PvDBP shows poorer binding to RBC expressing Fya compared to Fyb. We tested the hypothesis that blocking antibodies directed to PvDBP and the chemokine IL-8 and RANTES preferentially block binding of PvDBP to erythrocytes expressing either the Fya or Fyb polymorphism. Binding of Fya and Fyb genotyped human erythrocytes to PvDBP was measured by flow cytometry. All variants of PvDBP (AH, O, P, C and SAL1) consistently showed an average of 40-50% lower binding to Fya erythrocytes. Blocking the Duffy antigen with polyclonal rabbit antibodies or pooled human antibodies with binding inhibitory activity showed 2-4 fold greater inhibition of PvDBP binding to Fya than Fyb erythrocytes. While both Fya and Fyb erythrocytes had similar PvDBP binding and inhibition of RANTES, Fya binding to PvDBP was significantly inhibited by IL-8 (at concentrations similar to levels identified in patients with acute malaria) to a greater extent than Fyb erythrocytes. In conclusion, these results show that in addition to the inherent decreased binding abilities of the Fya Duffy antigen polymorphism, binding inhibitory antibodies as well as chemokines may be more effective in blocking parasite invasion of RBC expressing Fya versus Fyb. These studies suggest that testing of a vaccine targeting PvDBP will be more effective in populations predominantly expressing the Fya allele.

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DOES ENHANCED DETECTION AND ANALYSIS OF MALARIA INFECTIONS IN UMBILICAL CORD BLOOD SAMPLES EXPLAIN LOW BIRTH WEIGHT AND FETAL ANEMIA IN NEWBORNS OF THE KASSENA-NANKANA DISTRICT OF NORTHEASTERN GHANA?

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Even with bednet use during pregnancy by >50% of women, and Fansidar-based intermittent preventive treatment (IPTp) that reached 82% of pregnant women, fetal anemia (FA, Hb <12.5g/dL) characterized 21.5% of live births in the rural Kassena-Nankana District of northeastern Ghana and 18% of these newborns were underweight (LBW, <2500g). Malaria microscopy identified *Plasmodium falciparum* infections in only 47 of 2258 (2.1%) umbilical cord bloods (2.1%) but these few positives revealed borderline statistical associations with first born status (P = 0.12), LBW (P = 0.09), and FA (P = 0.12). We hypothesized that a more sensitive screening of umbilical cord bloods could validate these associations and explain the high levels of LBW and FA observed. We further conjectured that higher rates of fetal malaria infection, and mutations in malaria genes associated with Fansidar resistance might reveal additional associations that could be acted upon to improve health and survival. This retrospective study aimed to determine the rate of infectivity and molecular characterization of *P. falciparum* in cord/heel blood taken from >2200 live births enrolled in an IRB-approved cohort study during March 2006-March 2007. Detection of *P. falciparum* in filter paper blood blots was based on nested PCR targeting the 18S ribosomal RNA. Genetic diversity within the *P. falciparum* PCR-positive samples was determined by analysis of *msp1*, *msp2* and *glurp* (glutamine-rich protein). Based on single nucleotide polymorphisms (snp), we determined the frequency of *dhps* and *dhfr* point mutations associated with Fansidar resistance in the parasitemias. Results are discussed in light of birth characteristics, seasonality, malaria protection, and demography.