neglected tropical disease, affecting the health of over 12 million people throughout 88 countries worldwide. We tested four commercial adult mosquito traps marketed for homeowner use in residential settings against a CDC light trap, routinely used for sand fly surveillance for efficacy in collecting sand flies. Our traps included the Mosquito Magnet (MM) Pro, the Sentinel 360 mosquito trap, the BG-sentinel mosquito trap, and the Mega-Catch trap. The BG-sentinel and CDC light traps were baited with 2 kg dry ice nightly, Sentinel 360 and Mega-Catch traps were not, while the MM Pro produced its own CO_2. Traps were not baited with optional lures (lactic acid, octenol baits) that are recommended for some models. Traps were rotated through five sites in a 5x5 Latin square experiment in a small farming village in the Nile River Valley 10 km north of Aswan, Egypt. Four repetitions were conducted during the height of the sand fly season (June, August (2x) and September, 2007) at a site in which *P. papatasi* is abundant and *Leishmania*-free. 6,440 sand flies were collected over four trials, 6,037 of which were *P. papatasi* (93.7%). The BG trap collected significantly more ($P < 0.05$) *P. papatasi* than the Mega-Catch and Sentinel 360 traps and more than the MM Pro and CDC light trap. Order of success and trap means (±SE) were: BG trap, 142.1 (45.8) > MM Pro, 56.8 (40.1) > CDC trap, 52.3 (27.5) > Mega-Catch trap, 38.2 (28.5) > Sentinel 360 mosquito trap 12.6 (8.2). Results indicate new, commercial traps are a suitable substitute for CDC light traps in sand fly surveillance programs. Unlit, CO_2-baited commercial traps performed much better than lit commercial traps lacking CO_2 production.

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SEARCHING FOR MOLECULAR DETERMINANTS OF SPECIES SPECIFICITY IN SAND FLIES COLONIZED BY *LEISHMANIA* PARASITES

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Leishmania parasite development in the vector sand fly takes place solely within the confines of the midgut. *Phlebotomus papatasi* is a restrictive vector of *Leishmania*, meaning that only *Leishmania major* fully develops to an infective form. In contrast, *Lutzomyia longipalpis* is a permissive vector of *Leishmania*; numerous species of *Leishmania* can proliferate and be transmitted by this sand fly. We have recently shown that transcript abundance of several midgut molecules were altered when the sand flies *P. papatasi* and *Lu. longipalpis* were colonized by *L. major* and *L. infantum chagasi*, respectively. To further understand the temporal abundance of transcripts and the effects of *Leishmania* colonization of sand fly midgut we utilized quantitative RT-PCR. We found the up- and down-regulation of several midgut transcripts that occurred primarily during blood meal digestion and at late time points associated with the presence of metacyclic promastigotes. To better understand the molecular determinants of sand fly-*Leishmania* species specificity we performed temporal expression profiling of midgut transcripts on *P. papatasi* colonized with *L. major* or *L. infantum chagasi* and *Lu. longipalpis* colonized with *L. major* or *L. infantum chagasi*. Quantitative RT-PCR was used to profile transcripts encoding putative trypsin, chymotrypsin, carboxypeptidase, peritrophin-like and microvillar proteins. The abundance of specific midgut transcripts are influenced by different *Leishmania* species, alluding to highly complex species specific molecular interactions that may define permissive and restrictive vector sand flies.

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PERSISTENCE OF *PLASMODIUM* DNA IN DESICCATED *ANOPHELES* MOSQUITOES AS DETERMINED BY REAL-TIME PCR

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Reliable storage and transport conditions for mosquitoes collected during surveillance or field studies are necessary for subsequent laboratory processing and successful pathogen detection. However, maintenance of a cold chain is either impractical or impossible in many malarious regions. The current study examines the persistence of Plasmodium DNA in laboratory-infected *Anopheles stephensi* mosquitoes stored over desiccant for 0, 1, 3, and 6 months while being held at four temperatures (*i.e.,* 28, 37, -20 and -80°C). Parasite DNA persistence was determined using real-time PCR and novel primers designed to amplify a 116 bp region of block 4 of the merozoite surface antigen. Preliminary results indicate that there is no significant difference in detection of parasite DNA between the different holding temperatures for up to 3 months. Therefore, the maintenance of a rigid cold chain does not appear to be necessary for the detection of Plasmodium DNA for short time periods (e.g., less than 3 months). The implications of these findings and the results of continuing experiments will be discussed.

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IDENTIFICATION OF BLOODMEALS IN SANDFLIES BY ELISA, IN PERU

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Phlebotomine sandflies are involved in transmission of leishmaniasis, bartonellosis, phleboviruses, flaviviruses, orbiviruses, and vesiculoviruses. From 2005 to 2007, there 21,362 cases of leishmaniasisand 8,117 cases of bartonellosis registered in Peru. *Bartonella bacilliformis* is the causative agent of bartonellosis transmitted by the sand flie *Lutzomyia verrucarum and Lu. peruensis* inhabiting the Andean areas of Peru. The determination of the host source of blood meals in hematophagous insects is important for elucidation of the vector-host relationship and to understand the epidemiology of the diseases transmission. The objective of this study was to determine the host source of sand flies by ELISA in an area of the Peruvian Andes endemic for bartonellosis. The study was conducted in the Jangas district (9°24′ LS and 7° 35′ LW), at 3,024 meters above sea level with the collections being performed between November 2007 to June 2008. Sand flies were collected by CDC light trap in ten different houses. The collected sand flies were dried and stored for later identification. Abdomens containing blood meals were assayed for host blood meal identification by the antibody-sandwich ELISA designed to detect human, bovine, cat, chicken, dog, guinea pig, horse, rat and swine IgG. The microplates were read at 405 nm using an ELISA plate reader. Samples were considered positive if absorbance values exceeded two times the mean of six negative controls and one positive control that fed on animal. The preliminary results from 2,835 female sand flies were the following: 73% were *Lu. peruensis,* 26.4% were *Lu. verrucarum* and 0.3% were *Lu. noguchi.* Of these, 23% (659) contained a blood meal. Of the 420 blood fed *Lu. peruensis,* 80% fed on human, 4% onon dog, 4% swine, 2 on % cat, 2% on horse, 2% on chicken, 0.8% on bovine, 2% on guinea pig, and 3.2% had multiple source blood meals. Of the 240 *Lu. verrucarum* containing a blood meal, 83% had fed on humans, 4% dog, 6% swine, 2% cat, 2% horse, 0.6% chicken, and 1% guinea pig, and 0.4% had multiple source blood meals. None of the 660 samples tested positive for

rodent blood. These results suggest that *Lu. peruensis* and *Lu. verrucarum* have a strong preference for feeding on humans.

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MULTIPLE-DOSE POPULATION PHARMACOKINETICS OF PYRONARIDINE IN HEALTHY VOLUNTEERS

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A novel pyronaridine/artesunate (PA) combination (Pyramax®) is currently under development for the treatment of malaria. The purpose of this study is to determine multiple-dose population pharmacokinetics of oral pyronaridine in healthy adult volunteers. Twenty-four healthy Korean subjects participated in a Phase I trial of multiple oral dosing of pyronaridine tetraphosphate (6-15 mg/kg) or PA (in the ratio 6:2, 9:3, 12:4 and 15:5 mg/kg). A total of 503 pyronaridine blood concentration measurements were analyzed. Nonlinear mixed effect modeling (NONMEM) with first-order conditional estimation was used to develop a pharmacostatistical model for the population pharmacokinetics. Covariates (age, sex, weight, height and BMI) were entered by stepwise forward addition and backward elimination, and their significance was determined by the difference in objective function between hierarchical models. Final model selection was based on physiological plausibility of parameter estimates, minimum objective function, diagnostic plots and residual distributions. The model was validated by comparing final parameter estimates obtained from 1000 replicated data generated from bootstrapping. A two-compartment model with first order absorption and elimination best described the data. Inter-subject variability (ISV) of clearance (CL/F), distribution CL (Q), and volume of compartments 1 and 2 (V1, V2) were described using an exponential error model. A log error model best described residual variability. The ISV of V1 and CL could not be estimated. Typical model parameter estimates (%RSE) were CL/F 966 L/d (5.4%), KA 17.5 1/d (13.5%), V1 1290 L (5.5%), V2 6700 L (11.4%) and Q 2820 L/d (9.4%). None of the tested covariates were found to correlate with the pharmacokinetic model parameters. The final model provided estimates within the 95% confidence intervals obtained by 1000 bootstrap runs. In conclusion, 2-compartment model was well-fitted to pyronaridine data. None of the tested covariates were identified as important covariates for the pharmacokinetics of pyronaridine in healthy adult volunteers.

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MONITORING OF INTERNATIONAL OUTBREAKS WITH AN OUTBREAK SURVEILLANCE DATABASE

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The National Travel Health Network and Centre (NaTHNaC) in England was created in 2002 with the goal of *'Protecting the Health of British Travellers'*. NaTHNaC maintains an Outbreak Surveillance Database (OSD) of international disease events that have the potential to affect UK travellers; the database is linked to NaTHNaC's open-access website (www.nathnac.org). The OSD provides NaTHNaC staff, stakeholders, travel and tropical medicine specialists, and the public with a comprehensive report of international outbreaks. Multiple reports of the same outbreak are linked allowing individual outbreaks to be analysed. Each day, resources including country authorities, the World Health Organization, ProMED-mail and the media are reviewed. Outbreaks meeting set criteria are entered into the OSD. All outbreaks posted from April 2004 through March 2008 were analysed to determine trends in disease occurrence and reporting. Data from the OSD were extracted, analysed and mapped using Excel®, STATA® and ArcGIS®. From April 2004 to March 2008, 5,484 events were entered into the OSD and organised into 2,973 outbreaks. Outbreaks were recorded in 189 countries; 41% were in Asia and Oceania and 22% in Africa. The five most frequently reported diseases were H5N1 in birds (14%), cholera (11%), dengue (11%), H5N1 in humans (9%), and human rabies (3%). The OSD facilitates the monitoring and mapping of diseases: e.g. 337 outbreaks of cholera, 68% from Africa and 14% from Asia; unusual disease occurrence: Ebola and Marburg from Angola, Uganda and the DR Congo; and outbreak progression: yellow fever in South America in 2007 and 2008. NaTHNaC can identify disease outbreaks via its OSD, and alert health professionals and travellers by posting a summary of the outbreak and the appropriate risk management. The unique OSD can be used by individuals and health bodies throughout the world. Analysis of the data in the OSD allows identification of current outbreaks, investigation of changing patterns of disease, and identification of emerging global threats.

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THE FACTORS AFFECTING MALARIA PREVENTION AND TREATMENT DECISIONS FOR CHILDREN IN THE DEMOCRATIC REPUBLIC OF CONGO

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Though endowed with natural wealth, the Democratic Republic of Congo (DRC) is one of the poorest countries of the world with a Human Development Index of 0.43; ranking 152nd out of 175 countries. The economic, political and war crises in the Democratic Republic of Congo (DRC) have impacted negatively on public health risks for the population. In recognition of the deep crisis, the government of DRC is promoting expansionary strategies to combat malaria as an important component in the global strategy to fight poverty and improve the standard of living of the people. Malaria is one of the primary causes of mortality and morbidity in the country, especially among pregnant women and young children, accounting for 30% of child mortality. However, insofar the government has come up with various control strategies including appropriate case management in both community and health facilities, scaling up the use of insecticide treated nets among others, it is also necessary to think beyond supply. Specifically, we need to consider how individuals take decisions during episodes of malaria and what social, economic and environmental factors affect this behaviour. Therefore, the main objective of this paper is to provide quantitative and qualitative evidence on the importance of individual, household and environmental characteristics on care seeking decisions during episodes of malaria in children as well as factors affecting malaria prevention methods in the DRC. The analyses will be based on the DRC Multiple Indicator Cluster Survey, (MICS) of 2001 supported by UNICEF. It is expected that the results will throw more light on the demand side associated to the prevention and treatment of malaria in this war-torn country.

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USE OF INSECTICIDE SYNERGISTS IN INVESTIGATING PYRETHROID RESISTANCE IN *SARCOPTES SCABIEI*

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Synergists are commonly used in combination with pesticides to suppress metabolism-based resistance, and to increase the efficacy of the agents. They are also useful as tools for laboratory investigation of specific resistance mechanisms based on their ability to inhibit specific metabolic pathways. To determine the role of metabolic degradation as a mechanism for acaricide resistance in human scabies, PBO (Piperonyl butoxide), DEF (S,S,S tributylphosphorotrithioate) and DEM (Diethyl Maleate) were used

with permethrin as synergists in a bioassay of mite killing. A statistically significant difference in survival time of permethrin-resistant *Sarcoptes scabiei* variety *canis* mites ($p < 0.0001$) was noted when any of the three synergists were used in combination with permethrin compared to survival time of mites exposed to permethrin alone. These results indicate the potential utility of synergists in reversing tolerance to pyrethroid-based acaricides (i.e. the addition of synergists to permethrin-containing topical acaricide cream commonly used to treat scabies). To further verify specific metabolic pathways being inhibited by these synergists, enzyme assays have been developed to detect esterase, glutathione-S-transferase (GST) and cytochrome P450 activity in scabies mites. Results of *in-vitro* enzyme inhibition experiments showed lower levels of esterase activity with DEF, lower levels of GST activity with DEM while cytochrome P450 activity remained constant in the presence of PBO. These findings further validate a metabolic mechanism as mediating pyrethroid resistance in scabies mites.

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PHARMACOKINETICS, CLINICAL AND SAFETY OUTCOMES OF PYRONARIDINE/ARTESUNATE TREATMENT OF ACUTE *PLASMODIUM FALCIPARUM* MALARIA IN UGANDA

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Pyronaridine/artesunate (Pyramax®) is a novel treatment of *Plasmodium falciparum* malaria. A randomised, multicentre, Phase II, dose-ranging clinical study was conducted to assess the safety and efficacy of fixed dose, orally administered pyronaridine and artesunate in adult patients with acute uncomplicated *P. falciparum* malaria. Pyronaridine pharmacokinetics was studied in a sub-population of 16 Uganda patients. Treatment with pyronaridine/artesunate: 6+2 mg/kg (n=5), 9+3 mg/kg (n=5), 12+4 mg/kg (n=6), was once daily for 3 days being closely matched for demographic characteristics. Pyronaridine/artesunate treatment resulted in cure at Day 28 for all patients treated with each of the dose groups and this effect continued out to Day 42. All patients were clear of parasites by Day 2. The concentration pyronaridine in whole blood was measured using a previously validated LC-MS method. Noncompartmental pharmacokinetic analysis yielded mean (±SD) values for C_{max} of 91.9 ± 30.8, 156.8 ± 57.1 and 226.1 ± 157.5 ng/mL following 6, 9, 12 mg/kg body weight oral doses, respectively. The corresponding values for $AUC_{(0\text{-}infinity)}$, T½, and Tmax were 749 ± 603, 1036 ± 286, 1134 ± 624 ng/mL*d, 19.1 ± 5.9, 15.9 ± 5.0, 14.6 ± 6.6 d, and 5.3 ± 2.0, 6.2 ± 6.3, 7.6 ± 4.9 h, respectively. The pyronaridine blood level profile shows a very pronounced distribution and elimination phase. A prominent second peak was noted in the pyronaridine blood level profiles for some patients. The elimination half-life is longer than previously reported, resulting from a more sensitive pyronaridine assay methodology and prolonged blood sampling. The pyronaridine Cmax (following the third dose) was lower in malaria patients compared with healthy volunteers, suggesting that malaria patients have a larger pyronaridine volume of distribution. Pyronaridine/artesunate treatment was well tolerated in this study.

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A DOUBLE BLIND, RANDOMIZED, CONTROLLED, DOSE ESCALATION PHASE IB FIELD TRIAL IN 12 TO 24 MONTH OLD CHILDREN IN BURKINA FASO TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF THE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-3 LONG SYNTHETIC PEPTIDE (MSP 3-LSP) ADJUVANTED IN ALUMINIUM HYDROXIDE VERSUS ENGERIX B

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Development of an effective malaria vaccine could greatly contribute to disease control. The merozoite surface protein-3 long synthetic peptide (MSP3-LSP) is a blood stage malaria vaccine candidate which has been shown to be safe in non immune and semi immune adults, and therefore proceeded to further development in children. This study aimed at selecting the most appropriate dose in young African children through assessment of the vaccine safety and immunogenicity. We conducted a double-blind, randomized, controlled, dose escalation phase Ib trial in 12 to 24 month old children. Two groups of children were given different doses of the MSP3-LSP antigen (15 µg or 30 µg). In each group, children were randomly allocated either to the MSP3-LSP candidate malaria vaccine or the control vaccine administered at aschedule of 0, 1, and 2 months. Immunization of group 1 and 2 was staggered for safety reasons starting with the lower dose. The primary endpoint was safety and reactogenicity within 30 days post vaccination. Blood samples were obtained at different time points for immunological response measurements. Study duration was 13 months for all the participants and the primary analysis was intent-to-treat basis. A total of 45 children were enrolled, 15 in each of the MSP3-LSP groups and 15 in the control vaccine group. Induration, pain and swelling at injection site were more observed in the MSP3 group (any dose) than the control; there was no difference between the two MSP3 groups. No serious adverse event related to vaccination was reported. Both doses regimen were able to stimulate strong cytophilic IgG (IgG1 and IgG3) responses to the candidate vaccine with a dose response effect. In conclusion, the MSP3-LSP vaccine was safe, well tolerated, and immunogenic in young children. The 30 µg dose was more immunogenic than the 15µg dose with comparable safety profile. This dose is recommended for further development in children.

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IN VITRO HEMOLYTIC EFFECTS OF 8-AMINOQUINOLINES IN NORMAL AND GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENT ERYTHROCYTES

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Acute hemolysis is a well known adverse effect of primaquine in individuals with glucose 6-phosphate dehydrogenase (G6PD) deficiency. As this side effect has limited the utility of 8-aminoquoline (8AQ) class of drugs, it is very important to understand the biochemical pathways leading to the hemotoxicity. Redox cycling of hydroxylated metabolites of 8-AQs may cause oxidation of hemoglobin, accumulation of reactive

oxygen intermediates (ROIs) and depletion of thiols, which may trigger the pathways leading to cell death. Insufficient understanding of the mechanism of toxicities of these drugs has hindered further developments in this area. Numerous earlier studies have tried to estimate the hemotoxic endpoints with parent compounds, as the metabolites appear to be very unstable and reactive. But as the metabolite(s) are believed to be responsible for the toxicity it is essential to understand the metabolism-mediated toxicity. Microsomal metabolism-linked hemotoxicity assay(s) have been developed, which would allow *in situ* formation of potential toxic metabolites. Generation of methemoglobin, formation of ROIs, depletion of total reactive thiols and reduced glutathione (GSH) were monitored as biochemical markers predicting hemotoxicity in normal and G6PD deficient erythrocytes. Primaquine and a few other 8-aminoquinolines under development -namely tafenoquine and enantiomers of NPC1161, generated similar increases in the level of methemoglobin and reactive intermediates in normal and G6PD deficient erythrocytes, when evaluated by a human hepatic microsomal metabolism-linked *in vitro* assay. Basal level of GSH was markedly lower in G6PD deficient as compared to normal erythrocytes. 8-AQs caused more pronounced depletion of GSH in G6PD deficient than in normal erythrocytes. Further assessment of these markers and the assay with a battery of hemolytic and non-hemolytic drugs/toxicants shall help to evaluate prospective application of this assay for prediction of hemolytic potential of new candidates and development of non-hemolytic 8-aminoquinolines.

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PILOT TRIAL OF THE HECT-CL DEVICE AS THERMOTHERAPY FOR CUTANEOUS LEISHMANIASIS IN PERU

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Current standard chemotherapies (CT) for Cutaneous Leishmaniasis (CL) are expensive, toxic/allergenic, frequently ineffective, burdensome (20 days at a 3º care center), and often unavailable. Thermotherapy (TT), through a variety of modalities over the last 20 years, has demonstrated high CL treatment efficacy, with fewer complications and less treatment burden than CT. The only FDA-approved TT device utilizes "localized current field-radio frequency" (LCF-RC), is beyond the financial access of the mostly rural and impoverished affected populations, and has no theoretical benefit over a low-cost alternative TT modality. The study protocol aimed to provide safety and efficacy data for a novel, safe, low-cost, and efficacious TT modality, the HECT-CL (Hand-held ExoCrystal Therapy for CL) device, in Peru. This pilot study enrolled 25 laboratory confirmed CL patients who were not candidates for primary CT therapy (secondary to intolerance, failure, or contra-indication), aged 1-65, ≤3 lesions ≤4cm in diameter, and without prior treatment for 1 month. Participants received 1 treatment per week for 3 weeks with the HECT-CL device (60-90 seconds depending on age and skin area). Patients were followed up on days 30 and 90 for evaluation of burn grade, healing progress, and super-infection. Pregnant patients and those aged <1 or >65 years were treated on a compassionate use basis only and not included in the study. The HECT-CL device proved to be a safe, inexpensive, and efficacious treatment for CL in Peru without signs of systemic or local toxicity. Details are provided. In conclusion, the HECT-CL device has potential for early intervention in the primary care settings in CL endemic areas, and deserves to be studied in large head-to-head trials against standard chemotherapy.

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POPULATION PHARMACOKINETICS OF ARTESUNATE AND AMODIAQUINE IN AFRICAN CHILDREN

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Artesunate-amodiaquine is one of four artemisinin combination treatments (ACT) currently recommended by the World Health Organisation. Until recently it has been available only as a loose combination of the individual drugs, frequently as a co-blister. Where possible ACTs should be formulated as fixed dose combinations. A coformulated artesunate-amodiaquine product has been developed by the Drugs for Neglected Diseases Initiative, and this has now been registered. A prospective population pharmacokinetic study, in children 6 months - 5 years of age, was conducted as part of the development of this fixed dose formulation to assess bioavailability and characterize its pharmacokinetic properties. Participants came from a randomised, open-label clinical study to compare the new fixed-dose combination of artesunate and amodiaquine (AS/AQ) to the same drugs given separately (AS+AQ). Children were divided into two groups of 70, the first group participated in the study of artesunate pharmacokinetics and the second group participated in amodiaquine pharmacokinetics. Three or four blood samples were taken per child, respectively. The population pharmacokinetic models for desethyl-amodiaquine, dihydroartemisinin (DHA) and total anitmalarial activity, defined as the sum of the molar equivalent plasma levels of DHA and artesunate, were constructed using the non-linear mixed effects approach. This population pharmacokinetic evaluation indicates that the two regimens have similar pharmacokinetic properties in young children with acute malaria. The estimates of pharmacokinetic parameters are in broad agreement with those of previous studies. Artesunate is converted rapidly to DHA. The pharmacokinetic parameter estimates of DHA and the total antimalarial activity, indicated rapid absorption and elimination - with plasma levels which were significantly higher following the first dose - when the patient was acutely ill, than after subsequent doses when usually afebrile and clinically improved. Amodiaquine is converted rapidly to desethylamodiaquine in a first pass effect. The elimination kinetics estimated from sparse data may have missed a slower elimination phase and therefore underestimated the terminal elimination phase. But importantly the bioavailability from the fixed dose combination was similar to that of the separate tablet for desethylamodiaquine, DHA and the total antimalarial activity.

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SEROPOSITIVE WOMEN AND THEIR NEWBORNS DETECTED BY ELISA WITH ANTIGENS OF A LOCAL STRAIN OF *TRYPANOSOMA CRUZI* AND FOLLOW-UP TO IDENTIFY CASES OF CONGENITAL TRANSMISSION IN TWO MEXICAN STATES

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Mothers with Chagas' disease can transmit *Trypanosoma cruzi* to their fetuses, who are then at risk of developing severe cardiac disease later in the course of their lives. There are very limited data about congenital Chagas' disease in Mexico. In a previous study in two Mexican hospitals

(Merida, Yucatan and Celaya, Guanajuato), we reported seroprevalence rates of 0.7%-0.8% in venous (IV) samples and of 0.6-0.9% in umbilical cord (UC) samples using commercial tests (Stat-Pak rapid test, Chembio, New York, USA and Chagastest ELISA Recombinant v3.0 Wiener, Rosario, Argentina). We have expanded this study by performing an additional ELISA test using whole antigens of a local strains of *T. cruzi*. We also performed follow-up 10 months after delivery with mothers from Merida who were positive by at least two of three diagnostic tests (non-commercial ELISA, Chagatest ELISA or Stat-Pak), as well as with seropositive newborns to determine the presence of antibodies against *T. cruzi* as evidence of congenital infection. We also determined whether antibodies against *T. cruzi* were present among the infants' siblings. The frequency of positive ELISA tests using local antigens was 1% (10/988), which was not different from the commercial assays. Three of four women who were found to be positive at delivery by at least two tests were confirmed seropositive for *T. cruzi* infection at follow-up, while none of the newborns were found to be seropositive at 10-15 months of age. Similarly, none of the siblings were seropositive. One newborn from an infected mother died at two weeks of age from heart failure without confirmation of infection with *T. cruzi*. We conclude that *T. cruzi* seroprevalence is close to one percent in our sample, and that large-scale studies are warranted to identify cases of congenital transmission in Mexico. Commercial or non-commercial ELISA tests produced similar results and could be used for future studies.

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GMM AND THE LOSS OF ACQUIRED IMMUNITY: LESSONS LEARNED FROM HISTORY

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Despite initial success in reducing malaria, the effort at controlling malaria has faltered and malaria is in fact now been increasing, especially in the Sub-Saharan Africa. Approximately 200 million people from that region suffer from malaria each year. Thus, the international health community is exploring the radical method of genetically modifying local mosquito populations so that they can no longer transmit malaria. However, the loss of acquired immunity that would result from the eradication of malaria poses a severe threat if malaria were to return in areas where GMM would have eliminated malaria. Studies have shown that in areas of high transmission rate, young children experience the greatest mortality, while malaria is a relatively mild condition in adults. This results from the acquisition of specific immunity. There are two prominent cases in history that highlight the catastrophe that could ensue if malaria returns to areas where it was previously eradicated: the epidemic of Madagascar and Sri Lanka. In 1949, Madagascar implemented a malaria eradication program based on DDT spraying and by 1960 had successfully eradicated malaria, consequently discontinuing DDT spraying. However, malaria returned and in 1988-1991 caused an epidemic which killed many thousands, poignantly demonstrating how the lack of immunity of a population allows for exponential growth of malaria. Similarly Sri Lanka launched a DDT campaign that radically reduced malaria in 1963. However, in 1994, the development of DDT resistance enabled the occurrence of a malaria epidemic in 1994. These cases emphasize the need for serious consideration of the loss of immunity in devising a GMM strategy.

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EVALUATION OF DOD-GEIS OVERSEAS SURVEILLANCE PROJECTS: 1998-2007

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From 1998-2007, the Department of Defense Global Emerging Infections Surveillance and Response System (GEIS) invested over US$40 million in infectious disease surveillance through the DoD's five overseas medical research laboratories (OSLs), located in Peru, Egypt, Kenya, Thailand and Indonesia. The primary objectives of the program are surveillance, capacity-building, research, response and cooperation, adjusting for specific Host-Nation needs and DoD priorities. A study was undertaken to evaluate the GEIS overseas surveillance efforts, 1998-2007. The unit of measurement used was the single-year project, funded with stated objectives. Using internal annual progress reports, data were extracted on the types of projects funded at each OSL during each year. These were categorized based on their stated fidelity to GEIS strategic objectives and disease focus areas. The results of the analysis indicate a high degree of consistency between the types of projects funded and GEIS programmatic objectives. Sixty-three percent of projects engaged in infrastructure enhancement or training in a Host-Nation, while the number of Host-Nations increased by over 300% (13 to 55) from 1998-2007. Seventy-four percent conducted infectious disease surveillance and over 93% of surveillance projects focused on identified GEIS priority syndromes. However, this analysis offers limited information on the true value of these projects individually or in concert. Such limitations result partly from the broad GEIS mission. Yet, GEIS was created in response to a 1992 Institute of Medicine report calling for increased, broadly-based capacity to detect emerging infectious diseases globally. GEIS, and similar international disease surveillance programs, would benefit from focused, measurable objectives that can be adapted as global and regional priorities change. However, efforts to establish measurable surveillance objectives should not sacrifice capacity-building that can both enable detection of heretofore unknown threats and promote true collaboration with Host-Nation scientists. This study reinforces the difficulty inherent in evaluating surveillance programs for emerging diseases on criteria beyond technical execution. It suggests the need for more nuanced tools for evaluating such systems that can account for broad, long-term goals such as capacity-building in tandem with clearly defined epidemiological objectives.

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THE ADDED BURDEN OF MALARIA AND ITS HEALTH IMPLICATIONS IN RURAL WOMEN IN OKIGWE ONCHOENDEMIC AREA OF IMO STATE, NIGERIA

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A community-based study was conducted to ascertain the added burden of malaria in 693 confirmed onchocerciasis cases in Amachara, Isiokwe and Ogii communities of Okigwe Local Government Area. The prevalence rate of malaria of 43.1% was observed in the overall data. Age-wise, those below years showed the highest infection rate of malaria (66.7%) in the three communities. The rising tide of onchocerciasis and malaria infections poses enormous socioeconomic burden in women in their reproductive age. This requires urgent control measures to alleviate the burden.

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DEVELOPMENT OF *SCHISTOSOMA JAPONICUM* FAST-ELISA ASSAY FOR SCHISTOSOMIASIS DIAGNOSIS

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Schistosomiasis is caused by digenetic blood trematodes and is one of the most serious parasitic diseases in China. China has conducted an ongoing control program for over 50 years and now reports that transmission has been interrupted in some areas. We have developed a high through-put assay for use in epidemiologic surveys to assess the success of control program. We developed an enzyme linked immunoassay (ELISA) using the Falcon assay screening test (FAST) system for high throughout screening and employed an existing immunoblot assay for confirmatory testing. Both methods measure total immunoglobulin that is reactive with the microsomal fraction prepared from *Schistosoma japonicum* adult worms

(JAMA). For the FAST-ELISA assay, we used a standard curve to measure parasite-specific immunoglobulin, quantified in units/ml. We analyzed a panel of 382 sera composed of 231 sera from parasitologically confirmed cases, 135 sera known to contain *S. japonicum* reactive antibodies (as measured by the JAMA immunoblot), 96 sera from persons with other parasitic infections and 151 from persons with no documented illnesses (normal sera). We used the J-index to determine the optimum assay cutoff. The optimized assay has a sensitivity of 94.1% and specificity of 96.0%. We propose that this assay is suitable for large scale epidemiological studies and to assess sero-incidence in young children as an indicator of recent infection, thereby providing a measure of success of a control program.

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TREATMENT OF ACUTE *PLASMODIUM VIVAX* MALARIA WITH PYRAMAX® (PYRONARIDINE TETRAPHOSPHATE/ ARTESUNATE) IN A CONTROLLED PHASE III CLINICAL TRIAL

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A phase III multi-centre, randomised, double-blind, double-dummy, comparative clinical study was conducted in adult and children patients with acute *Plasmodium vivax* malaria. The study was conducted in Thailand, Cambodia, India and Indonesia and assessed the safety and efficacy of a three day course of Pyramax® tablets (pyronaridine tetraphosphate:artesunate, 180:60 mg) versus chloroquine tablets (155 mg). Over 450 patients aged 3- 60 years and weighing 20-90 kg were recruited with acute uncomplicated *P. vivax* mono-infection confirmed with fever and positive microscopy of *P. vivax* with parasite density ≥250/ µL of blood (including at least 50% of asexual parasites). Primary efficacy endpoint was cure rate on Day 14. All patients were followed up to Day 42. Secondary efficacy endpoints included: cure rate on Day 21 and 28, parasite clearance time, fever clearance time and the proportion of patients aparasitemic on Days 1, 2 and 3. Safety was assessed through regular assessment vital signs, physical examination, 12-lead ECG and clinical safety laboratory evaluations for haematology, biochemistry and urinalysis. Monitoring of all safety was ensured through a Safety Review Board and a centralised reading of ECGs was conducted concurrent with the trial to ensure quality of recording and interpretation. Quality control for microscope slides on the primary efficacy parameter was conducted both locally as well as centrally at an independent laboratory. The treatment was well-tolerated by patients. The pyronaridine/artesunate safety and efficacy results for this controlled Phase III clinical trial against acute *P. vivax* malaria will be presented.

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HUMANS FROM AN ENDEMIC AREA OF CUTANEOUS LEISHMANIASIS IN MALI PRODUCE IFN-γ TO SAND FLY SALIVARY PROTEINS

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Cutaneous leishmaniasis is a vector borne neglected tropical disease present in more than 80 countries and around one million cases occur annually. We studied two villages in Mali, West Africa with reported cutaneous leishmaniasis cases. To evaluate the *Leishmania* infection rate, *Leishmania* skin test (LST) was performed and a prevalence of 31% and incidence of 10% was found in the two years of follow up (IRB approved protocol 06-I-N121). This indicates that *Leishmania* transmission is actively occurring in these two villages. Data from mouse model of cutaneous leishmaniasis suggests that an immune response to salivary proteins protects mice from infected bites of sand flies and IFN-γ production was correlated with the protective response. Our goal is to study the cellular immune response to salivary proteins generated by the bites of the sand fly vector in individuals of this area. The suspected vector, *Phlebotomus duboscqi*, was captured and using transcriptomic and proteomics we identified and cloned the individual salivary proteins. We tested for the presence of anti-saliva antibodies and we observed that LST positive individuals had higher levels of IgG anti-saliva antibodies. Western blot analysis showed that these individuals recognize different proteins from the vector saliva. In order to analyze cellular immune response to sand fly saliva we stimulated PBMC from individuals of the area with salivary gland extracts and measured the IFN-γ concentration in the culture supernatants. We observed specific anti-saliva IFN-γ production in individuals from this population. Our future goal is to identify the individual salivary proteins from *P. duboscqi* responsible by the IFN-γ production. The identification of the salivary proteins that induce a Th1 immune response and an IFN-γ production may help us to assess the relationship between anti-saliva immunity and leishmaniasis.

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ESSENCE DESKTOP EDITION; A SELF-CONTAINED DISEASE SURVEILLANCE APPLICATION

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The ESSENCE Desktop Edition (EDE) was created to offer a self-contained disease surveillance tool that can be deployed efficiently at any location. EDE mimics the web application ESSENCE (Electronic Surveillance System for the Early Notification of Community-based Epidemics) flow and functionality. EDE provides savable query execution, user defined preferences, and mechanisms for data input from several types of databases. ESSENCE is used to monitor the health of populations and to detect disease outbreaks to prevent their spread, as reported previously. ESSENCE collects and analyzes a variety of data. It uses anomaly detection algorithms which yield alerts that flag unusually high counts of disease indicators. It allows users to view alerts, demographic details and geographic maps of reported cases. EDE utilizes the Eclipse Rich Client Platform (RCP). This is a customizable platform built with software units called 'plugins' that supports modular development. The EDE is developed to support a wide variety of user needs 'out of the box'. This includes configuration for different data sources and user performed queries on that data. The EDE provides results in a format similar to the ESSENCE web application. It includes graphs, charts, detailed data on individual illness reports, as well as geographic maps of location of individual illness reports. The initial deployment of EDE is as an add-in module attached to the national disease surveillance system in the Philippines called the Surveillance Tool for Analysis, Management, and Reporting data (STAMR). In this instance, STAMR will be used to monitor the temporal trend of diseases that are officially notifiable in the Philippines. In conclusion, EDE is an easily deployable, upgradeable and extendable stand-alone desktop application that provides similar functionality to the current web deployment of ESSENCE. EDE users can configure the system specifically for the variables included in their database. They then have access to a robust core set of features that can be easily extended and upgraded.

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EVALUATION OF A RAPID IMMUNOCHROMATOGRAPHIC TEST FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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An individual's glucose-6-phosphate dehydrogenase (G6PD) activity level should be known prior to prescribing certain drugs, such as primaquine, for malaria prevention or treatment of malaria, due to the risk of hemolytic anemia when such drugs are used in those with G6PD deficiency. Current screening methods for G6PD deficiency are impractical for use in rural areas where malaria is endemic because of the need for large equipment or highly trained personnel. The NOW G6PD immunochromatographic test (ICT) is a rapid screening device for G6PD deficiency appropriate for use in malaria endemic areas. G6PD deficient and G6PD normal subjects enrolled at Boston Medical Center completed a demographic survey and provided 2 tubes of blood. Blood preserved in both EDTA and heparin from each subject was analyzed using the NOW G6PD test. Analysis of heparinized blood was performed using the Trinity Biotech quantitative determination of G-6-PDH. Results from the two methods were compared using 4.0 U G6PD/g Hb (as measured by the standard Trinity Biotech assay) as the cut off for G6PD deficiency. Between June 2007 and February 2008, blood from 50 G6PD deficient and 196 control subjects was analyzed. The average G6PD activity of the deficient samples was 1.7 ± 1.6 U/g Hb; the average G6PD activity for the control samples was 8.1 ± 1.7 U/g Hb. After lysed blood was applied to the NOW G6PD test device, results were read for the heparinized samples after 5 minutes and after 7 minutes for the EDTA samples. The sensitivity, specificity, positive and negative predictive values of the NOW G6PD test using heparinized whole blood were 0.98, 0.98, 0.72 and 1.00. The sensitivity, specificity, positive and negative predictive values of the test using EDTA whole blood were 0.98, 0.97, 0.63, and1.00. In summary, the NOW G6PD rapid ICT is a sensitive screen for G6PD deficiency that requires minimal training and equipment, and allows for rapid diagnosis of G6PD deficiency.

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TWO CASES OF CUTANEOUS AND VISCERAL LEISHMANIASIS IMPORTED INTO GUATEMALA FROM SOUTH AMERICA AND ITS POSSIBLE PUBLIC HEALTH IMPLICATIONS

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Leishmaniasis is a zoonotic disease endemic to 88 countries with a yearly incidence of 1-1.5 million cases of cutaneous leishmaniasis and 500 000 of visceral leishmaniasis. One case of human cutaneous leishmaniasis and one case of canine visceral leishmaniasis imported from French Guiana and Brazil respectively were detected in Guatemala in 2008. The human patient referred to be infected while completing military exercises in Guiana's forests and the dog was infected in Ceara, Brazil. Clinical evaluations were performed by a medical and veterinary doctor for each case; samples for smears, cultures and PCR were obtained from the cutaneous lesion in both cases. Bone marrow and spleen aspirates were obtained from the dog. Diagnosis was performed following standard methods. The human patient showed a typical cutaneous leishmaniasis lesion on the left arm. The dog showed clinical signs of both cutaneous and visceral leishmaniasis such as extreme weakness, loss of appetite, emaciation and typical dermal lesions of cutaneous leishmaniasis in legs. Diagnosis was confirmed by the observation of parasites in different clinical samples. The human patient and dog were treated with pentavalent antimonials in a scheme of 10mg/kg for 10 days and 28mg/kg for 28 days respectively and both resolved in a clinical cure. To our knowledge these are the first reported cases of imported leishmaniasis to Guatemala from South America. Both cases were from a region where different species of *Leishmania* parasites other than those occurring in Guatemala can be found. Implications of the transmission of new strains can vary from more aggressive clinical presentations, a wider geographical distribution, and resistance to current treatment schedules to outbreaks of the disease. Consequently, examination of dogs imported from endemic countries and possible cases of imported human leishmaniasis is needed. We insist on the development of guidelines for the detection, clinical/laboratory examinations, quarantine and managing of leishmaniasis imported cases by the Ministry of Health and Agriculture.

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VILLAGE BASED MALARIA CONTROL IN UNDERPRIVILEGED COMMUNITIES-RWANDA: SHOWCASE OF RWANDA VILLAGE CONCEPT PROJECT IN MUYOGORO VILLAGE

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The aim of our study was to assess the impact of malaria symptoms knowledge, attitude towards preventive measures as well as treatment seeking behaviors among members of Muyogoro Village community in South province of Rwanda. Malaria cause 40% of consultations in health facilities; thus assessing and analyzing local malaria problems are a prerequisite for successful control interventions. A descriptive and cross section study was done. Sessions on malaria prevention methods and health promotion were carried out 3 years ago and are still on course. Subsidized insecticide treated net was offered to every participant at completion of the sessions with the support of Rwanda National Malaria Control program(PNILP). A total of 300 participants were considered. Among respondents, 92.5% recognize that mosquito bites was the real cause of malaria, 100% recognize fever as the main symptom in malaria. Headache,joint pain,stomach trouble, losing weight and obesity had respectively 96.8%,93.7%,79.4%,39.2%and 14% of respondents. 95.8% consider that cutting bushes as one of the most efficient method to prevent against malaria, 95% prefer the use of insecticide treated mosquito net and 94.6% believe that removing stagnant water as the main prevention method against malaria as well, while avoiding going outside when raining and sharing food with someone who has malaria were respectively responded by 67.6%,19.2%.Asked on what they will do first during malaria attack, 95.7% of respondents answered that they would seek immediate hospital treatment, with 2.6% who will ignore the signs and just rest in bed.This is to notice that responding on the importance of health insurance, 94.6% of respondents believe that it allows them to form cooperatives for the cost of health insurance and 88% recognize that health insurance allows them to get hospital treatment at cheaper price. In conclusion, treatment seeking practice in malaria was related to level of education, culture and religion.We suggest that malaria public enlightenment efforts should be intensified through a much mobilized community based sensitization with the support of local and health authorities in order to achieve behavioral impact regarding malaria prevention and treatment seeking;effective malaria preventive methods be made affordable and that support be provided to make malaria treatments at public hospitals free.

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PRELIMINARY STUDY ON THE INCIDENCE OF SNAKEBITES IN BOLIVIA

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Investigations on the incidence and mortality from snakebites in Bolivia were based on cases treated in health facilities as reported by Health Authorities and six household studies carried out in different regions of Bolivia (Departments of La Paz, Tarija, Cochabamba and Santa

Cruz), representing the main biotopes of Bolivia: Amazonia, piedmont and Altiplano. The investigation in health facilities concerned all the country between 1996 and 2000. An average of 600 bites were treated each years in health centers of Bolivia (national annual incidence = 10 bites per 100,000 people). We observed a great disparity of the incidences according to seasons (maximum during the southern spring which corresponds to the rainy season) and departments (maximum in the Amazonian Departments of Beni and Pando, and Department of Cochabamba). Altiplano areas were quite free of snakebites. The household surveys showed annual incidences from 30 bites per 100,000 inhabitants in the Departments of Tarija and Cochabamba to 110 bites per 100,000 inhabitants in the Department of Santa Cruz, with intermediate incidences in the Department of La Paz (44 in altitude and 75 in the Amazonian region). However, in some place, annual incidence may exceed 200 snakebites per 100,000 inhabitants. Annual mortality was between 0.1 per 100,000 inhabitants in the Chaco region of the Department of Tarija or in the Department of Cochabamba and 3.9 in the mountainous regions of the Department of Tarija or Santa Cruz. These investigations confirmed that the highest incidence is observed in male adults and that the case fatality rate seemed relatively low (about 2 %). They also showed that a majority of victims looked after traditional practitioners and did not consult in modern medical centers; we observed also that the availability of antivenoms was very poor. That probably explains the low incidence reported by the National Health Services.

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CLUSTERING OF HANSEN'S DISEASE (LEPROSY) IN A POPULATION IN NORTHEAST BRAZIL

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The introduction of Multidrug Therapy (MDT) for the treatment of Hansen's disease in 1981 led to a dramatic reduction in the global disease burden: In Brazil the prevalence has been reduced from 19 cases per 10,000 population in 1985 to 2.4 in 2004, but there is still around 50,000 new cases diagnosed yearly. The disease is spread throughout the country, but more clustered in the North and Western Central Regions. Here we analyze the spatial distribution of Hansen's disease (leprosy) in endemic area in Brazil, testing the hypotheses of nonrandom patterns and constant risk of disease. A random sample of 808 out of 1293 Hansen's disease cases diagnosed between 1995 and 2006 was selected and geocoded. Spatial autocorrelation and spatial cluster analysis were used to identify areas of risk of disease. Factor analysis was performed to adjust for socio-economic variables potentially influencing clusters. Hansen's disease cases were not distributed randomly, with disease risk varying markedly between districts. The incidence of disease was higher around population dense regions. A significant relationship between the geographic distribution of disease and the social condition of the population was observed. Cluster analysis identified two areas of high risk, one with relative risk of 5.9 (p=0.001) and the other 6.5 (p=0.001), respectively. Our study demonstrates the power of GIS and spatial analysis to identify and explain the epidemiology of transmissible disease as Hansen's disease This provide a powerful tool in designing strategies for disease control, in particular through allowing early recognition and prompt diagnosis, which in turn should lead to reduction in disease severity caused by delay in treating the disease. Early start of multi drug therapy will reduce the transmission of Hansen's disease to the community.

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ASSESSING THE CARDIAC EFFECTS OF ARTESUNATE (AS) AND MEFLOQUINE (MQ) IN HEALTHY VOLUNTEERS IN A SAFETY AND PK, SINGLE DOSE, RANDOMISED, TWO PHASE CROSS OVER STUDY OF A NEW FIXED DOSE AS/MQ COMBINATION AND LOOSE AS + MQ

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Evaluating QT prolongation as a risk marker for Torsades de Pointe ventricular tachycardia is an essential step for registering new drugs. The ECG effects of a new fixed dose combination of artesunate and mefloquine (AS/MQ) and loose AS+MQ were assessed in a safety and pharmacokinetic, two phase, cross over study in healthy adults. Doses received were: (i) AS 200 mg/MQ 400 mg and (ii) AS 200 mg + MQ 500 mg, given 90 days apart. ECGs were performed at baseline, 1h, 4h, 24h, Day 90 and repeated at cross-over. PK samples were taken on D0, 1, 2, 3 5, 7, 14, 28, 53, 76, 90. A QT correction formula (QTc) QT / $(RR)^{0.4}$ gave the best QT - RR regression line. Analysis was by ANOVA for repeated measures. There were no statistically significant differences between the two arms regarding the PR, QRS and QTc intervals over time. The mean baseline QTc values were 399 (range 367 to 425) ms for both arms. The mean and mean changes (vs. D0) in the QTc for all patients combined was not statistically significant at any of the time points. One female had a QTc flagging (≥ 430ms male, ≥ 450 ms female) value (453 ms). Another female had an increase of 38 (9.5%) ms to 439 ms at one time point. Mean PR and QRS intervals were normal at all time points. The ECG interval changes were small and clinically insignificant. Future ECG PK analyses are unlikely to find a drug effect.

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INTEGRATED MAPPING FOR TRACHOMA AND URINARY SCHISTOSOMIASIS IS MORE COST EFFICIENT THAN SINGLE DISEASE APPROACHES. A STUDY OF 'COST DRIVERS' IN PLATEAU AND NASARAWA STATES, NIGERIA

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Integrated approaches to neglected tropical disease control are thought to be more efficient than single focus approaches. The purpose of this study was to assess the cost of integrated mapping for trachoma and urinary schistosomiasis (SCH) compared to the costs of trachoma only and SCH only mapping. Three different mapping regimens were employed in Plateau and Nasarawa States at the district level: 1) trachoma only mapping using a cluster survey sampling method (13 districts); 2) SCH only mapping using school based survey methods (4 districts); and 3) an integrated mapping strategy for both trachoma and SCH using a combination of cluster and school based methods (8 districts). Costs were systematically collected for employed personnel, transportation, consumables/supplies and per diems and were allocated to training or field work activities. The cost per district of trachoma only mapping was $1,761 and for SCH only the cost per district was $3,630. The cost per district of integrated mapping was $2,196. Per diem costs were the largest

cost component for each mapping strategy, 53.7%, 54.5% and 63.6% respectively. Integrated mapping resulted in cost savings in districts where mapping was required for both trachoma and SCH because of more efficient use of two primary cost drivers of neglected disease control, personnel and transportation. Savings were achieved by fewer visits to the field thus reducing personnel time (opportunity cost) and fuel for transportation to districts and villages. These results give us preliminary evidence on efficiency for a critical activity in integrated program implementation. Assessing efficiency for other NTD activities requires the following: 1) Consistent cost data over time from all levels of the program and from all financing sources; 2) Clear measures of program outputs (activities); 3) Focus and assessment of the major cost drivers - personnel, transportation and supplies; and 4) Development of measures to assess management efficiencies, in addition to economic efficiency, that enhance performance at all levels of the health system.

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HIGH ACCEPTABILITY OF A NEW POU SAFE WATER SYSTEM FOR TANZANIAN RURAL HOUSEHOLDS

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A new point-of-use safe system for producing safe water for households was tested for acceptability by residents of a rural community in Mkuranga District of coastal Tanzania. AquaSure® units are a gravity fed system based on an initial filtration step followed by passage through a halogen bead bed into a collection tank. Twenty families were given water units (WU) and then the usage and the householder's opinions as to the quality of the water produced, and the ease of use of the system, assessed 6-8 weeks after the start of the study. All the households were in a rural village setting and were typical of the residences of this part of rural Africa. The questions were divided into four groups, with examples of responses as follows. A. Taste and smell: All participants found the taste and the lack of smell superior to previous water supplies. B. Usage activities: three of the participants had minor problems with cleaning the unit. C. Value to family: all participants found the units to be valuable to their daily life and put figures of $60-100 as a price they were willing to sell the unit for, however eight participants stated that they would never sell the unit. D. Associated issues: the source of the water in all cases was ground water from shallow wells. Overall there was a very positive acceptance of the Units by all participants in this study with the only negative comments being related to the type of plastic used (not strong enough), to minor difficulties in cleaning the pre-filter, and to the size of the unit with large families. The study showed that the AquaSure® system appears to be very suitable for use in villages in rural Africa.

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MALARIA MORTALITY AND MORBIDITY IN THE FIRST FIVE YEARS OF LIFE IN A BIRTH COHORT OF CHILDREN IN NORTHERN GHANA

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Effective design of vaccine efficacy trials requires background information on the incidence and prevalence of vaccine trial endpoints in the target population. The first five years of life poses the greatest exposure and risk of malaria in areas of intense transmission. This age group provides the best opportunity for the study of malaria epidemiology and immunology to help in vaccine development and assessment. We enrolled a cohort of 2,274 children into a five year prospective cohort study sited in the Kassena-Nankana District of Northern Ghana_an agricultural area with intense seasonal malaria transmission. Passive surveillance will monitor each child for clinical illness, clinical malaria, severe malaria, severe malaria anemia, and mortality. Twice yearly active surveillance will obtain blood samples at the start (Apr-May) and end (Oct-Nov) of the high malaria transmission season for analyses of immune responses and host factors associated with clinical immunity and severe malaria. Among newborns enrolled from March, 2006 to March, 2007 male:female ratio was 0.9:1.0 with mean birth weight 2.9 ± 0.5kg and 2.3% of children parasitemic at birth. Cohort rates of parasitemia at the end of the high and low malaria transmission time points was respectively 12.0% and 4.7%. Uncomplicated malaria accounted for 64.5% of clinical illness in the cohort. Severe malaria constituted 24.4% of inpatient admissions with a case fatality rate of 2.0%. Ninety nine deaths (99/2274 = 4.3%) have so far been reported, only twenty eight of which occurred in the hospital.

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AGE-SPECIFIC INCIDENCE OF CLINICAL MALARIA IN A POTENTIAL MALARIA VACCINE CANDIDATE TESTING SITE OF BURKINA FASO

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To explore the feasibility of field sites for malaria vaccine trials, we conducted a prospective study of clinical malaria incidence during one year period in children aged 0 to five years. A cohort of 550 children living in the rural health district were recruited and followed up through biweekly home visit for 1 year period. During each visit, after a brief history taken, (history of fever, self treatment, attendance to health facility), axillary temperature was recorded. If there was reported fever over the past 24h, or if the measured axillary temperature was ≥ 37.5 °C, a finger prick blood sample was collected and a blood slide were prepared for malaria diagnosis. Children with acute disease were referred to the nearest health facility for appropriate treatment free of charge. A malaria episode was defined as positive *Plasmodium falciparum* parasites density in presence of fever. In total 56716 home visits were performed. The children were seen during 49062 visits. A total of 381 malaria episodes were diagnosed. The overall incidence of clinical malaria was 0.78 (95% ci [0.7-0.86]). Age specific incidence of clinical malaria decreased with increasing age from 0.88 (95% ci [0.68-1.08] in younger children (0.5-1 year) to 0.53% (95% ci [0.38-0.68]) in elder children (4-5 years). In conclusion, our results suggest that total burden of the disease is higher in younger children who represent the most appropriate target for a potential malaria vaccine candidate.

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LEUKOCITURIA AND BACTERIURIA AS INDICATORS OF URINARY TRACT INFECTION

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The confirmation of an urinary tract infection requires the detection of a pathogen via a urine culture (105 UFC/mL) in 48 hours. However, it is desirable to have a rapid diagnostic test (in less than 1 hour) in order to start treatment, especially in acutely ill patients. Among the rapid

test there is the determination of leukocytes and bacteria in the urine. The aim of this study was to determine if leukocituria and bacteriuria could predict a positive urine culture. Urine samples were collected in an aseptic condition from the midstream clean catch. Samples were semi-quantitatively cultured and centrifuged for the microscopic exam. The number of urine leukocytes and bacteria were determine per field (400x) in at least six fields, and categorized as: 1+ (1- 5), 2+ (6 - 10), 3+(11 - 20), 4+ (>21). The statistical analyses were performed using Stata 9.0. We have analyzed 260 urine cultures and sediments. 35.3% (92/260) cultures were positive. The highest rate of positive cultures were for female patients 41.1% (83/202), patients > 61y, 34.8% (33/70), and samples from winter 42.6% (23/54). The most common isolated bacteria were *Escherichia coli* present in 79.3% (73/92) of samples, followed by *Enterococcus faecalis* in 5.4% (5/92) and *Klebsiella pneumoniae* in 4.3% (4/92). The sensitivity of leukocituria was 70%, the specificity 87%, the ROC 0.80, the positive likelihood ratio (LR+) 6.0 and the negative likelihood ration (LR-) 0.34, with a cutoff point of 2+ leukocytes. In the case of bacteriuria the sensitivity was 78%, the specificity 92%, the ROC 0.86, the LR+ 9.0 and the LR- 0.23, with a cutoff point of 2+ bacteria. In conclusion, bacteriuria predicts slightly better the results of the urine culture. This is a rapid, simple and cheap test, which could be complemented with a Gram stain to improve its sensitivity. Of interest, in this study the rate of positive urine cultures was higher in females, in older patients and in samples from winter.

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DEMODEX FOLLICULORUM COUNT ON EYELID SCRAPING FROM PATIENTS ATTENDING CAYETANO HEREDIA NATIONAL HOSPITAL

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Demodex folliculorum is an ectoparasite dwelling into mammal hair follicles and is related to eyelashes, eyelid Meibomio´s and sebaceous glands. Although found in asymptomatic patients, *Demodex* is attributed to play an important role on ophthalmologic pathology as shown for cases of conjunctivitis, blepharitis, chalazion, among others. Despite a high frequency of eyelid infestation by *D. folliculorum* and feasibility of a specific and simple diagnostic test, the infection finding is underestimated. This study was undertaken to determine *D. folliculorum* count by eyelid scraping. This retrospective and descriptive study enrolled 151 patients coming to Laboratory of Parasitology at Institute of Tropical Medicine Alexander von Humboldt (IMT AvH) for an eyelid and skin scraping to be performed from May 2005 to May 2008. Tello´s technique that implies pouring of glycerol solution over palpebral border was performed. Once applied, the solution was gathered and put onto a glass slide by scraping the wetted eyelid area. Eyelashes affected were also removed and embedded with glycerol on the slide. Adhesive tape helped to recover ectoparasites from skin and also to cover the slide. For statistical analysis, Pearson correlation for quantitative variables and Bonferroni test for comparison of *Demodex* counts according to seasonal periods, were carried out (Stata 9). Ninety eight cases were positive for *D. folliculorum*. The mean count was 5.08 (CI 4.14-6.02). Most of patients were female (77.83%) and mean age was 44.91± 20.91 (CI 40.71-49.10). Correlation coefficient between *Demodex* count and patient age was 0.2421 (p=0.0027). In conclusion, females were more frequently affected. *D. folliculorum* count was statistically related to patient age but was not associated to seasonal period during which patient was diagnosed with demodicidosis.

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ASSESSMENT OF RISK FACTORS FOR DRUG RESISTANT TUBERCULOSIS IN LOUISIANA, 1993-2005

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The United States had 13,767 reported tuberculosis (TB) cases in 2006, the lowest rate since national reporting began in 1953. Average annual percentage decline has decreased from 7.3% per year during 1993-2000 to 3.8% during 2000-2006. The TB control program in the US has set an elimination target for TB for <1 case per million by 2015. This study has a general aim to assess the progress towards this target for Louisiana, and a specific aim to assess risk factors for drug resistance. This study was undertaken to: (1) determine the incidence of drug resistance and assess risk factors for drug resistance in tuberculosis patients in Louisiana, (2) compare tuberculosis rates in Louisiana with national trends and (3) study differences in rates over periods 1993-1999 (period I) and 2000-2005 (period II) to monitor progress towards elimination goals. Our results indicated that Louisiana had a total of 4,448 TB cases during 1993-2005 of which 199 (4.5%) cases were drug resistant (DR). The incidence of drug resistant TB declined from 4.7% during Period I to 3.9% during Period II. Risk factors independently associated with resistance were male gender (76%), African-American race (49%), age 25-44 years (44%) and foreign born (23%). National data revealed a total of 239, 552 TB cases reported during 1993 to 2005, Louisiana contributed to 1.86% of this total. Case trends by race differed for Louisiana compared to the national statistics. African-Americans had the highest incidence of all TB cases in the US with about 65% during period I. While Louisiana also showed a high among this minority group with 58% cases, the number of African Americans affected declined significantly nationally for period II while it remained the same in Louisiana. Proportion of all cases occurring in foreign-born persons was 55% nationally, the top two countries of origin being Mexico and the Philippines. In Louisiana, Honduras and the Philippines were among the top for TB and drug resistance cases. Incidence of drug resistance in Louisiana among the US-born decreased by 8% during period II. Notably, there was an increase of drug resistance among female cases (21% to 32%) which needs to be controlled for Louisiana to meet its goal as they contribute to 31% of all TB cases. Analysis of drug resistant cases by parishes showed three parishes to have an increase. The general trend for TB incidence in Louisiana is decreasing and meeting targets moderately, but is still higher than national trends.

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ASYMPTOMATIC PARASITEMIA AND COMPLEX SPECIES ASSOCIATIONS IN MALARIA ENDEMIC SUB-SAHARAN AFRICA

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Asymptomatic parasitemia of varying *Plasmodium* levels has been described in individuals residing in and migrating from malaria endemic areas. There is evidence to suggest clinical progression if untreated. Evolving diagnostic modalities provide new tools for assessing parasitemia in asymptomatic individuals. We evaluated 289 asymptomatic children aged <7 years in Ghana. The study was carried out in 2/07 with the subjects seen in a rural community clinic. Each patient had a clinical assessment, malaria microscopy, Rapid Diagnostic Tests (RDT) for malaria utilizing the BinaxNOW® Malaria Test, and a locally sourced RDT. We utilized Real-Time PCR as the gold standard to determine the different *Plasmodium* species. Species-specific amplification products from a variable region of the 18S rRNA gene were generated using flanking primers, then detected by means of SYBR Green fluorescence and melt curve analysis on a real-time PCR machine. PCR data was available

for 248 subjects with a 33.9% overall prevalence for asymptomatic parasitemia. Mixed parasitemia was found in 32% of subjects while the different *Plasmodium* species had the following prevalence: *P. malariae* 32.7%, *P. falciparum* 32.4%, *P. ovale* 1.2% and no *P. vivax*. The Giemsa microscopy was associated with a sensitivity of 38%, specificity of 95%, positive and negative predictive values of 82% and 74% respectively. The locally sourced RDT had a sensitivity of 54%, specificity of 99% with a positive and negative predictive value of 98% and 80% respectively. The BinaxNOW had a sensitivity of 82%, specificity of 95%, a positive and negative predictive value each 91%. PCR detects the presence of significant asymptomatic parasitemia and the occurrence of mixed infections. RDT's provide intermediate sensitivity in assessing asymptomatic parasitemia. The decision to treat asymptomatic patients based on microscopy has been shown to be beneficial, however it remains to be seen if the same applies to parastiemia detected with RDT's.

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ARTEMETHER-LUMEFANTRINE (COARTEM)IN TREATMENT OF UNCOMPLICATED MALARIA AT KASANGATI HEALTH CENTRE, UGANDA

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The objective of his study was to monitor the efficacy and safety of national policy regimen, artemether-lumefantraine (coartem), for the treatment of uncomplicated falciparum malaria in Uganda, five years after introduction of the drug as cognate first-line therapy. A single-blind cohort trial was undertaken. Participants in this study werec hildren aged one to 11 years with uncomplicated *Plasmodium falciparum* malaria confirmed by blood smear microscopy. The Kasangati Health Centre, 20 km north of Kampala, Uganda is located in an area of low-level malaria transmission. Patients were treated with artemether-lumefantrine. We determined risks of recurrent symptomatic malaria and recurrent parasitemia at day 28. Findings were adjusted by genotyping in order to distinguish recrudescence and new infection. One hundred and two children were enrolled. Coartem was highly efficient and well tolerated. Serious adverse events were not observed. While we did not find any recrudescences, few cases of recurrent malaria due to new infections were seen. In conclusion, artemether-lumefantrine was highly effective for treatment of uncomplicated *falciparum* malaria. Nevertheless, in this low-transmission area, even with the observed high efficacy of coartem, few patients were ill with new infections. Thus, in order to take full advantage of coartem, the newly introduced first-line therapy, early detection and treatment should be linked to preventive control of malaria transmission.

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SAFETY AND IMMUNOGENICITY OF A NEW MENINGOCOCCAL A CONJUGATE VACCINE IN A HEALTHY AFRICAN POPULATION AGED 2-29 YEARS

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Recurrent severe epidemics of meningococcal disease strike the African meningitis belt extending from Senegal to Ethiopia. Annual incidences can reach 1,000/100,000 *vs*. 1/100,000 in developed countries. Group A meningococcus has remained unique in its ability to cause those large epidemics. A vaccine that confers long-lasting protection induces herd immunity and is affordable for widespread use in Africa, is urgently needed. The Meningitis Vaccine Project (MVP) was funded in 2001 as a partnership between WHO and PATH to develop and introduce an affordable meningococcal conjugate vaccine for elimination of meningococcal epidemics in sub-Saharan Africa. A new conjugate meningococcal A vaccine (PsA-TT), manufactured by Serum Institute of India Ltd Pune - India is currently being tested in a phase II/III clinical studyin an African population 2-29 years of age. An observer-blind, randomized, controlled study to assess safety, immunogenicity and antibody persistence up to 1 year after vaccination of one dose of PsA-TT vaccine in Africans children and adults aged 2-29 years is underway. A total of 900 participants were recruited in The Gambia, Mali and Senegal and were randomized to receive either a single intramuscular injection of PsA-TT vaccine [0.5 ml contains 10µg Ps, 10-33µg TT and $AlPO_4$ adjuvant], or of a meningococcal ACWY polysaccharide vaccine. The primary objective of the study is to evaluate the immunogenicity of a single injection of PsA-TT vaccine during 4 weeks post-vaccination with comparison to the men A component of the tetravalent polysaccharide vaccine. The immunogenicity responses are evaluated in terms of serum bactericidal antibody (SBA) activity and anti-polysaccharide group A (anti-PsA) IgG levels. Safety is assessed through an active and daily follow-up for 4 days after vaccination. All Adverse Events were collected up to 4 weeks after vaccination and Serious Adverse Events are collected for the entire study duration (1 year). Data analysis of safety and immunogenicity at 4 weeks after vaccination is ongoing and results will be presented at the meeting. Data from this study will document the safety and immunogenicity of the MenA conjugate vaccine (PsA-TT) in the 2-29 years old population, and support vaccine licensure and subsequent large scale introduction in Africa.

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FIELD VALIDITY AND COMPARATIVE PERSISTENT ANTIGENICITY OF HRP-2 RAPID DIAGNOSTIC TESTS FOR MALARIA IN A HYPERENDEMIC REGION OF UGANDA

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Microscopy, which requires skilled personnel, is unavailable at many lower health facilities, where the majority of malaria cases are managed in the public sector. Rapid diagnostic tests (RDTs), in contrast, require limited expertise, but their operational validity has not fully been evaluated in Uganda. In addition, there are concerns about the RDTs which use the antigen histidine-rich protein 2 (HRP2) to detect *Plasmodium falciparum*, because they can continue to give positive results weeks after effective treatment due to persistence of the antigen in the blood. The study had two phases and was based in an area of Uganda which is hyperendemic for malaria. Firstly we assessed the operational accuracy of the Malaria Pf. ™ ICT (ICT) RDT in terms of sensitivity and specificity in all ages of the population. Following this, in children under five, we evaluated the duration of prolonged positivity of the ICT and Paracheck® RDTs, both HRP2 based. For the sensitivity and specificity aspect, a total of 357 febrile patients were evaluated using ICT, with microscopy as reference. Two independent microscopy and RDT readings were used to assess validity of the device. For the second phase, we followed up 224 children at 7 day intervals until the RDT results became negative to describe persistent antigenicity of ICT and Paracheck. Of the 357 patients enrolled, 139 (40%) had positive blood smears for asexual forms of *P.falciparum.* ICT had an overall sensitivity of 98%, specificity of 72%, NPV of 98% and PPV of 69% (95%CI: 62-75). ICT and Paracheck were comparable in performance (kappa =0.982). The overall mean duration of persistent antigenicity, as measured by the RDT, was 32 days, and this duration varied significantly depending on day 0 parasitaemia. In patients with initial parasite density >50,000/µl, the mean duration of persistent antigenicity was 37 days compared to 26 days for parasitaemia less than 1,000/µl (log rank 21.9,

p<0.001). In conclusion, ICT was found to be a valid test and appropriate for field use. Persistent antigenicity reduces the accuracy of HRP2-based RDTs, and this study highlights important issues that need consideration when using RDTs for diagnosis in a hyperendemic setting for malaria.

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POTENTIAL HEALTH IMPLICATION OF CHRONIC PARACETAMOL EXPOSURE IN AFRICAN POPULATION STUDY

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Urine samples of 269 participants from 3 villages (state which) in Zanzibar were profiled by high-resolution nuclear magnetic resonance. Clear differences in urinary metabolic profiles were observed in participants from the 3 villages. In addition, 16 participants from the total 269 were found to excrete high levels of paracetamol metabolites. This finding highlighted the potential public health problem in terms of analgesic abuse since chronic ingestion of paracetamol has been linked to hepatic injury. Some of the participants in this study were mothers with young children who were still being breast-fed. Therefore, the health implication posed to these young children who were being chronically exposed to analgesic unnecessarily needs to be highlighted and addressed urgently.

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PASSIVE IMMUNIZATION WITH SERUM FROM SECONDARY DENV PROVIDES PARTIAL CROSS PROTECTION AGAINST WNV INFECTION

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West Nile Virus (WNV) was recently isolated in Puerto Rico (PR), but the effect on the human population is still unknown. Since PR is hyperendemic to Dengue viruses (DENV), which like WNV is a member of the Flaviviridae family of viruses, we hypothesized that immunity to DENV may provide protection against WNV-infection. In previous studies, we found that active immunization with DENV-2 virus conferred cross-protection against WNV-induced disease. In this study, we performed passive immunization with serum samples collected from individuals with either primary or secondary DENV infections, prior to WNV NY99 challenge. WNV-reactive serum and flavivirus non-reactive serum were used as positive and negative controls, respectively. We found a higher but not significant difference in survival in the secondary DENV group (50%) when compared to the primary DENV (20%) or the negative control group (20%). A significantly higher survival (80%) was observed in mice immunized with WNV-reactive serum in comparison to mice that received primary DENV serum (20%, p =0-011) or non-reactive serum (20%, p = 0.010). Viral load after WNV infection was measured in serum and brain. We found peak viremia on 2 days post-infection (dpi) with similar levels in all groups. However, statistically significantly higher viral RNA (vRNA) levels were detected in the brain of the negative control group (3.4 x 105 PFU/mg) in comparison with the other groups on 8 dpi (peak day for most groups). The low vRNA levels in mice brain correlated with the high survival rates observed in the secondary DENV and the positive control group. This data suggests that serum from a secondary DENV infection but not from a primary DENV infection can provide partial cross-protection against WNV infection.

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ANALYSIS OF ANTIBODY RESPONSE AGAINST DENGUE VIRAL RECOMBINANT PROTEINS IN SERUM SAMPLES OF PATIENTS WITH IN DF AND DHF

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Dengue (DEN) has become one of the most important arthropod-borne viral infections of humans with about 100 million cases per year. The high serumprevalence of DEN and the co circulation of multiple serotypes suggest that many countries may be at risk of a major dengue hemorrhagic. All dengue serotypes are able to induce antibody responses to the various components of the virus. However, there is insufficient information regarding antibody responses to nonstructural proteins even though they are involved in the pathogenesis of the disease. The antigens used in most of the reported works were prepared from cell cultures. In this work, we evaluate the differences in the antibody response in primary and secondary infections by using recombinant Dengue virus proteins E, NS1, NS3 NS2B and NS4B expressed in *Escherichia coli* in a ELISA assay. Sera from healthy individuals living in non-endemic areas for dengue were used as control. A 50% of sera from dengue primary infection were able to recognize the recombinant E protein, in contrast an 80% of the secondary infected patients recognized this protein. No differences were observed in between the groups of DF and DHF. When the specific antibody response to non-structural proteins were analyzed, we observed similarly results such as a higher response in the secondary infections versus a primary infection. Interestingly when the specific antibody response against NS4b protein was analyzed; the group of serum samples from a secondary DF disease gave a significant higher response when compare with primary or secondary DHF. The antibody response to NS2B was frequently of low magnitude. Consistent negative antibody responses to all proteins was found in sera from the control group.

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THE ROLE OF HUMAN FIBROBLAST IN THE INNATE IMMUNITY AGAINST DENGUE VIRUS

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Giving the fact that mosquito inoculates the DV into human skin while it is feeding, the potential target cell for dengue infection should rather be the dermal/interstitial DCs (i.e. Langerhans cells), keratynocytes and fibroblast cells that are localize in epithelia. A rapid initiation of innate host defense might a limiting step of DV infection. In consequence an important issue to solve is identify other non hematopoyetical cells infected in the early steps that may play a crucial role in antiviral innate immunity to DV after the virus inoculation. Using human skin explants, we previously have successfully established an ex-vivo model of Dengue infection. When the *in situ* cutaneous infection was performed to evaluate the presence of other DENV infected cells. We observe the presence of Dengue virus antigen-positive non-hematopoyetic cells, which by histological location, morphology and distribution, are most likely to be fibroblast. From this skin we have establish primary human skin fibroblasts, where we have studied Den-2 infection in an *in vitro* model. Our results showed, the presence of Dengue virus antigen-positive fibroblast cells assessed by cytometry and IF were detected as early as 6 h, with a maximum of 24 h post infection (pi). Moreover the infectivity rate between samples

is variable in each individual. At the same time Dengue virus-infected fibroblasts produced interferon-β (IFN-β), and over regulation of presence of IFNα as early as 12 hr post- infection. Additionally Dengue virus elicited an increasing IFN regulatory factor 3 (IRF3) nuclear translocation, compared with the mock infected fibroblasts at 24 h post-infection.

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ALTERED CD8+ T CELL RESPONSES TO *IN VITRO* SECONDARY STIMULATION WITH HETEROLOGOUS DENGUE VIRUS SEROTYPES

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The four serotypes of Dengue virus (DENV 1-4) are the most common cause of viral hemorrhagic fever worldwide. Epidemiological evidence suggests that severe disease is associated with secondary infection by a DENV serotype different from that of the primary infection. Cross-reactive memory T cells are hypothesized to play an immunopathological role in secondary heterologous DENV infection. We characterized the CD8+ T cell response to an HLA-A11-restricted epitope on the DENV NS3 protein in PBMC from naturally-infected donors and the recipient of a candidate live attenuated DENV vaccine. Epitope-specific CD8+ T cells were studied directly *ex vivo*, in short-term bulk culture, and at the clonal level, using tetramer staining, intracellular cytokine staining and cytoxicity assays. DENV-specific CD8+ T cells showed marked cross-reactivity, with many clones responding to heterologous peptides as robustly as to the homologous peptide. The pattern of cytokine production and cytotoxicity of individual T cell clones varied greatly and suggest avidity for particular epitope variants plays a large role in influencing the heterogeneity of the DENV-specific CD8+ T cell response. These data demonstrate the ability of individual CD8+ T cell clones to differentially respond to variations within a relatively conserved DENV epitope. Our results suggest that DENV-specific memory CD8+ T cells can respond to a heterologous DENV infection, leading to preferential expansion of memory T cells originally tailored for a fundamentally different virus. This enhanced, albeit skewed, response during secondary DENV challenge could lead to sub-optimal viral clearance as well as immune cell-mediated pathology resulting in more severe disease.

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THE SPATIAL DIMENSION OF DENGUE TRANSMISSION IN IQUITOS, PERU

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Understanding the spatial and temporal dimensions of dengue virus transmission would improve the design of surveillance and control programs to prevent disease. We examined spatial patterns of dengue infections detected in a longitudinal cohort study of >3000 participants in Iquitos, Peru for the period between January 1999 and August 2003. Participants provided blood samples at ~ 6 month intervals that were analyzed for the presence of serotype-specific antibodies using a PRNT. Over the term of the study, DV1 and DV2 circulated at low, endemic transmission levels (incidence < 10%, prevalence > 70%). We sought spatial structure at geographic scales beyond the household among individuals seroconverting to DV1 or DV2 by comparing the spatial distribution of seroconversions to a random sample of individuals who did not seroconvert during the same time-frame by partitioning the data into 24 week time intervals and calculating mean nearest neighbor distances, G estimates, and K estimates (indices of clustering). Relative to random individuals, seroconversions did not demonstrate spatial clustering ($p>0.05$), indicating a lack of spatial dependence that could partly be caused by high herd immunity. In 2001, DV3 invaded Iquitos and caused a significant epidemic (incidence > 30%) in the virgin population before an intervention halted transmission. Examination of DV3 cases again failed to indicate any spatial clustering, despite low herd immunity. These results, taken with those presented elsewhere documenting focal dengue transmission, suggest that dengue transmission occurred too quickly to detect spatial clustering at the temporal resolution and spatial intensity of our study design. Moreover, patterns of human movement likely played an important role in the rapid city-wide spread of the disease. To define the spatial and temporal dimensions of dengue transmission should use smaller sampling intervals, sample more densely within the study area, account for human movements, and include cluster investigations.

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PATTERNS OF CROSS-REACTIVITY AND SPECIFICITY IN THE SEROLOGICAL RESPONSE TO DENGUE INFECTION IN KAMPHAENG PHET, THAILAND

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Infection with dengue viruses can result in specific immunity to the infecting serotype and cross-reactive immunity to other dengue serotypes. This analysis explores variability in the neutralizing antibody response among individuals with a recent history of symptomatic dengue infection and how this variability may be associated with viral, immunological, and clinical factors. 2000 school children per year were followed for the occurrence of dengue infection in Kamphaeng Phet, Thailand over a 9-year period. The infecting serotype was identified using RT-PCR and pre- and post-epidemic sera were analyzed for the presence of neutralizing antibodies to DEN1-DEN4. Outcomes of interest were serotype-specific titers of the immune response to infection and the number of serotypes to which the individual seroconverted or experienced an increase in titers. Of 499 symptomatic dengue infections, 382 had the infecting serotype identified. Among individuals with primary-type responses (4% of symptomatic infections), 47% responded to 3 or more dengue serotypes. Among individuals seronegative to DEN1-4 prior to infection, 11% were primary infections. 89% of all seronegative individuals responded to 3 or more dengue serotypes with subsequent infection, versus 71% of individuals with baseline immunity to a single serotype and 40% of individuals with baseline immunity to 2 or more serotypes. There was no difference in the magnitude of the response to the infecting serotype by baseline immunity. However, individuals who were seronegative at baseline had higher aggregate heterotypic titers at 0-2 months post-infection and lower aggregate heterotypic titers at 5+ months post-infection as compared to individuals with broader baseline immunity ($p<0.05$). The clinical severity of dengue infection was not associated with the antibody response to infection. In conclusion, significant differences were observed in the composition and magnitude of the antibody response to dengue infection by baseline immunity and time since infection.

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PRECLINICAL EVALUATION OF DENVAX: A CHIMERIC TETRAVALENT DENGUE VACCINE

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Dengue viruses serotypes 1-4 (DEN1-4) cause dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS): the most important arthropod-borne viral infection of humans with over 100 million cases and 25,000 deaths annually. Here we describe the preclinical evaluation of DENVax, a chimeric tetravalent dengue vaccine. DENVax is based on the live-attenuated DEN-2 PDK-53 vaccine which has been shown to be safe and immunogenic, generating long-lasting neutralizing antibodies in human clinical trials. Using DEN-2 PDK-53 as the genetic backbone, candidate chimeric vaccine viruses that express the structural genes of DEN-1, DEN-3 and DEN-4 were engineered. Viral RNA transcripts of the cDNA infectious clones for the DEN-2 PDK-53 and the three chimeric viruses were transfected into validated, GMP-quality Vero cells. Candidate GMP-quality seed stocks for each of the four vaccine viruses were derived, plaque-purified and sequenced. The phenotypic properties of the resulting viruses were characterized in tissue culture. In addition, viral RNAs were sequenced to examine the presence of the three attenuating mutations located in the 5′ non-coding region, NS1 and NS3 genes. Preclinical studies in AG129 mice and non-human primates have been conducted. Several vaccine formulations containing different ratios of all 4 serotype vaccines are being tested for toxicity, safety and efficacy in these models. Based on these data, the leading formulations will be prepared for Phase 1 human clinical trials in the U.S. and in Colombia in early 2009. Development of an affordable, easily delivered, safe, and effective dengue vaccine will protect those most at risk of DF, DSS, and DHF.

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DYNAMIC HOST GENE EXPRESSION PROFILING OF SEVERE FORMS OF DENGUE VIRUS INFECTION

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Dengue virus (DENV) infection causes acute febrile illness with systemic symptoms (dengue fever, DF). Hemorrhagic manifestations develop on day four to six of the disease, in small part of the patients with DENV infection. This severe illness is called as dengue hemorrhagic fever (DHF). Further among them, severe plasma loss causes hypovolemic shock (dengue shock syndrome, DSS) which requires intensive medical care. Host response known as cytokine storm has been reported to accompany to these severe cases, but pathogenesis of severe illness remains to be elucidated. For the better understanding of host determinants for disease severity, RNA expression profiling was conducted in the present study. Ten children aged from nine months to 14 years who admitted to Nhi Dong Hospital No.2 in Ho Chi Minh City, Vietnam in August and November, 2007 because of clinical symptoms meeting WHO criteria of DHF grade II (DHF, 6 cases) or grade III (DSS, 4 cases) were enrolled. In addition to routine hematological and virological examinations during hospitalization, paired blood samplings for RNA of whole blood cells were done on the day of onset of severe illness and several days later when the most symptoms disappeared. Ten pairs of RNA samples were then analyzed for gene expression by Illumina Human-6 Expression Bead Array. Seven genes including complement component 2 (*C2*) gene were expressed more than two-fold and 9 genes including genes for Protein S (*PROS*) and von Willebrand factor (*VWF*) exhibited less than half on the day of onset in comparison to the recovery phase commonly in all ten pair comparisons among total of 48701 genes examined. The results suggested the presence of background abnormalities in blood coagulation and complement system elucidating hemorrhagic tendencies. Further analyses of these data will provide some valuable insights for determinants of disease severity between DHF (grade II) and DSS (grade III).

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USING GPS TECHNOLOGY TO STUDY DISEASE TRANSMISSION: WHAT DO POTENTIAL STUDY PARTICIPANTS THINK ABOUT THIS?

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As GPS technology becomes more affordable and units more easy to carry, use of GPS technology to study disease transmission will increase. It is important to examine the possible barriers to using GPS technology from the perspective of potential study participants. Fifteen focus group discussions, with a total of 140 adults (68 males, 72 females), were carried out between January 25-31, 2008 in the rainforest city of Iquitos, Peru. These focus groups were made up of: 3 groups of 18-30 year old men, 2 groups of 18-30 year old women, 2 groups of 31-45 year old men, 2 groups of 31-45 year old women, 2 groups of 46-59 year old men, 2 groups of 46-59 year old women, and 2 groups of mothers - 1 group of young children ages 3-8 and 1 group of older children ages 9-17. The sample was not meant to be representative for the region, but the issues that were raised by these groups should reveal some of the issues that will come up in the community. Most people had NOT heard of a GPS, however, in most of the men's groups, a few men had heard of or even seen a GPS, and in a few cases, had used one for work. The two main concerns that came up regarding wearing a GPS for two weeks were whether the GPS could tape or videotape the participants, and how to handle the unit and properly charge it and take care of it. However, other issues voiced by focus group participants included some concerns about prolonged use of GPS units and its effect on health (i.e., infertility, cancer, or even attract lightning bolts), responsibility for and caring of units (i.e., who pays if it is lost or stolen or breaks, how does one know when to charge it), confidentiality (i.e., whether researchers would track respondents wearing the GPS unit, whether others might be able to figure out where users have been), and possible interference with other electronic devices (i.e., do cell phones or TVs affect the unit or vice versa). Overall, in this urban population in Iquitos, from the participants' perspective, there were few barriers to using GPS as part of a study.

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DENGUE VIRUS TYPE-2 (VD2), INDUCE FILOPODIAL STRUCTURES DURING VIRAL ENTRY IN CELL LINE HMEC-1

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The infection with DV2, begins with the adherence of the virus receptor dependent, to carry out the processes of transport, replicación, assembly and liberation. The mechanisms involved in the penetration virus, as for the participation of the cytoeskeleton have not been clarified. In this study was investigated the role of the cytoskeleton of the host cell in

the infection of DV2. The cell line HMEC-1 was transfected with the pEGFP-actin vector, it became infected with DV2 and the reorganization of the cytoskeleton was observed by epifluorescence microscopy in fixed and living cells, as well as the analysis of the percentage of cells infected across inmunofluorescence and flow citometry. We have thought that the infection with DV2, induces actin reorganization, showing an increase of the F-actin after 48 hrs p.i. The infection turns out to be diminished when added citocalasin D. This reorganization is observed from the first minutes of interaction of the virus with the cell, inducing the formation of filopodial-like proyections in the cellular periphery, later to its adherence and interaction with its receptor, for its later endocytosis. Formation of these structures does not depend on the route of Rho GTPase, because when bloking this route with Y-27632, the viruses can adhere and penetrate to the cells, and finish to be carried out a productive infection. When we used NSC-23766 and Lovastatin, inhibitors of Rac and Cdc42 signaling pathways, resulted in a significant decrease in the formation of the filopodials structures, as well as of the penetration and infection of the virus. The inhibition of the ATPase activity from the myosin II with Blebblistatin during the first minutes of interaction, resulted in a decrease in the percentage of cells infected after 48 hrs pi. This information suggests strongly that the interaction of DV2 with the cells HMEC-1, needs of actin across the formation of filopodial structures, regulated by Rac1 and Cdc42 GTPases and the activity of myoin II, in the process of entry of DV2.

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EVALUATION OF HOUSEHOLD TRANSMISSION OF DENGUE USING A CLUSTER EPIDEMIOLOGY STUDY DESIGN IN WEST JAVA, INDONESIA

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We are conducting a cluster investigation study of dengue infections in two large cities in West Java (Jakarta and Bandung), with the goal of identifying patients with dengue early in the course of infection to study disease progression and evaluate factors associated with disease severity. We report here the interim analysis of serotype distribution and disease outcome. 2820 household and nearest neighbor contacts of 144 persons hospitalized with acute dengue infection were prospectively observed over the two weeks following identification of the neighborhood index case. Volunteers were visited every other day and evaluated for signs of disease. Phlebotomy was performed every four days or whenever volunteers were symptomatic. Dengue infections were confirmed by serotype-specific RT-PCR, virus isolation, and serology assays. 389 dengue infections were detected in household contacts during 1316 person-months of observation, for a total incidence of 296 per 1000 person months. The majority were recent, distinguished by presence of IgM but no other signs of infection. 73 were acute infections detected at or following enrollment, defined by detectable viremia and/or seroconversion during two weeks of observation. These rates suggest an incidence density of concurrent and recent infections among household contacts of hospitalized dengue cases of between 25.9 and 138 cases per 1000,substantially higher than the estimated rate for the general population (12 cases per 1000), as expected. 15 cases of DHF were identified for an incidence density among household contacts and neighbors of 5.3 per 1000 population. The predominant serotype was DEN-3 in both cities, accounting for 45% of serotypable infections. Assessment of the relationship between severity of dengue disease and infecting serotype revealed that DEN-3 infections were more likely to result in DHF than were other serotypes. Asymptomatic viremic infections were detected in six individuals, three each due to DEN-2 and DEN-4. No asymptomatic viremia due to DEN-1 or DEN-3 was observed. Our findings suggest that infecting serotype impacts disease outcome among persons with dengue disease in Indonesia, corroborating and expanding upon recent reports from other regions.

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LONGITUDINAL PROSPECTIVE STUDY OF DENGUE IN A COHORT OF INDONESIAN ADULTS REVEALS A SHIFT IN SEROTYPE PREDOMINANCE AND INCREASED DISEASE SEVERITY

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We have conducted two longitudinal prospective studies of dengue fever (DF) and dengue hemorrhagic fever (DHF) in cohorts of adult factory workers since 2000 in Bandung, West Java. 4278 volunteers comprising employees at three textile factories have been observed for a total of 13,080 person-years over the course of the studies. Serum specimens were obtained at baseline, every four months, and during febrile illnesses. Febrile episodes were evaluated by RT-PCR and IgM ELISA for diagnosis of acute dengue infection. During the first four years of study, dengue infections were diagnosed in 176 volunteers: 137 cases of DF, 12 of DF with hemorrhagic manifestations and 27 cases of DHF. In the last 2 years of observation, 46 dengue infections have been detected: 24 cases of DF, 6 of DF with hemorrhagic manifestations and 16 cases of DHF. Longitudinal evaluation of serotype-specific incidence reveals a shift in circulating viruses. From 2000-2004 DEN-3 was the least predominant serotype but is currently the most predominant, accounting for 43% of typable infections identified from 2006-2008. The annual incidence rate of symptomatic dengue infection declined significantly over the course of the study from 19.3 cases per thousand population (95% confidence interval: 16.7 - 22.5) during the period 2000-2004, to 11.6 (95% CI: 8.2 - 14.9) cases per thousand from 2006-2008. In contrast, the annual DHF incidence showed an increasing trend over the same period, from 3.01 (1.87 - 4.14) cases per thousand in 2000-2004 to 4.03 (2.06 - 6.01) cases per thousand in 2006-2008. The overwhelming majority of infections were secondary throughout the period of observation. The fraction of all dengue infections in the cohort complicated by DHF in 2006-2008 was twice as high as in 2000-2004 (0.348 vs 0.153). DEN-3 infection presented the greatest risk for DHF throughout the period of observation, suggesting that increased DEN-3 incidence has accounted for increasing DHF incidence in this adult cohort.

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DENGUE KNOWLEDGE AND PRACTICE, A PHYSICIAN SURVEY IN AN ENDEMIC AREA OF THE U.S

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In Puerto Rico dengue is endemic and reportable via a passive laboratory-based surveillance system. A physician survey of dengue knowledge and practice was done. Better understanding of current practices may lead to quality of care improvements. A 37-item survey was mailed to a random sample of 2,150 physician generalists and all 362 specialists in Puerto Rico. Medical specialists unlikely to diagnose and treat dengue patients

were excluded. The survey was mailed in the fall of 2007; a second survey was sent in early 2008. A postcard reminder was sent after each mailing. No incentive was given. Of the 2,512 surveys sent out, 197 (7.8%) were returned because of incorrect address or death of physician. Of the 2,315 who received a survey, 810 (35.0%) responded and of these, 700 (86.4%) were currently practicing. Practicing physicians were male (n=427, 61.0%), Puerto Rico (n=239, 34.1%) or Dominican Republic (n=195, 27.9%)-trained, who had practiced, on average, 21.8 years (range 0-55 years). The most frequently cited way to identify probable dengue cases was the platelet count (92.9%) followed by WHO case definition (88.4%) and white blood cell count (85.3%). Most were able to correctly identify laboratory methods to diagnose an acute dengue infection while some (n=115, 16.4%) reported use of only acute sera to detect antibodies for diagnosis. One third (n=244, 34.9%) reported testing all suspected dengue patients while forty percent (n=303, 43.3%) refer suspected patients to a hospital for laboratory testing. Nearly forty percent reported using corticosteroids to treat dengue. The most commonly reported criteria for steroid use was a platelet count <50,000/cmm. Few practicing physicians (n=213, 30.4%) were able to correctly identify the best early indicator of shock. Nearly forty percent were unable to identify early warning signs and symptoms for severe dengue such as "onset of severe abdominal pain" and "persistent vomiting". Nearly one third (n=204, 29.1%) stated that they never report suspected dengue cases to the Department of Health. In conclusion, of practicing physician respondents, most know how to diagnose dengue but few order confirmatory diagnostic testing. Few report cases so dengue incidence is likely to be underestimated in Puerto Rico. Gaps in knowledge of dengue were identified and need further investigation. Physician education on dengue may be warranted.

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FREQUENCY OF DENGUE FEVER AMONG FEBRILE PATIENTS PRESENTING TO AN URBAN HOSPITAL IN MEDELLIN, COLOMBIA: PILOT STUDY RESULTS

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In 2007, the incidence of dengue in Medellin, Colombia was 21.2/10,000. Medellin is a large metropolitan area with modern infrastructure making it an ideal site for dengue research. Peak dengue season in Medellin generally runs from September through January of the following year. Due to difficulties with clinical diagnosis and lack of funding to support laboratory testing, under reporting of dengue has been problematic in the past. We established fever surveillance in three facilities in 2007: A private clinic, a public clinic, and San Javier Hospital, the only hospital in a barrio of Medellin, Colombia. Health care providers at these three facilities were asked to refer all patients with a core temperature >38.0 C or a history of fever during the previous 7 days, to a study physician posted in the clinic. The study physician completed a medical history and physical examination using a standardized case report form and then collected an acute serum sample. A convalescent serum sample was collected, 14-21 days later. Because the study physician rotated between clinics, surveillance was not continuous at any one site. A dengue case was defined as a patient presenting to a study clinic with fever in the preceding 7 days and a serum sample positive for dengue by RT-PCR or MAC ELISA. During the pilot phase of the study (December 12, 2007-February 20, 2008), 138 patients were recruited; 2 patients were confirmed as dengue by RT-PCR and an additional 22 patients by MAC ELISA. Dengue-positive patients ranged in age from 2 months to 49 years, with a mean age of 18 years. None of the patients met diagnostic criteria for DHF. Four confirmed cases were correctly diagnosis by the treating physician. In conclusion, in the pilot phase of a fever surveillance study, 17% of patients presenting with fever in Medellin, Colombia were laboratory-test positive for dengue. The reason for the unusually high proportion of diagnosed Dengue is uncertain at the moment, but is under examination. Misdiagnosis was identified as one cause for past instances of under reporting. Dengue may be a major cause of acute febrile illness in Medellin, Colombia.

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USE OF HAND HELD COMPUTERS FOR DENGUE CASE REPORTING AND FOLLOW UP, MEDELLIN, COLOMBIA: PILOT STUDY RESULTS

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Hand-held computers use the same operating systems and applications as desktop computers. Their small size and weight makes them ideal for use in field epidemiological studies. Data entered in clinical settings is immediately available on downloading. However, replacing paper case report forms with hand held computer applications could raise multiple logistical, security, and regulatory issues during a clinical trail. We are conducting a fever surveillance study in Medellin, Colombia in preparation for possible clinical trails. In the pilot phase of the fever study, we introduced hand held computers to be used instead of paper forms. The hand-held computers were programmed with data entry software. Study physicians were provided with these devices which were programmed with the standardized history physical exams questions (in Spanish).We compared data entry time and completion rate before and after introduction of the devices. We chronicled the logistical and other issues that occurred with use of the devices and the solutions to these issues. During the pilot phase of the study (December 12, 2007-February 20, 2008), 138 patients were recruited. The case report data was collected on paper for 57 of these patients; the remaining 81 were gathered using a hand held. The time taken to complete a history and physical exam (30-40 minutes) did not change going from a paper form to a hand held computer. However the number of incomplete forms was greatly reduced from 40% to 0%. Data entry and accompanying transcription errors were eliminated by the introduction of the hand held computers. Logistical issues encountered included risk of data loss, device malfunction, and issues with data transfer and storage. In conclusion, in the pilot phase of a fever surveillance study in Medellin, Colombia we replaced paper forms with programmed hand held computers. Savings in time and staff occurred through the elimination of data entry from form to computer. Missing data was also greatly reduced. Use of this technology greatly improved data quality in the study.

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LIMITED EVIDENCE OF HCV TRANSMISSION IN STABLE HETEROSEXUAL COUPLES FROM BAHIA, BRAZIL

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HCV infected patients frequently ask their physician about the risk of transmission to their partners. Although it is easy to answer that the risk does exist, it is difficult to quantify it. We studied the transmission of HCV infection in stable heterosexual couples: anti-HCV positive patients in hemodialytic therapy and their partners. Thirty-four couples were tested by third generation ELISA and RIBA. Blood samples of anti-HCV positive patients were evaluated by RT-PCR and detected sequences were genotyped by restriction fragment length polymorphism. Patients with negative RT-PCR samples were retested after 12 months. The mean period of living together was 16.6 ± 13.7 years. Couples reported their sexual activity as: daily (n=2), within 2-3 days (n=12), weekly (n=5), biweekly

(n=5), monthly (n=4) and occasionally (n=6). Previous history of sex partners was: <6 partners (76% of patients and 73% of partners), 7-10 (15 and 23%) and >10 (9 and 4%). The use of condom was reported to be regular by one couple, sporadic by 11 couples and 23 couples reported that they have never used it. Sharing personal items was common in the population studied: 4 (12%) shared tooth brushers, 11 (32.5%) shared razor blades and 24 (71%) shared nail clippers and manicure pliers. Seven couples (21%) shared all these items and 2 (6%) none. Coexistence of infection was observed in only one couple in which both subjects had positive RT-PCR samples and were infected by a concordant genotype (genotype 3). This couple was the only one in which both partners were currently in dialytic therapy and shared history of blood transfusion. One other couple had the partner with two positive ELISA tests and an indeterminate RIBA, with negative RT-PCR, which may suggest a false positive or a previous resolved infection. Either sexual relations, sharing of personal items and history of parenteral exposure (hemodialysis, blood transfusion) could explain transmission. We observed, in accordance with previous reports, that the risk of HCV transmission is minimal or negligible in stable heterosexual couples.

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PRO-INFLAMMATORY CYTOKINES IL-1β, IL-8 AND TNF-α ARE ASSOCIATED WITH PROTECTIVE EVENTS WHEREAS IL-2 AND IFN-γ WERE MORE LINKED WITH THE INCREMENT OF THE BIOMARKER ALT IN HCV SEROPOSITIVE PRE-BLOOD DONORS

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Inflammatory and regulatory cytokines could account for distinct anti-HCV innate and adaptive immune response as well as the viral clearance/persistence. We explored a possible association between the pattern of seric cytokines with the anti-HCV profile as well as the virological status and the liver injury biomarker alanine-aminotransferase-ALT in HCV-seropositive-HCV^{+} pre-blood donors. Type-1/pro-inflammatory (IL-1β/IL6/IL-8/IL-12/IFN-γ/TNF-α) and Type-2/regulatory (IL-4/IL-5/IL-10) seric cytokine pattern were studied by cytokine-bead-array in non-viremic-HCV$^{+/-}$ and viremic-HCV$^{+/+}$ in comparison with HCV-seronegative-NI pre-blood donors. Our findings demonstrated enhanced frequency of IL-1β and IL-8 high-producers within HCV$^{+/-}$whereas enhanced frequency of IL-6, IL-10 and IL-12 high-producers was observed within HCV$^{+/+}$. Interestingly, increased frequency of IL-1β high-producers was selectively observed among HCV$^{+/-}$ with indeterminate anti-HCV confirmatory test-(RIBA) while enhanced frequency IL-8 high-producers was restricted to the HCV$^{+/-}$ subgroup displaying positive RIBA, which also showed besides increased frequency of IL-4 high-producers, enhanced frequency of IL-6, IL-10 and IL-12 higher-producers, likely the HCV$^{+/+}$ group. Additionally, we have also observed increased levels of IL-6, IL-10 and IL-12 particularly in HCV$^{+/+}$ with low HCV-viral load. The most outstanding finding was the increased levels of IL-1β, IL-8 and TNF-α observed in HCV$^{+/+}$ displaying normal ranges of ALT whereas IL-2 and IFN-γ was selectively increased in HCV$^{+/+}$ with elevated ALT levels. Taken together, our results suggested that pro-inflammatory cytokines (IL-1β/IL-8/TNF-α), mainly related with innate immune response, were more prone to be associated with protective events whereas IL-2 and IFN-γ, largely involved with the adaptive immunity cytotoxic profile, were more linked with the increment of the liver injury biomarker ALT.

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ENHANCED FREQUENCY OF CD56^{BRIGHT} NK-CELLS TOGETHER WITH CD3^{-}CD16^{+}CD56^{-} NK-CELLS AND ACTIVATED CD4^{+}T-CELLS OR B-CELLS PARALLEL WITH CD4^{+}CDC25^{HIGH} T-CELL REGULATORY MAY PLAY AN IMPORTANT ROLE CONTROLLING VIREMIA IN HCV SEROPOSITIVE PRE-BLOOD DONORS

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Herein was performed a detailed phenotypic analysis of major and minor circulating lymphocyte subsets from HCV seropositive (HCV^{+}) pre-blood donors, including non-viremic-HCV$^{+/-}$ and viremic-HCV$^{+/+}$ in comparison with HCV-seronegative-NI pre-blood donors. Despite no changes in the hematological profiles of both groups, the findings highlighted that increased levels of pre-NK-cells (CD3^{-}CD16^{+}CD56^{-}) and lower frequency of mature NK-cells (CD3^{-}CD16^{+}CD56^{+}) were the hallmark of the innate immunity in HCV$^{+/-}$. Although both HCV^{+} groups displayed high percentages of CD56^{Bright} NK-cells, this subset was particularly higher in HCV$^{+/+}$ with low HCV-viral load. Increased frequency of circulating NKT2 subset was particularly observed in HCV$^{+/+}$ bearing low HCV-viral load. Enhanced frequency of activated CD4^{+} T-cells (CD4^{+}HLA-DR^{+}) was a distinctive feature of HCV$^{+/-}$, whereas increased percentage of B-cells (CD19^{+}) besides enhanced levels of CD19^{+}CD86^{+} cells were the major phenotypic features of HCV$^{+/+}$, particularly those displaying low HCV-viral load. Although CD4^{+}CD25^{High} cells was expanded in both HCV^{+} groups, this regulatory T-cell subset (Treg) was predominantly enhanced in HCV$^{+/+}$ showing low HCV-viral load. Parallel increment of CD4^{+}CD25^{High} cells, pre-NK and activated CD4^{+} T-cells was observed in HCV$^{+/-}$ whereas the parallel enhancement of Treg and B-cells, hallmarks of HCV$^{+/+}$, was selectively found in low HCV-viral load. Taken together, these findings suggested that CD56^{Bright} NK-cells besides pre-NK cells and activated CD4^{+} T-cells parallel with T-cell regulatory cells may play an important role controlling viremia during HCV infection. Moreover, low HCV-viral load seems to be associated with enhanced CD56^{Bright} NK-cells and B-cell responses besides a T-regulated immunological profile.

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DO WE NEED TO USE LABILE SERUM FACTOR FOR DETECTION OF NEUTRALIZING ANTIBODIES IN ARBOVIRAL DIAGNOSTICS?

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The plaque reduction neutralization test (PRNT) has been widely accepted as the main confirmatory and the most virus-specific diagnostic test for determining the presence of specific antibodies in a patient's serum for arbovirus infections. Investigations conducted 30-40 years ago established that fresh normal human serum enhances neutralization of a variety of arboviruses by homologous antisera. In the PRNT, samples are first heated at 56ºC to inactivate complement. Because the component of fresh serum that enhances neutralization is heat-sensitive, it is commonly known as labile serum factor (LSF). The benefits of adding non-heat inactivated LSF to the heat-inactivated serum in the PRNT test have led to the widely accepted practice of including this factor in all arboviral diagnostic PRNTs, which presents a limitation for performing this test in many laboratories because of the absence of commercially available LSF. The present study was carried out to measure the effect of LSF on performing the PRNT with diverse arboviruses. The results demonstrated the beneficial effect of application of the LSF in testing neutralizing antibodies against alphaviruses (Chikungunya, eastern, western, and Venezuelan equine

encephalitis viruses); the California group of bunyaviruses (La Crosse encephalitis and Jamestown Canyon viruses); and one flavivirus (West Nile virus, WNV). No significant differences were detected with most flaviviruses, such as St. Louis encephalitis, yellow fever, Zika, Japanese encephalitis (JEV), dengue viruses (DENV, serotypes 2 and 4), and with the coltivirus Colorado tick fever virus (CTFV). LSF did not have a significant effect on the neutralizing antibody titer when the chimeric viruses ChimeriVax - WNV, - SLEV, - DENV (4 serotypes), and - JEV (Acambis, Inc.), were used in the PRNT. Generally, LSF was observed to significantly enhance neutralization of fast growing viruses, which require 1 to 2 days for plaque formation, with the exception of CTFV; whereas there was no significant effect with slow growing viruses, which require >3 days for plaque formation.

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EVALUATION OF NUCLEIC ACID AMPLIFICATION ASSAYS FOR DETECTION OF JAPANESE ENCEPHALITIS VIRUS RNA IN CEREBRAL SPINAL FLUID FROM ACUTE ENCEPHALITIS PATIENTS

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Japanese encephalitis virus (JEV) infection is the leading cause of pediatric encephalitis in Asia. IgM antibody capture enzyme-linked immunosorbent assay (MAC ELISA) is the primary test for diagnosing JEV infection, as anti-JEV IgM antibodies are detectable in cerebral spinal fluid (CSF) within 5 days of disease onset in the majority of patients presenting with encephalitis. Because of the brief, low level of viremia in JEV infections, virus isolation and viral nucleic acid detection methods are considered to be of low sensitivity. However, in very acute cases anti-JEV IgM antibodies may not yet be detectable, and a second convalescent specimen may not be available. Nucleic acid amplification testing (NAAT) may enhance diagnosis in this group of patients. The use of real-time RT-PCR to detect JEV RNA was evaluated in CSF which was collected from acute encephalitis patients as part of an acute meningitis and encephalitis surveillance project. Sera and CSF from these patients had previously been tested by JEV MAC ELISA. NAAT was shown to enhance diagnosis of JEV infection in patients in which the CSF was collected < 7 days from the date of disease onset, the majority of which were JEV MAC ELISA negative.

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MOLECULAR DETECTION OF FLAVIVIRUS IN ENDEMIC AREAS IN PERU

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Flaviviruses provide some of the most important emerging and resurging arboviral diseases in humans, the opportune detection of these viruses is really important in countries who has large endemics and tropical areas where vectors are located, able to transmit virus by bitting. Molecular detection of Flavivirus using degenerated primers designed to detect each member of Flaviviridae in nonstructural NS5 region, it will serve as a tool of epidemiologic alertness and surveillance programs. Four types of samples have been used in this study: human serum; liver tissue from humans who died with fever, jaundice and hemorrhage; vectors and tissue from captured mammals in tropical areas. Viral RNA was extracted and then applied a generic RT-nested PCR that is able to detect each member of the Flavivirus group; amplification of RNAs from different Flavivirus was visualized (143pb). The RT- nested PCR was standarized for detection of Flavivirus using two pair of degenerated primers previously described. *Aedes aegypti, Haemagogus sp. and Sabethes sp, Culex sp* were the principal vectors analyzed, of all 16 pooles, 05 pooles vectors were positives, the result of sequencing was Dengue 3 (DEN3). We did not find positive samples for Flavivirus in 45 tissues of mammals as *Oryzomys* sp.(30), *Rattus* sp (3). *Didelphis* sp.(7), pigs (2), sajino (1), dogs(1), owl (1) captured in a native community in the Amazonian forest, this would indicate that none of the mammals captured and submitted for the analysis weren`t natural reservories for Flavivirus in the moment of capture. Of 175 samples of human serum, 24 were positives of place as Iquitos (DEN3), Piura (DEN1), Huanuco (DEN3), Yurimaguas (DEN3), Lambayeque (DEN1), Junín (DEN3), Lima (DEN3) and San Martin (DEN1). We also analyzed 17 samples of human liver tissue, the result of sequencing was Yellow Fever virus. This study has allowed us to know Flavivirus's circulation in endemic areas and to establish the risk for continuous outbreaks by Flavivirus in Peru.

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THE NATURAL HISTORY OF YELLOW FEVER IN EAST AFRICA REVISITED

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Over 70 years have passed since the discovery of yellow fever (YF) in East Africa but the disease has remained enigmatic because of unpredictable focal periodicity, lengthy inter-epidemic periods, and a precarious potential for large epidemics. Paradoxically, urban outbreaks involving *Aedes (Stegoymia) aegypti* have never been reported but the region has also witnessed the largest epidemic ever reported worldwide (200,000 cases), which was vectored by *Aedes (Stegomyia*) *bromeliae*. Outbreaks of the disease in East Africa had not been reported for nearly 20 years until it emerged for the first time in Kenya (1992-93) and more recently in Sudan (2003 and 2005). In revisiting the natural history of this disease we performed a series of studies in Kenya including: a retrospective serological survey of approximately 900 wild nonhuman primates; 10 months of entomological studies along important forest fringe areas; and vector competence experiments using a local YF genotype (East genotype) and *Ae. aegypti* and *Ae. bromeliae* mosquito species. Overall, the results from these studies suggest limited enzootic YF activity in Kenya, a limited number of vector species bridging ecotone habitats, comparatively low number of domestic vectors in forest fringe areas, and a lower vector competence for *Ae. aegypti*. A summary of significant results are discussed in the context of broader historical trends and provide additional insight into the ecological and epidemiological dynamics at both local and regional levels.

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KINETICS OF THE NEUTRALIZING ANTIBODY RESPONSE TO THE VERO-CELL CULTURE DERIVED JAPANESE ENCEPHALITIS VACCINE, IC51

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Japanese Encephalitis is the most common viral encephalitis in Asia. An estimated 50.000 cases with 6.000 deaths are reported annually. In lack of an active treatment, vaccination is an important control measure. IC51 is a Vero cell-derived, SA_{14}-14-2 based Japanese Encephalitis vaccine that has been proven immunogenic and safe when administered i.m. in a Day 0, 28 schedule in adults. In the present study, immune response kinetics of the standard and a rapid immunization schedule were investigated. In this observer-blinded study, 374 subjects were randomized to receive either the standard schedule (2x6 mcg, Day 0/28), a single dose (1x6 mcg, Day 0) or the double dose (1x12 mcg, in two injections, Day0) of IC51. Immunogenicity was assessed by measuring anti-JEV neutralizing

antibodies. The primary endpoint for non-inferiority of the 1x12 mcg vs 2x6 mcg group was seroconversion rate (defined as anti-JEV neutralizing antibody titer ≥ 1:10) at Day 56. In a follow-up trial, booster doses were administered to subjects with antibody titers below the threshold for seroconversion. In the per protocol population, in the 2x6 mcg group GMTs and SCR were 8.4 and 21.1% and in the 1x12 mcg group 16.7 and 53.9% 10 days after the first vaccination. On Day 28, in the 2x6 mcg group GMTs and SCR were 11.2 and 39.8% and in the 1x12 mcg group 22.8 and 65.8%. On Day 35, 7 days after the second vaccination, GMTs and SCR had increased to 265.8 and 97.3% in the 2x6 mcg group but remained at 17.6 and 58.8% in the 1x12 mcg group who had received Placebo at day 28. On Day 56, GMTs and SCR were 218.0 and 97.3% in the 2x6 mcg group and 11.2 and 41.2% in the 1x12 mcg group. The results of this study confirm the standard schedule with a second dose of the inactivated JE vaccine (2x6 mcg). While the immune response kinetics in the 1x6 mcg and 1x12 mcg groups were similar, the second dose administered at day 28 in the 2x6 mcg group led to a rapid increase in antibody response, resulting in seroconversion rates of > 97% already one week after the second dose.

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PREVALENCE OF CANDIDIASIS AMONG WOMEN USING CONTRACEPTIVES IN BENIN CITY NIGERIA

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The risk factors of contraceptive use among women has been a factor limiting against its use one of which is the inconveniences posed and secondary microbial infection of which *Candida albicans* is a major agent. Moreover, candidiasis is very common among women particularly those of child bearing age. The determination of the risk of candidiasis infection among women using contraceptive becomes very significant to be able to support or discourage the use of contraseptives among women. A total of 120 women on contraceptives attending family planning clinic in University of Benin Teaching Hospital, Benin City, Nigeria were randomly recruited into the study.Their consent was verbally obtained and strict confidentiality was assured and Ethics Committee approval of the Ethics committee of the University of Benin Teaching Hospital were obtained before the commencement of the study. High vaginal swabs were collected from the women and culture for the isolation of *C. albicans*. Using routine methods of culture in Medical Microbiology Department of University of Benin Teaching Hospital Benin City, Nigeria. 52 species of *C. albicans* were isolated from all three specimens cultured, giving an overall prevalence of 45.38% The highest isolate of *C. albicans* women using injectable methods 83.33%, IUD 26.92%, diaphragm, 3%. Prevalence was also found to increase with the increase in duration of usage. We present in this study the prevalence of *C. albicans* infection am ong women using different types of contractseptives in Benin CIT, Nigeria.and that the prevalence is higher among those using inject able methods as compared with those using other methods.

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ANALYSIS OF GENETIC DIVERSITY WITHIN A STABLE ENZOOTIC FOCUS OF POWASSAN VIRUS IN NORTHERN WISCONSIN

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Deer tick-lineage strains of Powassan virus (POWV, *Flaviviridae:Flavivirus*) were originally isolated in 1996 from *Ixodes scapularis* collected in Connecticut. Since then, they have been isolated from deer ticks collected from dense infestations in several Northeastern and North Central US sites. The epidemiological significance of these strains (frequently termed Deer Tick virus - DTV) is not well characterized, but may be great: deer ticks efficiently maintain several important zoonotic pathogens, including the agents of lyme disease, human babesiosis and human granulocytic anaplasmosis. Recent reports suggest an increase in POWV/ DTV incidence over the last eight years. Powassan virus may therefore be an emerging tick-borne threat to human health in North America. Accordingly, we sought to determine whether the prevalence of POWV infection in deer ticks in a Northern WI focus changed significantly between 1997 and the present. In addition, we determined whether within-host genetic diversity of this agent is similar to West Nile virus (WNV, *Flaviviridae:Flavivirus*). Adult *Ix. scapularis* were collected in the Fall of 2007 at two sites outside of Hayward, Wisconsin. Pools of tick homogenates were tested for DTV by RT-PCR and ticks from the positive pools were analyzed individually. Virus isolation was attempted from RT-PCR positive ticks. We isolated POWV from 7/299 (2.3%) adult *Ix. scapularis*, including four females and three males. Analysis of viral genetic diversity within four ticks revealed that POWV has on average 0.010% mutations/nucleotide sequenced whereas WNV populations isolated from mosquitoes typically have twice this amount. These data strongly suggest that DTV/POWV is maintained in a stable enzootic focus in WI, and that POWV genetic diversity within ticks is restricted relative to WNV within mosquitoes.

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ONE STEP RT-PCR FOR DETECTION OF ZIKA VIRUS

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Zika virus (ZIKV) is a flavivirus transmitted by mosquitoes and circulating mainly in Africa and Asia where a major outbreak occurred in Micronesia in 2007. Human infection induces influenza like syndrome and retro-orbital eye pain, oedema, lymphadenopathy and diarrhea. In Senegal, ZIKV is regularly isolated by the entomological surveillance program of arbovirus in the South Eastern of the country while his human impact is unknown. Routine laboratory diagnosis for ZIKV are virus isolation or serological methods which are impaired by time consuming and cross-reactivity with others flaviviruses namely dengue, Chikungunya. The aim of this study was to develop a rapid, sensitive and specific RT-PCR method to detect ZIKV in human serum and cell culture medium. A set of sense and antisense primers was designed from conserved region among nucleotide sequence of the envelope gene of ZIKV. Thirty seven strains of ZIKV were used to validate the one step RT-PCR assay and confirm with nucleotide sequencing. Thirty one strains of 19 other flavivirus were tested for the specificity. Serial dilutions were tested for the sensitivity of the assay. ZIKV RNA was detected in all strains. The assay was specific for ZIKV, since no amplification was detected in other strains of flavivirus. The amplicons sequence showed 92 to 99 % nucleotide sequence homology with the published sequence of ZIKV Uganda 1947 strain MR-766 (AY632535). For sensitivity assay, the detection limit of the method was 7.7 pfu/reaction in serum and cell culture medium. The one step RT-PCR assay described here is rapid, specific and sensitive method for detecting ZIKV strain. This assay could improve differential diagnostic of ZIKV from other co-circulating arbovirus since the clinical symptoms are not specific. Further studies using human serum naturally infected by ZIKV are needed to validate it. To improve detection of ZIKV, a real time PCR method is ongoing.

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ONE STEP RT-PCR FOR DETECTION OF ZIKA VIRUS

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Zika virus (ZIKV) is an emerging mosquito-borne flavivirus circulating in Asia and Africa. Human infection induces an influenza-like syndrome that is associated with retro-orbital pain, oedema, lymphadenopathy or diarrhea. Diagnosis of Zika fever relies on virus isolation and serology,

which are time consuming or cross-reactive. The objecive of this study was to develop a one-step RT-PCR assay to detect ZIKV in human serum. An assay targeting the envelope protein coding region was designed and evaluated for its specificity, detection limit, repeatability, and capacity to detect ZIKV isolates collected over a 40 year period from various African countries and hosts. The assay's detection limit and repeatability were respectively 7.7 pfu/reaction and 100% in serum and L-15 medium, while none of 19 other flaviviruses tested were detected. In conclusion, the assay is rapid, sensitive and specific to detect ZIKV in cell culture or serum, but needs to be validated for diagnosis using clinical samples.

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THE BLOOD-BRAIN BARRIER IN THE CEREBRUM IS THE INITIAL SITE FOR THE JAPANESE ENCEPHALITIS VIRUS ENTERING THE CENTRAL NERVOUS SYSTEM

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Japanese encephalitis (JE) virus is a member of the encephalitic flaviviruses and frequently causes neurological sequelae in a proportion of patients who survive the acute phase of the infection. In the present study, we molecularly identified viral infection in the brain of mice with rigidity of hindlimbs and/or abnormal gait, in which JE virus particles appeared within membrane-bound vacuoles of neurons throughout the central nervous system. Deformation of tight junctions (TJs) shown as dissociation of endothelial cells in capillaries, implying that the integrity of the blood-brain barrier (BBB) has been compromised by JE virus infection. BBB permeability evidently increased in the cerebrum, but not in the cerebellum, of JE virus-infected mice intravenously injected with the tracer of Evans blue dye. This suggests that the permeability of the BBB differentially changed in response to viral infection, leading to the entry of JE virions and/or putatively infected leukocytes from the periphery to the cerebrum as the initial site of infection in the central nervous system (CNS). Theoretically, the virus spread to the cerebellum soon after the cerebrum became infected.

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YELLOW FEVER VACCINE VIRUS AND IGM ANTIBODY DETECTION IN URINE AND CEREBROSPINAL FLUID IN PATIENTS WITH YELLOW FEVER VACCINE-ASSOCIATED VISCEROTROPIC DISEASE

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In August 2007 four lethal cases of yellow fever vaccine-associated viscerotropic disease occurred in Ica region, amongst 42,742 persons immunized against yellow fever with a vaccine from a batch using the 17DD strain, and there also was a non-lethal case in Lima in a person immunized against yellow fever who received a vaccine from a batch using the 17D-204 strain. We report these findings considering that there has never been any report indicating presence of yellow fever (YF) virus and IgM antibodies against YF in urine and cerebrospinal fluid (CSF) in YF and in yellow fever vaccine-associated viscerotropic disease cases.We determined the presence of YF virus in serum, urine, and tissue samples, as well as the presence of IgM anti-YF antibody in serum and urine from four patients who died because of yellow fever vaccine-associated viscerotropic disease, three women 23-, 24-, and 49- years old, and a 79- years old male patient, respectively. The antibody was also found in CSF of a 60-year old woman who developed a neurological condition but survived. RT-PCR and cultures in C6-36 cells were used for detecting YF virus; and a MAC-ELISA test was used for detecting IgM-anti YF antibody. The vaccine virus was found in the first case using RT-PCR in serum, urine, and tissues (liver, brain and kidney); and IgM anti-YF was found in serum. The vaccine virus was found in the second case using RT-PCR in serum, tissues (liver and kidney), and urine; and IgM anti-YF was found in serum and urine. The third case was positive for YF using RT-PCR testing in liver tissue. The fourth case was positive for yellow fever using RT-PCR testing in serum and liver tissue, and IgM anti-YF was found in serum. In the 60 year woman we determined the presense of YF virus using RT-PCR and IgM YF antibody in CSF. Histopathology using H-E and immunohistochemistry was compatible with yellow fever in the four fatal cases. In conclusion, this is the very first time that the presence of the vaccine virus and IgM anti-YF antibody is reported in urine and CSF from patients with yellow fever vaccine-associated viscerotropic disease or patient with neurological condition, and this may be useful for making a diagnosis according to the time with the disease.

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TH1/TH2 DIFFERENTIATION IN CHRONIC AND RECURRENT AMERICAN CUTANEOUS LEISHMANIASIS AND ASYMPTOMATIC INFECTION WITH *LEISHMANIA VIANNIA PANAMENSIS*

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The pathogenesis of dermal leishmaniasis is immunologically mediated. Contrary to the polarized response in susceptible and resistant mouse models of cutaneous leishmaniasis, a mixed Th1/Th2 cytokine response characterizes asymptomatic human infection as well as non-healing disease caused by *Leishmania* of the *Viannia* subgenus. The cell populations participating in this mixed Th1/Th2 response and their relative contribution to the secreted cytokine profile and outcome of infection is unknown in American cutaneous leishmaniasis. The obective of this study was to determine whether the proportion and phenotype of cells producing Th1 and Th2 cytokines and the cytokines secreted in response to *L. panamensis* distinguishes asymptomatic infection and non-healing disease. IFNγ, TNFα, IL-10 and IL13 producing cells from endemically exposed asymptomatic donors, patients with active chronic and recurrent lesions and healthy controls were identified in mononuclear cells responding to live *Leishmania panamensis* promastigotes *in vitro*. Cells were co-cultured with promastigotes during 72 hours in the presence or absence of hIL-2r. Intracellular cytokines and cell phenotype were determined by multiparameter flow cytometry. In parallel, secreted cytokines were quantified in supernatants by ELISA. A higher proportion of IFNγ producing CD4+ and CD8+ LT were observed in active chronic disease (P<0.05) and asymptomatic infection than healthy controls. The proportion of IL-10 producing CD8, and CD4 LT producing TNFα was significantly higher for active chronic disease than asymtomatic infection (P<0.05). Exposure to live *Leishmania* resulted in loss of expression of CD14 by macrophages. IL-10 and TNFα were mainly produced by CD14 negative cells. Patients with chronic dermal leishmaniasis presented significantly higher proportions of cells secreting proinflammatory IFNg and TNFa and anti-inflammatory IL-10 in response to live *Leishmania* than Asymptomatically infected individuals. CD8 lymphocytes appear to be the principle source of IL-10 and CD4 lymphocytes and macrophages the source of TNFα in active Chronic disease.

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IDENTIFICATION AND CHARACTERIZATION OF SECRETED PROTEINS OF *LEISHMANIA CHAGASI*

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Visceral leishmaniasis (VL) affects 500,000 new individuals each year, primarily in less developed countries. It is caused by vector-borne

protozoan parasites *Leishmania donovani* and the synonymous organisms *L. infantum* and *L. chagasi (Lci)*. It is well known that excreted/secreted (ES) proteins of pathogenic organisms often play a major role in successful establishment of the pathogen within the host, by facilitating invasion of host cells, immunomodulation, maintenance of the pathogen in hospitable host cell compartments, and nutrient acquisition. Despite their potential to provide insight into the mechanisms by which the parasite survives in the host, ES proteins of *Lci* have not been extensively studied. Therefore, the *L. infantum* genome was screened using a set of standard algorithms to predict the suite of ES proteins of *Lci*. Five of the identified candidate genes were chosen for further study based on their putative function or their homology to known virulence proteins of other pathogens. The genes encode a P1/S1 nuclease, a peptidyl-prolyl cis-trans isomerase (PPIase), a protein disulfide isomerase (PDI), a cathepsin L-like protease, and a surface antigen-like protein. The P1/S1 nuclease homolog has been shown to be secreted in *L. donovani* and hence is the positive control of our system of ES protein identification. The PPIase has been shown to be secreted from, and required for virulence of *Trypanosoma cruzi*. Both PDI and the cathepsin proteases have been implicated in *Leishmania* virulence, and the extracellular presence of cathepsins in *L. donovani* has been suggested. Genes encoding these proteins were cloned into the pDEST17 *E. coli* expression vector and expressed by induction with arabinose. Rat antisera has been successfully raised against the P1/S1 nuclease, and sera against the remaining proteins are being generated. These antibodies will be used to analyze gene expression in different life stages of *Lci*, to confirm their secretion, and for intracellular localization of the secreted proteins within the infected macrophage.

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IDENTIFICATION, CHARACTERIZATION, AND EVALUATION OF THE *TRYPANOSOMA BRUCEI* CA^{2+} CHANNEL (TBCC1) AS A POTENTIAL DRUG AND VACCINE TARGET

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Trypanosoma brucei is a protozoan parasite that causes Human African Trypanosomiasis (HAT, sleeping sickness). 2-300 million people are affected by this disease and there are an estimated 50,000 deaths annually in Sub-Saharan Africa. Thus, new drugs or an effective vaccine that targets membrane proteins and is capable of protecting against infection are sought. Existing drugs used to treat HAT are toxic and often times lethal and vaccines developed against HAT have been unsuccessful due to parasite evasion of the host's immune system by antigenic variation. A pilot *in vitro* drug study using commercially available Ca^{2+} ATPase inhibitors inhibited parasite proliferation and survival at micro Molar concentrations. However, this inhibition inadequately suppressed proliferation in the long term. Further analysis indicated that parasites could possibly use Ca^{2+} channel(s) to offset inhibition of the Ca^{2+} ATPases. Molecular bioinformatics analysis indicated the presence of a putative L-type *T. brucei* Ca^{2+} channel (TBCC1) which was located in pericellular and flagellar pocket regions by immunocytochemistry. The hypothesis is that inhibition of the *T. brucei* Ca^{2+} channel by L-type Ca^{2+} channel blockers or anti-TBCC1 antibodies induced via a novel *Vibrio cholerae* ghosts vaccine TBCC1 construct will interfere with $[Ca^{2+}]$ homeostasis and proliferation in parasites. To test this hypothesis we performed drug inhibition assays using Ca^{2+} channel blockers: Nifedipine, Nimodipine, and Verapamil, and Pentamidine as control against blood stage parasites *in vitro*. Mice were vaccinated with or without recombinant *V. cholerae* ghosts expressing TBCC1 to assess the level of antibody and cytokine production and subsequently challenged with *T. brucei* to assess parasitemia and survival. Results indicate that Nifedipine, Nimodipine, and Verapamil are potent inhibitors of *T. brucei* at μMolar concentrations that are comparable with Pentamidine controls. This novel shot gun approach to testing trypanostatic drugs or antibodies may lead to development of new drugs and vaccines against HAT.

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LUTZOMYIA LONGIPALPIS RECOMBINANT SALIVARY YELLOW-RELATED PROTEIN (LJM11) CONFERS PROTECTION AGAINST LEISHMANIA INFECTED SAND FLIES

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Sand fly salivary proteins are injected in the host skin during blood feeding. Some of these molecules have been shown to be immunogenic. Previous work has shown that immune response to sand fly bites or salivary gland homogenate from *Phlebotomus papatasi* conferred protection against *Leishmania major* infection. The protection was correlated with a delayed type hypersensitivity (DTH) response in the presence of IFN-γ. In the present work, we vaccinated C57BL/6 mice with LJM11 (44 kDa salivary protein) recombinant protein from *Lutzomyia longipalpis* saliva, a molecule able to induce DTH in mice. The recombinant protein was obtained using a mammalian cell expression system yielding a soluble protein. C57BL/6 mice were vaccinated intradermally, three times at two weeks intervals with 500 ng of LJM11. *Lu. longipalpis* can be colonized with *L. major*. We tested if vaccination with LJM11 could protect mice against bites by *L. major*-infected *Lu. longipalpis* sand flies. LJM11 vaccinated mice controlled the parasite load in comparison to a control group. Furthermore, spleen cells from mice pre-exposed to *L. longipalpis* bites were restimulated with LJM11 protein and induced IFN- γ production *in vitro*. The identification and characterization of a sand fly salivary protein able to protect against infected sand fly bites validates the development of sand fly salivary-based vaccines against leishmaniasis.

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EFFECT OF THIADIAZOLE AND ARIL-SYDNONE DERIVATIVES ON A CONSTITUTIVE NITRIC OXIDE SYNTHASE OF *LEISHMANIA AMAZONENSIS*

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Nitric oxide synthase (NOS) is an enzyme that has been much studied. This enzyme competes by L-arginine with arginase because both use it as substrate. This enzyme catalyzes the hydrolysis of L-arginine to L-citrulline and nitric oxide (NO). Data from our laboratory revealed the existence of the NO pathway and a constitutive NOS isoform (cNOS) in *Leishmania* sp. was identified previously. In *L. amazonensis*, cNOS is essential during parasite-macrophage interaction, as reported previously. This pathway has been regarded as promising target for experimental drug therapy anti-*Leishmania*. The purpose of his study was to verify the effect of mesoionic compounds (thiadiazole and aril-sydnone derivatives) on the cNOS-*L. amazonensis* activity. *L. amazonensis* (MHOM strain / LTB 0016) were cultured (promastigotes to 26 ºC in Schneider's medium / pH 6.9 / 10% of fetal bovine serum and axenic amastigotes to 32 ºC in Schneider's medium / pH 5.5 / 20% of fetal bovine serum) in absence/presence of three thiadiazoles (MISAL-R=OCH_3, NO_2 and H) and three sydnones (SID-R = OCH_3, NO_2 and H). After 24 hours of incubation, nitrite (μM) was measured by Griess reaction, five times, in the supernatant obtained after centrifugation at 1000 x g at 4ºC for 15 min. For promastigotes, compared with the untreated control group (1.0μM±0,001), results obtained in tests with thiadiazole derivatives were 0μM for all compounds. For sydnone derivatives (SID-R=OCH_3, NO_2 and H), results for promastigote were 0.3μM±0.002, 1.5μM±0.001 and 0.3μM±0,006 respectively. For axenic amastigotes, results, compared with the untreated control (4 μM±0.002), were 2.23μM±0.002, 1.73μM±0.003 and 1.61μM±0.006 for cultures treated with thiadiazole derivatives (MISAL-R = OCH_3, NO_2 and H) and 2.23μM±0.007, 1.48μM±0.002 and 0.49μM±0.005 for sydnone derivatives (SYD-R = OCH_3, NO_2 and H). In conclusion, the thiadiazoles

and aril-sydnones derivatives used in this work were able to inhibit significantly the cNOS-*L. amazonensis* activity. Thus, new tests are being conducted to evaluate the effect of these compounds on the inducible NOS of macrophage, seeking to verify the possible effect of them on the modulation of cNOS- *L. amazonensis* / iNOS-macrophage during the infection.

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THERAPEUTIC AND IMMUNOLOGICAL EFFECTS OF PYRAZOLE CARBOHYDRAZIDES DERIVATIVES ON THE MOUSE MODEL OF *LEISHMANIA AMAZONENSIS* INFECTION

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Leishmaniasis is an important parasitic disease in the tropical and subtropical regions of the world. The derivatives pyrazole carbohydrazides are synthetic compounds with *in vitro* anti-*Leishmania* activity. In this study, the 1-(4-X-phenyl)-N`-[(4-Y-phenyl) methylene]-1*H*-pyrazole-4-carbohydrazides were investigated concerning immunological and therapeutic effects on mouse model infection of leishmaniasis. The animals infected with *Leishmania amazonensis* and treated with those compounds were evaluated trough different assays such as: body weight, amine transferases and creatinine levels, leukometry, *Leishmania* specific anti-body levels, cytokines, PGE production, cutaneous lesion size and parasitic burden. In order to evaluate the toxicity, the possible immunoregulation and therapeutics effects of compounds, no infected mice were also treated. *Leishmania* specific anti-body levels were assayed in plasma by ELISA methodology. Nitric oxide (NO) production was measured by Griess reagent, while cytokines and PGEs were also evaluated by ELISA in cells culture supernatant from infected/treated-CBA. Toxicity parameters as the body weight, plasmatic concentrations of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST) or urine-creatinine levels were not affected, after oral administration. It was observed that treatment with those compounds controlled footpad cutaneous lesion evolution and parasite dissemination to draining lymph node, and also promoted a drop of blood neutrophils. They have therapeutic action by controlling lesion size and parasitic burden development comparable with ketoconazole, the reference drug. *Leishmania* specific anti-body levels were analyzed by ELISA and the results would suggest an important immunomodulation in Th1 and Th2 pathway and nitric oxide concentrations which were elevated after treatment. Cytokines levels and PGE production have been assayed to observe the immunomodulatory and anti-inflammatory effects of those compounds. These results provide new perspectives on the development of drugs with activity against leishmaniasis.

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CHARACTERIZATION OF THE EARLY INFLAMMATORY RESPONSE TO BITES OF *LEISHMANIA MAJOR* INFECTED *PHLEBOTOMUS DUBOSCQI* SAND FLIES IN NAÏVE AND PRE-EXPOSED MICE

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During bloodfeeding, infected sand flies inoculate the parasite into the skin together with a variety of molecules that are capable of modulating the host's immune response. Previous studies have shown that mice pre-exposed to sand fly saliva develop a delayed type hypersensitivity response (DTH) at the site of a recall response to saliva that was correlated to protection against *Leishmania* infection. Here, we further explore the early inflammatory response in the skin of mice resulting from the bite of *Leishmania* infected sand flies. Naïve and pre-exposed (10 uninfected *Phlebotomus duboscqi* bites three times at one week intervals) C57BL/6 mice were challenged with bites of 8-10 *P. duboscqi* infected with *L. major*. Mice were sacrificed at different time points to follow the kinetics of the inflammatory response. Skin cells were recovered from the ear for phenotypic characterization of leukocytes by flow cytometry, and RNA was extracted and hybridized to a macroarray (Oligo GEArray® Mouse Inflammatory Cytokines and Receptors) to identify cytokines and chemokines pertinent to this response. Two-six hours post bite, naïve mice showed an increased expression of chemokines that attract macrophages, granulocytes and NK cells (MCP-1, MCP-3, eotaxin, KC, IP-10, GRO-β, PF-4) while pre-exposed mice showed an increased expression of chemokines related to the migration of NK cells, macrophages and granulocytes but also to the recruitment of activated T cells and dendritic cells (TCA-3, BRAK, ELC, I-TAC). The early expression of these chemokines reflected the phenotype of cells detected subsequently at 24 hours post bite. There was an increased presence of macrophages (12.9% vs. 9.6%) and CD4+ T cells (24% vs. 4.3%) in the pre-exposed compared to the naïve group. Our results suggest that the nature and kinetics of cytokines and chemokines are altered by anti-saliva immunity thus influencing the development of anti-*Leishmania* immunity. Understanding these early events will help clarify the mechanism and key immune molecules involved in saliva-induced protection against *Leishmania* parasites.

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EVALUATION OF THE CHRONIC PHASE IN DOGS NATURALLY INFECTED BY *TRYPANOSOMA CRUZI*

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Chagas disease is caused by *Trypanosoma cruzi* and is a major parasitic disease. The disease develops through three phases including the acute, followed by asymptomatic and symptomatic chronic phases. Dogs are considered a good experimental model for the study of Chagas disease. However, little is known about the presentation of natural infection with *T. cruzi* from lineage I of Mexico. Thus we examined 9 naturally infected *T. cruzi* seropositive and 10 seronegative dogs for clinical-pathological, immunological and parasitological evaluation. High lymphocyte and low monocyte counts were observed in peripheral blood of seropositive dogs. Electrocardiograms indicated alterations in 3/9 seropositive dogs, including right bundle branch block (RBBB), sinusal block and QRS complex changes. These 3 seropositive dogs were considered in the symptomatic chronic phase. High levels of IgG2 antibodies compared with low levels of IgG1 suggested a bias towards a Th1 immune response in seropositive dogs. Visceromegalia, megaesophagus, megacolon were not observed in seropositive or negative dogs at the necropsy and only one seropositive animal presented cardiomegaly. Histopathologic analysis of heart sections revealed inflammation, particularly in the right ventricule and the septum wall. This is the first evaluation of *T. cruzi* natural infection in dogs in Mexico, which provides a good framework for the practicing veterinarians as well as for the further use of this animal model.

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TRYPANOSOMA CRUZI STRAINS INDUCED DIFFERENTIAL DETACHMENT OF THE PLACENTAL TROPHOBLAST THROUGH OXIDATIVE STRESS AND COULD PARTICIPATE IN THE CONGENITAL CHAGAS INFECTION

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Chagas disease is caused by *Trypanosoma cruzi* and can be transmitted through placenta causing congenital infection. The mechanisms, by this infection occurred, remains as elusive knowledge. Maternal conditions, immunological competence and structure integrity of placenta and strains of *T. cruzi* could contribute to infect intrauterine concepts. The objectives of this study were: a) To analyse infection and structural alteration of chorionic villi with different *T. cruzi* strains *in vitro*. b) To correlate Nitric Oxide (NO) production, endothelial Nitric Oxide Synthase (NOSe) expression and nitrosylation rate with infection and parasite viability. Placental villi explants co-cultured for 24 h with $1x10^6$ trypomastigotes of Tulahuen and Lucky strains (isolated from a congenital case). Histological and immunohistochemical analysis: of NOSe and Nitrotyrosine (NT); semiquantification of RNAm of NOSe; measurement of amastigotes per nest, infection areas, detachment of syncytiotrophoblast. In culture media: quantification of NO, hCG and live parasites. Both strains had a similar area of infection and amastigotes per nest ($p>0.05$). Percentage of live parasites in co-culture supernatants was significantly higher with the congenital strain than with Tulahuen. Explants co-cultured with the Lucky strain showed higher detachment of STB, smaller CTB proliferation and hCG levels than controls; Tulahuen co-cultures showed higher detachment of STB and CTB proliferation and decreased levels of hCG than controls. Nitrites concentration did not show significant differences ($p>0.05$), but NOSe and NT positive areas were higher than controls and NOSe RNAm amount was greater than controls. In conclusion, *T. cruzi* does not produce sustained infection in placental tissue, and promotes an increment in oxidative stress probably associated with a rise in NO, NOSe transcription and translation. These findings could be involved in the alterations of villi structure such us STB detachment. These phenomena and the differential *T. cruzi* stocks survival, could be related to a successful infection and some clinical forms of the congenital Chagas disease.

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TRYPANOSOMA CRUZI UP-REGULATES HUMAN DEFENSIN α-1 IN EPITHELIAL CELLS TO CAUSE TRYPANOSOME MEMBRANE PORE FORMATION AND REGULATE CELLULAR INFECTION

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Human defensins play a fundamental role in the initiation of innate immune responses to some microbial pathogens. Here we show that trypomastigotes up-regulate human defensin α-1 expression in epithelial cells to regulate cellular infection. Human defensin α-1 displays a trypanocidal role against trypomastigotes and amastigotes via apoptosis. The toxicity is mediated by membrane pore formation, membrane blebbing and induction of DNA fragmentation resulting in reduction of trypanosome infection in human cells. Human defensin α-1 significantly reduced trypomastigote motility and viability. Human defensin α-1 enters the trypanosome when membrane pores are present and is associated with later intracellular damage and rupture of the flagellar axoneme. Trypanosome membrane depolarization abolished the toxicity of defensin α-1 against the parasite. Pre-incubation of trypomastigotes with sub-lethal concentrations of defensin α-1 followed by exposure to human epithelial cells significantly reduced *Trypanosome cruzi* infection in these cells. Thus, human defensin α-1 is an innate immune molecule that causes severe toxicity to *T. cruzi* and plays an important role in reducing cellular infection. This is the first report showing that human defensin α-1 causes membrane pore formation in a human parasite leading to trypanosome destruction.

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GENETIC POLYMORPHISM IN THE VISCERALIZING GENE SEQUENCE OF *LEISHMANIA TROPICA* ISOLATED FROM THE SOLDIERS RETURNING FROM IRAQ

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Human leishmaniasis is manifested by three different forms; cutaneous, visceral and mucocutaneous. *Leishmania tropica* is primarily responsible for the cutaneous leishmaniasis in the Old World. In addition to its usual cutaneous manifestation, *L. tropica* has been reported as following a different tropism behavior and results in visceralizing disease in some patients. In order to see if there was a genetic factor behind this tropism behavior, a visceralizing gene was sought. This study was undertaken to find out genetic differences in a visceraling gene and its possible relationship in the tropic behavior using recently isolated strains of *L. tropica* isolated from soldiers returning from Iraq. Primer for the visceraling gene region was designed and used to amplify DNA from samples, isolated and maintained in our Leishmania Diagnostic Laboratory. The species diagnosis of all the representative strains used in this study, were confirmed by isoenzyme analysis before they were cryo-preserved. The gene was cloned from the representative samples of *L. tropica* and sequenced to find the sequence similarity among *L. tropica* strains and compared with *L. major*. Genetic variation in the visceraling gene sequence of *L. tropica* were found which indicates that more than one haplotype is present and it is polymorphic in nature. It is still not clear if any particular haplotype is responsible for the tropism changes from cutaneous to visceral or vice-versa. More studies are underway using additional samples to find a possible link between them.

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STUDY OF TRYPANOSOMATID VIRULENCE FACTORS USING BIOINFORMATIC AND EXPERIMENTAL APPROACHES

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The main three trypanosomatid parasites causing human disease, *Leishmania sp.*, *Trypanosoma cruzi* and *T. brucei* are protozoa that complete their life cycle in an insect vector and a variety of vertebrate hosts. These pathogens have developed various strategies to modify their environment, influence host immune responses, or invade target cells. Materials secreted by these parasites are involved in such processes and may represent targets for vaccines and rational drug design. Taking advantage of the recently sequenced genomes of these three trypanosomatids, we designed an experimental approach based on bioinformatic analyses to identify hypothetical conserved trypanosomatid proteins involved in the endoplasmic reticulum/Golgi-dependent secretory pathway. The method we designed allowed us to identify three new trypanosomatid conserved proteins, demonstrating the utility of this approach for the identification of *bona fide* secreted proteins by trypanosomatids. Current studies of the biological properties of these proteins suggest that some are directly involved in a process increasing survival and replication of the parasite inside its target cell.

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IDENTIFICATION OF *PLASMODIUM* GENES INVOLVED IN THE PROTECTIVE PRE-ERYTHROCYTIC IMMUNE RESPONSE

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Several lines of evidence support the feasibility of producing an anti-malarial vaccine targeting the pre-erythrocytic stages of *Plasmodium*. For example, immunization with radiation attenuated sporozoites (RAS) of *Plasmodium* produces sterile protective immunity, in humans and rodents. Infected hepatocytes are targeted by this immune response, suggesting infected hepatocytes have protective *Plasmodium* antigens presented by MHC molecules on their surface. Recently, using the *P. yoelii* murine malaria model, activation of naïve CD8+ T cells was shown to occur within 24hrs after RAS immunization; primarily in the lymph nodes draining the site of RAS injection. Since sporozoites are the only parasite form with access to these areas, the protective antigens must be expressed by RAS before and after sporozoite entry into hepatocytes, up until parasite developmental arrest. Furthermore, wild-type sporozoites given to mice under chloroquine treatment can illicit protection from wild type sporozoite challenge, suggesting that wild-type sporozoites, to a variable degree, are immunogenic. Together these observations imply that the *Plasmodium* antigens important in the protective anti-*Plasmodium* immune response are expressed by RAS, wild-type sporozoites, and liver stage parasites. The long term goal of this research project is to identify *Plasmodium* antigens involved in the protective pre-erythrocytic immune response. The central hypothesis of this project is that comparison of the transcriptomes of the pre-erythrocytic stages of *Plasmodium* will identify a set of commonly expressed genes among which will be the protective anti-plasmodial antigens. To test this hypothesis, the global gene expression of early and late liver stage parasites, RAS, and wild type sporozoites will be compared using a whole genome *P. yoelii* microarray. Preliminary microarray analysis of early (24hr) and late (48hr) *P. yoelii* liver-stage parasites has revealed ~500 differentially regulated genes.

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REAL-TIME *IN VIVO* IMAGING OF LIVER STAGES OF *PLASMODIUM YOELII*: GFP/LUCIFERASE REPORTER PARASITES

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The health and socioeconomic impact of malaria is rising due to the spread of drug resistant parasites, and the lack of an effective vaccine. After *Plasmodium* sporozoites are deposited in the dermis by a bite of an infected mosquito, they go through an obligatory development stage in hepatocytes. When this stage is blocked, clinical manifestations and transmission are prevented. Moreover, infections by sporozoites induce protective immunity thus studies exploring intra-hepatocytic stages are the current standard for vaccine development. However, identification of new drug targets and vaccine candidates are hindered by the limited number of tools available to evaluate the development of liver stages *in vivo*. In an effort to overcome this obstacle, we are developing a more efficient method to study *Plasmodium* liver stages using bioluminescence imaging (BLI). We have generated *P. yoelii* YM parasites (rodent model) that express firefly luciferase under a constitutive promoter (PyLuc). PyLuc parasites complete the life cycle in both mice and mosquitoes while maintaining the expression of luciferase. These parasites have similar growth and virulence patterns to wild type *P. yoelii* YM. Using BLI, we can visualize PyLuc dissemination *in vivo* in erythrocytic stages. In addition, for the first time, we can image intra-hepatocytic stages 44 hours after infection of mice with PyLuc sporozoites. The signal correlates with parasite load as confirmed by real time qRT-PCR of *P. yoelii* 18s rRNA, amplified from total RNA extracted from livers of mice infected with PyLuc sporozoites. This bioluminescence signal is undetectable when mice are treated at the time of infection with Atovaquone. On the contrary, bioluminescence is detectable when PyLuc infected mice are treated with Chloroquine, which has no effect in liver stages. The bioluminescence signal is quantifiable, thus PyLuc can also be used to evaluate partial effects in liver stages, an important aspect in drug and vaccine development. Furthermore, host immune factors affecting hepatocytic development can be explored. From the above observations, we propose that PyLuc parasites will provide a powerful tool that will lead to greater understanding of *Plasmodium* intra-hepatocytic stages. In addition, PyLuc parasites and BLI will contribute to efforts in identifying new effective chemotherapy and vaccine candidates to combat malaria infections.

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EFFECT OF CHLOROQUINE, METHYLENE BLUE AND ARTEMETHER ON THE HEPATIC OXIDATIVE STRESS AND ANTIOXIDANT DEFENCE SYSTEM OF *PLASMODIUM YOELII NIGERIENSIS*-INFECTED MICE

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Malaria is a major public health problem in the tropics. Malaria, like other infection activates the immune system of the body thereby causing release of reactive oxygen species (ROS) as an antimicrobial action. During malaria infection, both host and parasite are under oxidative stress. Haemoglobin degradation by the malaria parasite produces the redox active by-products, free haem and hydrogen peroxide, conferring oxidative insult on the host cell. Most antimalarials are thought to be pro-oxidative in action, thus affecting the antioxidant defense system of both host and parasite. However, little is known of the effect of these drugs on the cellular antioxidant defense system and extent of lipid peroxidation in the hepatic tissues of the host during malaria chemotherapy. This study therefore aims at evaluating the antimalarial efficacy of chloroquine (CQ), methylene blue (MB) and artemether (ART) plus their effect on the malondialdehyde (MDA) level, glutathione (GSH) level and glutathione-S-transferase (GST) activity in hepatic tissues of the host during *P.yoelii* infection. One hundred and twenty mice were grouped into six treatment groups and CQ (10mg/kg), MB (10mg/kg) or ART (4mg/kg) was administered to both the infected and uninfected mice for three consecutive days after established *P. yoelii* infection. Two groups of animals were used as positive (with malaria) and negative (without malaria) controls respectively. Lipid peroxidation and antioxidant status were determined in liver samples using standard procedures. CQ, MB and ART caused significant increase (CQ→MB →ART) in MDA level in both infected and uninfected mice. Similarly, GSH level and GST activity increased during administration of the three drugs in both *P. yoelii*-infected and uninfected mice. In conclusion, malaria infection as well as CQ, MB and ART induce oxidative stress and disrupt the antioxidant defense system of the host.

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ERYTHROCYTE INVASION AND VARIATION IN MEROZOITE LIGAND GENE EXPRESSION IN *PLASMODIUM FALCIPARUM*

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Specific receptor-ligand interactions involved in the invasion of erythrocytes are central to malaria parasite replication and virulence. By changing the levels of expression of some of these ligands, such as the erythrocyte binding antigenic (EBAs) proteins and reticulocyte binding protein homologues (Rh), cultured adapted *Plsasmodium falciparum* lines have been shown to use different erythrocyte receptors to mediate alternative pathways of invasion. In a case-control study of severe and mild malaria in The Gambia we have determined erythrocyte invasion

phenotypes and the expression profiles of the *eba* and *rh* ligand genes of 166 clinical isolates. Considerable heterogeneity was seen in the invasion profiles, and parasites from mild malaria controls were more dependent on trypsin-sensitive receptors than those from severe malaria cases. Expression profiles showed a high degree of variation with distinct clusters indicating coordinated expression among ligands. There were no significant associations between expression profiles and invasion pathways or disease severity, suggesting that variant expression of merozoite ligands may be a means to escape acquired immune responses rather than defining alternative virulence phenotypes.

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HIGH-THROUGHPUT, QUANTITATIVE DISSECTION OF INTRA-ERYTHROCYTIC GROWTH OF THE HUMAN MALARIA PARASITE, *PLASMODIUM FALCIPARUM*, USING FLOWCYTOMETRY

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Accurate measurement of parasite growth is of utmost importance in malaria research while studying the growth-inhibitory effects of therapeutic compounds, inhibitory antibodies and genetic manipulations. While several methods, other than the standard isotopic hypoxanthine assay, have been recently described to measure parasite growth rates, they all fail to address the biological phenomena underlying the observed growth defects. Here, we have modified previously described flow cytometry-based protocols for quantitative analysis of parasite growth. Important attributes of this new protocol include: (1) precise estimation of parasite growth rates; (2) calculating the length of the parasite asexual cycle; and (3) determining the erythrocyte invasion efficiency of daughter merozoites generated at the end of the asexual cycle. We were also able to adapt our methods to a high-throughput, automated system, thereby allowing screening of multiple parasite clones simultaneously. Such thorough evaluation of *Plasmodium falciparum* growth defects will reveal critical information about parasite biology that will contribute significantly towards designing novel antimalarials.

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GAMETOCYTOGENESIS IN *PLASMODIUM FALCIPARUM*

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Even though the first malaria parasite ever seen by the human eye was an exflagellating male gametocyte, remarkably little is known about the cell biology of this stage of the parasite's life cycle and of the developmental changes leading to the production of gametocytes within the human host. In particular, we know little about what triggers the switch from the asexual pathway to the production of the sexual stages within the host's blood stream in a process termed gametocytogenesis. While there is overwhelming evidence that commitment to gametocytogenesis relies on a switch which is sensitive to environmental stimuli and is therefore dependant on a signaling mechanism between the environment and the parasite with input into pathways leading to transcriptional control, we know little of how this signaling pathway works. Studies on such pathways in *Plasmodium falciparum* have previously been hampered by the lack of a robust model for detecting their activity. The differentiation pathways that are activated during gametocytogenesis lead to morphological changes that provide a scorable phenotype and are not essential for parasite proliferation. Standard gametocyte assays have not been suitable for the study of early gametocyte biology. Therefore we have developed a new approach; a high throughput assay that allows us to rapidly quantify and detect changes in commitment to gametocytogenesis. We are using this as a reporter system to biochemically elucidate components of the parasite's intercellular signaling and to determine the point in the parasite life cycle at which commitment to gametocytogenesis occurs.

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DIVERSITY OF *PLASMODIUM FALCIPARUM* PLASTOME IN GAMBIAN ISOLATES

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Research towards mapping the population genetic structure of the malaria parasite is being revolutionized by advances in genome sequencing and analysis. Towards more strategic sampling for extensive genome wide surveys of natural parasite populations, basic information on the population structure and evolution will be relevant. In view of this, we are analysing the 35kb apicoplast (plastid) genomes of different *Plasmodium falciparum* natural populations. Plastid genome sequence information will also be vital for intra-specific phylogenetics as it could resolve malaria population sub-structure and evolution otherwise obscured by high levels of diversity and recombination in mitochondria and nuclear sequences respectively. We amplified and sequenced the plastid genome from 16 *P. falciparum* isolates (8 wild isolates from the Gambia and 8 laboratory cultured isolates). Of 29.430bp sequenced (excluding the inverted repeat), contig lengths of wild isolates ranged from 29,415 to 29,425bp. The *Plasmodium* plastome has very limited diversity, including a number of nucleotide substitutions, small insertions, deletions and repeats. We have identified 6 nucleotide polymorphisms that are non-singletons and 24 putative indels. A microsatellite (TAA) size polymorphism (16-22 bp) was found on the RNA polymerase gene, rpoD. We will also analyse plastid sequences of *P. falciparum* isolates from west, central and eastern Africa and test for recent phylogenetic structure and signatures of population explosion.

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INFLUENCE OF THE PREGNANCY-ASSOCIATED HORMONE HUMAN CHORIONIC GONADOTROPHIN ON GROWTH OF *PLASMODIUM FALCIPARUM IN VITRO*

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Pregnant women are more likely to be infected with *Plasmodium falciparum* than non-pregnant women. Pregnancy-associated hormones are thought to be important because they can modulate innate and acquired immune responses. Human chorionic gonadotropin (hCG) is a glycoprotein produced by syncytiotrophoblasts, NK cells and macrophages during pregnancy. hCG has immunomodulatory properties and is believe to help establish maternal tolerance to the fetus. hCG levels peak around 8 to 12 weeks after conception which correspond to the period when the prevalence of malaria is highest in pregnant women. A report in 1989 suggested that hCG might increase the growth rate of *P. falciparum in vitro*, thus providing a possible explanation for the increased susceptibility of pregnant women. However, results from this study have never been confirmed. Accordingly, we evaluated the effect of hCG on *in vitro* growth of *P. falciparum*. Two strains of *P. falciparum* parasites in human erythrocytes (3D7 and FVO) were cultured for 7 days with different concentrations, ranging from 12.5 mIU/ml to 200 mIU/ml, of purified hCG obtained from CellSciences, Calbiochem and Sigma-Aldrich. Parasitemias were determined by microscopy and flow cytometry using Vibrant DyeCycle Orange which stains nucleic acids. Since some commercially available preparations of hCG are reported to be contaminate with other bioactive molecules, *P. falciparum* infected erythrocytes were co-cultured with choriocarcinoma cells (BeWo) which naturally produce hCG following induction with forskolin. Results shows that hCG from commercially available sources did not increase parasites growth rate *in vitro*. Furthermore, co-culturing *P. falciparum* with BeWo did not promote parasite growth, even though BeWo cells were confirmed to be secreting hCG. In conclusion, it does not appear that hCG is responsible for elevated

P. falciparum parasitemias found early in pregnancy when hCG levels are elevated.

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MALARIA IN PREGNANCY IN INDONESIA: CHARACTERIZATION OF VAR2CSA TRANSCRIPTS, ANTIBODY RESPONSE TO *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN (PFEMP1), AND PLACENTAL HISTOLOGY

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Malaria in pregnancy (MiP) contributes to anemia in mothers and to low birth weight babies. The pathogenesis of MiP is partly due to parasite accumulation in the placenta. var2CSA has so far been identified as the dominant var gene that encodes for *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP1), a protein mediating parasite adhesion to the placenta. Antibody to variant suface antigen (VSA) demonstrated cross reactivity of parasites from pregnant women living in different area. We carried out a study of MiP in a highly endemic malaria area in Timika, Papua, through collection of blood and placenta from pregnant women and non-pregnant individuals infected with *P. falciparum*. var gene expression was examined and parasite accumulation in the placenta was confirmed by histology. Current findings showed diagnosis of peripheral parasitemia is not always associated with placental parasitemia. Approximately 40% of women in whom peripheral parasitemia was detected harbored no parasites in their placenta as confirmed by histology. The effect of malaria infection in pregnancy outcomes such as low birth weight will be examined. Results of var2CSA analysis demonstrated that placental isolates express higher levels of var2CSA transcripts compared to peripheral isolates of the same pregnant women. This result extends to the Asia Pacific Region previous findings from Africa that var2CSA/PfEMP1 is the important ligand mediating parasite adhesion in the placenta. Antibody reactivity of sera taken from pregnant women infected with malaria and non-infected individuals were analyzed using Fluorescence Activating Cell Sorter (FACS). The results showed that pregnant women infected with malaria have higher antibody response to PfEMP1 than the non-pregnant individuals, thus confirming the previous findings. In summary, all the results above will be put together to get better picture of MiP impact on pregnancy outcomes in Indonesia. The findings will also be important to be used as a baseline data if such malaria vaccine containing var2CSA component is to be implemented.

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INFLAMMATORY MEDIATORS AS BIOMARKERS FOR MALARIAL ANEMIA SEVERITY IN PEDIATRIC POPULATIONS RESIDING IN HOLOENDEMIC *PLASMODIUM FALCIPARUM* TRANSMISSION AREAS

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Although the etiology of severe malarial anemia (SMA) is multi-factorial, dysregulation in inflammatory mediators is associated with enhanced pathogenesis. However, the inflammatory profile associated with susceptibility to SMA is not fully understood. To further investigate the role of these biomakers in conditioning SMA (Hb<6.0g/dL), a Cytokine 25-plex assay was used to measure IL-1β, IL-1ra, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, TNF-α, IFN-α, IFN-γ, GM-CSF, MIP-1α, MIP-1β, IP-10, MIG, Eotaxin, RANTES, and MCP-1 levels in Kenyan children residing in a holoendemic *Plasmodium falciparum* transmission area (n=282). Relative to aparasitemic controls (AC), children with SMA had significantly increased plasma levels of IL-2R, IL-6, IL-10, and MIG, while IL-4, IL-12p70, IL-17, IFN-α, and RANTES levels were significantly reduced (P<0.05). Since intra-leukocytic deposition of hemozoin correlates with disease severity, we determined the association between pigment-containing monocytes (PCM) and inflammatory mediators in *P. falciparum*-infected children. These results revealed that PCM was significantly associated with increased levels of IL-2R, IL-6, TNF-α, and MIP-1β, while IFN-γ levels were significantly reduced relative to PCM negative children (P<0.05). Further analyses in parasitemic children demonstrated that hemoglobin levels were inversely correlated with IL-2R levels (r=-0.274, P<0.001) and positively associated with IFN-γ levels (r=0.243, P=0.002). In addition, only IL-2R and IL-6 levels were associated with PCM levels (r=0.245, P=0.002; and r=0.156, P=0.047, respectively). These results reveal that measurement of circulating inflammatory mediators in children residing in malaria holoendemic areas may be important biomarkers for malarial anemia severity.

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DECREASED PEDIATRIC SEVERE MALARIAL ANEMIA IS ASSOCIATED WITH REDUCED INTRA-MONOCYTIC HEMOZOIN DEPOSITION

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Previous studies in *Plasmodium falciparum* holoendemic transmission areas show that severe malaria anemia (SMA; hemoglobin, Hb<6.0 g/dL) decreases with increasing age in children 1-4 years. Additional studies illustrate that increasing pigment-acquisition by monocytes is associated with increasing parasitemia. The association between age, pigment-containing monocytes (PCM) and SMA was therefore investigated in children (n=271; aged 2-32mos.) presenting at hospital with acute malaria in western Kenya. Complete hematological and parasitological measures were determined. The median (Q1-Q3) PCM levels in children with *P. falciparum* infection decreased with age (P<0.0001): 1-5mos. [2726 (870-5097)]; 6-11mos. [2138 (838-3797)]; 12-23mos. [1058 (457-2463)]; and 24-32mos. [1056 (461-3360)]. Post-hoc analyses revealed that PCM levels were higher in the 1-5mos. (P<0.0001) and 6-11mos. (P=0.002) groups vs. the 12-23mos. group. Median Hb levels progressively increased with age (P=0.006): 1-5mos. [5.4 (4.3-6.4)]; 6-11mos. [5.6 (4.6-7.1)]; 12-23mos. [6.2 (5.2-7.6)]; and 24-32mos. [6.2 (5.0-7.4)]. Consistent with increasing Hb levels, prevalence of SMA decreased (P=0.008) with age: 1-5mos. (68.9%); 6-11mos. (63.1%); 12-23mos. (43.0%); and 24-32mos. (50.0%). Results presented here show that reduced prevalence of SMA with increasing age is associated with decreased intra-monocytic hemozoin deposition.

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SPECIFIC INHIBITION OF THE PHOSPHOETHANOLAMINE METHYLTRANSFERASE OF THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM* BY AMODIAQUINE

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The phosphoethanolamine methyltransfease, PfPMT of the human malaria parasite *Plasmodium falciparum* is a member of a new family of phosphoethanolamine methyltransferases found in some protozoa, worms and plants. There are no known human homologous of this enzyme, making it an ideal target for drug therapy. PfPMT is involved in the synthesis of the major membrane phospholipid, phosphatidylcholine. Its synthesis is necessary for the generation of new parasite membranes, a critical step in the *P. falciparum's* intra-erythrocytic life cycle. The PfPMT enzyme catalyzes a three-step S-adenosylmethionine (SAM)-dependent methylation of the nitrogen atom of phosphoethanolamine to form phosphocholine. This activity represents a limiting step in the synthesis of phosphatidylcholine from host serine via the serine decarboxylation-phosphoethanolamine methylation (SDPM) pathway. We have adapted and optimized an enzyme-coupled non-radioactive *in vitro* methyltransferase assay to measure PfPMT activity and screen for possible inhibitors of this enzyme. Using this assay we have found that the antimalarial drug and histamine methyltransferase inhibitor amodiaquine specifically inhibits PfPMT activity. NMR studies of the free enzyme and as a function of amodiaquine concentration demonstrated the specificity of biding of the compound to the enzyme. Conversely, the antimalarial aminoquinolines, chloroquine, quinacrine, quinine and quinidine and several histamine methyltransferase inhibitors were ineffective against PfPMT activity, further demonstrating the specificity of the interaction between amodiaquine and PfPMT. The inhibition of PfPMT could thus contribute to the antimalarial activity of amodiaquine. Together these findings will set the stage for the development of inhibitors of PMT enzymes and possible future control of protozoan and worm parasitic infections**.**

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PRODUCTION OF RETICULOCYTES FROM HEMATOPOIETIC STEM CELLS FOR DEVELOPMENT OF A CONTINUOUS *IN VITRO* CULTURE SYSTEM FOR *PLASMODIUM VIVAX*

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Among *Plasmodium* species that infect humans, *Plasmodium vivax* is the most widespread. *Plasmodium vivax* infection causes severe illness and significant economic harm in endemic countries. Unlike *P. falciparum*, *P. vivax* exclusively infects reticulocytes (immature red blood cells) which constitute only a small percentage of human adult blood cells. There is currently no convenient culture system that yields enough reticulocytes to maintain *P. vivax* parasites *in vitro*, which has been a major obstacle for vaccine development and study of the mechanisms of anti-malarial drug resistance. Here, we report the production of reticulocytes from hematopoietic stem cells for *in vitro* culture of *P. vivax*. Purified CD34+ cells from human cord blood were purchased from AllCells (Emeryville, CA, USA). The cells were grown in QBSF-60 (Quality Biological, Gaithersburg, MD, USA) or StemSpan SFEM (StemCell Technologies, Vancouver, BC, Canada) under sequential culture conditions with different combinations of cytokines, such as IL-3, stem cell factor and erythropoietin. The cell number expanded by more than 10^5 during 15 days of culture. Flow cytometry showed a decrease and increase in the number of CD34+ and CD36+ cells, respectively. Quantitative reverse transcription PCR showed increased expression of β- and γ- globins and glycophorin A, suggesting that the cells were undergoing erythropoietic differentiation. Light microscopy with Cresyl Brilliant Blue staining showed that up to 12 % of the cultured cells became reticulocytes during the culture period. Purified mature *P. falciparum* parasites were incubated overnight with the erythroid cells to test for cytoadherence. We observed merozoites on the erythrocyte surface, indicating that appropriate receptors are present for parasite attachment. We are currently testing co-culture of the erythroid cells with a mouse stromal cell line to enhance erythrocyte development. Infection experiments with *P. vivax* parasite are also planned.

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ANALYSES OF THE *PLASMODIUM FALCIPARUM VAR* GENE FAMILY IN PARASITE ISOLATES FROM ZAMBIA

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Antigenic variation is a process that allows malaria parasites to rapidly change the molecules on the red cell surface to avoid the host's immune response. These molecules are encoded in the parasite's genome by multicopy, nonallelic gene families. One of these families, the *var* gene family, encodes for about 50-60 *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) proteins. These proteins have been associated with both antigenic variation and cytoadherence of infected erythrocytes at blood microvasculature sites throughout the body. Since some members of this gene family are current vaccine candidates, understanding the levels of diversity and conservation of these genes and the extent that they are expressed during disease are crucial. We are testing existing and developing new tools to estimate *var* gene repertoires from multiple field isolates collected in Zambia. These data are allowing us to make broad comparisons and gain insight into the mechanisms driving the evolution of the *var* gene family. These analyses will also help us elucidate the mechanisms of antigenic variation and may lead to the identification of new conserved *var* genes that may have important functional roles in disease.

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TYROSINE NITRATION OF PROTEINS BY A PUTATIVE NITRATE REDUCTASE IN SEXUAL AND ASEXUAL *PLASMODIUM FALCIPARUM* PARASITES

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We had previously identified endogenous nitric oxide (NO) signals in food vacuoles of *Plasmodium falciparum* trophozoites and in gametocyte stage parasites. However, intraerythrocytic trophozoites do not produce NO by an arginine-dependent mechanism. Instead, PF13_0353, a NADH-cytochrome b5 reductase, might be responsible for the endogenous generation of NO in *P. falciparum,* from either nitrate or nitrite, using a nitrate reductase mechanism. Nitrate and nitrite anions are a readily available in human erythrocytes and could be utilized by the parasite as substrates for this synthetic activity, moreover, we observed that *P. falciparum* parasites can grow for extended periods of time in nitrate-free RPMI 1640. We have previously shown that antibodies raised against the PF13_0535 gene product recognized epitopes in the food vacuole region of mature trophozoite stage parasites. The presence of NO and superoxide in the intracellular parasite may generate peroxinitrite, leading to tyrosine nitration of proteins, which is a marker of NO production. To investigate this possibility we used anti-nitrotyrosine antibodies in parasite lysates. We observed positive tyrosine nitration in lysates of both asexual (trophozoites) and sexual forms (stage III-V gametocytes), confirming endogenous reactive nitrogen species (RNS) generation at these stages. To further investigate the nitrogen metabolism in *P. falciparum* we incubated hemoglobin-free, food vacuole-rich trophozoite fractions and gametocyte lysates in phosphate /EDTA buffer with nitrite (NO_2^-) added. We observed

that the nitrite (NO_2^-) concentration in the cell preparation supernatants decreased after 30 min incubation, suggesting that this substrate was consumed. These findings provide further evidence of the possible generation of endogenous NO in asexual and sexual *P. falciparum* and suggest that nitrite (NO_2^-) could be one of the substrates of the enzymatic activity that generate RNSs in this organism.

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GROWTH-INHIBITORY EFFECT OF A FUCOIDAN FROM BROWN SEAWEED UNDARIA PINNATIFIDA ON *PLASMODIUM* PARASITES

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The present study was undertaken to investigate the inhibitory effects of fucoidan, a sulfated polysaccharide isolated from the edible brown seaweed *Undaria pinnatifida,* on the growth of *Plasmodium* parasites. In order to assessment of antimalarial activity of fucoidan, growth inhibition activities were evaluated using cultured *P. falciparum* parasites *in vitro* and on *Plasmodium berghei* infected mice *in vivo*. Fucoidan significantly inhibited the invasion of erythrocytes by *P. falciparum* merozoites, and its 50% inhibition concentration was similar to those for the chloroquine-sensitive *P. falciparum* 3D7 strain and the chloroquine-resistant K1 strain. Four day suppressive testing in *P. berghei* infected mice with fucoidan resulted in a 37% suppressive effect versus the control group and a delay in death associated with anemia ($P < 0.05$). In addition, fucoidans had no toxic effect on RAW 264.7 cells. These findings indicate that fucoidans from the Korean brown algae *U. pinnatifida* inhibits the invasion of *P. falciparum* merozoites into erythrocytes *in vitro* and *in vivo*.

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HEMATOLOGICAL EFFECTS IN PATIENTS WITH *PLASMODIUM VIVAX*, TIERRALTA-CÓRDOBA, COLOMBIA

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The malaria is a disease that represents a threat for the human life not only for the high morbidity and mortality, but because also generates a great socio-economic impact in the sub-tropical and tropical countries where the disease is highly endemic. In addition, it is considered to be a re-emergent disease in diverse areas of the world, where it was thought initially eradicated during the eradication campaign during 60´s. The malaria has clinical manifestations that can change from short duration fever episodes, if the diagnosis is opportune and the treatment is effective, up to systemic severe complications and death. The hematic changes associated with malaria are well recognized, but the specific changes can to vary according to the endemicity levels of the malaria, history of hemoglobin disease, nutritional condition, demographic factors and malaria immunity. The intention of this study was to characterize the hematologic alterations in patients with *Plasmodium vivax* in an endemic area of Colombia that is catalogued as an area of high risk in the country. For such purpose, there was obtain blood samples of 100 positive patients for *P. vivax* and 100 control patients from the same area, to was carry out IV generation hemogram, thick smear from peripheral blood and coprology examination for concentration technique. In addition, there was realized the clinical characterization of the patient, including variables as age, weight, height, sex, origin, race and number of previous episodes for malaria, in order to evaluate and to correlate with the hematologic finds. The results suggest that individuals with *P. vivax* show tendency thrombocytopenia, others hematic parameters were normal.

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A RANDOMIZED CLINICAL TRIAL OF THE PROTECTIVE EFFICACY OF TRIMETHOPRIM-SULFAMETHOXAZOLE PROPHYLAXIS AGAINST MALARIA IN HIV-EXPOSED CHILDREN

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Malaria is a leading cause of death in Africa for children under 5 years of age. Trimethoprim-sulfamethoxazole (TS) prophylaxis is used throughout Africa to prevent opportunistic infections in HIV-infected and HIV-exposed (HIV-uninfected children born to HIV-infected mothers) children. Recent studies show that TS prophylaxis also protects HIV-infected children against malaria. However, there are no studies regarding the protective efficacy of TS prophylaxis in HIV-exposed children. We are conducting a randomized clinical trial in an area of perennial high malaria transmission in Uganda. We enrolled 201 HIV-exposed infants aged 6 weeks-9 months. All children were breastfeeding and taking TS prophylaxis at enrollment. We provided insecticide-treated nets at enrollment and follow the children for all their healthcare needs until the age of 21 months. Per World Health Organization and Uganda Ministry of Health recommendations, each HIV-exposed child was continued on TS prophylaxis until DNA PCR confirmation of negative HIV status 6-8 weeks after cessation of breastfeeding. Each child was then randomized to continue or discontinue TS prophylaxis. Malaria was diagnosed when a child presented with a new episode of fever and a positive thick blood smear. Generalized estimating equations were used to measure the association between TS and the risk of malaria adjusting for repeated measures. Among 201 HIV-exposed infants enrolled, 110 have stopped breastfeeding, been confirmed HIV-uninfected, and been randomized to continue or discontinue TS prophylaxis. The median age at randomization is 9 months (range: 6.2-16.5). There have been 32 malaria cases among 57 HIV-exposed children randomized to continue TS after 12.0 person-years of follow-up time (2.66 cases/person-year). There have been 51 malaria cases among 53 HIV-exposed children randomized to discontinue TS after 11.5 person-years of follow-up time (4.44 cases/person-year). After adjusting for age, TS prophylaxis was associated with a 38% reduction (95%CI= 2%-61%, p=0.04) in the risk of malaria. These findings suggest that TS prophylaxis is modestly protective against malaria in HIV-exposed children when continued beyond the period of exposure to HIV. Data will continue to accrue through the next six months and will update prior to the annual ASTMH meeting.

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THE COST-EFFECTIVENESS OF RECTAL ARTESUNATE FOR TREATING SEVERE CHILDHOOD MALARIA AT THE COMMUNITY LEVEL

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Severely ill malaria patients cannot swallow anti-malarial medicines. In remote areas, access to health facilities providing parenteral treatment is limited. Hence, safe and effective treatment of most severe childhood

cases is greatly delayed or not achieved. A randomized, double-blinded, placebo-controlled, community-based trial has shown a 25% reduction in mortality using a single dose of rectal artesunate as emergency treatment of children who could not take drugs by mouth prior to standard parenteral treatment at a referral health care facility. These results support the World Health Organization's recommendation for use of artesunate suppositories for pre-referral treatment of severe malaria. Using a decision tree model and the trial data, we compare the outcomes and costs of standard parenteral treatment of cases with those of pre-referral treatment with rectal artesunate before standard care for a hypothetical cohort of febrile children with malaria living in an area with intense, perennial transmission. We assume 30-60% of children's fevers are due to malaria. Using a probabilistic framework to account for important factors affecting treatment-seeking behaviour of caregivers, we estimate the clinically-cured proportion of children with malaria at 6% (range 2-14%), which is in agreement with alarmingly low results of studies assessing community effectiveness of antimalarial treatment. We assume that 2-5% of treatment failures would progress to severe malaria. Outcomes measured include child deaths, neurologic sequelae, and disability adjusted life years averted. We consider the incremental direct costs of artesunate suppositories and their administration to patients in villages by community health workers. Pre-referral treatment with rectal artesunate is a very cost-effective adjunct to standard parenteral treatment of severe malaria cases in high transmission areas; the cost per death averted ranges between $1.31-12.83 when access to rectal artesunate in the community is 10-30%. Sensitivity analysis results will be presented to establish the effect of varying parameter estimates and assumptions on our results.

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IN VITRO ACTIVITY OF A DICHLOROMETHANE FRACTION OF *LANSIUM DOMESTICUM* LEAVES AGAINST *PLASMODIUM FALCIPARUM* CLONE 3D7

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Plants have been used as medicine for as long as humans have learned of their healing properties. Reduced efficacies of several common frontline drugs to treat malaria and the limited choice of synthetic compounds have shifted the search for novel antimalarial compounds from synthetic chemistry to scientific validation of activity in medicinal plants traditionally used to treat malaria. Surveys of indigenous peoples in Borneo revealed the use of teas prepared from various parts of *Lansium domesticum* (langsat). Langsat, a tree that is native to this region, can be found growing in the wild as well as being cultivated for its edible fruit. Exhaustive methanol extraction of langsat leaves followed by solvent partitioning resulted in four fractions (hexane, dichloromethane, ethyl acetate, methanol). The highest bio-activity was found in the dichloromethane (DCM) fraction (IC_{50} of 25 µg/ml), as determined by *in vitro* assessment of schizont maturation of *Plasmodium falciparum* clone 3D7. The IC_{50} for the total crude methanolic extract was 50 µg/ml. The inhibitory effects that were observed in synchronised early trophozoite cultures exposed to 100 µg/ml of the DCM fraction for 12 or 24 hours was not seen when exposure was initiated at the late trophozoite stage. Therefore the bioactivity demonstrated in the langsat leaf DCM fraction was early trophozoite-stage specific. The potential of the langsat tree as a sustainable source of antimalarials will be discussed.

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ADHERENCE TO ARTEMETHER LUMEFANTRINE AS FIRST-LINE TREATMENT FOR UNCOMPLICATED MALARIA IN TANZANIA

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Artemether/Lumefantrine (ALu) has replaced the less effective sulfadoxine/pyrimethamine as first-line treatment policy for uncomplicated malaria in Tanzania beginning of 2007. In the ALIVE (Artemether/Lumefantrine In Vulnerable Patients: Exploring health impact) project, we assessed patients' adherence and perception of ALu, as well as safety under programmatic conditions. Malaria patients were prescribed ALu and gave consent to be enrolled at a rural health facility in Kilombero District, Tanzania. Each patient was randomized for followed up and impromptu visit at home after either dose 2,3,4,5 or 6 (patient blinded to the assessed dose) and administered a structured questionnaire. Between February and April, 2007, 552 patients were recruited. 64% were children under 13 years whose questionnaires were administered to relatives or care takers. Median age was 4 (1.6-18.0 IQR) years and children of 5 years and below were 53%. Based on patients' responses and blister pack checks, nobody missed a dose. 112 patients were assessed after dose two. 95% of these took dose 2 at hour 8±1 (recommended 8h); the rest, (440), >80% took subsequent doses at the correct hour ±4. 92% found the pictogram in the ALu pack useful for them to take doses at appropriate time. All subjects did find clustered packing useful as a reminder on how to take ALu. Overall 4% (n=24) preferred quinine injection. One death due to pneumonia occurred in an infant two days after last dose of Alu. In conclusion, dherence to standard ALu regimen was outstanding. 100% of the patients took the expected dose prior to the monitoring visit and timing was very satisfactory. This confirms that patients are able to comply with the twice daily dose of ALu regimen. Reasons for this high adherence include patients' conviction that the drug is effective, good understanding of pictorial dosing instructions on packaging, which were also used by NMCP in training the prescribers before launching the new policy.

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EVALUATION OF THE ANTIMALARIAL AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT OF *NIGELLA SATIVA* IN MICE INFECTED WITH *PLASMODIUM YOELLI NIGERIENSIS*

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Antimalarial activity and effect of methanolic extract of *Nigella sativa* (black seed) on oxidative stress and antioxidant defense system were investigated in mice infected with *Plasmodium yeolli nigeriensis*. Thirty adult albino mice were divided into five treatment groups. Three groups were inoculated by intraperitoneal injection with $1x10^7$ infected erythrocytes on day 0. After 72 hours of inoculation, group 1 were administered 1.25g/Kg body weight *N. sativa* extract orally for 5 days, group 2 received chloroquine 10mg/Kg for 3 days and group 3 received normal saline. Groups 5 and 6 consisted uninfected mice but treated with extract alone and normal saline respectively. The Rane test procedure was used to evaluate antimalarial activity. Parasitaemia was monitored daily in the animals for 7 days. Oxidative status was evaluated by estimating malondialdehyde (MDA) as an index of lipid peroxidation, reduced glutathione (GSH) and glutathione-S-transferase (GST) activity in the

liver as well as catalase (CAT) and superoxide dismutase (SOD) in blood 24hours after treatment. The extract and chloroquine produced 99.2% and 94.6% chemosuppression respectively relative to untreated control. *P. yoelli* infection caused a significant (P<0.05) elevation of MDA level and reduction in GST and GSH. N. sativa extract significantly (p<0.05) decreased the elevation of MDA and also inhibited the depression of GST activity and GSH level in infected mice. The extract contrary to chloroquine caused a significant increase in SOD and CAT activities in both infected and uninfected mice. *N. sativa* extract has appreciable antimalarial activity *in P. yoelli* infected mice and caused an alteration in the antioxidant defense system in mice. This may have some implication for its mechanism of action.

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PHARMACOKINETIC AND CLINICAL DETERMINANTS OF RESPONSE TO CHLOROQUINE TREATMENT IN NIGERIAN CHILDREN WITH ACUTE UNCOMPLICATED FALCIPARUM MALARIA

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Resistance to chloroquine (CQ) is widespread and has necessitated the use of artemisinin based combination therapy in many endemic areas. There are many potential causes of CQ treatment failure which include resistance-conferring mutations in the parasite and inter-individual variation in host pharmacokinetics (PK) which all influence drug concentrations at the active sites. This study was designed to investigate the relationship between post treatment whole blood concentrations of CQ and clinical response in Nigerian children with acute uncomplicated falciparum malaria. Ninety children aged 6months to 12 years with acute uncomplicated falciparum malaria were enrolled into the study. Each child received 25mg/kg body weight of CQ given over 3 days and was followed up for 28 days to monitor clinical and parasitological response. Filter paper blood samples (100µl) were obtained from each patient on D0 prior to treatment and on days 1,2,3,4,5,6,7,14 and 28 for determination of whole blood concentrations of CQ by standard HPLC techniques. Eighty three children completed the study. Infection in 45% of the patients responded adequately to treatment, while infections in 16.8%, 9.6% and 28.9% exhibited early treatment failure, late clinical failure, and late parasitological failure respectively. Mean maximum concentrations of CQ was significantly higher in patients with CQ sensitive infection (3.33µg/ml vs 2.42µg/ml P= 0.007). Day 3 and 7 CQ concentrations were significantly higher in the group of patients with CQ sensitive infections (Day 3; 2.15 µg/ml vs 1.42 µg/ml P= 0.005, Day 7; 1.83µg/ml vs 0.99µg/ml P= 0.030 respectively). The Parasite reduction ratio (PRR) on D2 was significantly higher in children with CQ sensitive infection (P=0.004). The parasite reduction ratio and a day 3 CQ concentration of less than 1.6µg/ml were significantly associated with an increased risk of CQ treatment failure. Post treatment concentrations of chloroquine either in plasma or red cells are useful indicators of clinical response. The results show an association between day 3 and 7 CQ concentration and treatment outcome. In addition, the parasite reduction ratio and Day 3 concentration appear to be useful predictors of treatment outcome.

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PHARMACOKINETICS OF SULFADOXINE-PYRIMETHAMINE ADMINISTERED ALONE OR IN COMBINATION WITH AMODIAQUINE OR ARTESUNATE IN CHILDREN UNDER FIVE IN MALI

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Sulfadoxine-Pyrimethamine, in combination with either artesunate or amodiaquine, is recommended for the treatment of uncomplicated malaria in Africa. In addition, sulfadoxine-pyrimethamine monotherapy is being used or evaluated for intermittent preventive treatment at the community level during pregnancy (IPTp), in infants (IPTi), in children (IPTc) or in school children (IPTsc). Yet, the pharmacokinetic parameters of these drugs in these key target populations are poorly documented. In a randomized controlled trial using the WHO 2003 protocol children aged 6-59 months with uncomplicated falciparum malaria, received either one dose of SP alone (SP), one dose of SP plus three daily doses of amodiaquine (SP+AQ) or one dose of SP plus 3 daily doses of artesunate (SP+AS). We collected exactly 100 µl of whole blood on a filter paper before drug administration at day 0 and at days 1, 3, 7, 14, 21 and 28 after drug administration. We analyzed samples from 41, 39 and 33 children in the SP, SP+AQ and SP+AS arms, respectively. The mean concentrations of pyrimethamine (ng/mL) on day 7 were 66.59 (6.48 - 162), 76.70 (24.20 - 222) and 68.23 (17.10 - 160) in SP, SP+AQ and SP+AS arms, respectively. The mean concentrations of Sulfadoxine (mg/mL) on day 7 were 33.79 (5.18 - 50.40), 35.11 (4.01 - 71.30) and 34.76 (11 - 60.50) in SP, SP+AQ and SP+AS arms, respectively. None of these comparisons showed statistically significant differences (kruskall wallis p >0.05). Further analysis of pharmacokinetic parameters such as maximum concentration (C_{max}), time to maximum concentration (T_{max}) and area under the concentration time curve (AUC) are underway. Pharmacokinetic interactions between the respective drugs and the effect of age on these pharmacokinetic parameters will be investigated and discussed.

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IMPACT OF PROGRAMMATIC USE OF A NEW FIXED-DOSE COMBINATION OF ARTESUNATE-MEFLOQUINE FOR THE TREATMENT OF FALCIPARUM MALARIA IN THE JURUÁ VALLEY, ACRE, BRAZIL

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A new fixed-dose combination of artesunate-mefloquine (ASMQ, Farmanguinhos, Brazil) was developed by a DNDi-led international consortium. ASMQ is formulated in paediatric and adult strength tablets for once daily administration of 1 or 2 tablets over 3 days. Due to the high burden of disease and concerns about increasing antimalarial resistance to quinine-doxicycline (QN/DX) first line treatment, the Brazilian National Malaria Control Programme decided in 2006 to evaluate the impact of the programmatic use of ASMQ within 3 priority municipalities (Cruzeiro do Sul, Mâncio Lima, and Rodrigues Alves; total population: 96,496) in the Juruá Valley, Amazon Basin. The effectiveness of ASMQ vs QN/DX was evaluated through the following outcome measures: incidence of

falciparum malaria, *Plasmodium vivax / P. falciparum* ratio, proportion of slides with gametocytes, rate of recrudescence of falciparum malaria 40 days after treatment and rate of adverse events. All patients in the study area were evaluated for entry. Inclusion criteria were: age > 6 months, assexual *P. falciparum* parasitaemia of 250 to 100,000/µl or < +++ and consent for participation. Patients were excluded in case of pregnancy or amenorrhoea > 1 month, mixed malaria and/or with the presence of signs and symptoms of severe malaria or danger signs**.** Data was collected through the national malaria surveillance system, with the use of the standard notification sheets, data entry system and software (SIVEP Malaria). The national pharmacovigilance forms were used for reporting adverse events. Analysis consisted of comparison of outcomes within the municipalities before and after the intervention, and with remaining municipalities in Acre state without intervention. In the first year of evaluation, a total of circa 17,000 patients were treated. Preliminary results show a significant impact of the introduction of ASMQ with a 69.8% reduction in the number of cases of falciparum malaria and 62.1% reduction of malaria-related hospital admissions in the state of Acre. Complete results will be presented.

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EFFICACY AND SAFETY OF ARTESUNATE + AMODIAQUINE (AS+AQ) IN COMPARATIVE TRIALS IN SOUTH-SAHARAN AFRICA: A SYSTEMATIC REVIEW AND AN INDIVIDUAL PATIENT META-ANALYSIS

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AS+AQ is widely used for treating uncomplicated falciparum malaria. A systematic search identified 29 comparative trials with 28-day follow-up conducted during 1999-2006 at 43 sites in south-Saharan Africa. The analyses of efficacy were derived from published and additional data by site within studies (total 12097 patients) and were conducted on crude (modified intent to treat n=11753; per-protocol n=11216) and PCR-corrected (n=11,315) Day28 outcomes. Heterogeneity was investigated (funnel and Galbraith plots, Cochrane Q test); treatment effects were estimated using a multilevel random effect and a logit model. Efficacy of AS+AQ ranged widely (20-100%). The weighted mean and median were 72.9% and 85.4% for crude and 94.1% and 95.2% for PCR-corrected Day28 success rates. There was no significant difference in the efficacy of AS+AQ and comparators on either analyses except an advantage over AQ alone (Relative Risk = 1.2 and 1.29) and chloroquine+SP (RR= 1.99 and 1.95). We obtained individual patient data (IPD) from 21 comparative trials done at 29 sites in 15 countries enrolling 10,609 patients in the AS+AQ (n=4896) or comparator arms (n=5713). Efficacy, estimated by survival analysis (Kaplan-Meier) over 28 days on intent-to-treat basis, was 76.1% (95%CI 74.9-77.4) for crude and 94.0% (93.3-94.7) for PCR-adjusted rates. For the latter (multivariate analysis stratified by site), the risk of recrudescence with AS+AQ was lower than AQ alone (Hazard Ratios, HR=3.02), AS alone (HR=8.67), AQ+SP (HR=1.98) and CQ+SP (HR=6.76), not different from AS+SP and artemether+lumefantrine, and higher than dihydroartemisinin-piperaquine (HR=0.48) Effects on parasite, fever and gametocyte clearance and tolerance, as well as risk factors for failure are analysed. Implications for research and policies are discussed.

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CLINDAMYCIN PLUS QUININE FOR TREATING UNCOMPLICATED FALCIPARUM MALARIA: A META-ANALYSIS

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Artemisinin-based combinations are currently the recommended treatment for uncomplicated falciparum malaria, but are in limited supply and may not be affordable. Clindamycin plus quinine, a non-artemisinin-based combination, is a cheaper, readily available alternative treatment for uncomplicated falciparum malaria. The objective of this study was to compare the efficacy of clindamycin plus quinine with other antimalarial drugs in the treatment of uncomplicated falciparum malaria. We included randomized controlled trials comparing the efficacy of clindamycin plus quinine with other antimalarial drugs for treatment of uncomplicated falciparum malaria. We searched the Cochrane Infectious Diseases Group Specialized Register (January 2008), CENTRAL (*The Cochrane Library 2008,* Issue 1), MEDLINE (1966 to January 2008), EMBASE (1988 to January 2008), LILACS (January 2008), and conference proceedings. Two authors independently assessed study eligibility, extracted data and assessed the methodological quality. The primary outcome measure was treatment failure by day 28. We computed the relative risk (RR) for dichotomous data and weighted mean difference for continuous data, and combined the data using a fixed effects model. Seven trials (929 participants) were included. The risk of day 28 treatment failure was significantly reduced by clindamycin plus quinine compared with quinine (RR 0.14, 95% CI 0.07, 0.29), quinine plus SP (RR 0.17, 95% CI 0.06, 0.44), amodiaquine (RR 0.11, 95% CI 0.04, 0.27), or chloroquine (RR 0.11, 95% CI 0.04, 0.29). There was no difference in failure when the combination was compared with quinine plus tetracycline or doxycycline (RR 0.78, 95% CI 0.20, 3.02), artesunate plus clindamycin (RR 0.57, 95% CI 0.26, 1.24), or chloroquine plus clindamycin (RR 0.38, 95% CI 0.13, 1.10). Adverse events were similar across treatment groups and severe adverse events were rare. In onclusion, clindamycin plus quinine should be considered in the treatment of uncomplicated falciparum malaria. Larger trials are required to compare the efficacy of clindamycin plus quinine with artemisinin-based combinations.

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APPLYING A REAL TIME PCR ASSAY TO THE ROUTINE LABORATORY DIAGNOSIS OF FALCIPARUM MALARIA

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Real time polymerase chain reaction (rt-PCR) is an increasingly attractive method for the diagnosis of malaria. It is rapid, simple to use, does not rely on a skilled microscopist, and does not require handling of post-amplification products. We compared a rt-PCR assay with blood film microscopy for the diagnosis of *Plasmodium falciparum* malaria at the Hospital for Tropical Diseases, London. Between 10th July and 10th August 2007, 310 samples were examined by Giemsa-stained blood film for the presence of malaria parasites. All patients had arrived from malaria-endemic countries with a clinical presentation compatible with malaria. Out of the 310 samples, 208 were initial blood films and were analysed; the remainder constituted repeat blood films on the same individual. rt-PCR was performed on samples in duplicate using *P. falciparum* primers (protocol and primers developed by S Sharp). The investigator was blinded to microscopy results. Out of 208 samples analysed by microscopy, 14 were positive for *P. falciparum*, 3 for *P. vivax*, 2 for *P. ovale* and 1 for *P. malariae*. All 14 samples positive by microscopy for *P. falciparum* were also positive for *P. falciparum* DNA by PCR. One sample which was negative

on blood film microscopy was positive for *P. falciparum* DNA by PCR. All of the non-*falciparum* species diagnosed by microscopy were negative for *P. falciparum* DNA by PCR. The patient with a negative blood film and positive PCR presented 4 days later with a *P. falciparum* parasitaemia of 2.9%. It is likely that the level of *P. falciparum* parasitaemia in the initial blood sample was below the threshold for detection by microscopy, but not by rt-PCR. This result was therefore interpreted as a 'true positive'. Sensitivity of rt-PCR was therefore 100% (greater than blood microscopy), with specificity 99.5%, positive predictive value 93% and negative predictive value 100%. With a turn around time of 3 hours including DNA extraction, this method is an increasingly practical option where resources permit for first line diagnosis of *falciparum* malaria.

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DETECTION OF *PLASMODIUM KNOWLESI* BY REAL-TIME PCR

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Recent studies have shown that natural transmission of the simian parasite *Plasmodium knowlesi* to humans occurs frequently in Southeast Asia. *P. knowlesi* infections were misidentified as *Plasmodium malariae* in 70% of cases in Malaysia because of morphologic similarity between the two plasmodium species on stained blood smears. The goal of this study was to determine if a real-time PCR assay used in our laboratory which detects and discriminates all four species of human *Plasmodium*, could also detect *P. knowlesi* and other simian species. Real-time PCR was performed on genomic DNA obtained from ATCC and on DNA extracted from filter paper blood spots using sterile water followed by automated extraction on the MagNA Pure LC System. Based on melting curves, *P. knowlesi, P.cynomolgi, P. inui, P. fragile, P. simiovale, P. simium* were indistinguishable from *P. vivax* while *P. brasilianium* was indistinguishable from *P. malariae.* None of the simian plasmodium species tested had melting curves similar to *P. falciparum* and *P. ovale*. Probes specific for *P. knowlesi* were designed and tested with both *P. knowlesi* and *P. vivax* strains. Preliminary results show that these probes are able to differentiate between the two species. We have established that our real-time PCR assay can detect several simian *Plasmodium* species without differentiating them from either *P. vivax* or *P. malariae*. We have designed a set of probes that will permit a distinction to be made between *P. vivax* and *P. knowlesi*.

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VARIABLE SENSITIVITY OF MALARIA RAPID DIAGNOSTIC TESTS IN HOUSEHOLD SURVEYS-TANZANIA, 2006

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Rapid Diagnostic Tests (RDTs) represent an alternative to microscopy for malaria diagnosis and have shown high sensitivity and specificity in a variety of studies. As household surveys become more frequent to monitor progress of intervention scale-up, RDTs are increasingly being used in these surveys for parasite prevalence assessment and immediate treatment decisions. As part of the Interdisciplinary Monitoring Project for Antimalarial Combination Therapy in Tanzania (Impact-Tz), randomly selected households in three districts were visited between May and September 2006 by teams of experienced interviewers. One team was assigned to Ifakara and one to Rufiji and Morogoro. Interviewers collected data on socioeconomic status, knowledge of malaria, careseeking for febrile illness, and use of malaria preventive methods. Blood smears and RDTs (Paracheck®, Orchid Biomedical Systems, Mumbai, India) were performed on 14,334 consenting members of surveyed households. Blood slides were read by two experienced microscopists at a central laboratory site, with a third microscopist's reading for discrepancies. Sensitivity and specificity of RDTs were measured against reference microscopy. Overall, 13.8% of blood smears performed were positive. There were 5348 blood smear/RDT pairs from Ifakara, 4719 from Morogoro, and 4267 from Rufiji. The sensitivity of RDTs based on reference microscopy varied significantly across the three districts. RDTs performed well in Rufiji and Morogoro, with sensitivities of 98.3% and 84.3% respectively, but in Ifakara, RDT sensitivity was only 58.4%. In Ifakara, over 4,000 parasites per microliter were required to reach 80% sensitivity. Overall RDT specificity was 78.2% and did not vary greatly by site. Quality control is essential to reliable field use of rapid diagnostic tests for malaria. Use of RDTs in household surveys is valuable for immediate treatment decisions, but must be deployed such that performance can be monitored.

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VALIDATION OF MICROSCOPE EQUIPPED WITH A VERSATILE ILLUMINATOR (THE EARL-LIGHT) IN DETECTING MALARIA PARASITES

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This study was to validate monocular microscope equipped with a versatile illuminator (the EARL-Light) in detecting malaria parasites.We compare the efficacy of the equipment with field microscopy using monocular microscopes using natural light source for active surveillance of malaria parasite in northern Thailand. Field and EARL-light microscopy consisted of approximately five minute read (100 fields) of Thick film at x 1,000. Diagnostic values of these microscopic experiments was determined by comparing with expert microscopy which is 10- minute read, counting number of parasite per 200 leucocytes, at x1,000 using a high-quality, well maintained microscope with an artificial light source. All discordance and 20 % of concordance of either experimental results were cross-check blindly. A total of 800 blood films collected in June 2007 were included in the study, of which 39 (4.88 %) were positive for *Plasmodium falciparum*, 55 (6.88 %) for *P. vivax* by expert microscopy. A total of 7.5 % (60 of 800) of the *P. falciparum* and *P. vivax* positive slides had a parasitemia of less than 500 per µl. Earl light microscopy showed less inferior sensitive than natural light microscopy (80.6 % and 83.3 %) but showed more or less the same specific (98.6 % and 99.2 %) for diagnosis of *P. falciparum* malaria, with a positive predictive value (PPV) of 72.5 %, 81.1 %, and negative predictive value (NPV) of 99.1 % and 99.2 % respectively. The Kappa of both tests showed more or less the same (0.731 and 0.790 respectively). The corresponding sensitivity and specificity for the diagnosis of *P. vivax* malaria were 76.1%, 78.8 and 99.5, 99.5, respectively, with a PPV of 93.1 %, 93.3 %, and an NPV of 97.7 %, 98.0 % respectively. EARL-light microscopy, as defined in this study, is not more effective than natural light microscopy, and microscopy of Giemsa- stained thick and thin blood films by skilled microscopist has remained the standard laboratory method for the diagnosis of malaria. However, most of microscopists preferred the EARL-light to the Natural Light microscopy but the major disadvantageous factor might be due to the unaccustomability of the users to this tool. Most of experimental users felt advantage to this tool but minors needed to improve the tool's attribution. The tool would give some sort of extremely benefit to strengthen the routine diagnostic method to detect malaria cases in non electricity remote areas.

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THE VALIDATION OF THE DMSC MALARIA PF./PV. RAPID DIAGNOSTIC DEVICE FOR THE DETECTION OF F*ALCIPARUM* AND NON *FALCIPARUM* MALARIA IN THAILAND 2006

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An effort of validating newly developed rapid and specific rapid diagnostic kit, DMSC Malaria Pf./Pv. was done to identify individual infected with *Plasmodium falciparum* and *P. vivax* at peripheral areas in Thailand. The study aimed to validate an alternative tool being used to control the severe public health impact of this disease. The kit was developed by utilizing Gold particle linked monoclonal antibodies against the intracellular metabolic enzyme parasite lactate dehydrogenase (pLDH). Malaria parasites were differentiated by based on antigenic differences between the pLDH isoforms.The test could differentiate live from dead organisms as pLDH is produced only by live *Plasmodium* parasite. To validate this test, a gold standard, 100 fields of traditional Giemsa-stained thick-smear blood films examination was used to compare with the DMSC test's result. 369 patients suspected of having malaria were enrolled for this validation. Ten µl of each individual's whole-blood were diagnosed by this test and found a total of 101 samples (27.4 %) were positive by blood films, while 103 (27.9 %) were positive by DMSC test. Barring the blood film examination, it indicated that 35.6 % (36 of 101) of the patients infected with *P. falciparum* and the others, 64.4 %(65 of 101) infected with *P. vivax*. The DMSC test showed that 36.9 % (38 of 103) were positive for *P. falciparum* and 63.1 % (65 of 103) were positive for *P. vivax*. This study was demonstrated that the DMSC test had sensitivities of 77.8 and 87.9% and specificities of 97.0 and 97.4 %, respectively, comparing with the Gold standard test for detecting *P. falciparum* and *P. vivax* malaria. In addition, patients parasitemia less than 100 µl of blood could not be identified for malaria positive by the DMSC test. This study could be concluded that the DMSC test is an effective tool for rapid diagnosis of malaria even it could not replace the tradition blood film examination.

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MAPPING EPITOPES RECOGNISED BY MONOCLONAL ANTIBODIES AGAINST PFHRP2 AND IMPLICATIONS TOWARDS OPTIMISATION OF MALARIA RAPID DIAGNOSTIC TESTS

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The ability to rapidly and reliably diagnose malaria infections is key to both the management of individual patients as well as public health efforts to control the disease. Malaria rapid diagnostic tests (RDTs) offer the potential for such rapid and reliable diagnosis. However, field studies have reported variable sensitivities in different settings, mostly relating to patients with low level parasitemia. One possible cause of this variation in sensitivity is variation in the epitopes recognised by detecting monoclonal antibodies (MAB), both in their composition and copy number, factors that may affect the antigen-antibody binding affinity/avidity. This is particularly relevant to RDTs targeting PfHRP2 as this protein is highly polymorphic. To investigate the role of epitope variability we undertook detailed mapping of the epitopes recognized by a panel of 13 HRP2-specific MAB using overlapping synthetic peptide technology. Results showed that the MABs recognized distinct epitopes of different length. With sequence data from over 400 distinct field isolates from around the world, bioinformatics analysis was used to determine a relationship between the epitope identity and the copy number of epitopes and geographic distribution of the parasite isolates. The mapping results provide possible explanation for why the same MAB recognizes different strains with different sensitivity and why different MABs recognized the same strain with different strength. The outcome of this analysis will guide efforts to improve current RDTs for malaria.

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FIELD EVALUATION OF A RAPID MALARIA DIAGNOSTIC TEST (PARASCREEN™) FOR MALARIA DIAGNOSIS IN THE PERUVIAN AMAZON

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Immunochromatographic rapid malaria diagnostic tests (RMDT) constitute a fast and opportune alternative for the malaria diagnosis in areas where microscopy is not available. Parascreen™ is a RMDT that detects *Plasmodium falciparum* Histidin-rich protein 2 antigen for *P. falciparum* diagnosis and pan-malarial antigen for *Plasmodium spp* diagnosis. The objective of this study was to validate a RMDT ParaScreen™ under field conditions in Loreto-Perú. The RMDT ParaScreen™, was applied to individuals with symptoms related to malaria who attended to the health services between October and December 2006 (n = 332). The results obtained by RMDT were compared with Polymerase Chain Reaction (PCR) and expert microscopy (EM). The following indicators were calculated for Parascreen™: sensibility (S), especificity (E), positive (PV+) and negative predictive values (PV-), positive (LR+) and negative likehood ratio (LR-). Compared with PCR, Parascreen™ had for *P. falciparum* malaria a S= 81,8%, E= 99,1%, PV+= 75%, PV-= 99,4, LR+= 87,27 and LR-= 0,18; and for non-*P. falciparum* malaria a S= 76,1%, E= 99,2%, PV+= 97,1%, PV-= 92,0%, LR+= 92,51 and LR-= 0,24. Compared with EM, Parascreen™ had for *P. falciparum* malaria a S= 53,5%, E= 98,7%, PV+= 66,7%, PV-= 97,8%, LR+= 42,27 and LR-= 0,47; and for non-*P. falciparum* malaria a S= 77,1%, E= 97,6%, PV+= 91,4%, PV-= 92,7%, LR+= 32,0 and LR-= 0,22. In conclusion, Parascreen™ is valid and acceptable for malaria diagnosis under field conditions as we shown during its use in the Peruvian Amazon. Its use must consider the incidence and predominance of *Plasmodium* species.

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ISOLATION AND CHARACTERIZATION OF THE MSP1 GENE FROM *PLASMODIUM MALARIAE* AND *OVALE*

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The Merozoite Surface Protein 1 (MSP1) is the principle surface antigen of the blood stage form of the *Plasmodium* parasite. It is a primary target of the host immune system, and antibodies recognizing MSP1 are normally present following Plasmodium infection. In particular, the relatively conserved C-terminal portions of MSP1 (p19, p33 and p42) harbor immunodominant epitopes, making this region a significant component of malaria vaccines and diagnostic tests. Although the MSP1 gene has been reported for multiple Plasmodium species, including *P. falciparum* and *P. vivax*, this gene has not been described for the other two major human-infective species, *P. malariae* and *P. ovale*. Portions of the MSP1 protein from all four species could play a vital role in a broad-based malaria vaccine or immunoassay. This study describes the isolation and characterization of the complete *P. malariae* and *P. ovale* MSP1 genes, and the *E. coli* expression of the p19 portion of these genes. A PCR assay was used to screen for the presence of Plasmodium DNA in whole blood samples from Cameroon, and to identify the infecting species. Nucleic acid extracted from *P. malariae* and *P. ovale* infected samples was used for PCR amplification of a short region near the 5'-end of the genes. Specific primers within the 5'-regions were used in combination with degenerate primers near the 3'-end to amplify the near full-length gene for each species. Remaining sequences at the extreme termini of

each gene were obtained using PCR walking methods. The MSP1 genes isolated from *P. malariae* and *P. ovale* were 5256 and 5193 base pairs in length respectively, encoding proteins of 1751 and 1730 amino acids. The percent identities of the deduced MSP1 amino acid sequences between *P. falciparum, P. vivax, P. malariae* and *P. ovale* ranged from 43% to 53%. Recombinant *P. malariae* and *P. ovale* MSP1-p19 proteins were expressed and purified from *E. coli*.

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SEROPREVALENCE OF *PLASMODIUM FALCIPARUM, VIVAX, MALARIAE* AND *OVALE* ANTIBODIES AMONG BLOOD DONORS FROM CAMEROON

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Serological testing is not recommended in the diagnosis of acute malaria but can be useful for diagnosis of malaria in, for example, previously non-immune individuals, for determination of chronicity rates, and in the screening of blood donors or donors deferred for potential exposure to malaria. Construction of a robust EIA for detection of plasmodium antibodies requires identification of plasmodium antigens that elicit an antibody response early after infection and that persists for months or years. The present study was conducted to compare the immunoreactivity rates of various plasmodium antigens among blood donors from a malaria endemic area to aid in the selection of antigens for EIA development. Proteins were coated onto polystyrene beads and used in indirect EIAs employing goat-anti-human IgG peroxidase conjugate. There were 7 proteins derived from *Plasmodium falciparum* (Pf), 3 from *P. malariae* (Pm), 5 from *P. vivax* (Pv), and one from *P. ovale* (Po); these included, for the first time, the MSP1-19 antigens from each species. Results were compared to reactivity rates using a commercial ELISA. There were 212 donor sera available for testing; of these 205 were antibody positive by the commercial test and 211 were IgG positive in at least one bead EIA. Only one donor was negative in all assays. Immunoreactivity rates by species were: 209 Pf (98.5%), 161 Pm (76%), 159 Pv (75%), and 96 Po (45%). Highest IgG prevalence for any single marker was Pf-MSP1-19 (187/212, 88.2%). Of the 7 commercial assay negative donors, 5 were MSP1-19 IgG positive (3 Pm, 2 Pf) and one was Pf-HRP-2 IgG positive. These results indicate that the majority of blood donors from Cameroon exhibit falciparum antibodies and that anti-vivax prevalence was much higher than expected. A single assay employing MSP1-19 proteins from all four plasmodium species would detect antibodies in 96.2% of all donors from this malaria endemic country.

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UTILITY OF MSP1-19 RECOMBINANT ANTIGENS FOR DETECTION OF ANTIBODIES TO *PLASMODIUM FALCIPARUM, OVALE, MALARIAE* AND *VIVAX*

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Detection of antibodies elicited by infection with one of the four major *Plasmodium* species that cause malaria in humans can be used to assess the level of exposure in various populations. Blood donors in some countries are deferred from donating if they had malaria in the past, or have lived in or recently traveled to a malaria endemic area. In Australia and several European nations, donor eligibility is restored if antibodies to plasmodium antigens are not detectable after the deferral period. Currently available commercial plasmodium antibody assays do not include (or do not disclose) antigens derived from *P. ovale* (Po) or *P. malariae* (Pm). We have cloned the entire MSP1 gene from Po and Pm and expressed the immunodominant carboxyl-terminal P19 regions in *E. coli*. MSP1-19 antigens from *P. vivax* (Pv) and *P. falciparum* (Pf) were also cloned and expressed. The utility of these antigens to detect plasmodium antibodies were evaluated individually in research EIAs and results compared to that of a commercial ELISA. A panel of 24 human sera from individuals with blood smear and IFA confirmed infections were tested. The commercial test detected 8/8 and 4/4 individuals with Pv and Pf infections, respectively, as did the corresponding species-specific MSP1-19 EIAs. In contrast, the commercial ELISA detected antibody in 0/2 and only 5/8 individuals with Pm or Po infections, respectively, while species-specific MSP1-19 EIAs detected all individuals with blood smear and IFA confirmed Pm and Po infections. These data demonstrate enhanced antibody assay efficacy by use of MSP1-19 recombinant antigens from *P. ovale* and *P. malariae* in addition to *P. vivax* and *P. falciparum*.

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OPTIMAL-IT® AS AN ALTERNATIVE TO MICROSCOPY FOR MALARIA DIAGNOSIS IN REMOTE AREAS UNABLE TO ACCESS GOOD LABORATORY SERVICES IN BURKINA FASO.

Innocent Valea

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We assessed the performance of OptiMAL-IT®, for the detection of malaria infection in comparison with microscopy in 464 children under 5 years of age with uncomplicated malaria attending the Nanoro district hospital, Burkina Faso. A finger prick was done and two blood drops collected, one for the OptiMal-IT® rapid diagnosis test (RDT) and the other for the microscopy. The RDTs were performed according to the manufacturer's recommendations and blanked to the microscopy slides readers. The results were classified, using the thick film microscopy as the gold standard. The primary outcomes were the test sensitivity, specificity, positive predictive value and negative predictive value. The prevalence of malaria infection determined by microscopy and by OptiMAL-IT® was respectively 82.8 % and 82.3%. The sensitivity and specificity of OptiMAL-IT® for the detection of Plasmodium spp (for any parasite density) were respectively 98.70 (CI 95% = 97.56 - 99.84) and 96.25 (CI 95% = 94.35 - 98.15) with a positive likelihood ratio of 7.89. However we observed a decrease of sensibility when the parasites densities were less than 500 parasites/µl. From the results reported in this study, OptiMAL-IT® could be a good alternative for malaria diagnosis in Burkina Faso, though the issue of conservation in peripheral health facilities should carefully be considered before their implementation. However, the decrease of sensibility for low parasite densities limits his use for the follow-up of malaria cases.

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PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2 ELISA FOR USE IN MALARIA INTERVENTION TRIALS

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Microscopy is the gold standard for detection and quantification of malaria asexual parasitemia. Unfortunately, a number of factors mitigate utility of malaria microscopy. i.e its inability to detect the sequestered late stages parasites, the method is poorly reproducible and even then, requires considerable expertise for correct diagnosis and quantification. Due to these reasons, parasite biomarkers such as *P. falciparum* Histidine Rich Protein 2 (PfHRP2) are increasingly being used to resolve problems of malaria diagnosis. In a series of studies, we have sought to develop PfHRP2 ELISA as a quantitative assay that could ultimately benefit malaria intervention trials. The dynamic range of PfHRP2 ELISA has been determined as 3.91-250 ng/mL for rPfHRP2 (CV of 0.29-7.56%) and 11.7-750 infected RBC/µL (iRBC/µL) for spiked iRBC (CVs of 0.29-7.56%). The same spiked samples evaluated by microscopists had a similar

sensitivity, but CVs were unacceptably high (20.7-161.6%). PfHRP2 is known to persist in circulation for up to 28 days even after a successful anti-malarial chemotherapy. We therefore designed experiments to determine blood compartment survival of PfHRP2. Compartment analysis by ELISA, flowcytometry and immuno-fluorescence indicate that the bulk of persistent PfHRP2 is inside the RBC and not in plasma. We conclude that PfHRP2 ELISA is a suitable adjunct to microscopy and could benefit malaria intervention trials. Finally, the compartment survival findings imply that PfHRP2 persistence is a reflection of iRBC ½ life rather than plasma clearance.

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EVALUATION OF 3 RAPID DIAGNOSTIC TESTS (CARESTART™ MALARIA 3 LINE PLDH (PAN, PF), OPTIMAL-IT® PLDH (PAN, PF) AND CARESTART™ 2 LINE PLDH (PAN) FOR THE DIAGNOSIS OF MALARIA IN MYANMAR

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Obtaining biological confirmation of the diagnosis is considered an essential element of the detection and treatment of malaria in MSF programmes. Several Rapid Diagnosis Tests (RDTs) using monoclonal antibodies against histidine-rich protein 2 (HRP-2) produced by *Plasmodium falciparum* (*Pf*), have shown reliable results when evaluated. A second type of RDT, targeting parasite lactate dehydrogenase (pLDH), produced by all *Plasmodia* species, is appearing on the market increasingly, but few studies support the use of these new tests. In an MSF-Switzerland programme in Dawei, Myanmar, 3 pLDH based RDTs were evaluated in patients presenting with clinically suspected malaria. A subset of patients with microscopically confirmed malaria had their RDTs repeated on days 2, 7 and then weekly until negative. Each RDT was read twice. At the end of the study samples of study RDTs were sent for temperature stability and quality control testing. Between Aug and Nov 2007, 1004 patients were enrolled in the study. Slide microscopy diagnosed 214 *P. vivax* (*Pv*), 99 *Pf* and no malaria in 650 cases. The sensitivities (Se) and specificities (Sp), of the RDTs for the detection of malaria were: *OptiMal-IT®*: *Pf*: Se 95.2% [CI^{95} 87.5-98.2], Sp 94.7% [92.8-96.2]; non-*Pf*: Se 89.6% [83.6-93.6], Sp 96.5% [94.8-97.7]; *Pv* alone: Se 91.4% [85.3-95.2]. *CareStart Malaria™ 3 line*: *Pf*: Se 93.5% [CI^{95}85.4-97.3], Sp 97.4% [95.9-98.3]; non-*Pf*: Se 78.5% [71.1-84.4], Sp 97.8% [96.3-98.7]; *Pv* alone: Se 80.6% [72.9-86.5]. *CareStart Malaria™ 2 line*: *Pf*: Se 89.1% [CI^{95} 84.2-92.6], Sp 94.7% [92.5-96.3]; *Pf* alone: Se 95.6% [87.7-98.5]; *Pv* alone: Se 91.0% [92.5-96.3]. Inter-observer agreement was excellent for all tests (kappa > 0.9). The median time for the RDTs to become negative was 2 days for the CareStart™ tests and 7 days for OptiMAL-IT®. Only *CareStart Malaria™ 2 line* passed all heat stability evaluation. In conclusion, in this study OptiMAL-IT® RDT and the CareStart™ 2 line pLDH (Pan) test met the 95% threshold of Se for detection of falciparum malaria set by WHO. The Se of both tests to detect vivax malaria exceeded 90%. However any decision to implement one of these tests should take into account the heat stability results, positive predictive value in the context in which it would be deployed and cost-effectiveness.

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FEASIBILITY OF THE RAPID DIAGNOSTIC TESTS (RDTS) FIELD USE FOR MALARIA CASE MANAGEMENT IN SENEGAL

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In 2006, a pilot study on the feasibility of the RDT use in the field was carried out by the Service of Parasitology of the University C A Diop Dakar, in collaboration with the National Malaria Control Program. Eleven medical districts and two reference hospitals were selected according to the epidemiology of Malaria in Senegal to use RDT's for management of malaria cases. Each district was compared with a district control (without intervention) located in the same area. The Paracheck® test detecting HRP2 was selected. The operational feasibility was measured using questionnaire. At the end of one year of intervention, analysis of the results show that the biological confirmation of cases allowed the real estimate of malaria morbidity and a reduction in the consumption of ACTs in the majority of the medical structures. The acceptability of the TDR by health workers and the patients was good. The introduction of RDTs allowed an improvement of the diagnosis and the management of malaria cases in Senegal.

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DETECTION OF VIVAX MALARIA BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) METHOD IN THE REPUBLIC OF KOREA

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Loop-mediated isothermal amplification (LAMP) of DNA is a novel technique that rapidly amplifies target DNA under isothermal conditions. LAMP method for rapid malaria detection previously reported was used for evaluating the sensitivity and specificity of LAMP versus microscopic examination and nested PCR from *Plasmodium vivax* patients in the Republic of Korea. LAMP was performed for vivax malaria diagnosis and compared with results from microscopic examination and nested PCR. Total 131 blood samples were collected from patients, who came to a clinic in hospitals and field clinics. Out of 131 samples, total 101 microscopically positive blood samples were determined by LAMP method, which detected malaria parasites in 99 of 101 microscopically positive blood samples (sensitivity, 98.0%; specificity, 100%), in good agreement with the results of nested PCR. The LAMP reactions yielded results within about 60 min and results were determined by naked eye. Accordingly, in comparison to the results obtained by microscopy, LAMP had a similar sensitivity and specificity and LAMP yielded results similar to those of nested PCR in a shorter turnaround time. Because it can be performed with a simple technology using water-bath, heat-processed template and the ease in results readout, LAMP may be applicable diagnosis method for vivax malaria in the Republic of Korea.

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MALARIA SLIDE-READING FOR QUANTITATION OF PARASITEMIA IN MALARIA INTERVENTION TRIALS: A BETTER TRANSITION POINT FROM THICK TO THIN FILMS

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People in western Kenya, an area with high malaria attack rates, experience extremely high parasite densities when they are sick with falciparum malaria. In such holoendemic areas, calculation of parasite density is exceedingly time-consuming and error prone, as current practice requires manually counting parasites and blood cells on thick or thin blood films. Specifically, a microscopist starts the process by reading a thick blood film. If the ratio of parasites to WBCs is too high (e.g., 2 asexual parasites per WBC), the reader switches to a thin film and counts parasites among RBCs. Once parasite density surpasses a certain threshold, thin films are preferable because reading thick films cannot distinguish superimposed parasites. On the other hand, reading thin films for lower parasite densities is more time-consuming due to the requirement to review more microscopic fields for infrequent events. Therefore, finding an optimal transition point to choose between thick and thin films would balance the problems inherent in each method. A recent randomized Phase 2 field trial of a malaria vaccine showed a bimodal distribution of parasite densities in both the vaccine and comparator groups rather than a smoother, continuous distribution. This bimodality largely reflected the type of film that was read (i.e., low densities came from thick films and high densities came from thin films, but there were no "middle" densities) in that there were fewer parasite densities observed at the transition point used to switch from thick to thin film. Microscopists re-read both thick and thin films in a blinded manner according to a detailed SOP from clinical episodes of malaria that occurred during this trial. Analyses of the new data suggest that the original transition point from thick to thin films -- at least 400 parasites in the presence of 200 WBCs -- was, in fact, too low, and that a higher threshold would have been more appropriate. Based on these new readings, we have determined a better range of transition points that may help to prevent this type of bimodality in future trials.

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ANTIMALARIAL ACTIVITY OF PHENYLTHIAZOLYL-HYDROXAMATE-BASED HISTONE DEACETYLASE INHIBITORS

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The antimalarial activity and pharmacology of a series of phenylthiazolyl-hydroxamate-based histone deacetylase inhibitors (HDACIs) was evaluated. In *in vitro* growth inhibition assays approximately 50 analogs were evaluated against four drug resistant strains of *Plasmodium falciparum* (Pf). The range of 50% inhibitory concentrations (IC_{50}s) was 0.0005 - > 1 μM. Five analogs exhibited IC_{50}s < 3 nM, and three of these exhibited selectivity indices > 600. The most potent compound, WR301801 (YC-2-88) was shown to cause hyperacetylation of Pf histones which is a marker for HDAC inhibition in eukaryotic cells. WR301801 did not exhibit cures in *P. berghei*-infected mice at oral doses as high as 640 mg/kg/day x 3 or in *P. falciparum*-infected *Aotus lemurinus lemurinus* monkeys at oral doses of 32 mg/kg/day x 3, despite high relative bioavailability. The failure of monotherapy in mice may be due to a short half-life, since the compound was rapidly hydrolyzed to an inactive acid metabolite by loss of its hydroxamate group *in vitro* (half-life of 11 min in mouse microsomes) and *in vivo* (half-life in mice of 3.5 h after single oral dose of 50 mg/kg). However, WR301801 exhibited cures in *P. berghei*-infected mice when combined at doses of 52 mg/kg/day orally with sub-curative doses of chloroquine. Next generation HDACIs with greater metabolic stability than WR301801 may be useful as antimalarials if combined appropriately with conventional antimalarial drugs.

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IN VITRO ANTIMALARIAL ACTIVITY 4(1H) PYRIDONE DERIVATIVE GSK932121

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GSK932121 is the selected compound of a novel class of antimalarial agents that act as potent inhibitors of *Plasmodium* mitochondrial function. Results from a variety of experiments provide strong evidence that the site of action of 4-(1H) pyridones is cytochrome b, a critical element of the respiratory complex III or bc1 complex. This target is inhibited by several compounds, some of them already in clinical use, such as atovaquone. However, although atovaquone and pyridones have the same target, pyridones in contrast to atovaquone seem to have a different mode of binding. Because of this, GSK932121 is fully active against isolates carrying resistance determinants to marketed compounds and shows no cross resistance with atovaquone. The compound was also a selective inhibitor in terms of whole-cell acitivity, it was at least 1000-fold more active in the parasite than in human cell lines. Factors such as the rate of antimalarial action and the recrudescence of *P. falciparum* parasites after incubation were also investigated. Exposure of parasites to a concentration of 2 μg/ml of GSK932121 resulted in a 50% inhibition of growth after 2 hours and 90% inhibition after 10hrs of incubation respectively. Parasites incubated for 3 days with 2 μg/ml of GSK932121 were detected after 17 days of incubation, indicating an important reduction of the initial parasitemia because of the effect of the drug. These results taken together indicate that GSK932121 is a promising new antimalarial drug.

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ATORVASTATIN, A HMG-COA REDUCTASE INHIBITOR, AS A NEW THERAPEUTIC STRATEGY IN *PLASMODIUM FALCIPARUM* MALARIA

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Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, influence a broad array of pathogenic microorganisms. In vitro activity of atorvastatin was assessed against 22 *Plasmodium falciparum* strains, from a wide panel of countries. Inhibitory concentration 50% ranged from 4.0 to 14.1 μM. The capacity of atorvastatin for reversing resistance in *P. falciparum* to quinoline antimalarial drugs, such as chloroquine, quinine, mefloquine and monodesethylamodiaquine, was assessed against these 22 strains. Atorvastatin had no effect on chloroquine or monodesethylamodiaquine activity. Atovastatine potentiated the activity of quinine in some strains and that of mefloquine in all strains. In parallel, each strain was genotyped to observe polymorphism on quinoline resistance-associated genes such as

pfcrt, *pfmdr1* and *pfmrp* or *pfnhe-1*. Analyses of potential association of polymorphism and synergy of quinine or mefloquine effects by atorvastatine are in progress.

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PRE-CLINICAL MOUSE TOXICITY STUDY OF THE THIRD GENERATION ANTIFOLATE, JPC-2056-I

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JPC-2056-I is a third generation orally active folic acid antagonist that is effective against resistant strains of *Plasmodium falciparum* and *Plasmodium vivax*. A pre-clinical toxicology study using CD-1 mice was conducted under an approved Animal Care and Use Committee protocol. The animals were administered drug in rodent food pellets containing JPC-2056-I (14, 42, 70 or 98 mg/kg/day) or Proguanil (positive control; 168 mg/kg/day). Animals in the negative control arm of the study were provided food pellets without drug. Blood samples were collected periodically for hematology and blood chemistry determinations and the results were reported earlier. Upon sacrifice, organs and tissues were harvested and submitted to Charles River Laboratories for gross pathology and histopathology. The NOAEL and LOAEL and the major pathology finding (lack of weight gain) were presented previously. Histopathology has been completed and the details of the results will be the subject of the presentation. Additional data related to a cell-based assay for hERG activity will also be disclosed.

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COMPARISON OF SYBR GREEN I, PICO GREEN AND [^{3}H]-HYPOXANTHINE INCORPORATION ASSAY ASSAYS FOR *IN VITRO* ANTIMALARIAL SCREENING OF MEDICINAL PLANTS FROM NIGERIAN ETHNOMEDICINE

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The standard method for *in vitro* drug screening is based on incorporation of [^{3}H]-hypoxanthine and the standard method for *in vitro* drug screening is based on incorporation of [^{3}H]-hypoxanthine, an expensive assay that utilizes radioactive materials and this poses safety and disposal problems in developing countries. Recently, non-radioactive screens have emerged using DNA stains as a reporter to measure parasite growth and their uses have been evaluated for antimalarial screening. This study compared the SYBR Green I (SG), PICO green® (PG) and the standard [^{3}H]-hypoxanthine (HP) incorporation assays for *in vitro* antimalarial screening of medicinal plants. A side-by-side comparison of SG, GP and HP methods was evaluated by determining the antimalarial activity of 24 medicinal plant extracts and standard antimalarial drugs against *Plasmodium falciparum* strain NF54. Parasite growth was determined using SG (0.01%), PG (0.3%) or incorporation of [^{3}H]-hypoxanthine (0.1µCi). The 50% inhibitory concentration (IC_{50}) of the plant extracts and the standard drugs were calculated from the three assays. IC_{50} values for all the extracts and antimalarial drugs tested using the three methods yielded similar results. Of the 24 plant extracts, the ethyl acetate extract of stem bark of *Cassia siamea* showed the highest *in vitro* antimalarial activity with IC_{50} values of 2.70 ± 0.63, 2.61 ± 0.77 and 2.83 ± 0.63 4 µg/ml in SG, PG and HP based assays respectively (P = 0.96). In contrast, the methanol extract of leaves of *Jatropha carcus* was inactive, IC_{50} values were 34.64 ± 2.12, 46.23 ± 5.19 and 33.07 ± 4.79 µg/ml in SG, PG and HP based assays respectively (P = 0.16). Furthermore, IC_{50} values for chloroquine in SG, PG and HP based assays were 2.72 ± 0.53, 3.33 ± 0.69 and 2.64 ± 1.31 ng/ml (P = 0.37) respectively. Also, IC_{50} values for artemisinin were 1.54 ± 0.12, 1.41 ± 0.57 and 1.13 ± 0.57 ng/ml (P = 0.87) in SG, PG and HP based assays respectively. The HP based assay exhibited the most robust signal-to-noise ratio of 100:1, compared to signal-to-noise ratios of 5:1 for SG and 6:1 for PG. The SG based assay is less expensive than the PG and HP based assay. SG appears to be a cost effective and safe alternative for antimalarial drug screening and a viable technique that may facilitate antimalarial drug discovery process especially in developing countries.

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DEVELOPMENT OF REVERSED CHLOROQUINES AS ANTIMALARIAL DRUGS: EFFICACY IN AN ANIMAL MODEL

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We have developed a class of molecules, termed Reversed Chloroquines (RCQs) that are hybrid molecules made up of a chloroquine (CQ) like moiety and a chemosentisizer (Reversal Agent, RA) against CQ-resistance (CQR). RCQs have been shown to have low-nanomolar *in vitro* IC_{50} values against either CQR or CQS malaria, often surpassing the activity of even CQ against CQS strains of *P. falciparum*. Here we report on an expanded set of RCQs, some of which surprisingly have stronger potency against CQR than CQS *P. falciparum*. A subset of these RCQs has been tested in a mouse model of malaria, and found to be capable of reducing the parasite burden to below detectable limits, and able to effect a cure by the oral route.

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IN VITRO AND *IN VIVO* EVALUATION AND EARLY TRANSITIONING STUDIES OF NOVEL QUINOLIZIDINYL- AND QUINOLIZIDINYLALKYL- DERIVATIVES OF 4-AMINOQUINOLINE WITH POTENT ANTIMALARIAL ACTIVITY

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New quinolizidinyl and quinolizidinyl-alkyl derivatives of 4-aminoquinolines have been synthesised. A terminal bulky bicyclic basic moiety has been introduced to prevent the metabolic oxidation that limits the usefulness of quinoline compounds. Leads have been obtained either by a semi-synthetic route starting from l-lupinine (a quinolizidine alkaloid extracted from *Lupinus luteus* or *L. hispanicus* seeds), leading to an optically active compound, or by a completely synthetic route leading to racemates. Three of these compounds are highly effective *in vitro* against both CQ-S, CQ-R or multi drug resistant strains of *Plasmodium falciparum*. No relevant cytotoxicity is detected against human or murine cell lines, as reported previously. They all inhibit β-haematin formation in the BHIA (b-Haematin Inhibitory Activity) assay suggesting interference with haem detoxification as mechanism of action, similarly to CQ. One of this compound, named AM1 has been selected for further characterisation. *In vivo*, AM-1 inhibits

parasitemia with ED_{50} of 5.1 or 4.7 mg/kg, per os against *P. berghei* or *P. yoelii* in a murine standard 4-day test, a dose that is very similar to that of CQ (ED_{50} po 3.8 mg/kg). Preliminary data on the pharmacokinetic parameters when given orally to CD1 mice, on inhibition of P450 isoforms *in vitro*, on *in vivo* toxicity and metabolism confirm that AM1 can be considered a promising lead to develop an effective antimalarial agent suitable for artemisinin-based combination therapy (ACT).

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USE OF THERMAL MELT ASSAYS TO IDENTIFY POSSIBLE CELLULAR TARGETS OF ANTI-*PLASMODIUM* COMPOUNDS

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Screens of large chemical libraries for inhibitors of *Plasmodium falciparum* growth have led to the identification of hundreds of compounds with ED_{50}'s below 5 μM, tractable chemistry, and limited toxicity. However, hit-to-lead development with such compounds remains difficult as long as their cellular targets remain unknown. To address this problem, we tested for possible interactions among 89 of these compounds and 26 recombinantly expressed *Plasmodium* proteins using thermal melt assays. Compounds and proteins were combined in 96-well plates along with a probe that fluoresces when bound to hydrophobic regions of proteins. Plates were heated from 20 to 90°C in a Real Time-PCR machine, and proteins' melting temperatures (Tm's) were measured for each well. Tm's could be determined for 24 of 26 Plasmodium proteins tested. Tm's of 4 proteins -- 6-pyruvoyl tetrahydropterin synthase (6PTS), adenosine deaminase (ADA), methionine aminopeptidase 1 (MAP1), and S-adenosylhomocysteine hydrolase (SAHH) -- were increased by >2°C by at least one of the 89 compounds, implying that these compounds bind to, stabilize, and possibly inhibit the proteins. Enzyme activity assays confirmed inhibition of ADA and MAP1 by their respective ligands, with IC_{50}'s ranging from 1 to 10 μM. Additional work is needed to determine whether these proteins are primary targets of the corresponding ligands *in vivo*. Nevertheless, thermal melt screening appears to be a promising way of identifying possible cellular targets of anti-*Plasmodium* compounds.

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IN VITRO HEPATIC METABOLISM STUDIES OF PRIMAQUINE AND RELATED ANTIMALARIAL COMPOUNDS

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The hepatic metabolism of primaquine (PQ), an 8-aminoquinoline with broad spectrum antimalarial activity yet an incomplete metabolic profile, was evaluated *in vitro* using commercially available primary human hepatocytes and microsomal preparations. In addition CYP450 isoenzymes were used to determine the potential for metabolism-based drug interaction of PQ and carboxy-PQ (its known *in vivo* metabolite) as well as the closely related quinine, quinacrine, and pamaquine. To ascertain the metabolic activity of the liver cells in suspension, sub-populations of these were used to metabolize markers specific for CYP450 1A2 (phenacetin), 2C19 (mephenytoin), 2D6 (propranolol), and 3A4 (testosterone) isoenzymes in parallel with PQ and carboxy-PQ. At 2 and 4 hours the reactions were stopped and the samples analyzed using tandem mass spectrometry. Compared to time and temperature controls, all marker concentrations were reduced to <50% their original concentrations after 4 hour incubations. Similarly, PQ was metabolized to 38% its original concentration after 4 hours. The disappearance of PQ was accompanied by the appearance of carboxy-PQ, in the same samples, at equimolar concentrations consistent with half of the missing PQ. Pooled human liver microsomes also metabolized PQ to carboxy-PQ in a reaction moderately inhibited by 2C19 and 2C9 selective inhibitors, but extensively inhibited by CYPs 3A4 and surprisingly 1A2 selective inhibitors. All antimalarials tested were metabolically stable (half-life > 60 min) in human liver microsomes except for pamaquine (half-life = 34 min). Quinacrine and pamaquine showed increased potential (IC50 < 1μM) for CYP2D6, while PQ showed increased potential for 1A2 and moderate potential (IC_{50} < 10 μM) for 2C9 and 3A4. The contribution of plasma and blood to PQ metabolism along with the significance of the drug interactions described are being evaluated. This work provides plausible metabolism-based explanations to reports of drug interactions *in vivo*.

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IN VITRO AND *IN VIVO* EVALUATIONS OF NEW QUINOLINE METHANOL ANALOGS OF MEFLOQUINE

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Quinoline methanol analogs of mefloquine are being evaluated by the U.S. Army for development as an antimalarial prophylaxis. This work provides *in vitro* metabolism and disposition data and *in vivo* efficacy data for a series of newly synthesized analogs. The compounds were selected based on their relatively high antiparasitic potency when tested *in vitro*. The IC_{50}s against four strains (W2, D6, C235, C2A) of *P. falciparum* ranged from <0.5 to 120 ng/ml or <1 to 270 nM. Metabolic stability was measured following incubations with liver microsomes in a high throughput assay. LCMS analysis of the extracted microsomal samples yielded median half-life values of >60 and 47 minutes for human and mouse, respectively. The potential for drug interaction was measured for the principal P450 metabolic enzymes (1A2, 2D6, 2C9, 2C19, 3A4) using expressed isoenzymes and fluorescent markers of enzymatic activity specific for the given enzyme. Most compounds displayed low potential (IC_{50}s >10μM) for interactions with CYPs 2C9 or 3A4, while several compounds showed a moderate interaction potential (IC50s between 1-10μM) for CYPs 1A2 and 2C19. Approximately one-third of the compounds tested had an increased (IC50 < 1μM) interaction potential for 2D6. Metabolite identification exhibited masses consistent with hydroxylated and dealkylated products as the most abundant metabolites in microsomal preparations for all four species (human, monkey, rat, mouse) evaluated. Determinations of apparent permeability coefficients using Caco-2 cells showed most of the compounds tested were moderately permeable. When using an *in vivo* challenge monkey model, six of the compounds resulted in better than 90% decreased parasitemia. Preliminary cure data in mice and monkeys are being evaluated in light of the compounds' pharmacokinetic profiles. In general, this work characterizes metabolism and disposition parameters utilized in identifying lead compounds for development.

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METABOLISM OF CYP450 MARKERS OF ENZYMATIC ACTIVITY AND PRIMAQUINE BY THE INDUCED HC-04 IMMORTALIZED HEPATIC CELL LINE

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HC-04, the immortalized cell line of hepatic origin known to sustain *Plasmodium falciparum* and *P. vivax* growth *in vitro*, was evaluated for the inducibility of its CYP450 enzymes along with its capacity to metabolize markers of CYP450 activity. Enzyme induction was determined by quantitative PCR while substrate metabolism was determined by tandem mass spectrometry. When compared to untreated cells, treatment of HC-04 cells for 72 hours with the CYP inducer 3-methylcholantrine (3MC) resulted in 25-50 fold increases in expression of CYPs 1A2, 2D6 and 3A4. The expression of CYP1A2 was comparable to that observed for cryopreserved normal human hepatocytes, while the 2D6 and 3A4 expression was less than 10% that of normal cells. Treatment of the cells with the inducer rifampicin resulted in smaller (2 to 10 fold) increases in CYPs 2D6 and 3A4 but not 1A2. Previously induced HC-04 cells failed to significantly metabolize phenacetin or mephenytoin, markers of CYPs 1A2 and 2C9 enzymatic activity, respectively. However, a 24% metabolism of propranolol (marker of 2D6 activity) was observed after a 4 hour incubation using HC-04 cells pretreated with 3MC. In addition, the metabolism of testosterone (marker of 3A4 activity) was greater than 95% after a 2 hour incubation with induced cells compared to non-induced. Similarly, 32% metabolism of the 8-aminoquinoline primaquine was observed after its incubation with HC-04 cells for 4 hours, compared to control incubations. Combined, the data demonstrate the potential of HC-04 for development of an *in vitro* liver stage antimalarial efficacy model.

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ANTIMALARIAL ACTIVITY OF SEMISYNTHETIC ANALOGS OF STEROID ISOLATED FROM *SOLANUM NUDUM* (SOLANACEAE)

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Given the antimalarial activity of steroids isolated from *Solanum nudum* plant is necessary to identify the part of the molecule involved in the activity through study of structure-activity relationship guided by antiplasmodial activity and cytotoxicity assays that supporting the potential use antimalarial of compounds steroidal Diosgenone was modificated in OH-1, Met-1, NB-1, PTSN-1, diosgenine, dicarbonilic diosgenine, and both reduced diosgenin and diosgenone. Also SN-2 was modified (diacetate, aldehyde, ketone-aldehyde, aldehyde-alcohol and alcohol-ketone). Using bromide 3 - (4.5 Dimethylthiazole-2il) -2,5-diphenyl tetrazolium (MTT) was evaluated its cytotoxic activity and its antimalarial activity *in vitro* strains FCB-2 and NF-54 (chloroquine resistant and chloroquine sensitive, respectively) using incorporation of hypoxanthine were made It was found that diosgenone analogs had low toxicity similar to natural compound, except for the derivative dicarbonilic (47 µg/ml Vs. 100.9 µg/ml) and toxicity in SN-2 steroid derivatives (924 µg/ml for natural steroid Vs 19.3. for the derivative Aldehido-ketone). Antimalarial activity *in vitro* for derivatives diosgenone less than the natural compound was found when replacing the carbonyl and antimalarial activity in diosgenin, which has no activity antimalarial after of addiction carbonyl group. Also an IC_{50} of 0.3 µg/ml for derivative diacetate SN-2 and 4.1 µg / ml for acetate present in the reaction of SN-2 compared to 222.8 ug / ml for natural compounds was found. All derivatives of SN-2 that undergone changes in their functional group acetate showed an antimalarial activity better than natural compound. In conclusion, the carbonyl group might be necessary for the action of the antimalarial diosgenona. It is necessary to repeat corroborate the antimalarial activity of derivatives of SN-2 using a different reagent oxidation.

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RISK FACTORS OF POOR TREATMENT OUTCOME IN PATIENTS TREATED WITH ARTEMETHER/LUMEFANTRINE (COARTEM®) AS FIRST-LINE TREATMENT FOR UNCOMPLICATED MALARIA IN SOUTH-EASTERN TANZANIA

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A three day's six dose regimen of Artemether/Lumefantrine (Coartem®) is efficacious to treat uncomplicated plasmodium malaria and is currently prioritized by the World Health Organization as a replacement for failing antimalarial monotherapies. Tanzania mainland started the implementation of new first-line antimalarial policy with Coartem® beginning of 2007. Within the frame of the Tanzanian National Malaria Control Programme' operational research platform, Ifakara Health Research and Development Centre, investigated the factors impeding good treatment outcome in the Coartem® treated vulnerable patients. An *in vivo* follow up study included risk assessment component to investigate under five years malaria patients who were prescribed Coartem® and gave consent to be followed up for one month. Patients presented to health facilities in Ifakara town and were randomized in to two groups to receive Coartem®. One group was administered treatment at the health facility (observed group) and the second (non-observed) group was given full dose of Coartem® for self administration at home. Patients were followed up for 28 days to assess parasite clearance and factors associated with poor treatments outcome assessed by questionnaire at the end the 6th dose on day three. Overall 272 patients were recruited (beginning in May 2007): In observed group 138 and 134 in non-observed group. Nearly all (99%) reported to have been properly instructed on how to administer Coartem®. There was 18% treatment failure in non-observed group compared to 5% in observed group. Self administration of drug (Odds ratio [OR] 0.24; 0.10-0.59; 95% confidence intervals [CI] and leaving far away beyond normal walking distances from health facility OR 0.11; 0.03-0.48 CI were associated with poor outcome of parasitaemia clearance. In conclusion, in malaria endemic countries good coverage of therapeutical outcome with efficacious antimalarial is likely to be compromised by lack of health facility system within possible close distance to the patient for proper treatment supervision.

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IN VITRO ANTIMALARIAL DRUG SENSITIVITY TRENDS IN KENYAN *PLASMODIUM FALCIPARUM* ISOLATES USING NON-RADIOISOTOPIC SYBR GREEN I FLUORESCENCE ASSAY AND *PF*MDR COPY NUMBER ESTIMATION

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Plasmodium falciparum in vitro drug susceptibility testing, coupled with molecular analysis, are valuable tools for predicting *in vivo* drug susceptibility. Newer non radio-isotopic technology like SYBR green offers field expedience. 118 blood samples containing *P. falciparum* parasites

from untreated volunteers in West Kenya were screened for sensitivity to 6 antimalarial drugs using SYBR green I fluorescence assay. Controls were chloroquine (CQ) and mefloquine (MQ) resistant and sensitive clones. Drug sensitivity was expressed as 50% inhibitory concentrations (IC_{50}). Copy numbers of *Pf*MDR1 were estimated. Drug susceptibility results were obtained for 80 (68%) samples: 63 immediate *ex vivo*, 17 cultured. Approximately 50% of the isolates had IC_{50} values suggestive of resistance to CQ and MQ. For *Pf*mdr1, about 30% of tested isolates showed 2 or 3 copy numbers. In conclusion, for *P. falciparum in vitro* drug sensitivity profiles, we have established the SYBR green I-based fluorescence assay, a 1st in East Africa. Preliminary findings substantiate worrying clinical observations of growing resistance to some antimalarials, beyond CQ and Fansidar.

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CHLOROQUINE AND SULPHADOXINE-PYRIMETHAMINE RESISTANT GENOTYPES OF *PLASMODIUM FALCIPARUM* IN MILD MALARIA AND CEREBRAL MALARIA PATIENTS IN INDIA WITH EVIDENCE OF SELECTIVE SWEEPS

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Treatment of *Plasmodium falciparum* is complicated by the emergence and spread of parasite resistance to many of the first line drugs used to treat malaria. Anti-malarial drug resistance has been associated with specific point mutations in a number of genes, suggesting that these single nucleotide polymorphisms can be useful in tracking the emergence of drug resistance. In addition, the use of neutral genetic markers, such as microsatellites, can be used to address changes in population diversity and levels of gene flow which can then be used to assess the strength of selective pressures and origins and spread of drug resistance. Determination of the prevalence and spread of these mutations is critical to developing localized drug policy and contributing to a global map for anti-malarial drug resistance. In India, *P. falciparum* can manifest itself as asymptomatic, mild, or severe malaria, with or without cerebral involvement. We tested whether chloroquine and antifolate drug resistant genotypes would be more commonly associated with cases of cerebral malaria than with cases of mild malaria in the province of Jabalpur, India by genotyping the genes *dhps*, *dhfr*, *pfmdr1*, and *pfcrt* using pyrosequencing, direct sequencing, and real time PCR. Further, we used microsatellites surrounding the genes to determine the origins and rate of spread of the drug resistant genotypes in this area. Approximately 50 and 60% of the *P. falciparum* associated with mild malaria and cerebral malaria cases were mutants of *dhfr* and *pfcrt* loci, respectively while less than 15% of the parasites were mutants of *dhps* and *pfmdr1* loci. Drug resistant genotypes were equally likely to be associated with cerebral malaria as they were with cases of mild malaria. We found evidence of a selective sweep in *pfcrt* and, to a lesser degree, *dhfr*, indicating high levels of resistance to chloroquine and sulphadoxine-pyrimethamine. Microsatellites surrounding *pfcrt* indicate that the genotypes were most similar to those found in Papua New Guinea while those surrounding *dhfr* were most similar to haplotypes found in Southeast Asia.

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CLEARANCE OF DRUG RESISTANT MALARIA PARASITES IS ASSOCIATED WITH HOST GENETIC DETERMINANTS

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The extension of *Plasmodium falciparum* strain parasite poses a big challenge in malaria endemic countries in term of effective treatment against acute malaria and the tools for a monitoring of this resistance. *In vivo* tests, *in vitro* assays and molecular test do not provide full understanding that a number of patients cleaned resistant parasites while others were unable. Human genetic may participate in eliciting the mechanism. We treated 189 patients suffering from uncomplicated Falciparum malaria with Amodiaquine (3 days course) in Burkina Faso and followed up for 28 days. The point mutation Pfcrt T76K is found in 59.3% (112/189) of the patients. Clinical outcome is known for all these patients: Ninety nine over one hundred and twelve (88.4%) patients cleared their parasites compared to 11.6% who did not. All patients carrying the marker of drug resistant were successfully typed for 67 SNPs (Single Nucleotide Polymorphism) located on 17 chromosomes. The Single Nucleotide Polymorphism (SNPs) was analyzed by primer-extension and MALDI-TOF mass-spectrometry in different immune or inflammatory genes and/or promoter regions. Preliminary analysis showed that the SNP rs 17140229 (polymorphism of the gene Cystic Fibrocis Transmembrane Conductance Regulator (ATP-binding cassette sub-family C member 7)) was associated resistant parasite clearance (OR=0.30 95% CI [0.14-0.97]) while the SNP rs 229587 (polymorphism of the gene Spectrin β, erythrocytic includes spherocytosis clinical type I) was associated with the non clearance phenotype (OR=3.89 95% CI [1.10-20.97]). There was no statistically significant association of parasite clearance phenotype with the other SNPs but analysis of more extend sample is needed. This analysis is providing additional light in the participation of the host genetic in the elimination of drugs found to be resistant in *in vivo* and *in vitro* tests.

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STATUS OF THE ARTEMISININ RESISTANCE-ASSOCIATED *PF*ATPASE6 S769N MUTATION IN *PLASMODIUM FALCIPARUM* INFECTIONS OF LUSAKA URBAN DISTRICT, ZAMBIA

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Artemisinin derivatives constitute a key constituent of present-day treatment for *Plasmodium falciparum* malaria. In Zambia artemisinin-based combination therapy (ACT) has been implemented since chloroquine mono-treatment was replaced in national malaria policy revisions of 2003. Resistance to artemisinin is associated with a S769N point mutation in the sarcoendoplasmic reticulum calcium ATPase6 (SERCA-*Pf*ATPase6) gene of *P. falciparum*. However, the baseline or current levels of this mutation in Zambia remain unknown. Using a simple nested PCR and allele-specific restriction enzyme digestion strategy, *P. falciparum* infections from 10 sites in Lusaka urban district were assayed for the prevalence of the *Pf*ATPase6 S769N mutation. The availability of current first line ACT drug regimen (artemether-lumefantrine) and the extent to which it has been used since introduction were assessed using interview by questionnaire. Of 104 infections that were analyzed, 100% carried the artemisinine-sensitive wild type allele, 769S. Artemether-lumefantrine was available in both health centres and private chemists of Lusaka urban district. Of 119 respondents that were interviewed at least 38 (31.9 %) had been affected by malaria since artemether-lumefantrine introduction, and all had been treated with the ACT regimen. It was concluded that the artemisinin resistance mutation is absent from Lusaka district, suggesting full *P. falciparum* sensitivity to artemisinin, unless a different resistance mechanism occurs

in the area. These data can serve as a base-line for future surveillance of artemisinin susceptibility in Lusaka urban district. ACT's appear to be both widely available and utilized as first line malaria treatment in the district.

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ARTEMISININ-BASED COMBINATIONS VERSUS AMODIAQUINE PLUS SULFADOXINE-PYRIMETHAMINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN FALADJE, MALI

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Because of the emergence of chloroquine resistance in Mali, artemether-lumefantrine or artesunate-amodiaquine (AS/AQ) are recommended as first-line therapy for uncomplicated malaria, but not been available in Mali until recently because of high costs. From July 2005 to January 2006, a randomized open-label trial of 3 oral antimalarial combinations AS/AQ, artesunate plus sulfadoxine-pyrimethamine (AS/SP), and AQ plus SP (AQ/SP) was conducted in Faladje, Mali. We enrolled 397 children <5 years of age with uncomplicated *falciparum* malaria, and followed them for 28 days to assess treatment efficacy. Baseline characteristics were similar in all three treatment groups. The uncorrected rates of adequate clinical and parasitologic response (ACPR) were 55.7%, 90.8%, and 97.7% in AS/AQ, AS/SP, and AQ/SP, respectively (p<0.001); after PCR correction ACPR rates were similar among treatment groups: 95.4%, 96.9%, and 99.2% respectively (p=0.17). Mean hemoglobin concentration increased across all treatment groups from Day 0 (9.82 ±1.68 g/dL) to Day 28 (10.78± 1.49 g/dL) (p<0.001), with the greatest improvement occurring in children treated with AQ/SP. On Day 2, the prevalence of parasitemia was significantly greater among children treated with AQ/SP (50.8%) than in children treated with AS/AQ (10.5%) or AS/SP (10.8%) (p<0.001). No significant difference in gametocyte carriage was found between groups during the follow-up period except on Day 3 where a greater proportion of children treated with AS/SP were gametocytemic (p=0.03).The combination of AQ/SP provides a potentially low cost alternative for treatment of uncomplicated *Plasmdium falciparum* infection in Mali, and appears to have the added advantage of longer protective effect.

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THE PREVALENCE OF THE *PF*CRT-76 POINT MUTATION ON *PLASMODIUM FALCIPARUM* MALARIA INFECTIONS OF LUSAKA URBAN DISTRICT, ZAMBIA

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Malaria is a leading cause of morbidity and mortality in Zambia, where 95% of the cases are caused by *Plasmodium falciparum.* Chloroquine, the erstwhile drug of choice for uncomplicated malaria treatment, was replaced with sulphadoxine-pyrimethamine interim treatment in 2002, followed by artemisinine-based combination therapy in 2003. Nation-wide sentinel-based *in vivo* efficacy studies at the time documented prevailing chloroquine therapeutic failure rates ranging from 25-52%, with up to 90% prevalence of the *P. falciparum* chloroquine resistance-conferring K76T mutant. The current study assessed the prevalence of the K76T mutant in Lusaka urban district four years after the suspension of chloroquine use. A total of 161 filter paper blood spots were collected from patients visiting 10 randomly selected government owned health centres around Lusaka between September 2006 and April 2007. Parasite DNA was extracted by the chelex method and assays for the K76T mutant were performed by nested PCR and restriction enzyme digestion. The K76T mutation occurred in 53.8% of the infections, suggesting a decline in chloroquine resistance was taking place. However, the reintroduction of chloroquine, either, alone or in combination therapy, is not recommended at this time.

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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAYS FOR DETERMINING CHLOROQUINE IN WHOLE BLOOD SPECIMENS

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The overall aim of this project is to apply pharmacokinetic-pharmacodynamic (PK-PD) models in a longitudinal trial of repeated treatment with chloroquine (CQ) combinations to learn how best to combine antimalarial drugs to deter the emergence and spread of resistance. Selection of drug resistant *P. falciparum* is proposed to occur in a drug concentration range that extends from the minimum inhibitory concentration (MIC) of susceptible parasites to the MIC of the resistant parasites. We term this concentration range the 'window of selection'. No antimalarial drug has a window of selection defined, other than pyrimethamine. Using a combination of PK-PD data from a CQ combination efficacy study and *in vitro* studies, we shall identify PK-PD characteristics of drug combinations that will deter emergence and spread of resistance. The initial part of this project involves measurement of whole blood CQ concentrations in patients treated with CQ alone or in combination with artesunate, azithromycin or atovaquone-proguanil. We report an HPLC assay validation for CQ concentration measurement and initial *in vitro* assay results. A reversed-HPLC method has been revalidated for the analysis of CQ in 100 µL whole blood with an aim to improve assay sensitivity. CQ was eluted with a 2.5 ml Hexane / Methyl tert Butyl Ether (MTBE) mixture (ratio1:1, v/v) and was assayed on a Phenomenex Spherclone BDS C18 5µ 15cm x 4.6mm analytical column. The mobile phase comprised 0.1% Triethylamine in Acetonitrile, 85:15 (pH adjusted to 3.0 with orthophosporic acid). CQ recovery was 84%. The CV between 10 ng/ml and 25 ng/mL was 13 % and the limit of quantitation was 25 ng/mL with a CV= 5%. The limit of CQ detection was 10 ng/mL. The assay uses a smaller volume of sample, and can more accurately measure low blood CQ concentrations than published assays which also use UV detection. The essay will be used to measure blood CQ level in children who were given a standard oral dose of CQ as treatment for uncomplicated malaria. A preliminary HPLC analysis of 300 specimens is due to start.

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INVESTIGATING THE GENETIC BASIS OF 8-AMINOQUINOLINE SENSITIVITIES IN A *PLASMODIUM FALCIPARUM* GENETIC CROSS

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The increasingly rapid and widespread development of drug resistance among global malaria populations emphasizes the need for novel therapies. Combinations of existing and new anti-malarial compounds are recommended to slow the evolution and spread of drug resistance. Ideal drug partners should target independent parasite pathways under

the supposition that selection for mutations conferring resistance to distinct compounds will not occur simultaneously at multiple loci. Genetic loci carrying molecular determinants of resistance can be identified and profiled using quantitative trait loci (QTL) mapping. Progeny of a genetic cross exhibit a range of drug sensitivities as a result of inherited allelic variation at loci involving drug target pathways and/or mechanisms of resistance. Primaquine (PQ) and its recent derivative tafenoquine (TQ) are 8-aminoquinolines developed for treatment of malaria. Primaquine is used to treat liver-stage *Plasmodium vivax* and exhibits gametocidal activity. However, this drug is contraindicated for *P. falciparum* due to cytotoxicity, a short half life and inactivity against blood stages at pharmacologically attainable concentrations. Alternatively, TQ is effective at lower doses, has a longer half life and is active against all erthrocytic stages. We report the results of in-vitro drug assays performed using PQ and TQ, alone and in combinations with chloroquine (CQ) and verapamil. QTL analysis indicates a strong influence from the chromosome 7 locus carrying *pfcrt* and an inverse relationship between CQ response and that of the 8-aminoquinolines. The sensitivity of CQR parasites to the 8-aminoquinolines qualifies them as potential candidates for combination therapy. .

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RELATIONSHIP BETWEEN PERSISTENCE OF SUBPATENT ASEXUAL *PLASMODIUM FALCIPARUM* INFECTIONS AND SUBSEQUENT RECRUDESCENCE AFTER ANTIMALARIAL TREATMENT

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Malaria control relies mainly on efficacious treatment of uncomplicated disease episodes. Emerging or spreading parasite resistance to commonly used drugs is associated with failure of chemotherapeutic regimens to completely eliminate primary asexual blood stage infections. The rate of inadequately treated primary infections is determined by the *in vivo* test. This test aims to detect persistent sub-microscopic blood stage infections by capturing subsequent recrudescences (patency) up to 8 weeks after treatment. In high transmission areas PCR-based molecular techniques are required to distinguish recrudescent from re-infections. These techniques are error-prone leading to unreliable treatment outcome estimates but it also raises general questions about the determinants of survival of asexual blood stage infections, e.g. the minimal infectious population size. We hypothesized that persistent, subpatent asexual blood stage infections can be detected on day 7. Moreover, we postulated that this could be used to predict subsequent patency (recrudescence). We analyzed venous blood samples on day 7 from 34 children who were treated with a supervised 3-day course of amodiaquine for uncomplicated *Plasmodium falciparum* malaria. mRNA was isolated and transcribed into cDNA, which was used to amplify a ring stage-specific transcript (*pfresa*) using primers designed to distinguish PCR products originating from transcripts or genomic DNA. The pre-determined detection threshold was ≥10 parasites/mL corresponding to approximately 10^5 ring stage parasites in the peripheral circulation. In 11/34 (32%) samples we detected metabolically active persistent asexual parasites 7 days after start of treatment (i.e., 4 days after last dose). A similar proportion (38%; 13/34) of primary infections recrudesced until day 28. Persistence of infection on day 7, however, poorly agreed with subsequent recrudescence at the individual level with a low positive predictive value (PPV) of 36% (95% CI 11%-69%). In young children (≤2 years), the PPV increased to 60% (95% CI 26-88%). This points to a large stochastic element in the risk of persistent infections to recrudesce - probably determined by unknown host-parasite interactions. We will present additional data from patients treated with fast-acting artemisinin-based combination and with a second time point (day 14) to minimize measurement errors due to asexual parasite sequestration.

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MALARIA IN INDIA AND CONSEQUENCES OF CLIMATIC CHANGES

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India contributes about 80% of total cases in South East Asia (SEA). In India, malaria morbidity and mortality are major public health concerns with around 2 million confirmed cases and 1000 deaths reported annually. The distribution and epidemiology of malaria varies in different parts of the country. The reasons for continued transmission are both due to technical and operational constraints. The technical constrains include drug resistance in parasite, insecticide resistance in vectors, high cost of effective drugs and insecticides, improper environmental management etc. The operational constraints are inaccessibility of endemic areas, rapid urban development, migration across borders and prevailing socio-cultural habits. Recent climate changes has also been identified as a key player in the increase vulnerability of populations to malaria. Present strategies for the control of malaria in India include early detection and prompt treatment of cases and vector control. Out of the reported 58 species of Indian anophelines, nine are known to transmit malaria. *Anopheles culicifacies* is responsible for about 60% of new cases of malaria each year in the rural plains of the country followed by *An. fluviatilis* (~15% in hilly forested regions). Information generated on species complexes in malaria vectors in India has given a new dimension to malaria transmission. GIS technology has been used to map malaria receptivity, distribution of Indian anophelines, identification of risk factors and development of GIS based malaria Information System for decision support in malaria control. Analysis of present climate trends in relation to malaria in India has predicted that in 2050s' the duration of the transmission windows is likely to widen in northern and western states and shorten in the southern states. However, malaria is likely to persist in Orissa, West Bengal and southern parts of Assam, bordering north of West Bengal. But, it may shift from the central Indian region to the south western coastal states of Maharashtra, Karnataka and Kerala. Also the northern states, including Himachal Pradesh and Arunachal Pradesh, Nagaland, Manipur and Mizoram in the northeast may become malaria prone. Therefore, it is important to provide resources for the study of malaria related problems in India, thus helping in planning appropriate control strategies.

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NATURAL TRANSMISSION BLOCKING IMMUNITY TO MALARIA: SPECIFICITY AND DURATION OF EFFICACY

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Immunity to the sexual stages of *Plasmodium falciparum* can be induced during natural infections and has been shown to significantly reduce the infectivity of parasites to mosquitoes. This study was conducted in an area hypoendemic for *falciparum* malaria with <5 infectious bites per person per year in lower Moshi: a) village of Msitu wa Tembo (perennial) and b) TPC sugar plantation (seasonal: mainly migrant workers living in factory houses). The objective of this study was to study generation and longevity of immune responses to sexual stage antigens. Samples were collected from the patients diagnosed with uncomplicated malaria. After treatment, slides were read at day 0, 7, 14, 28, and 84. A total of 306 samples from 102 human subjects (all age groups from TPC and < 16 years from MT) were serologically analysed by *Pfs230* and *Pfs48/45* ELISA.

Transmission-blocking (TB) immunity was assessed using the standard membrane feeding assay (SMFA). Pfs230 and Pfs48/45 ELISAs were positive in 53/116 subjects from TPC and MT (OD≥0.150) at day 28. Of these 53 subjects, 26 out of 44 were positive from TPC (59%) whereas for MT 27 out 72 (37.5) were positive. The mean OD-value at day 28 was significantly higher ($p<0.01$) compared to day 0 and day 84 for both tests. The OD values in the Pfs230-ELISA was higher compared to Pfs48/45-ELISA but not significantly different ($p=0.44$). There are no differences between the villages for ELISA results. Fine specificity for TB-epitopes will be further evaluated in an ELISA with the recombinant Pfs48/45-10C antigen and SMFA. In conclusion, these preliminary results so far indicate a high prevalence of the *P. falciparum* sexual stage antibodies with longevity of up to 84 days post-exposure in the naturally infected people in this hypoendemic area.

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MALARIA EPIDEMIOLOGY IN VIETNAM: LOW INTENSITY OF TRANSMISSION AND HIGHLY COMPLEX PARASITE POPULATION

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In Vietnam, malaria occurs nowadays mainly in the forested and mountainous provinces of central Vietnam and the main species identified by standard microscopy are *Plasmodium falciparum* and *P. vivax.* Other species as well as mixed represent usually less than 5% of the infections. Species specific PCR (semi-nested multiplex PCR, SnM-PCR) was performed on human blood samples collected in 2 malaria endemic provinces (Binh-Thuan and Ninh-Thuan), situated in the southern central coast of Vietnam. A pre-defined subset of microscopically positive and negative blood samples were selected in each province and results were compared to SnM-PCR. A total 484 blood samples on filter paper (289 from Binh-Thuan and 195 from Ninh-Thuan), were analyzed by SnM-PCR. Though *P. falciparum* and *P. vivax* were the predominant species, either as mono- or mixed infections, several *P. ovale* and *P. malariae* infections, either as mono- or mixed infections with *P. falciparum* and *P. vivax* were detected. All *Plasmodium ovale* infections identified in Binh-Thuan were confirmed by sequencing. Among the microscopically negative samples, a substantial proportion was positive by PCR, 16% in Binh-Thuan and 25.6% in Ninh-Thuan. Microscopy was unable to identify *P. ovale* and *P. malariae* infections or complex mixed infections. In both study sites, the species distribution was different than expected: all 4 malaria species and a certain number of mixed infections, some of them carrying 3 species, were detected. The confirmation of several *P. ovale* infections indicates that in Vietnam this species is more prevalent than originally thought. Sub-patent infections might play a non-negligible role in maintaining malaria transmission and in challenging any control efforts aiming at malaria elimination.

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DETECTABILITY OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS AT 24H RESOLUTION: EXTENSIVE VARIATION, BUT NO PERIODICITY

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Even the most sensitive methods for diagnosis of *Plasmodium falciparum* can only detect a fraction of all infections present in a host. In asymptomatic patients, this fraction - the detectability q - amounts to roughly 50%. It can be estimated by means of repeated sampling using molecular typing methods. Accurate measurements of detectability are desirable since its value affects estimates of multiplicity of infection, prevalence, and of the frequency of breakthrough infections in clinical drug trials. However, it has been argued, that individual infections may exhibit complicated patterns of appearance on a time scale comparable to the duration of the 48 hour erythrocytic cycle of *P. falciparum*. This would imply that the length of the intervals between consecutive samples may influence estimates of q - or, equivalently, the chance to detect a particular infection. In order to test for the presence of such non-random behaviour of infections, and to determine an appropriate method for estimating q, we have carried out a longitudinal molecular study in northern Ghana. From each of the 111 participants, 4 blood samples were collected over a period of 8 days, and tested for presence of different msp2 genotypes. We analyse these data by comparing various statistical models and find no deviation from randomness in the patterns of appearance of infections. Instead, extensive variation of detectability among infections becomes apparent. We then use our insights to justify a simple algorithm for obtaining reasonably robust estimates of q, and of the extent to which it varies.

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EVIDENCE OF *PLASMODIUM* SPECIES INTERACTIONS IN AN ENDEMIC POPULATION IN COASTAL KENYA

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Malaria is holoendemic along the Indian Ocean coast in southern Kenya with *Plasmodium falciparum* (Pf), *P. malariae* (Pm), and *P. ovale* (Po) infections causing disease. The prevalence of mixed species infections and associated morbidity may be influenced by interactions among *Plasmodium* species. Existing evidence of species-specific interactions varies by region, by season, and by endemic species. Previous research has shown an overabundance of mixed Pf-Pm infections in sub-Saharan Africa, but few studies have appropriately controlled for potential confounders. This cross-sectional study used PCR/sequence-specific oligonucleotide probe hybridization to identify asymptomatic *Plasmodium* species infections in participants age 8 and older in Kingwede, Kenya. Results of multivariate general estimating equation (GEE) analyses accounting for household clustering of participants suggest that infections are not randomly distributed. Specifically, odds of Pf infection in an individual co-infected with Pm are 3.5 times higher than in a Pm negative individual (95% CI= (2.1, 6.0)) controlling for age, having a regular income, household socio-economic position (SEP) and an age-household SEP interaction. In age-stratified, multivariate analyses, a marginally significant Pf-Po association was observed (OR=4.0; 95%CI (1.0-17.0)) in adults (18 and older) controlling for SEP variables and for Pm infection but this association was not seen in children. Factors that best explain mixed *Plasmodium* species infection distribution in this population, identified by GEE models, include age, and a history of recent treatment for malaria. These findings suggest that *Plasmodium* species infection distributions in this population are not independent and highlight the possibility of heterogeneous immune response profiles in *Plasmodium* endemic regions. Such heterogeneities could complicate vaccine development and merit further study.

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VALIDATING SEROLOGICAL METHODS TO ESTIMATE MALARIA TRANSMISSION IN CAMBODIA

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Malaria transmission rates appear to be falling in many areas in the world. Whether this is due to changes in the environment or behaviour, or due to malaria control interventions, the resulting lower levels of transmission can only be measured by very sensitive methods. Traditional measures of transmission, including EIR and parasite prevalence, tend to be time consuming, labour intensive, costly and inexact. They are also subject to bias through seasonality and heterogeneity of infection, as well as being insensitive in lower transmission areas. Serology offers a comparatively cheap and convenient additional method to estimate transmission in all areas and it reflects cumulative exposure over time and is therefore less influenced by short-term fluctuations in transmission, as reported previously The method uses anti-malarial antibody prevalence as an estimate of transmission. Acquisition of anti-malarial antibodies increases with age and they accumulate more quickly in areas of high transmission[2]. The data obtained can be used to fit a model of the dependence of sero-prevalence with age by maximum likelihood to generate an antigen specific estimate of force of infection (λ), which has been shown to correlate with EIR. We are currently evaluating the method in various transmission settings with different pre-erythrocytic and blood stage antigens. Here we present data from samples collected for a malaria baseline survey in Cambodia where both *Plasmodium vivax* and *P. falciparum* are transmitted at very low levels. Seroprevalence correlates with parasite prevalence and is highest in communities near forested areas, as expected, since the major malaria vectors are restricted to forested areas, as reported previously. Furthermore, although we find an increase in seroprevalence with age, seropositivity is essentially restricted to individuals over the age of 15 and is significantly higher in males than females, suggesting that behavioural factors (such as travelling into the forest to work) are a major risk factor for infection. Importantly, the much higher sensitivity of serological measures of transmission should allow us to identify areas of very low but continued risk of infection, which may not be detected through parasite prevalence data.

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THE TANZANIAN NATIONAL VOUCHER SCHEME (TNVS): EVIDENCE ON CORE BEDNET AND MALARIA INDICATORS FOR PREGNANT WOMEN AND INFANTS AFTER THREE YEARS OF IMPLEMENTATION

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The Tanzanian National Voucher Scheme (TNVS) delivers a discount voucher for bednets to pregnant women and infants via routine antenatal services. One strength of delivery systems that use routine health services is their continuing accessibility. To optimise coverage, however, a better understanding is needed of the timing of the delivery of malaria in pregnancy interventions. The TNVS comprehensive approach to monitoring and evaluation included annual nationally representative household and Reproductive and Child Health (RCH) facility surveys in 2005-07. Changes in coverage over 3 years and the effect of timing and gestation on pregnancy coverage estimates for bednet are investigated. Between 2005-07, net and ITN use by Tanzanian pregnant women increased by 56% and 109%, respectively, and 70% and 115%, respectively for infants. Inequities in household ownership improved but coverage in the lowest quintile was only 44% that of the highest quintile after three years. Use of ITNs increased with gestational age, and after delivery of the infant. In 2007 21% and 26% of women in their first and third trimester respectively used an ITN, rising to 34% for infants. In 2007 83% of women received a bednet voucher by the end of pregnancy but only 50% of women got one at their first visit to RCH. Mean gestation at first visit was 20 weeks. By multiplying remaining pregnancy weeks by coverage achieved at discrete antenatal visits we calculate "percent of optimal coverage achieved". We observe that, on aggregate, 55% of the pregnancy period was covered by vouchers in 2007. In conclusion, there were sustained gains across the bednet indicators after 3 years of implementation of a clinic based voucher delivery system for bednets. Measurement of bednet coverage in terms of the "percent of optimal coverage achieved" demonstrates how both early attendance and prompt delivery by antenatal staff are needed to maximise public health gains. There is a need for clarity about when in pregnancy it is desirable to achieve high bednet coverage.

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IFN-γ AND IL-4 RESPONSES INDUCED BY PROMISCUOUS T-CELL EPITOPES OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN 9 (PVMSP9) IN MALARIA NATURALLY EXPOSED INDIVIDUALS IN BRAZIL

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Plasmodium vivax MSP9 is a merozoite surface protein which stimulates both cellular and humoral immune responses in naturally exposed individuals. To identify immunodominant human T-cell epitopes in PvMSP9, we used the MHC class II binding peptide prediction software ProPred that contains 51 quantitative matrices for human MHC. Five peptide sequences (including 3 overlapping regions) were predicted to bind to the largest number of HLA molecules at a 1% threshold within the N-terminal region of PvMSP-9. Five synthetic peptides [pE (147-159), pH(438-449), pJ(325-339), pK(434-448) and PL(443-456)], representing the predicted putative promiscuous T cell epitopes were tested in IFN-γ and IL-4 Elispot assays using peripheral blood mononuclear cells (PBMC). 142 individuals naturally exposed to malaria infections from Rondonia State Brazil were included and HLA typing performed on the study cohort using multiplex PCR and Luminex technology. The synthetic peptides tested elicited a robust IFN-γ and IL-4 recall responses. The overall frequencies of IFN-γ and IL-4 responders to at least one of the promiscuous peptides were 62% (88/142) and 46% (60/129), respectively. None of the healthy controls, from non-endemic areas of malaria, recognized the PvMSP9 peptides. The frequencies of IFN-γ responders to each peptide were 50.7% for pE, 36.6% for pH, 27.4% for pJ, 38.7% for pK and 50.7% for pL and the frequencies of IL-4 responders were 29.4% for pE, 33.3% for pH, 25.4% for pJ, 26.3% for pK and 30.4% for pL. This response was not associated to a particular HLA-DR allelic group since most of the peptides induced a response in individuals of 12 out of 13 allelic groups. The

prediction of promiscuous epitopes using ProPred led to the identification of immunodominant epitopes recognized by PBMC from a significant proportion of a genetically heterogeneous population exposed to malaria infections. The combination of several such T cell epitopes in a vaccine may increase the frequency of responders and the overall effectiveness of the immunization of genetically distinct populations.

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MATERNAL MALARIA AND DOPPLER INTERROGATION OF FETOPLACENTAL CIRCULATION: A LONGITUDINAL STUDY

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Malaria infections during pregnancy cause adverse maternal and fetal outcomes including maternal anemia, fetal growth restriction, low birthweight, and preterm birth. However, little is known about the effects of malaria on the fetoplacental circulation. In order to determine the effect of concurrent malaria infection on changes in the mean umbilical artery resistance index over time, hierarchical linear models were fitted to data for the 897 follow-up visits of 176 women that occurred from 22 to 40 weeks gestation in Kinshasa, DR Congo. Of the 176 study participants, 72 (40.9%) did not experience a malaria episode, 66 (37.5%) experienced one malaria episode, and 38 (21.6%) experienced two or more malaria episodes. While previous studies have reported an independent effect of concurrent malaria infection on fetoplacental hemodynamics, including uterine and umbilical artery resistance, we did not find a significant effect of concurrent malaria infection on umbilical artery resistance in preliminary analyses of the current sample ($\beta = 0.00943$, 95%CI: -0.0168, 0.0357). However, the effect of malaria infection on umbilical artery resistance is modified by maternal body mass index, age, and gravidity. This is consistent with our previous observation that the effect of malaria on the fetus is modulated by maternal nutritional status. Results of final hierarchical linear models examining the effect of concurrent malaria infection on fetoplacental circulation (including uterine and umbilical arteries) will be presented. The results of this study will contribute to a better understanding of the pathogenesis of maternal malaria over the course of pregnancy and will have implications for reduction of morbidity and mortality due to maternal malaria.

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MALARIA MORTALITY AMONG UNITED STATES RESIDENTS, 1990-2005

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Malaria is an important and often serious infection in United States (U.S.) travelers. To assess the burden of malaria mortality among U.S. residents we examined national mortality records for the years 1990-2005. A malaria-related death was defined as any death in which malaria was reported as the underlying or contributing cause of death. A total of 165 malaria-related deaths were identified from 37 states over the 16 year period studied. Age-adjusted malaria mortality rates were highest in Asians (22 cases, adjusted rate ratio [ARR]=6.3, 95% CI 5.1, 7.7) and blacks (37 cases, ARR=3.2, 95% CI 2.5, 3.8) relative to whites and in males (116 cases, ARR=2.8, 95% CI 2.1, 3.8). Foreign-born persons accounted for 67 (41.2%) of the deaths. All but one of the fatal cases in Asians, 70% of the deaths in blacks and 4of the 5 Hispanic cases were foreign-born. No discernable temporal or seasonal trends were observed. Principal co-morbidities listed as contributing to death included respiratory distress syndrome in 38 (23%), cerebral conditions in 19 (11.5%) and renal failure in 12 (7.4%) persons. Malaria deaths routinely occur in U.S. residents and may be more common than previously recognized. Travel-acquired malaria infections and fatalities are readily preventable through the use of appropriate chemoprophylaxis, personal protection measures and the rapid recognition and treatment of infections.

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PREVALENCE AND LONGEVITY OF SUB-CLINICAL *PLASMODIUM FALCIPARUM* INFECTIONS AMONG SCHOOL CHILDREN FROM A HIGHLAND AREA OF KENYA

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To determine the dynamics and duration of *Plasmodium falciparum* infections in a highland area of unstable transmission, we carried out a molecular epidemiological study in a cohort of malaria infected school children over a period of 12 months. Overall, microscopy grossly underestimated parasite prevalence, detecting only approximately one third of infections in comparison to the polymerase chain reaction (PCR) method ($P < 0.0001$). A trend of decreasing prevalence and infection duration with age was observed. Parasite prevalence among age groups 5-9 and 10-14 years assessed by both microscopy and PCR was high (34.0% and 33.6%, respectively), but it was significantly lower in the older children (7.3%, $P < 0.0001$). Within the highland site, there was substantial micro-geographic variation in infection complexity. The mean number (± SE) of infected samples per child from the valley bottom (5.4 ± 0.4) was about twofold higher than that for children living mid-hill (2.7 ± 0.38) and the hilltop (2.6 ± 0.32). Malaria prevalence at the valley bottom (52.4%) was significantly higher than mid-hill and the hilltop (25.8% and 23.4%, $P < 0.0001$). Malaria infections among children living at the valley bottom lasted longer than those from mid-hill or the hilltop. Our results are consistent with gradual acquisition of immunity with age upon repeated malaria-parasite exposure, and also show that malaria transmission risk is highly heterogeneous in the highland area. The implications of these findings for malaria control in the highlands will be discussed.

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INDIVIDUAL, HOUSEHOLD, AND ENVIRONMENTAL RISK FACTORS FOR MALARIA INFECTION IN AMHARA, OROMIA AND SNNP REGIONS OF ETHIOPIA

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Malaria remains a serious and unstable health problem in Ethiopia. We assessed malaria infection in relation to age, altitude, rainfall, socioeconomic factors and coverage of control measures in Amhara, Oromia and SNNP regions of Ethiopia in Dec 2006-Jan 2007, before completion of net distribution scale-up. Surveys were conducted in 224 randomly selected clusters of 25 households in malarious areas (overall sample of 27 884 people in 5 708 households, the majority of which were located below 2000m). In 11 538 blood slides examined, malaria prevalence in persons of all ages was 4.1% (95% CI 3.4 to 4.9%) overall and 4.2% at altitudes below 2000m, with 56.5% of infections being *Plasmodium falciparum.* At least one mosquito net or one long-lasting insecticidal net (LLIN) was present in 37.0% (95% confidence interval

[CI] 31.1 to 43.3%) and 19.6% (95% CI 15.5 to 24.5%) of households, respectively. In multivariate analysis (N=11 437) for risk of parasitaemia, significant protective factors were: number of LLINs per household (odds ratio $[OR]_{per\ additional\ net}$=0.60; 95% CI 0.40 to 0.89), living at higher altitude ($OR_{per\ 100m}$=0.95; 95% CI 0.90 to 1.00), and household wealth ($OR_{per\ unit\ increase\ in\ asset\ index}$=0.79; 95% CI 0.66 to 0.94). Malaria parasite prevalence was positively associated with the peak monthly rainfall in the year before the survey ($OR_{per\ additional\ 10mm\ rain}$=1.10; 95% 1.03 to 1.18).

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DNA SEQUENCE ANALYSIS OF INTERGENIC SPACER REGIONS AMONG *ANOPHELES ARABIENSIS* POPULATIONS AT MULTIPLE GEOGRAPHIC SITES IN MALI

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Countries such as Mali provide several ecological niches for anopheline breeding. In the humid southern Savanna, members of the *Anopheles gambiae* complex live under sympatric conditions. Conversely, in the dry northern region, ponds provide more isolated environments. In these studies, we compared the intergenic spacer (IGS) regions of *An. arabiensis* collected in the humid savanna region (rainfall-related breeding sites) to the IGS regions of *An. arabiensis* from ponds in the dry northern region (Menaka). The sites selected for this study were in the Savanna zone of Kolokani (Missira), a suburb of Bamako (Gbakoro Droit) and the dry northern region (northeastern Menaka - Anderamboukane). Morphology was used to identify 124 members of the *An. gambiae* complex from these sites, and PCR to distinguish *An. gambiae* from *An. arabiensis*. Dideoxy nucleotide sequencing of the IGSs was performed using the CEQ8000 Beckman Coulter sequencer. Nucleotide sequences for the IGS region of *An. arabiensis* were aligned and compared using editSeq and SeqMan; phylogenetic trees were prepared using MEME and MAST. Based on PCR, 58 of the 124 anopheline mosquitoes collected were *An. arabiensis* (47%); 66 were *An. gambiae* (53%). Therefore, sequencing was performed on 37 *An. arabiensis* from the southern Savanna (Missira), 14 from the dry northern region (Menaka) and 7 from suburban Bamako (Gbakoro Droit). These sequencing results reveal a high degree of diversity within the IGS region of *An. arabiensis* in Mali.

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MALARIA INCIDENCE IN INFANTS IN BANCOUMANA, MALI

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Infants in malaria endemic areas are the primary target for a blood-stage malaria vaccine. To properly plan for Phase 2 trials, the incidence of malaria infection in the target population and factors that may affect malaria incidence must be known to appropriately power the trial and determine the sample size needed. This observational study is being performed to determine the incidence of malaria infection and disease, correlate malaria rapid diagnostic test (RDT) and malaria microscopy results, and determine the effect of hemoglobin type on malaria infection in infants in the area of the Bancoumana Vaccine Center. 105 infants aged 6 weeks to 6 months were enrolled and received a baseline evaluation, 6 monthly visits during the rainy season and weekly home visits. At monthly visits, and if ill or febrile, infants were examined and determinations of hemoglobin, blood films, and an RDT for malaria were performed. Malaria treatment was given per Mali national guidelines. Preliminary results indicate that the incidence of clinical malaria is 0.41. The average hemoglobin at entry was 10.1 g/dl and dropped to 9.2 g/dl by October. The incidence of grade 1 anemia (7.5 - 8.4 g/dl) was 20%, grade 2 anemia (6.1 - 7.4 g/dl) was 8% and grade 3 anemia (5 - 6 g/dl) was 4%. There was judged to be 50% use of Insecticide Treated Nets and the concurrence in this population of blood film and RDT results was 99.3%. Hemoglobin typing revealed 76.5% AA, 11.2% AS, and 11.2% AC. Final clinical data from this study and the effect of hemoglobin type on malaria infection and disease will be presented.

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BURDEN OF DISEASE DUE TO MALARIA IN PREGNANCY AMONG WOMEN ATTENDING ANTENATAL CLINICS AND HOSPITALIZED FOR MALARIA IN THE STATE OF JHARKHAND, INDIA

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A substantial proportion of the population of India is at risk for malaria including pregnant women. Although past studies have suggested a moderate burden of disease due to malaria in pregnancy, they included only symptomatic pregnant women and thus may have overestimated the proportion of women with malaria. In order to better define the burden of malaria in pregnancy in India, we performed cross-sectional surveys at antenatal clinics (ANC) in the state of Jharkhand, which is considered to be highly malaria-endemic, in central-east India. Pregnant women admitted to the hospital for malaria were also evaluated. Enrolment occurred over a 12 month period at three health facilities in rural, semi-urban, and urban locations. Malaria was diagnosed by Giemsa-stained blood smear and/or rapid diagnostic test (RDT). A positive diagnostic test for malaria was obtained in 1.8% (43/2382) of pregnant women attending ANCs. 53.4% were infected with *Plasmodium falciparum*, 37.2% *P. vivax* and 9.3% mixed. Peripheral parasitemia was significantly more common in pregnant women in the semi-urban and rural ANCs ($p<0.001$) and in primigravidae and secundigravidae relative to multigravidae ($p=0.0042$). Parasitemia was more common in pregnant women with a history of fever within the last week or who were febrile at the time of the study visit (5.5% vs. 1.1%, $p<0.001$). Anemia was common among ANC participants whereas severe anemia was rare. Anemia was not associated with malaria ($p=0.55$); however, severe anemia was more common among women with parasitemia ($p=0.0078$). Only 0.6% (14/2386) women acknowledged the use of malaria chemoprophylaxis. There were 27 pregnant women admitted to the hospital with malaria (19.2% of all non-delivery admissions). All except two were confirmed with microscopy or RDT. Most (21/25, 84%) were infected with *P. falciparum,* two (8%) had both *P. falciparum* and *P. vivax* and two (8%) had only *P. vivax*. Severe malaria (severe anemia, cerebral malaria) was diagnosed in 29.6% (8/27). Malaria occurred relatively infrequently among pregnant women attending ANCs in this region of India although it was associated with an increased risk of severe anemia and was responsible for a clinically relevant proportion of hospitalizations. Since many pregnant women were symptomatic, control efforts should be focused on preventive measures such as insecticide-treated bednets and improved malaria case management.

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CHANGES IN VECTOR DENSITY PREDICT MALARIA INCIDENCE IN HIGHLAND KENYA: IMPLICATIONS FOR MALARIA EARLY WARNING SYSTEMS

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Malaria early warning systems (MEWS) based on rainfall and temperature patterns have had variable success in predicting changes in malaria incidence. Since weather affects malaria transmission through the Anopheles vector, vector density might serve as a more specific predictor for MEWS. Malaria incidence was assessed by active surveillance from April 2003 to March 2005 in the highland areas of Kipsamoite and Kapsisiywa, Kenya (elevation >1800 m; population 7000). Clinical malaria was defined as fever, chills, headache or severe malaise with the presence of *Plasmodium falciparum* on blood smear. Indoor resting Anopheles vectors were captured using pyrethrum spray capture from 120 randomly selected households every 2 weeks and were identified taxonomically by trained field workers. Vector density was assessed as the average number of mosquitoes per household in each cluster area over a 2-week period. Daily rainfall and maximum and minimum temperature were measured in both sites. Predictors of malaria incidence were assessed with a longitudinal negative binomial regression model using generalized estimating equations and an auto-regressive correlation structure. Sinusoidal covariates were included to account for seasonality. Temperature, rainfall and vector density collected during a 2-week period were compared to malaria incidence 2-10 weeks later. The highest (>0.4 vectors per house) as compared to lowest levels of vector density (0 vectors) was associated with a 3.1-fold increase in malaria incidence 2 weeks later (P= 0.003) and 2.1-fold increase in incidence 4 weeks later (P=0.01). In contrast, the highest levels of rainfall correlated weakly with malaria incidence 10 weeks later (P=0.04), and temperature did not correlate with malaria incidence at any time lag. In conclusion, vector density predicts malaria incidence in this highland area of western Kenya better than rainfall or temperature. To develop vector-based MEWS for areas of unstable transmission, further studies will be needed to identify optimal vector density warning thresholds.

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BURDEN OF DISEASE DUE TO MALARIA IN PREGNANCY AMONG PREGNANT WOMEN ATTENDING DELIVERY UNITS IN THE STATE OF JHARKHAND, INDIA

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A substantial proportion of the population of India is at risk for malaria including pregnant women. Although past studies have suggested a moderate burden of disease due to malaria in pregnancy, they included only symptomatic pregnant women and thus may have overestimated the proportion of women with malaria. In order to better define the burden of malaria in pregnancy in India, we conducted cross-sectional surveys at delivery units (DU) in the state of Jharkhand, which is considered to be highly malaria-endemic, in central-east India. Enrolment occurred over a 12 month period at three hospitals in rural, semi-urban, and urban locations. Malaria was diagnosed by Giemsa-stained blood smear and/or rapid diagnostic test (RDT) of peripheral and placental blood. Of the 718 women enrolled, only 1.7% had peripheral parasitemia. The majority of the pregnant women (83%) had untreated bednets in their homes and had used them recently (74%). *Plasmodium falciparum* was identified in 75% (9/12), *P. vivax* in 17%, and mixed infections in 8%. Although a greater proportion of women presenting to semi-urban and rural DUs were parasitemic (4/183, 2.2% and 6/280, 2.1% vs. 2/254, 0.8% in urban areas), this difference was not significant (p=0.39). More than half (58%, 7/12) of women with peripheral parasitemia were asymptomatic. Placental parasitemia was present in 2.4% (17/712) overall. Placental parasitemia was significantly associated with fever (p=0.001), yet 59% (10/17) of women with placental parasitemia were asymptomatic. Birth outcomes were similar between pregnant women with and without parasitemia. For DU participants with peripheral parasitemia, 100% were anemic as compared to 58.9% of those who did not have parasitemia (p = 0.004). The overall burden of malaria in pregnant women attending DUs was relatively low in this region of India. Malaria contributes to the substantial burden of anemia among pregnant women. Rather than using intermittent preventive therapy in pregnant women, as is being done in many African countries, efforts should be focused on preventing malaria and anemia in India.

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MADAGASCAR DIAGONAL FUNDING STUDY

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There are anecdotal reports that malaria control efforts are decreasing the number of malaria patients presenting to clinics in Madagascar. The objectives of the Madagascar Diagonal Funding Study are to assess whether malaria control activities reduced demand for therapeutic malaria services from 2003-2008, and whether this reduction allowed a refocusing of clinical efforts as anecdotally reported. Twelve facilities were visited in three districts on the east and west coasts with varying levels of malaria control activities: Soanierana Ivongo (high), Maintirano (medium), and Morafenobe (low). All clinical diagnoses of malaria, diarrhea, pneumonia and other diseases in children less than five years of age were collected from the patient registers from 2003-2007. The number of diagnoses was used as a proxy for the demand for clinical services. The ratio of diagnoses due malaria, diarrhea and pneumonia may represent a change in service uptake and was used as a gauge of any potential "diagonal effect." Findings from the study demonstrate that in the low activity district: the number of malaria and pneumonia diagnoses stayed relatively stable; the percentage of diagnoses due to malaria decreased slightly while pneumonia increased. In the medium activity district: the number of malaria and pneumonia diagnoses decreased; the percentage of diagnoses due to malaria and pneumonia stayed constant. In the high activity district: the number of malaria diagnoses greatly decreased; the percentage of diagnoses due to malaria decreased; the number of pneumonia diagnoses decreased; and the percentage of diagnoses due to pneumonia increased. In all three districts the burden of diarrhea did not change significantly. In conclusion, in both the medium and high activity districts, there was a decrease in the number of diagnoses for malaria, suggesting that scale up of malaria activities may have decreased malaria burden. In addition, in the high activity district, the percentage of diagnoses due to malaria decreased while the percentage due to pneumonia increased; this may reflect a change in service uptake due to a "diagonal effect." This diagonal phenomenon may be due to an underlying change in disease incidence, or an increased ability to differentiate and diagnose malaria and pneumonia

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A COMPARISON OF THE IMPACT OF MALARIA CONTROL ACTIVITIES IN CAMBODIA MALARIA SURVEY BETWEEN 2004 AND 2007

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Malaria is one of the leading public health problems in Cambodia. The 2007 Malaria Survey is assessed the performance and impact of malaria control activities in Cambodia in comparison with the results of the 2004 baseline. While the baseline survey had three domains only two of these will be included in the 2007 survey but add an extra risk zone beyond 2 kilometers from the forest to collect malaria-related data especially from the people who usually visit the forest. This change is based on the low malaria prevalence rate in domain 3, so it was decided to focus efforts and limited resources on the areas where data on malaria prevalence will be useful for action. The overall slide positively rate in higher risk regions, was 2.9%, the positive rate by *Plasmodium falciparum* was 1.6%, 0.9 % by *P. vivax* and 0.3% for mix infection. The infection was mostly high affected with the poorest people group if classified by socio-economic group and attacked to all age group. People more than 90% who know malaria transmission by mosquito bite when they went to forest and could be prevent by use of mosquito net. Up to more than 80%, from the poorest to less poor people in at risk was sleep under bed net. Around 70% households who recognize sign and symptom of malaria; 100% know well about danger sign of malaria and 93.3 % know where to go testing and treatment. But only 46.7% they were seeking treatment within 24 hours. The important finding of the survey is the similarity of epidemiological malaria data obtained from routine surveillance. It is around 40% reduction of malaria incidence among total population from year 2004 to 2007. People gained more knowledge on malaria prevention if compared to baseline result. In both survey results, It is significantly reduction from 4.4% (2004) to 2.9% (2007). There were significantly relationship between positive blood slide and risk areas, socio-economic and people who had fever. It is increased of percentage household sufficient net from 17.5 to 58.6. It is increased the percentage of awareness of anti-malaria drug among target population at risk from 47.3 to 72.The percentage of target groups who know that Malarine treatment is effective only if entire course is taken 10.3 in 2004 compared to 38.9 in 2007 survey. Based on result finding it showed that successfully for program implementation.

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ANTIBODY RESPONSES TO THE MEROZITE SURFACE PROTEIN (MSP) COMPLEX OF *PLASMODIUM FALCIPARUM* IN MALARIA PATIENTS FROM CENTRAL INDIA

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The merozoite surface protein -1 (MSP-1) is an important malaria vaccine candidate antigen. In this study we investigated antibody responses to the MSP-1 complex (recombinant antigens of MAD20 allelic forms of 19D, 30D, 38D, 42D, 83D, and K-1 allelic forms of 42F and 83F subunits) proteins among *Plasmodium falciparum* infected patients enrolled from primary and tertiary hospitals of Madhya Pradesh, Central India. A total of 386 plasma samples including 60 cerebral malaria, 57 severe malaria (including patients with anemia and other organ involvement but no cerebral malaria) and 269 uncomplicated malaria cases were used to determine the total IgG antibody prevalence and levels against the various subunit of MSP-1 antigen. Enzyme linked immuno sorbent assay (ELISA) was used for total IgG antibody estimation. Sera of 16 individuals from non endemic area were used as negative control and the mean optical density plus 2 standard deviations of the control sera was used as cut off to score positive response. The prevalence of total IgG response varied for each antigen ranging from 72% to 92%. The total IgG antibody levels were lower for all subunits of MSP-1 complex, except for D19 and F42, in the severe malaria group when compared to cerebral malaria and uncomplicated malaria group. Total IgG antibody levels for the 30D, 42F, 83D and 83F subunits were significantly higher in uncomplicated malaria group as compared to cerebral malaria group. A strong correlation was observed in the antibody responses between the 19D versus 42D subunits (r2 = 0.89) and the two allelic forms of 83 kDa: 83D and 83F (r2=0.82). In conclusion, the current study provides insight into the acquired humoral responses to the MSP-1 complex in an endemic area of malaria in India with low seasonal transmission of malaria. The study suggests that there may be a dysregulation in the generation of optimal antibody response to some of the MSP-1 protein fragments in severe malaria and cerebral malaria patients and it remains to be determined if such differences contribute to susceptibility of individuals to disease severity.

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IS ACQUISITION OF ANTI-MEROZOITE SURFACE PROTEIN 3 ANTIBODIES RELATED TO PROTECTION AGAINST FALCIPARUM MALARIA?

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Current malaria control strategies include among others, the deployment of different malaria vaccine candidates among which is the Merozoite Surface Protein 3 (MSP3). Merozoite surface protein is a polymorphic malaria parasite protein that may have a role in *Plasmodium falciparum* parasite invasion of erythrocytes. Studies have shown MSP3 antigen to be a potential malaria vaccine candidate against *P. falciparum* asexual blood stage parasites. We conducted a cross-sectional malariometric study from both high and low malaria transmission areas of Northeastern-Tanzania with the aim of determining human humoral immune responses to MSP3 antigen and the association of anti MSP3 antibodies against malaria attack. Study individuals were aged less than 20 years. Collected samples were analysed by using indirect enzyme linked immunosorbent assay (ELISA) to find out reactivity of total IgG, IgM and IgG subclasses to the MSP3 antigen. We noted that, acquisition of anti MSP3 antibodies is age related and varied with transmission intensity for the cytophilic antibodies (IgG1 and IgG3) which have been shown to be protective against malaria attack. Conversely, in the low transmission areas (highland), the level of total IgG and IgM were higher. Further analyses will correlate both clinical and parasitological data to find out if there are some protective effects against malaria attack in individuals with higher IgG1 and IgG3 levels. These preliminary findings indicate that MSP3 antigen could act as a potential malaria vaccine candidate thereby calling for its further evaluation in a wider population.

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MACROPHAGE MIGRATION INHIBITORY FACTOR IN PLACENTAL INTERVILLOUS BLOOD PLASMA AND ITS ASSOCIATION WITH BIRTH OUTCOMES IN *PLASMODIUM FALCIPARUM* INFECTED WOMEN IN CENTRAL INDIA

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Malaria during pregnancy is associated with the delivery of low birth weight (LBW) babies and preterm deliveries (PTD). While the mechanisms involved in the development of these adverse outcomes are not fully understood, host inflammatory responses have been proposed as possible mediators. Macrophage migration inhibitory factor (MIF) is a unique proinflammatory cytokine with both hormonal and enzymatic properties and it is expressed in very high levels in the placental intervillous blood than in the peripheral blood. It is involved in the activation of macrophages and killing of intracellular parasites. MIF may play a role in immune responses to malaria during pregnancy by virtue of its ability to activate macrophages and to overcome the immunosuppressive effects of glucocorticoids. This study investigated whether MIF levels in the intervillous blood plasma are associated with adverse outcomes of malaria during pregnancy in central India. Commercially available ELISA kits (RandD systems) were used to determine the level of MIF in 18 *Plasmodium falciparum* infected and 54 non-infected placentas collected from women who participated in a burden estimate study. Overall, elevated geometric mean of MIF levels were found in women with stillbirths (16924.76 ng/ml), PTD (12062.28 ng/ml) and women who delivered LBW babies (11788.7 ng/ml) compared to women with term normal deliveries (9456.32 ng/ml) regardless of malaria infection. As previously demonstrated, infected women had elevated levels of MIF compared to uninfected women although this was not statistically significant in this population. Among the malaria infected women, higher MIF levels were significantly associated with the delivery of LBW babies ($p = 0.004$). PTD was significantly associated with higher MIF levels in falciparum infected sample than in uninfected sample ($p = 0.011$). Primigravidae women had the highest levels of MIF, followed by secundigravidae and then multigravidae women. Results from this study suggest that high intervillous blood MIF levels are associated with adverse birth outcomes in *P. falciparum* infected pregnant women residing in a low endemic area of malaria in central India.

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EVALUATION OF IGG AND IGM ANTIBODY RESPONSES THAT RECOGNIZE T AND B CELL EPITOPES IN SEVERAL VACCINE CANDIDATE ANTIGENS OF *PLASMODIUM FALCIPARUM* VACCINE STRAIN 3D7 IN SERA FROM PATIENTS WITH NATURALLY ACQUIRED MALARIA LIVING IN THE PERUVIAN AMAZON BASIN

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Currently, there is little information regarding naturally acquired immune responses from patient populations living in hypoendemic areas such as the Peruvian Amazon basin. To address this short fall, we measured by indirect ELISA, IgG and IgM antibody responses directed against synthetic peptides in sera samples collected from 105 patients with naturally occurring cases of *Plasmodium falciparum* from Loreto. The synthetic peptides corresponded to eight T and B cell epitopes found in four of the major vaccine candidate proteins (AMA-1, CSP, LSA-1 and TRAP), and were designed from the 3D7 vaccine strain sequence. Twenty-seven of the isolates were collected in years 1998-1999 from patients diagnosed with severe and complicated malaria. Twenty-four samples were from patients enrolled in a sulphadoxine-pyrimethamine *in vivo* study conducted in 1999. Also, fifty-four samples came from individuals presenting with malaria-like illness at clinics located in different communities near Iquitos during 2006. The IgG test using CSP Th2R and the IgM test using AMA-1 R-1 had the highest sensitivity to detect positive responders in *P. falciparum* sera: 54.3% (57/105) and 85.7% (90/105), respectively. The specificity of the test was calculated as the percentage of *P. vivax*-positive sera that tested negative for *P. falciparum*. The IgG tests using LSA-1 (90%) and LSA-J epitopes (90%) and the IgM test using TRAP P1 (90.9%) had the highest specificities. In addition, a positive correlation between antibody responses to the eight epitopes was found (Spearman's rank test) for IgM and IgG titers. Overall, antibody responses to the eight epitopes tested were low for IgG; however, significant differences in titers between the three study groups were observed for IgM (Kruskal-Wallis test, $p<0.05$). The frequency of positive responders among the study groups for each of the eight epitopes was minimal for IgG (35.1% ± 14.8) and higher for IgM (85.6% ± 16.1). In conclusion, in this low transmission area, IgG responses were poorly identified perhaps due to the low exposure to the parasite. In contrast, the evaluation of IgM allowed us to differentiate specific responses to the eight epitopes tested among the study groups, which could aid in the identification of well recognized and strongly correlated epitopes to be considered in a subunit vaccine.

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CELL MEDIATED IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN PREGNANT CAMEROONIAN WOMEN

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Pregnant women are more likely to have *Plasmodium falciparum* infections than non-pregnant women. Their increased susceptibility is thought to be due to down-regulation of antimalarial cellular immune responses by pregnancy-associated hormones; however, the precise nature of the alteration(s) is unknown. Our goal was to monitor changes in immune responses in pregnant Cameroonian women during the course of pregnancy. A total of 154 pregnant women were enrolled during the first trimester and 46 non-pregnant women served as controls. Blood samples were collected monthly for parasitological studies, including PCR-based parasite genotyping to determine multiplicity of infectivity (MoI). Peripheral blood mononuclear cells were collected every other month and cultured *in vitro* with an extract of asexual-stage parasites and pools of Class I and II-restricted peptides from CSP, LSA-1, and MSP-1. INFγ and IL-10 were measured by ELISPOT and IL-4, IL-13, IL-6, and TNFα by Luminex-based multiplexing. As expected, an increase in prevalence, parasitemia and MoI were found between 14-21 weeks of pregnancy. Compared to responses of non-pregnant women, INFγ responses to a pool of Class I promiscuous epitopes from CSP and LSA-1 ($p=0.02$) and pools of conserved epitopes from CSP ($p=0.003$) and LSA1 ($p= 0.03$) were significantly reduced in during the first, but not second or third, trimester. Additionally, IFNγ responses to Class II-restricted peptides of MSP1 also were decreased during the first trimester. Since INFγ plays an important role in controlling liver- and asexual-stage parasites, suppression of IFNγ responses to CSP, LSA1 and MSP1 during the first trimester, may contribute to the increased susceptibility observed in pregnant women early in pregnancy.

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ALTERED MALARIA ENDEMICITY IN RURAL COMMUNITIES IN THE GAMBIA AND IN GUINEA BISSAU

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In the Gambia and Guinea Bissau, epidemiological studies previously showed high malaria prevalence in rural areas, with peak among young children, aged 2 - 5 years. However, recent health facility based analysis suggest substantial decline in malaria incidence over the past years. As a prelude to a longitudinal immunological study on parasite persistence, it was necessary to determine the prevalence of malaria in a cross-sectional study of 12 villages in 2 different areas of The Gambia and one area in Guinea Bissau. In January - February 2008 (early dry season), age stratified randomization was performed and a total of 2615 participants were enrolled. The presence of parasitaemia was tested in blood samples using a rapid malaria diagnostic test (OptiMAL®), microscopy of Giemsa stained thick films and a qualitative PCR assay. Levels of total IgG antibodies to MSP1-19 antigen - a potential tool for estimating malaria endemicity at a population level - were measured. We found very low parasite prevalence detected using the rapid diagnostic test (0.9 ± 1.5 %) compared to the microscopy (11 ± 10 %) and PCR (25 ± 15 %). The prevalences are considerably lower than previously described in these areas. In addition to documenting the decline of malaria in the Gambia, the data also indicate that an age shift has occurred: regardless of the method used, the highest parasite prevalences were among children aged 11 - 15 years. This age shift may reflect a delay in the acquisition of immunity to malaria. Modeling and evaluation of the serological data will also be presented, and discussed in relation to prospective surveillance of these and similar communities.

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IGG RESPONSES TO THE N- AND C- TERMINAL DOMAINS OF THE CS PROTEIN AND PROTECTION AGAINST CLINICAL MALARIA IN MALARIA ENDEMIC SETTING IN BURKINA FASO (WEST AFRICA)

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Identification of antigens with reliable and reproducible immune correlates of protection against *Plasmodium falciparum* infection could be important in the development and testing of malaria vaccines candidates. IgG against two synthetic constructs (MR48 and MR178) representing the N- and C-terminal domains of the CS were used to assess the seasonality and the relationship between antibody levels and the protection against clinical malaria. Study was carried out with children less than five years from 4 villages belonging to Saponé Health District, in Burkina Faso. We performed two clinical and parasitological cross-sectional surveys at the low and the peak of malaria transmission seasons. During each survey, thick and thin blood films were prepared for parasites check and 5 ml of venous blood taken and plasma used for total IgG measurement by ELISA. Children were then actively followed by being home visited twice a week to record malaria cases for a year. No relationship was found between the IgG levels and age for both peptides (P=0.69 (low transmission) and 0.16 (peak of transmission) for MR48 and P=0.86 and 0.93 for MR178). Geometric means of IgG levels to CS N-terminus were similar at the low and at the peak of transmission (1.1, 95%CI: 1.0-1.2 vs 1.2 95%CI 1.1-1.2), P=0.78); however, IgG response to CS C-terminus was high during the low season compare to the peak season (1.1, 95%CI: 1.0-1.1 vs 1.0, 95%CI 0.9-1.0, P=0.02). Mean episodes number per child was 0.5 (95%CI 0.5-0.6).Geometric mean of IgG levels at the peak were similar in children without malaria and those with at least one malaria episode during the year follow up period (1.4 95%CI:1.2-1.4 and 1.2, 95%CI:1.1-1.3; P=0.51 for MR48 and 1.2, 95%CI:1.0-1.3 and 1.1 95%CI: 1.0-1.1; P=0.24 for MR178). In conclusion, IgG to these two constructs may be associated with protection; however investigation on the IgG subclass responses may help to better understand the type of the induced protective immunological responses.

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IMMUNIZATION WITH A SMALL PEPTIDE (CEL-1000) PROTECTS AGAINST RODENT MALARIA BY MODULATING INNATE IMMUNE RESPONSES IN LIVER

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A small immunomodulatory peptide derived from the second domain of human MHC-II β chain (CEL-1000, DGQEEKAGVVSTGLIGGG) confers sterile protection against challenge with *Plasmodium yoelii* sporozoites. Protection is likely mediated by mechanisms of innate immunity as there is no homology between CEL-1000 peptide and known malarial proteins. Furthermore, boosting with CEL-1000 did not induce memory responses which are intrinsic to adaptive (antigen-specific) immunity. We thus investigated the changes of various immune cell types in the liver and spleen of mice immunized with CEL-1000. Protection correlated with a significantly lowered frequency of (i) IFNγ- and TNFα-secreting NKT cells ($CD3^+$,$TCR\alpha\beta^+$, $CD4^{lo}$,$CD8^{lo}$,$DX5^+$,$CD11c^+$), and (ii) IL-12-producing dendritic cells and Kupffer cells in the liver. However, the frequency of these cell subsets in spleen was similar to that in control mice. We conclude that in the absence of adaptive immunity, protection against malaria can be achieved by modulating the innate immune responses within the liver.

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MULTIPLEX ANALYSIS OF CYTOKINE RESPONSES TO PRE-ERYTHROCYTIC AND ERYTHROCYTIC MALARIA ANTIGENS IN A HIGHLAND KENYA POPULATION

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Host immunity to malaria in low transmission areas differs considerably from that in areas of stable, seasonal malaria transmission. Here we evaluated antigen-specific cytokine responses in preliminary sample of adults (n=29) from a low-transmission highland area of Kenya using a multiplex microsphere-based assay (Bio-Plex, Bio-Rad Laboratories, Inc.). Specifically, we evaluated frequency and levels of IFN-g, TNF-a, IL-6, and RANTES production following PBMC exposure to pre-erythrocytic (CSP, LSA-1, and TRAP), blood-stage (MSP-1 and MB2), and pre-erythrocytic/blood stage (AMA-1) peptides predicted to be *P. falciparum* T cell epitopes. In general, cytokine production was more pronounced in response to blood stage antigens. Levels (geometric mean, range) of MSP-1-specific IFN-γ (7.4 pg/ml, 0-191 pg/ml, P=0.02), TNF-α (0.8, 0-68; P=0.04), and IL-6 (55.7, 0-9,833; P=0.008) and MB2-specific TNF-α (1.3, 0-36; P=0.04), IL-6 (37.5, 0-12,251; P=0.01), and RANTES (7.1, 0-446; P=0.01) were significantly elevated in residents from this low transmission area