Myxothiazole (30-fold) and Antimycin (>40-fold). Resistant clones $3D7_{V284L}$ and $K1_{G133S}$ were grown for 8 month in culture media with the selecting pyridone at their respective IC_{50} (approximately 6-fold higher than the parental strain). After this period, cytochrome b gene was amplified and sequenced. An additional mutation was found in the case of $3D7_{V284L}$ clone (S196G) and two extra mutations in the case of $K1_{G133S}$ (R95K and S196N) clone. Interestingly, in both clones one of the aminoacids modified with these new mutations is the same. When sensitivity to standard antimalarials, pyridones and known cytochrome b inhibitors was studied in the new double and triple mutants, differences found were not significative, suggesting a compensatory role of the newer mutations in cytochrome b. These mutants grew better than the original ones, robustly enough to allow us to isolate mitochondria. When ubiquinol: cytochrome bc1 activity on isolated mitochondria was tested for sensitivity to inhibitors, the same degree of resistance was observed than in the whole cell assays. These results strongly support the hypothesis that cytochrome b is indeed the primary target for the antimalarial activity of 4-(1H)-pyridones.

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TWO-STAGE FRACTIONAL FACTORIAL DESIGN TO INVESTIGATE FACTORS INFLUENCING THE EFFICACY OF PAFURAMIDINE MALEATE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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In the early stages of clinical drug development there are often multiple factors, such as dose and frequency of administration, that are hypothesized to have an impact on efficacy. It is important to determine the impact of each of these factors prior to moving to a large phase 3 study. The fractional factorial design provides a means for investigating the relative impact of multiple factors in a phase 2 study. Determining which of the factors has the greatest impact on the efficacy of the drug will increase the chances of a successful phase 3 study. A full factorial study design has a separate treatment group for all possible combinations of the factors of interest. For example if there are 3 factors that each have 2 levels (low and high) then there are a total of 8 different combinations of the low and high levels of the 3 factors. Due to limited resources it is often not feasible to study 8 different treatment groups so the fractional factorial design will study only a subset of the 8 groups. If 4 of the 8 groups are studied then the difference between the 2 levels (high - low) for each of the 3 individual factors can still be estimated. Our specific factorial was based on the phase 1 development of Pafuramidine Maleate for the treatment of uncomplicated *Plasmodium falciparum* malaria. Total daily dose (400mg vs 600 mg), dosing frequency (QD vs BID) and artesunate use (Yes vs No) all appear to have an impact on efficacy. In order to determine the optimal combination of these 3 factors. A fractional factorial design is being used in a randomized, open label, phase 2b study. The 4 dose groups in the study are: 1) 600 mg QD pafuramidine maleate without artesunate; 2) 400 mg divided BID pafuramidine maleate without artesunate; 3) 400 mg QD pafuramidine maleate with artesunate; and 4) 600 mg divided BID pafuramidine maleate with artesunate. Incorporation into 2 stageswas done. There are 2 stages in this study. The first stage will randomize 60 patients (15/group) to the 4 dose groups outlined above. At the end of the first stage, the difference between the high and low levels of each of the 3 factors (total daily dose, dose frequency, and artesunate use) will be estimated. The IDMC, sponsor and principal investigator will review the results from the first stage and determine which regimens from the possibilities in the full factorial design should be further studied with an additional 80 patients in stage 2.

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PRE-CLINICAL MONKEY TOXICITY STUDY OF JPC-2056-I, A THIRD GENERATION ANTIFOLATE

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JPC-2056, 1-(3-(2-chloro-4-trifluoromethoxy)phenoxy)propyloxy)-5-isopropylbiguanide, a third generation oral active folic acid antagonist, is currently in pre-clinical development as an anti-malarial agent effective against resistant strains of *Plasmodium falciparum* and *P. vivax*. A toxicology study was conducted and completed under an approved Animal Care and Use Committee protocol utilizing fifteen *Macaca fascicularis* monkeys. The animals were dosed daily by oral gavage with JPC-2056, Proguanil (positive control) or vehicle control. Blood samples were collected periodically for hematology and blood chemistry determinations. Upon sacrifice, organs and tissues were harvested and submitted to Charles River Laboratories for gross pathology and histopathology. An oral NOAEL of 7.5 mg/kg and a LOAEL of 15 mg/kg were determined and the major pathology identified was gastrointestinal effects leading to soft/loose stools or diarrhea. An antimicrobial panel was conducted to explore alterations in intestinal flora as a source of the observed GI effects. The full results of this study will be presented.

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

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JPC-2056 is a third generation oral 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. Upon sacrifice, organs and tissues were harvested and submitted to Charles River Laboratories for gross pathology and histopathology. NOAEL and LOAEL were determined and the major pathology identified was lack of weight gain. The full results of this study will be presented.

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MALARIA CONTROL IN THE MUNICIPALITY OF SAN ESTEBAN, HONDURAS, CENTRAL AMERICA

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Malaria is an important public health problem in the municipality of San Esteban in the Department of Olancho, Honduras, Central America. During the period of January 2005 through July 2006 there were 538 slide-confirmed malaria cases (*Plasmodium*. *vivax*: 454, *P. falciparum*: 84) identified from 6,007 blood smears submitted to the municipal laboratory (slide positivity rate = 9.0%). During May-July 2006, at the request of municipal health personnel, we assessed the burden of malaria in San Esteban and provided recommendations for control. Epidemiologic data from slide-confirmed malaria cases in 2005 and 2006 were reviewed. A Knowledge, Attitudes and Practices Survey (KAP) was conducted in households in San Esteban communities to assess malaria-related diagnostic, treatment and prevention practices. In 2005 and 2006 538 cases of *P. vivax* and *P. falciparum* malaria occurred in San Esteban in all age groups and both sexes, with increased frequency during May - October. We administered 112 KAP surveys in 19 communities in San Esteban. Seventy percent of respondents reported a history of malaria in a household member in the past, with the highest frequency reported in mothers (45%) and children < 14 yrs (37%). Most respondents were familiar with malaria symptoms (96%) and knew about mosquito transmission (80%). However, almost half reported waiting 4 or more days before seeking care, and also reported taking treatment for a range of 0-15 days (recommended: chloroquine for 3 days with primaquine for 14 days). Most households did not have mosquito protection such as bednets or screens and had not recently been sprayed with indoor residual insecticide. In conclusion, malaria is a significant problem in San Esteban equally affecting all ages and both sexes. Gaps were identified that could improve malaria prevention and control. We recommend: 1) improve timing and coverage of indoor residual spraying; 2) increase promotion and funding for insecticide-treated bed nets; 3) incorporate lessons learned about community malaria practices into education sessions; Improved prevention and treatment practices can reduce morbidity from malaria in these communities.

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CONGENITAL *PLASMODIUM FALCIPARUM* INFECTION IN NEONATES IN MUHEZA DISTRICT, TANZANIA

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This study aimed to determine the magnitude of congenitally acquired malaria infection among infants (<3 months old) in a malaria holoendemic area of north-eastern Tanzania. PCR amplification was used to amplify MSP2 gene as a marker to determine the genetic relatedness of *Plasmodium falciparum* detected in the placenta blood, cord blood and blood from infants born of mothers diagnosed with placental malaria and presented clinical malaria. Placental malaria was observed in 95 (9.3%) of mothers, of whom, 40 (42.1%) were primigravidae, 31 (34.7%) secundigravidae and 23 (24.2%) multigravidae and 18 (19.1%) of infants born from mothers with placental malaria developed clinical malaria below three months of age. A total of 1022 placental and cord blood samples were examined for malaria infection. The prevalence of *P. falciparum* by microscopy was 9.8% and 0.4% in the placenta and cord blood samples, respectively. The prevalence by PCR in the placenta was 9.3 % (95/1017) and cord was 6% (61/1017). Eighteen (19.1%) of infants born from mothers with placental malaria developed clinical malaria below three months of age. Six (40%) out of 14 pairs of placental blood and cord blood samples that shared band size and fragments size were, genetically unrelated while eight pairs (60%) were genetically related indicating congenital malaria infection. One pair (14.3%) of sequenced placental and infants samples were genetically related. Infants born from primigravidae were significantly more likely to be infected with *P. falciparum* (P< 0.001) as compared to infants from secundigravidae and multigravidae, and it was detected that infants from multigravidae mothers do get *P. falciparum* infection early in life with the relative risk of 1.43, than those from secundigravidae and primigravidae. Malaria parasitemia of the placenta has strong likelihood to result in congenitally acquired malaria.

(ACMCIP Abstract)

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MALARIA PARASITE PREVALENCE IN THE ARTIBONITE VALLEY OF HAITI DURING THE RAINY SEASON, 2006

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Haiti is one of only two countries in the Caribbean with endemic transmission of *Plasmodium falciparum* malaria. However, reliable population based estimates on the exact distribution and burden of malaria within Haiti are scarce, with existing data coming primarily from confirmed malaria cases reported through the health system. We conducted a population based survey to estimate the prevalence of malaria parasite infections among individuals older than one month in the Artibonite Valley during the high transmission season in 2006. Results from PCR among 714 individuals showed the prevalence of *P. falciparum* malaria to be 3.1% (95% Confidence Interval: 0.6-5.7%). Individuals with malaria infections ranged in age from 1 to 62 years old, with males representing 65.2% of infections. The majority of infections were febrile. Malaria transmission was highly localized, with all 23 infections coming from only 8 villages of the 20 sampled (40%), suggesting transmission is potentially based on a set of discrete ecological determinants. To our knowledge, this is the first population based estimate of malaria parasite prevalence in Haiti using PCR diagnosis. While modest, a 3.1% prevalence represents as many as 198,000 cases that likely occur each year during the rainy season within low-lying areas of Haiti. This is a significant level of morbidity, especially when one considers that the severity of the disease is likely high given the low level of acquired immunity among the Haitian population. We argue that future malaria interventions in Haiti be geared towards controlling malaria in the context of a moderate transmission setting, thus large scale distribution of ITNs or widespread use of indoor-residual spraying may be less cost effective than enhanced surveillance with effective case management or focused larval control.

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FACTORS ASSOCIATED WITH THE PROVISION OF APPROPRIATE ANTIMALARIAL THERAPY FOR CHILDREN UNDER FIVE IN NIGERIA

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Malaria continues as a significant health problem in Nigerian children under five and most caregivers fail to provide appropriate treatment in a timely manner. The primary objective of this analysis was to determine which factors are related to the prompt and appropriate treatment of febrile illness and therefore, presumptive malaria, in Nigerian children under five years of age. Analyses were performed using the Nigeria Demographic and Health Survey (NDHS); a cross-sectional survey of women 14-49 years conducted in 2003 by Measure DHS. The 1603 children of these women who had fever in the 2 weeks preceding the survey comprised our sample population. The outcome variable was

prompt and appropriate treatment of fever. Factors thought to be related to the outcome of correct treatment were compared by chi squared statistics, simple logistic regression and multivariate logistic regression. A total of 431 (26.9%) children with fever in the two weeks prior to the survey received prompt and appropriate treatment for their febrile illness. The odds ratio (OR) for correct treatment if treatment was sought at a drug shop was 0.49 95% CI [0.39, 0.69] as compared to the public sector. Children whose mothers sought treatment for fever through a traditional practitioner had an OR of 0.07 95% CI [0.02, 0.22]. Maternal education modified the effect of socioeconomic status on the outcome; the OR for middle income families and mothers with no primary education was 2.92 95%CI [1.73, 4.92] and the OR for rich families with no primary education was 8.52 95%CI [2.99, 24.24] compared to poor families with a mother who did not have primary education. Individuals who lived in the northcentral and southern regions had lower odds of correct treatment as compared to the combined northeastern and northwestern region. Older children had higher odds of correct treatment with an increase in odds of 1.15 per year increase in age 95% CI [1.03, 1.29]. In conclusion, this analysis was conducted to identify factors that might relate to the appropriate management of fever with the thought that the identification of these factors may lead to interventions designed to improve the case management of febrile children under five. Source of treatment is one such important factor to target in order to improve the level of correct treatment of febrile illness in Nigeria.

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PREVALENCE OF MALARIA AT BOOKING AMONG ANTENATAL PATIENTS IN A SECONDARY HEALTH CARE FACILITY IN IBADAN, NIGERIA

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Malaria and pregnancy are mutually aggravating. One of the most common complications of malaria in pregnancy is anemia which also has a negative impact on the outcome of pregnancy. This study aims to determine the prevalence of malaria parasitaemia at booking and its impact on the gestational age at booking. In a cross sectional design, thick and thin blood smears were prepared from all pregnant women who reported for booking and provided informed consent. Demographic and obstetric details were also obtained. The main outcome variables were the presence of malaria parasites in blood film and fever. Both were categorized as binomial variables yes or no. Categorical variables were analyzed using the chi-squared test while continuous variable were analyzed by t-test both for equal and unequal variance using the variance ratio function of the Stata software to determine the appropriate use of the Satterthwaite's correction for the degrees of freedom. Multivariate analysis was by multiple linear regression. Level of statistical significance was at $p< 0.05$ for all the analyses.The prevalence of malaria parasitaemia in the study population using the blood film as evidence of malaria was 8.4%, 3.6% had low parasitaemia, 4.1% had moderate parasitaemia and only 0.7% had high parasitaemia. The prevalence of asymptomatic malaria was 89.04%. The mean gestational age at booking had significant association with the incidence of fever ($p = 0.0052$). Febrile subjects booked at a mean gestational age of 22.7 weeks (95% CI 21.6-23.8) while the afebrile subjects booked at a mean gestational age of 24.2 weeks (95% CI 24.1-24.6), it was 24.25 ($\pm$ 6.17) week for all patients. The prevalence of anemia was 58.1% among patients with patent parasitemia compared with 22.6% among those without patent parasitemia ($p<0.0001$. In conclusion, malaria parasitaemia is low in this study; symptomatic malaria was associated with early booking for antenatal care. Malaria parasitemia was a significant determinant of anemia at booking.

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ASSOCIATION OF MALARIA INFECTION WITH MOTOR AND LANGUAGE DEVELOPMENT AMONG PRESCHOOL CHILDREN IN ZANZIBAR

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Malaria continues to be a major public health issue, particularly in sub-Saharan Africa where children suffer 200 million clinical malaria episodes per year. While cognitive impairments from severe malaria have been documented, the potential developmental consequences of chronic parasitemia remain unclear, and literature about preschool children is especially scarce. This study analyzed data from children under five in Zanzibar, an area holoendemic for malaria, who participated in a factorial trial of iron supplementation and mebendazole on growth, anemia, and development. Gross motor and language development were assessed by parental report at baseline and one year later. The average increase in score over the year was 5 units. Three hundred and fifty nine children comprised the language cohort and 255 children constituted the motor cohort. All children in the study were randomly assigned to one of two follow-up protocols: measurement of malaria parasitemia on either months 0,1,3,5,7,9,11, and 12 or months 0,2,4,6,8,10, and 12. Multivariable linear regression was used to assess the association between the change in development score over the one year period and average parasite count over the year. While language development was not associated with parasitemia, for every 1000 parasites/microliter increase in average parasite count, there was a.23 unit increase in motor development (adjusted for age and height for age Z score at baseline). The results may suggest that children who are able to withstand higher parasite loads have a survival advantage that enables them to have similar motor development as other children. Alternatively, the results could reflect a treatment effect because all children in the study with suspected malaria were promptly treated.

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MULTIPLEX EVALUATION OF SINGLE NUCLEOTIDE POLYMORPHISMS TO DIFFERENTIATE BETWEEN NEW AND RECRUDESCENT INFECTIONS IN CLINICAL TRIALS OF ANTIMALARIAL DRUGS AGAINST *PLASMODIUM FALCIPARUM*

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The World Health Organization recommends that anti-malarial clinical drug trials include genotyping to distinguish new from recrudescent strain infections following treatment. Common methods used for genotyping involve amplification of alleles in the polymorphic genes glutamine-rich protein (*glurp*), merozoite surface proteins 1 (*msp1*), and *msp2*. New technologies expand opportunities for developing more efficient strategies to evaluate the susceptibility of malaria species and strains to drug treatment. Here we present a new method for genotyping infections that can be performed by a multiplex strategy using single nucleotide polymorphisms (SNP) distributed throughout the *Plasmodium falciparum* (Pf) genome. Marker loci were chosen where the diallelic SNP alleles were each observed in at least 5 strains as shown in PlasmoDB.

PCR primers were developed and confirmation that the two expected alleles were present in Papua New Guinea (PNG) Pf strains was tested by sequencing locus-specific products from a pool of 10 DNA samples from infected people, and from screening individual samples (Wosera region; n=93) by a ligase detection reaction-fluorescent microsphere assay (LDR-FMA). Of 26 SNP tested to-date, 4 were confirmed as polymorphic in PNG. Comparison of pre- and post-treatment samples is confounded when all alleles are detected at each marker locus; the probability of this outcome was predicted to be $1.4x10^{-3}$. Blood samples from 12 patients enrolled in a clinical trial were then genotyped to determine if new vs. recrudescent infections could be distinguished. Samples from 8 patients showed new infections at 42-days post treatment, while 2 patients showed recrudescent infections, and 2 patients responded to treatment and had no infection at day 42. This shows that LDR-FMA of SNP is a viable alternative to current approaches; technical efficiency of LDR-FMA introduces important advantages to evaluating large sample sizes. Analytical methods are under development to assess the level of confidence that SNP-based genotyping differentiates new vs. recrudescent infections.

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SEASONAL VARIATIONS IN *PLASMODIUM* SPECIES. CASE STUDY OF A RURAL PERIPHERAL HOSPITAL FROM CENTRAL INDIA

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Malaria in central India is complex because of the vast tracts of forest with tribal settlement. Satna (Pop. 1870104, ethnic tribe 14%) was not considered a malarious district as it contributes 3 % malaria and 1% falciparum infection in the state. Due to rapid socio-economic and ecological changes and high mobility of population for a variety of reasons, the malaria situation has been deteriorated recently in most part of state. The number of reported cases remained very low due to low accuracy of malaria microscopy and irrational clinical practices. In view of this a malaria clinic was established at the Maihar civil hospital, Satna by National Institute of Malaria Research FS Jabalpur (ICMR) to screen all fever cases clinically suspected to be malaria. The main objective of the study was to determine malaria prevalence rates caused by each species of Plasmodium, and the annual and seasonal changes in the prevalences of infection. Analysis of data (September 2005-April 2007) revealed that malaria was present throughout the year and both *Plasmodium vivax* and *P. falciparum* were prevalent in all age groups but their prevalence was highly seasonal. Season wise distribution of malaria cases (all age group combined) showed the lowest prevalence (160/2530) in hot dry summer season which was mainly due to *P.vivax* (84%) followed by monsoon (581/3696) where the relative contribution of *P. vivax* and *P. falciparum* were 59 and 41% respectively (OR 2.8; 95% CI 2.3-3.3) and highest prevalence (1333/5175) was recorded in post-monsoon/ autumn (OR 5.1; 95% CI 4.3-6.1), which was due to *P. falciparum* (82%). The age specific data on malaria revealed that children between >4-14 yrs were more at risk of developing malaria (695/2684) as compared to other age group (OR 1.8; 95% CI 1.6-2.1, p<0.0001). Further analysis revealed a linear decreasing trend in *P.vivax* with increasing age (β-coefficient-0.128; F 34.72; p<0.00001). We conclude that systematic inclusion of laboratory based diagnosis should be promoted in the health facilities showing low or high seasonal endemicity. At this stage the necessary technical skills for microscopy based examination may be difficult to scale up immediately, therefore, rapid diagnostic tests based on the detection of *Plasmodium* antigen may be the most efficient approach to manage malaria by prompt diagnosis and treatment.

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MALARIA IN FOREST VILLAGES OF MANDLA AND DINDORI DISTRICTS IN CENTRAL INDIA (MADHYA PRADESH)

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Malaria remains a major public health problem in Madhya Pradesh particularly among ethnic tribes of forest villages which contribute >50 malaria and >70% *Plasmodium falciparum* in the state. The limited resources of National Vector Borne Disease Control Programme necessitate that they be directed towards most at risk. A longitudinal study on malaria was undertaken in forest villages of Mandla and Dindori districts from 2004-2007 with the objective to determine the pattern of malaria in forest villages and to record annual changes in the prevalence of infection. The long term objective was to study the effects of antimalarial measures. These two districts contribute 29% of malaria and 40% *P. falciparum* in the state, while their population is only 2.4% of the state population. For this study, five villages were selected from Bajag PHC, Dindori (Pop. 2800) and five from Bichia, Mandla (Pop. 2059). The surveillance of fever cases or with history of fever was done fortnightly. The pattern of malaria and vectors were different between the two sites, though *P. falciparum* was the dominant species at both site (>90%). Logistic regression analysis revealed significantly more malaria (1183/3971) at Bajag (Exp β=4.05, 95% CI 3.38-4.85 p<0.0001). Analysis of age specific data revealed that prevalence of malaria is very high in all age groups including infants (<1yrs) at Bajag. The highest malaria (237/518) was recorded in young children of 1-4 yrs (OR 3.29, 95% CI 2.69-4.02) followed by older children (OR 2.85, 95% CI 2.34-3.47) as compared to adults (>14 yrs) (p<0.0001). While at Bichia highest prevalence was recorded in children of >4-8 yrs (OR 1.6; 95% CI 1.01-2.53, p<0.005). Entomological surveillance revealed the presence of two vectors at both the sites i.e. *Anopheles culicifacies* (Average per man hour density 5.4:12.7) and *An. fluviatilis* (MHD 0.66:0.33) at Bajag and Bichia respectively. The density of *An. culicifacies* was significantly higher at Bichia (p<0.0001) while *An. fluviatilis* at Bajag (p<0.025). Analysis further revealed that as a result of intensive intervention measures at Bajag there was a linear declining trend in malaria prevalence from 2004 to 2007 (70%) while at Bichia because of irrational intervention measures malaria further increased by 30%. Knowledge of malaria risk at a local level may prove more informative for formulating control strategies by supporting resource allocation decisions at the district level.

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MALARIA RELATED KNOWLEDGE, PERCEPTIONS, AND PRACTICES IN THE ARTIBONITE VALLEY OF HAITI

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Low to moderate levels of *Plasmodium falciparum* malaria prevalence have been documented in low-lying areas of Haiti. A two-stage cluster sample probability proportional to cluster size (n = 200 households) was conducted in the Artibonite Valley of Haiti during the high malaria transmission season in November-December 2006. Knowledge, perceptions and practices related to malaria were obtained from household representatives using a standardized questionnaire. Blood drops on filter paper were obtained from all household members older than one month (n = 714) for ascertaining malaria parasite prevalence using PCR. The normalized differential vegetation index (NDVI) was calculated using Landsat 7 Enhanced Thematic Mapper data; elevation data for selected villages (n = 20) were obtained from the US Geological Survey. Individual,

household and community-level determinants of malaria infections and correct malaria related knowledge were assessed using logistic regression. Only one in five (21.3%) of the household respondents knew that malaria was transmitted by mosquitoes, knew that both children and adults are at risk, and knew that ITNs can reduce transmission (i.e. had correct knowledge). Results suggest that respondents residing in households with a high wealth index were 27 times more likely than those with a low wealth index to have correct malaria-related knowledge (OR = 27.4, 95% CI 4.0 - 188.54). Respondents from households with at least one malaria infection were significantly less likely to have correct malaria-related knowledge as compared to those in households with no malaria infections (OR = 0.16, 95% CI 0.03 - 0.87). Significant determinants of malaria infections among household members included fever greater than 37.5°C (OR = 12.78, 95% CI 4.64 - 35.24) and being aged 5-9 years old (OR = 2.40, 95% CI 1.26 - 4.61). Elevation and NDVI were not significantly associated with malaria infections. Further research on how socio-behavioral and ecological factors are related to malaria infection and cost-effective control strategies is needed.

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SIMULATION OF MALARIA TRANSMISSION AMONG HOUSEHOLDS IN A THAILAND VILLAGE USING REMOTELY SENSED PARAMETERS

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We have used discrete-event simulation to model the malaria transmission in a Thailand village with approximately 700 residents. Specifically, we model the detailed interactions among the vector life cycle, sporogonic cycle and human infection cycle under the explicit influences of selected extrinsic and intrinsic factors. Some of the meteorological and environmental parameters used in the simulation are derived from Tropical Rainfall Measuring Mission and the Ikonos satellite data. Parameters used in the simulations reflect the realistic condition of the village, including the locations and sizes of the households, ages and estimated immunity of the residents, presence of farm animals, and locations of larval habitats. Larval habitats include the actual locations where larvae were collected and the probable locations based on satellite data. The output of the simulation includes the individual infection status and the quantities normally observed in field studies, such as mosquito biting rates, sporozoite infection rates, gametocyte prevalence and incidence. Simulated transmission under homogeneous environmental condition was compared with the prediction from a SEIR model. Sensitivity of the output with respect to some extrinsic and intrinsic factors was investigated. Results were compared with mosquito vector and human malaria data acquired over 4.5 years (June 1999 - January 2004) in Kong Mong Tha, a remote village in Kanchanaburi Province, western Thailand. The simulation method is useful for testing transmission hypotheses, estimating the efficacy of insecticide applications, assessing the impacts of nonimmune immigrants, and predicting the effects of socioeconomic, environmental and climatic changes.

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MALARIA KNOWLEDGE, ATTITUDE AND PRACTICES AND PREVALENCE OF *ANOPHELES* IN THE FOREST OF REGION OF GUINEA, WEST AFRICA

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Understanding communities' perceptions on malaria transmission, treatment and prevention is essential for the design of culturally adapted and effective control measures. We assessed the knowledge, attitude and practices (KAP) of an urban community on malaria, the mosquito vectors, the use of insecticide-treated bednets (ITNS), and anti-malarial drugs. Methods: A cross-sectional study of 336 households was conducted in the city of N'Zérékoré, Guinea (population: 300,000) in 2006. Households were randomly selected using a geographical sampling survey conceived and implemented by Keating *et al*. in 2003. Heads of households were interviewed and demographic information, household and environmental characteristics, and malaria-related knowledge, attitude and practices were assessed. Only 37% of respondents knew that malaria was transmitted by mosquitoes. Other explanations for malaria included dirty water, rodents, eating bananas or mangoes, kissing or touching someone infected, acts of god, or working in the rain. Fifty-seven percent had no explanation. Most people obtained knowledge about malaria from the radio and health facilities. Burning material (34%), window screens (24%), spraying of insecticides (24%), ITNs (22%), and removal of stagnant water near the house (9%) were the most frequent methods cited to prevent malaria. Fourteen percent said they took no preventive measures. Thirty percent of households reported owning at least 1 ITN. ITNs were obtained from markets (65%), health facilities or hospitals (28%), relief organizations (4%), refugee camps (1%), or received as gifts (1%). Stated reasons for not using ITNs were too expensive (58%), too small (34%), didn't know where to find them (27%), and don't work (16%). Seventy-eight percent of respondents reported an episode of malaria and 74% were treated, including chloroquine (23%), quinine (17%), and sulfadoxine-pyrimethamine (3%). *Anopheles* were found in only 1 of the 336 households. In conclusion, although malaria was common in this community, overall knowledge of malaria and practices for its prevention were poor. Interestingly, *Anopheles* were almost completely absent from the peridomestic environment, perhaps indicating breeding in sites more distant from the home. The significance of these findings for improving malaria control will be discussed.

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HIGH PREVALENCE OF ASYMPTOMATIC MALARIA INFECTIONS IN THE PAPUA NEW GUINEA DEFENCE FORCE

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A malaria survey in the Papua New Guinea Defence Force (PNGDF) was undertaken to better understand the epidemiology and drug resistance of malaria in soldiers. Papua New Guinea has a complex geography which results in different malaria transmission patterns. The PNGDF has several bases throughout the country and the data was collected from soldiers living in three separate areas. The survey was conducted with asymptomatic PNGDF volunteers from Lae, Wewak and Port Moresby between June and July 2006. 408 soldiers (27% of the PNG army) were tested for malaria parasites by PCR. Eighty-seven volunteers (21%) had an infection which corresponds to the following distribution: 44/139 (32%) Lae, 24/90 (27%) Wewak, 19/179 (11%) Port Moresby. *Plasmodium vivax*-only infections accounted for 62% of all infections and *P. falciparum*-only infections for 20%, while *P. vivax*/*P. falciparum* mixed species infections accounted for 17% and *P. falciparum*/*P. malariae* for 1%. Twenty *P. falciparum* infections were separated into 10 different genotypic patterns as determined by *Hinf*I digestion of *pfmsp2*, while 46 *P. vivax* infections could be separated into 18 different genotypic patterns as determined by *Alu*I or *Hha*I digestion of *pvmsp3*α. Drug resistance markers, such as *pfcrt*, *pfdhfr*, and *pvdhfr*, have been examined, and the prevalence of drug resistant alleles will be presented.

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SPECIFIC *PLASMODIUM* ELIMINATION DURING A SECOND INFECTION IN CBA/CA MICE

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In malaria endemic areas individuals are susceptible to several infections before they develop a protective immunity, the basis of this susceptibility and the precise nature of the immune mechanisms that control the parasitaemia during the first and subsequent infections are still not well understood, however, they are relevant to understand the immunity to malaria. In this work we studied how a first infection with *Plasmodium yoelii* 17XL affect the dynamic of the parasitaemia during a second infection with the homologous parasite, or with *P. chabaudi* AS or with the mixture of *P. chabaudi* AS and *P. yoelii* 17XL in CBA/Ca mice using a nested PCR. A batch of CBA/Ca mice was infected with $5X10^4$ parasitized erythrocytes with *Plasmodium yoelii* 17XL. On day 7 post-infection, mice were treated with a therapeutic dose of pyrimethamine, 8 weeks later, mice were split into three groups, the first was re-infected with $5X10^4$ parasitized erythrocytes of the homologous parasite, the second group was re-infected with *P. chabaudi* AS and the third with the mixture of both parasites. Parasitaemia was measured in Giemsa stain blood films and by using a specific nested PCR. We also studied some of the pathology parameters as weight lost, splenic index and haemoglobin concentration in the re-infected mice. The results show that there was a specific parasite elimination since mice reinfected with the homologous *Plasmodium* developed lower parasitaemia levels than mice infected with the heterologus parasite. Interestingly mice reinfected with the mixture of both parasites cleared the parasitaemia almost at the same time than mice infected with the homologous *Plasmodium*. In spite of the ability to clear the parasitaemia developed by mice infected with the homologous parasite, this group of mice showed almost the same levels of haemoglobin concentration and weight lost that mice infected with the heterologous parasite. The results of splenic index showed an increment in all groups of mice, at the beginning of re-infection it was higher in mice re-infected with *P. yoelii* 17XL but at the end of re-infection the splenic index was higher in mice infected with *P. chabaudi* AS. In this work we showed that during a re-infection even when protection against the homologous parasite has been developed still remains strong spleen activation probably due to exposition to different or new *Plasmodium* antigens, which explains at least in part the need of several infections to get a solid immunity.

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ROLE OF COMPLEMENT AND COMPLEMENT REGULATORY PROTEINS IN SEVERE ANEMIA CAUSED BY *PLASMODIUM CHABAUDI*

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Malaria accounts for about 1-2 million deaths per year, with the majority due to complicated *Plasmodium falciparum* malaria, such as severe malaria-associated anemia. Not much is understood about the pathogenesis of this anemia. C57BL/6 mice inoculated with 10^6 *P. chabaudi* AS-infected red blood cells experience a primary peak of high parasitemia and severe anemia at approximately 6-7 days post infection. After one week of recovery, a secondary peak with much lower parasitemia occurs with an additional hematocrit drop. We will present data on the role of complement (C3), red cell complement regulatory proteins and anti-malarial antibody levels in the development of anemia in this model by comparing the infection in wild-type animals and complement (C3) and complement regulatory protein knockouts.

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FCγ RECEPTOR POLYMORPHISMS IN GHANAIAN CHILDREN WITH CLINICAL MALARIA

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Multiplication of the blood-stage of the malaria parasite and host factors are responsible for the pathogenesis of malaria. Host factors include immune factors and genetic factors such as polymorphisms polymorphisms in Fcγ receptors. Acquired immunity to malaria is dependent on age and exposure. Cytophilic antibodies (IgG1 and IgG3) play a major role in effecting acquired immunity however, in some studies IgG2 have been found to be associated with protection against clinical malaria because of its high affinity for certain polymorphisms of FcγRIIA and FcγRIIIB. Fcγ receptors, which are found on myeloid cells such as mononuclear phagocytes and neutrophils, bind to the Fc portion of immunoglobulin (Ig), leading to phagocytosis and parasite killing thus mediating immune responses. The gene that codes for this receptor is polymorphic and is associated with predisposition to malaria infection. In this study we investigated the roles of the FcγRIIA and FcγRIIIB polymorphism in the pathogenesis of clinical malaria in Ghanaian children. The study population consisted of a cohort of 210 children aged, three to ten years from Dodowa in Ghana, who were followed up in longitudinal cross sectional study under the Afro-immunoassay morbidity surveillance for eight months. PCR-RFLP was used to characterize these polymorphisms in our study population. The data was related to the clinical data from the morbidity surveillance, and also to immune responses against some vaccine candidate blood-stage antigens. We present the results and discussion of the study.

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IMMUNOEPIDEMIOLOGY OF PVRII, A PUTATIVE VACCINE CANDIDATE REPRESENTING *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN, IN SRI LANKA

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Study of naturally acquired antibody responses to a potential vaccine candidate is imperative to provide insights to vaccine development. Recombinant protein PvRII, a putative vaccine candidate representing region II of native *Plasmodium vivax* Duffy Binding Protein (DBP), was used in ELISA to examine the total (IgG+IgM), IgM and IgG isotype antibody responses. A reduction sensitive ELISA was preformed for total antibodies. Sera were collected from acute vivax malaria patients from two endemic areas (EAs) where low and unstable malaria conditions prevail, and from a non-endemic area (NEA) in Sri Lanka. Prevalence of total antibodies was 60% from Colombo (NEA; N=111), 46% from Anuradhapura (EA; N=94) and 41% from Kataragama (EA; N=106). Significantly higher prevalence (Chi square, $P<0.05$) and magnitude (ANOVA, $P<0.01$) of total antibodies were recorded from NEA compared with EAs. Total antibody parameters in all test populations were independent of age of individuals, parasite density and previous exposure. Conformation sensitive anti-PvRII monoclonal antibody 2H10 reacted only with non-reduced PvRII but not with its reduced form. Test sera partially reacted with reduced PvRII, and this indicating the recognition of linear B cell epitopes. A parallel increase in total antibody response to PvRII linear epitopes with increasing exposure was detected in residents of Kataragama. No significant difference was detected among test populations either in anti-PvRII IgM prelavence (Chi square, $P>0.05$) or magnitude (ANOVA, $P>0.05$). Prevalence of IgG3 (Chi

square, P<0.05) and magnitude of IgG1 (Wilcoxon Signed Rank test, P<0.01) were significantly higher compared with other IgG isotypes in all test areas. An increase in prevalence of IgG isotypes in parallel with increasing exposure was recorded from Kataragama. An antibody shift in the prevalence of a combination of IgM+IgG1+IgG3 to a cytophilic IgG1+IgG3 with increasing exposure detected in both endemic areas for PvMSP-1_{p19} and PvAMA-1 using the same battery of serum, was not observed for PvRII.

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INTERFERON Γ RESPONSE TO A T-CELL EPITOPE OF *PLASMODIUM FALCIPARUM* GLUTAMATE RICH PROTEIN (GLURP) CORRELATES WITH PROTECTION FROM CLINICAL MALARIA IN GHANAIAN CHILDREN

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Glutamate Rich Protein (GLURP) is a potential blood stage malaria vaccine candidate antigen. T-cell response against this antigen is important for generation and maintenance of long-lasting antibody response. In this study, cytokine responses to peptides from the conserved region of GLIURP were assessed in relation to protection from clinical malaria. Randomly selected stored cell samples from a cohort of Ghanaian children aged 3-15 years were used. They were actively and passively followed for a complete malaria season and categorized as having at least one episode of malaria (susceptible), and those who did not have any episode of malaria (protected). Cryo-preserved Peripheral Blood Mononuclear Cells (PBMC) of 56 children were stimulated in a 6-day culture with 4 overlapping peptides of GLURP (LR129, LR130, MR186, MR187) and cytokines (IL-10 and IFNγ) were measured in culture supernatants. PBMC's from 14 non-exposed Danes were used as controls.IFNγ responses to GLURP LR129 and LR130 stimulated cultures in the Ghanaian children were significantly higher (P<0.050) than levels in Danish controls. Of the 4 GLURP peptides tested and cytokines assessed in culture supernatant, IFNγ responses to MR186 correlated with protection (p=0.003), suggesting the importance of this epitope in the induction of protective memory responses. This epitope of GLURP should therefore be included in a future GLURP specific vaccine.

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T CELL RESPONSES TO MODIFIED *PLASMODIUM FALCIPARUM* $MSP1_{19}$ ANTIGENS IN PEOPLE PREVIOUSLY EXPOSED TO NATURAL MALARIA INFECTION

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Epitopes on the 19kDa C-terminal region of the merozoite surface protein 1 ($MSP1_{19}$), recognized by processing-inhibitory and blocking monoclonal antibodies had been previously modified to yield proteins that can bind MSP1 processing inhibitory antibodies but not blocking antibodies. It was unclear whether these modifications would affect critical T cell epitopes on $MSP1_{19,}$ therefore, *in-vitro* assays for T cell responses to wildtype $MSP1_{19}$ and a panel of site-directed mutagenetically modified antigens was studied in individuals naturally exposed to *Plasmodium falciparum* infection. All the mutants tested stimulated T cells from exposed individuals but not in malaria naïve individuals. Interestingly, stimulation indices for the T cell responses induced by some of the mutants were at least twofold higher than those elicited by the wildtype MSP1-protein. A mutant protein with four-amino-acid-substitution: Glu27◊Tyr, Leu31◊Arg, Tyr34◊Ser and Glu43◊Leu (27+31+34+43; residues numbered from the N-terminus of $MSP1_{19}$) had the highest T cell stimulation index (SI=360) with prevalence of 57.1% responders. This study suggests that this specific mutant $MSP1_{19}$ 27+31+34+43, which also abrogate the binding of all known blocking monoclonal antibodies without affecting the processing-inhibitory antibody epitope, might be a better choice for an MSP1-based malaria vaccine.

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ANTIBODY RESPONSES TO THE MSP-1 COMPLEX PROTEINS IN CEREBRAL MALARIA PATIENTS IN INDIA

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Humoral immune responses to the merozoite surface protein-1 (MSP-1) of *Plasmodium falciparum* are known to be protective against malaria. MSP-1 consists of a complex of four major non-covalently bound polypeptides; p30, p38, p42 and p83. In addition, MSP-1 complex proteins associate with MSP-6 and MSP-7 and form a functional complex in mediating some functional activity. Studies of the MSP-1 sequence obtained from different *P. falciparum* isolates demonstrated the presence of several conserved regions but also major dimorphic portions belonging to either the K1 (f) or the MAD20 (d) allelic families. To date, a few studies have examined the humoral immune responses to the MSP-1 complex as a whole in humans. In this study, we investigated if antibody responses to the MSP-1 complex, MSP-6 and MSP-7 have any protective correlation in cerebral malaria patients. To address this, we utilized subjects recruited in Central India which included three categories: healthy controls (HC), mild malaria (MM) and cerebral malaria (CM) patients. Plasma collected at enrollment of subjects were tested in an ELISA assay using recombinant proteins of all the MSP-1 complex proteins, including the two dimorphic families (d and f), MSP-6 and MSP-7, for the presence of antibody responses. We observed differential prevalence of anti-MSP-1 complex antibodies depending on the antigen. For all the antigens, the levels of total IgG increased in the MM category compared to HC. However, the antibody levels in general declined in CM patients as compared to MM and this reduction was significant for anti-MSP-1_{f83} (p<0.05). The predominant subclasses of IgG observed were IgG1 and IgG3 with negligible amounts of IgG2 and IgG4 for most subunits. We observed an increase in the prevalence and levels of both IgG1 and IgG3 in MM patients as compared to HC. In CM patients, IgG1 and IgG3 antibody levels were generally reduced compared to MM reaching significant reductions for anti-MSP-1_{d83} and MSP-1_{f83} IgG3 (p<0.05). These results suggest that there may be a deficit in the generation of optimal antibody response to some of the MSP-1 protein complex that may be relevant for protection in CM patients.

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NATURAL RESISTANCE AGAINST SEVERE MALARIA IN GHANAIAN CHILDREN DEPENDS ON TOLL-LIKE RECEPTORS

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Toll-like receptors (TLRs) are involved in innate immune responses against infections by the induction of cytokines and have never been studied in children with acute malaria in Ghana. We assessed the *in-vitro* capacity of leukocytes of children with malaria to produce inflammatory cytokines (TNF-α, IL-6, IL-10 and IL-1ra) in response to TLR-mediated ligands. Cytokines in supernatants, obtained from whole blood cultures of children with severe malarial anaemia (SA), cerebral malaria (CM) and uncomplicated malaria (UM), were measured by ELISA. Significantly higher levels of TNF-α, IL-6 and IL-1ra were measured in response to bacterial lipoprotein (TLR-2) in severe malaria (SA, CM) patients than in UM patients ($p<0.05$). Similarly, significantly higher levels of IL-1ra was measured in response to Gram-negative bacteria (TLR-4) in children with severe malaria than in UM patients ($p<0.05$). Likewise, significantly higher levels of TNF-α was measured in response to unmethylated bacterial DNA (TLR-9) in children with severe malaria ($p<0.05$), whereas levels of IL-10 was significantly higher only in children with SA than in UM patients ($p<0.05$). In addition, significantly higher levels of IL-10 and IL-1ra were measured in response to bacterial lipoprotein and Gram-positive bacteria (both TLR-2) in SA than in UM patients ($p<0.05$). In response to *E. coli* lipopolysaccharide (TLR-4), significantly higher levels of TNF-α, IL-6, IL-10 and IL-1ra were measured only in SA, whereas levels of TNF-α in supernatants of CM was significantly reduced as compared with UM ($p<0.05$). Our data suggest that TLRs may be involved in the pathogenesis of severe malaria.

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MALARIA DEFERRED BLOOD DONATIONS: ARE THEY REALLY A THREAT?

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The scientific community is aware of the ongoing loss of blood donors due to increasing numbers of malaria deferrals. The American Red Cross looses over 1% of presenting donors each year to malaria deferrals, resulting in an estimated annual loss of more than 93,000 donations. There are three deferral categories; 12 month deferral for travel to an endemic area, and 3 years deferral for residence (5 years or more) in an endemic area or previous malaria infection/symptoms. While there has been only 5 transfusion-transmitted malaria cases reported in the US since 1998, the criteria used to exclude potential blood donors need to be reevaluated due to excessive donor loss. Blood donors, in a single region, deferred for travel or residence in malaria endemic areas, or a previous history of malaria, were randomly selected and recruited for the present study. They were asked to donate 2 blood tubes (EDTA) and complete a malaria exposure questionnaire. 724 malaria deferred donors and 3,229 non-deferred donors were tested by a commercial EIA (Newmarket Laboratories, UK) for *Plasmodium* spp. antibodies. Samples found repeat reactive (RR) by EIA were considered positive and also tested by Real-Time PCR for parasite DNA. Eight (1.1%) deferred donors and 11 (0.3%) non-deferred donors were RR by EIA; none were positive by PCR. Among the 8 positive deferred donors, 3 were deferred for residency in India and 5 were deferred for travel to endemic areas (4/5 was for travel to India). Of the 711 travel deferrals, 75% had visited Central America and the Caribbean, but only 1 was RR. Nine of the 19 RR donors had previously been diagnosed with malaria and 70% of these were probably exposed to *Plasmodium* in India or Africa. Seropositives were found among deferred donors and non-deferred donors; many previously had malaria most likely acquired in regions of higher risk than Central America and the Caribbean. PCR was ineffective at demonstrating parasitemia, due to low parasitemia and small extraction volumes, thus the possibility of transmission is unknown. Given the known relationship of donors with a history of malaria and transfusion-transmission, consideration should perhaps be given to permanent deferral of donors who once had malaria. Similarly, serious consideration should be given to serological screening of malaria travel deferred donors, so that they may be re-entered in the donor pool.

(ACMCIP Abstract)

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IMMUNOREGULATION IN MILD AND SEVERE *PLASMODIUM FALCIPARUM* MALARIA

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The initial need for inflammatory responses to control parasite replication in human *Plasmodium falciparum* malaria, and the paramount importance of IFN-γ in this response, is well established. Equally, sustained or excessive inflammatory responses have been conclusively linked to severe pathology. This has led to the hypothesis that the ability to down-regulate inflammatory responses once parasitaemia is under control is crucial to avoid immune mediated pathology, and may therefore be an important feature of clinical immunity. The mechanisms by which this cytokine balance is maintained are poorly understood. To study the association between Th1/regulatory cell balance and disease severity, we are conducting a case-control study in Gambian children with severe or mild malaria, respectively. We hypothesise that blood from severe malaria cases contains more Th1, or fewer regulatory T cells, than blood from mild malaria controls. Samples for immunoassay are being collected at admission and four weeks thereafter. The phenotype, absolute numbers, and relative proportions of IFN-γ to IL-10 or TGF-β producing cells are determined by flowcytometry either directly or after re-stimulation with malarial and control antigens. By means of RT-PCR the proportion of Th-1, Th-2 and regulatory T cells will be assessed and compared for severe and mild malaria cases.

(ACMCIP Abstract)

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CLINICAL AND IMMUNOLOGICAL MARKERS FOR THE PROGNOSIS OF MALARIA IN GHANAIAN CHILDREN

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The pathophysiological mechanisms involved in the pathogenesis of severe malaria are still obscure. These are local elements mainly due to *Plasmodium falciparum* sequestration, as well as systemic components. Malarial infections have been diagnosed by the peripheral density of the *P. falciparum* parasite, thus providing limited information about the disease severity, its manifestation or outcomes. The study sought

to identify certain prognostic markers for severe/complicated malaria in Ghanaian with and without concurrent bacteraemia. Bacteria were isolated from blood cultures and identified using standard bacteriological methods. The plasma cytokines were measured using Biosource Multiplex Beads Assay and ELISA. Pigmented neutrophils and monocytes were determined by microscopy.The frequently isolated pathogenic bacteria species was *Salmonella typhi*. The levels of TNF-α, IL-6, IL-10 and IL-1ra were significantly higher in both severe malaria (SM) and non-severe malaria (nSM) cases than in non-malaria (NM) group. IL-6 and IL-1ra were also significantly elevated in concurrent bacteraemia (CB) compared to NM. IL-8 levels were higher in SM than in nSM ($p< 0.05$). The levels of IFN-α in NM were higher than in SM ($p< 0.05$) and nSM ($p< 0.05$). IL-10/TNF-α ratio was lower in SM ($p< 0.001$) and nSM ($p= 0.001$) compared to NM. IL-10/IL-6 ratio was lower in CB compared to SM ($p= 0.01$) and nSM ($p= 0.02$). This ratio was also lower in NM compared to SM and nSM groups. Total pigmented neutrophils and monocytes were higher in the SM compared to nSM and NM. Higher numbers of pigmented neutrophils were seen in children with malaria and bacteraemia compared to those without malaria. Presence of malaria pigment in phagocytic cells and elevated plasma levels of certain cytokines may be used as markers of severe malaria.

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B CELL ACTIVITY IN CHILDREN WITH SEVERE MALARIAL ANEMIA

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The B cell complement receptor CR2 (CD21) plays an important role in the generation of humoral immune responses. The C3dg fragment of complement is the ligand for CD21, and covalent incorporation of C3dg fragments in antibody-antigen immune complexes enhances antigen binding, processing and presentation by cognate B cells. In this study we have used flow cytometry and fluorescent microscopy to analyze the expression level of CD21 on B cells, and we have also measured complement fragment C3dg associated with B cells in children with severe malarial anemia enrolled in a case control study. Children with severe malarial anemia had more CD20+ B cells (29 % ± 10 % of total lymphocytes) compared to controls (21 % ± 6.7 %, P = 0.001). Likewise, the % CD-21 positive B cells were higher in cases (26 ± 10 of total lymphocytes) than in controls (18 ± 5.7, P = 0.003), but, the mean fluorescent intensity of CD21 was lower in cases (255 ± 88) compared to controls (347 ± 114, P = 0.004). More B-cells were associated with C3dg deposition (20 % ± 12 %) compared to their controls (13 % ± 8.8 %, P = 0.002). We think that the high numbers of CD20+ B-cells in cases is in keeping with the theory of polyclonal B-cell stimulation by malarial antigens. The decreased level of expression of CD21 may be linked to processing of C3dg-opsonized substrates (presumably immune complexes) on B cells, mediated by macrophages of the mononuclear phagocytic system.

(ACMCIP Abstract)

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CALCITONIN GENE POLYMORPHISM (-624 T/C) IS ASSOCIATED WITH SUSCEPTIBILITY TO MALARIAL ANEMIA IN INFANTS AND YOUNG CHILDREN

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Previous studies illustrate that plasma procalcitonin (PCT) levels are associated with disease severity in bacterial and *Plasmodium falciparum* malarial infections. Although the role of procalcitonin in the pathogenesis of severe malarial anemia (SMA) remains undefined, polymorphic variability in the calcitonin gene (CALCA -624 T/C) conditions disease outcomes in infectious and autoimmune diseases. To examine the role of genetic variation in the calcitonin gene on conditioning severe malaria disease outcomes in children in a holoendemic *P. falciparum* transmission area, the relationship between the CALCA -624 T/C polymorphism and high-density parasitemia (HDP >10,000 parasites/μL), SMA (Hb<6.0 g/dL) and MA (Hb<8.0 g/dL) was investigated. Children with acute malaria (n=310) were enrolled at Siaya District Hospital in western Kenya. Genotyping was carried out using a Taqman 5′ allelic discrimination by real time PCR. Prevalence of TT, TC, and CC genotypes were; 40.0%, 40.6%, and 19.4%, respectively. Multivariate logistic regression revealed that heterozygous (TC) individuals had significantly higher risk of SMA (OR 1.89, 95% CI 1.12-3.19, P=0.01) and MA (OR 1.76, 95% CI 1.01-3.05, P=0.04) relative to the TT genotype. Homozygous C alleles (OR 0.80, 95% CI 0.39-1.63, P=0.54) and heterozygous individuals (OR 0.86, 95% CI 0.49-1.53, P=0.61) also had a non-significantly decreased risk of HDP. Taken together, these results demonstrate that variation in the CALCA promoter is associated with susceptibility to severe disease outcomes in children with malaria.

(ACMCIP Abstract)

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GENETIC DIVERSITY IN MSP-1 GENE OF *PLASMODIUM FALCIPARUM* IN AN ENDEMIC AREA OF CENTRAL INDIA

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Genetic diversity presented by *Plasmodium falciparum* field isolates, the occurrence of variant forms of the parasite in different geographic areas, and occultation of multiple genotypes during a single mosquito, constitute one of the main obstacles to the design of a malaria vaccine. Merozoite surface protein (MSP)-1, proteins causing immune responses in humans and are important candidates for development of blood stage malaria vaccines. The block 2 MSP-1 is particularly polymorphic and 3 distinct allelic families have been described as MAD 20, K1 and RO33. Allelic forms of these antigen genes have been reported from different parts of the world and further characterization of the degree of polymorphism in the antigen would help us to understand the design of malaria vaccine. Samples were collected on filter paper (Whatman no 3) from the highly malarious tribal population of Baigachak (Dindori district, Madhya Pradesh). Diagnosis of *P.falciparum* was confirmed by light microscopy on thick and thin blood smears. DNA was extracted from the blood sample by using the Tris EDTA buffer. The extracted DNA was used for amplification of MSP-1 gene block 2. PCR was performed following a 2-step amplification scheme. Sequencing was performed using ABI Big Dye Terminator Ready Reaction Kit Version 310 (Applied Biosystems). Sequence obtained were translated analused using BioEdit software and translated sequences were then aligned using the MEGALIGN programme (DNASTAR, INC., Madison, WI). Out of 118 samples 72 samples were amplified and sequenced. Majority (38 of 72) of sequences were under K1 family while 19 in MAD 20 family and remaining 15 sequences lying in RO33 family. Further we found extensive variations in K1 family, 24 subtype of alleles were found and among this family isolates were classified as 6 isolates in K1- I, 4 in K1-II, 3 inK1-XIV, 2 in each subtype of K1-IV, K1-V, K1-XIII and K1-XVI and rest were belongs in single subtype allele. In the family MAD 20, we found 11 subtype of alleles and among the family isolates were classified as 5 isolates in MAD20-III, 2 isolates in each subtype of MAD20-I, MAD20-VII, MAD20-IX, MAD20-XI and rest were single subtype allele. RO33 family was most conserved and only two

type of alleles were found, 8 belong RO33- I and 7 isolates showed RO33 II. The extensive polymorphism was observed for each marker (K1, MAD 20 and RO33) and the MSP-1 K1 repeat was the most diverse that could be considered in designing malaria vaccine.

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TOWARDS *PLASMODIUM VIVAX* ANTIGENIC GENES HAPMAP OF INDIAN ISOLATES

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Sequence polymorphism in six potential antigenic loci of *Plasmodium vivax* has been investigated to understand the haplotype number and distributions in different geographical regions. These loci are: Apical Membrane Antigen-1 (AMA-1), Duffy Binding Protein (DBP), Circumsporozoite protein (CSP), Merozoite Surface Protein-1 (MSP-1), and two sexual stage antigens ookinite surface proteins 25S and 28S. Fifty single clone *P. vivax* field isolate covering five distinct geographical regions of India namely Delhi, Panna (Madhya Pradesh), Nadiad (Gujarat), Chennai (Tamil Nadu) and Sonapur (Assam) were used for the construction of antigenic gene Haplotype map. A total of 50 haplotypes were found after combining all the sequences at six antigenic loci. Data revealed high degree of sequence polymorphism and highly diverse nature of Indian *P. vivax* isolates. Asexual stage antigens display very high number of nucleotide substitutions compare to the sexual stage antigens. Higher rate of non-synonymous substitutions compare to synonymous substitutions (dn/ds) indicates these genes are under positive selection pressure, however degree of selection pressure is not uniform at all the six loci suggesting differential host immune response against parasite surface proteins. Distribution of haplotypes was very unique to each population and was not shared in the same or different populations. This study would provide very meaningful information for the designing of multi-unit malaria vaccine based on the geographical distributions of the haplotypes and this multi loci approach provide a better insight to resolve recrudescence infections in therapeutic efficacy studies.

(ACMCIP Abstract)

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GENETIC DIVERSITY ASSOCIATED WITH VACCINE CANDIDATE ANTIGENS IN *PLASMODIUM FALCIPARUM* AND *P. VIVAX* ISOLATES FROM THE AMAZON REGION OF PERU

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Plasmodium falciparum and *P. vivax* are the major malaria species found in the Amazon region of Peru. We sought to characterize the allelic variability in vaccine candidate antigens since even minor variations in the peptide can alter the effectiveness of a vaccine. We analyzed T and B cell epitopes in vaccine candidate antigens from 56 samples of *P. falciparum* and 78 samples of *P. vivax* collected during years 2003-2006 in Loreto. For *Pfcsp*, we identified 3 different alleles with nucleotide diversity values (π) for Th2R and Th3R of 0.013 and 0.02, respectively. We identified three *Pflsa*-1 alleles for the N terminal peptide T1, with π =0.004. For *Pfmsp-1* we found six alleles, four from K1 type and two from MAD 20 type. A total 24 SNPs were found in domain I of *Pfama-1*, which is a site that has been implicated for antibody recognition. *Pfssp* contained the most conserved regions in all of the high-activity binding peptides. Sequence analysis of *Pvssp2* also revealed a low level of nucleotide diversity while a total of 11 polymorphic sites were identified in domain I *of Pvama-1*. *PvMSP-1*$_{20}$ and *PvMSP-1*$_{14}$ fragments, were also analyzed showing four amino acid substitutions in the first fragment and none in the latter. In conclusion, the study indicated that Peruvian Amazon populations exhibit less genetic polymorphisms than African populations shown by nucleotide and allelic diversity values and that B and T cell epitopes of *Plasmodium falciparum* are well conserved in Peruvian populations.

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A SNP-BASED MOLECULAR BARCODE FOR *PLASMODIUM FALCIPARUM* IDENTIFICATION AND TRACKING

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Single nucleotide polymorphism (SNP) genotyping provides the means to develop a practical, rapid, inexpensive assay that will identify uniquely any *Plasmodium falciparum* parasite. Such an assay can be used to distinguishing re-infection from recrudescence, to monitor the frequency and distribution of specific parasites in a patient population undergoing drug or vaccine-induced selective pressure, or for tracking samples in the laboratory. Uses in the lab include determining whether isolates are pure and differentiable, and monitoring specific parasites during culture adaptation and cloning. Based on whole-genome sequencing and Affymetrix chip-based genotyping of a worldwide collection of strains we have identified a panel of SNP markers with high minor allele frequencies (>30%), for which we could construct a robust TaqMan genotyping assay. Twenty-four such independent markers, selected at random from across the genome, can provide nearly 17,000,000 different signatures. To date, no two parasites known to be of independent origin have been found to have the same allele signature. The TaqMan genotyping assays can be performed on DNA from cultured parasites, white blood cell-depleted frozen whole blood, frozen whole blood, or dried filter paper spotted whole blood with a success rate >99%. Less than 25 ng of parasite DNA is needed to complete a panel of 24 markers. We compare the ability of this SNP panel to detect and identify parasites to other standard molecular methods including MSP1 and MSP2 typing, and will report on the ability of the assay to distinguish re-infection from recrudescence in patient samples. Six of 20 parasites culture adapted from patient samples from Senegal that appeared to be single genotypes by MSP typing were shown to be mixed cultures using the SNP barcode. It is our intention to make available a field-deployable universal typing tool, useable without special skills, with ordinary lab equipment, and at a reasonable cost, that will unambiguously and permanently identify and track *P. falciparum* parasites both in patient samples and in the lab.

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THE IMPACT OF DISSOCIATION ON TRANSPOSON-MEDIATED DISEASE CONTROL STRATEGIES

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There has been much recent interest in the use of transposable elements (TEs) to drive refractory genes into vector populations to control malaria and Dengue fever. Effective disease control requires, among other things, that the refractory gene is tightly linked to the TE. Rare recombination events can lead to loss of linkage between the drive system and refractory gene. It has also been shown that some TEs, such as the *P* and *hobo* elements, produce internal deletion derivatives at a significant rate following excision or transposition. This raises the concern that a similar process could lead to loss of the refractory gene following a transgenic release. Here, we show that any transposon-mediated gene drive strategy must have an exceptionally low rate of dissociation if it is to be effective.

As a minor consolation, the model predicts that if dissociation inhibits the success of the project then the introduction of the refractory gene is reversible within a human time frame. A refractory gene that compromises the rate of replicative transposition or confers a fitness cost to the host places stricter constraints on the dissociation rate required for disease control. This constraint is considerably relaxed if the refractory gene confers a fitness benefit to the host, emphasizing the significance of recent experiments which show that transgenic mosquitoes have a fitness benefit when fed on *Plasmodium*-infected blood.

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HIGH GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* AND LOW COMPLEXITY OF INFECTION IN THE PERUVIAN AMAZON

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Several studies from malaria endemic areas have used molecular epidemiology tools to generate policies for the malaria control disease. In the present study we carried out the genetic characterization of *Plasmodium falciparum* populations analyzing samples from 521 symptomatic patients enrolled during the years 2003-2005 in the Peruvian Amazon (Iquitos, Peru). For the genetic characterization we performed molecular genotyping using three highly polymorphic genes (MSP1, MSP2 and GLURP) finding a high genetic diversity of the parasite population in the study area. By other hand, we found low prevalence of polyclonal infections and a complexity of infection equal to 1 (mean COI = 1). The more polymorphic molecular marker, in this area during this period of time, was MSP1, followed by GLURP and MSP2. We found statistical differences in the allelic distribution between high/low malaria transmission and host age in monoclonal infections. Furthermore, we found higher risk to have polyclonal infections in children until 10 years old. The low complexity of infections found in this study supports, partially, the rapid rise and spread of drug resistance to the mono-therapies of first line given in the past in this area, as well as the high prevalence of monoclonal infections (90%) supports the theory for a more efficient immunity acquisition with large percentage of asymptomatic infections which are very frequent in the Peruvian Amazon.

(ACMCIP Abstract)

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PREVALENCE AND MOLECULAR BASIS OF α-THALASSAEMIA IN A MALARIA ENDEMIC REGION OF VIETNAM

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Alpha thalassaemia is the most prevalent haemoglobinopathy worldwide and may be important in malaria protection. Most forms of α thalassaemia result from the deletion of one or both copies of the α globin gene on chromosome 16. Hetero- and homozygotes for single gene deletion mutations are asymptomatic, and such mutations appear to be moving to fixation in certain populations. Whilst heterozygotes for 2 gene deletion mutations are asymptomatic, the homozygous state is incompatible with life. Compound heterozygotes for single and double gene deletions have haematological disease of variable severity, which limits the selective advantage of single gene deletion mutations in populations in which both are prevalent. We have assayed the prevalence of known Southeast Asian α thalassaemia mutations in southern Vietnam to assess the protective effect of haemoglobinopathies against malaria. Subjects were selected at random from volunteers attending a series of malariometric surveys. The prevalence of $\alpha^{3.7}$, $\alpha^{4.2}$, α^{SEA}, α^{FIL}, α^{THAI}, α^{CS} and α^{Pakse} were assessed by molecular methods. Three ethnic groups were genotyped: 302 Kinh, 109 Tay-Nung and 957 S'Tieng individuals. The vast majority of α thalassaemia in Vietnam is due to three mutations, α^{SEA}, $\alpha^{3.7}$ and α^{CS}. The S'Tieng have asignificantly higher prevalence of α thalassaemia (28.5%) than the Kinh (3.1%) or Tay-Nung (5%) (P=≤0.001). The higher prevalence of α-thalassaemia among the S'Tieng ethnic group is consistent with a theory of selection through protective effect against malaria. Patterns of malaria prevalence and disease in the S'Tieng suggest exposure to higher rates of malaria transmission, and they have traditionally lived in the upland areas historically associated with greater malaria morbidity. However, the ethnic roots of the S'Tieng are close to the Khmer of the SouthEast Asian mainland while the Kinh and Tay-Nung groups are related to Southern Chinese groups. Further clinical studies are underway to investigate the protective effect of these haemoglobinopathies in malaria.

(ACMCIP Abstract)

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DISRUPTION OF 2-CYS PEROXIREDOXIN TPX-1 GENE IN *PLASMODIUM BERGHEI* HINDERS THE SPOROZOITE DEVELOPMENT

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As *Plasmodium* spp. actively proliferate in the vertebrate and insect hosts, the parasites are subjected to the toxic effects of reactive oxygen species through their development. Since the parasites lack classical glutathione peroxidase and catalase, they are likely to utilize peroxiredoxin (Prx) as the principal factor for reducing cellular peroxides. To investigate the physiologic role of cytosolic 2-Cys Prx (PbTPx-1) during the insect stage of the rodent malaria parasite *Plasmodium berghei*, we infected the vector mosquito *Anopheles stephensi* with a parasite carrying a targeted knockout (KO) of Prx (Prx-KO) and found that disruption of *pbtpx-1* affected oocyst maturation and sporozoite formation of the parasite in the mosquito. The number of Prx-KO midgut-oocysts at 14-15 days post-feeding (pf) was comparable to that of the wild-type (WT) parent strain; however, the percentage of mature oocysts in Prx-KO-infected mosquitoes at the same time point was lower (1-4%) than that of WT-infected mosquitoes (15-32%). The numbers of sporozoites that formed in midgut oocysts and accumulated in the salivary gland of Prx-KO-infected mosquitoes by 21 days pf were decreased to 10-20% and 3-10%, respectively, of those values in WT-infected mosquitoes. Reduced development of Prx-KO oocysts was also observed *in vitro*, and a higher frequency of DNA strand breaks was detected in Prx-KO than in WT cultured oocysts by comet assay. Immunoelectron microscopy revealed increased formation of 8-hydroxy-2'-deoxyguanosine, a marker

of oxidative DNA damage, in nuclei of Prx-KO oocysts at an early developmental stage. Although the specific mechanism remains to be elucidated, the present findings suggest that Prx is involved in oocyst maturation and sporozoite development of malaria parasites.

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TRACING THE ORIGIN, MOLECULAR IDENTITY AND GENETIC DIVERSITY OF THE *PLASMODIUM FALCIPARUM* FCR3/ GAMBIA FAMILY

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Plasmodium falciparum FCR/Gambia is one of the first malaria parasite isolates adapted to *in vitro* cultivation (by Trager and Jenson in 1976). A few sub-lines of FCR3/Gambia and the parental isolate were deposited to ATCC by William Trager in 1981-82. Recently, the genetic diversity and the origin of the FCR3/Gambia isolate were questioned in various science forums. The malaria research community questioned this because the low coverage genome sequence of FCR3/Gambia is very similar to three other popular *P. falciparum* isolates, namely FCB/SE Asia, ItG2F6/SE Asia and 106/1/Sudan. In order to gain insight into this important question in the malaria field, the multigene family RIFIN microsatellite amplicon profiles were analyzed, and were found to be similar in these four parasite isolates. Moreover, the DNA sequence of the two highly polymorphic and leading malaria vaccine candidates, MSP-1 and MSP-2, were also analyzed, and all four isolates have identical MSP-1 and MSP-2 DNA sequences. Importantly, in 1989-90 two different labs confirmed that the sub-lines of the FCR3/Gambia isolates do not express Ring Infected Erythrocyte Surface Antigen (RESA) because the 5' end of the RESA gene has been inverted and partly deleted, and a telomere has been added to it, as reported previously. Specific primer sets were designed to identify the deletion and inversion in the RESA gene of FCR3/Gambia and its sub-lines that were deposited by Trager into the ATCC. By PCR, the primers amplify a 180 bp amplicon from the genomic DNA of the RESA inversion/deletion isolates, but not in RESA-positive isolates including the parental FCR3/Gambia, FCB, ItG2 and 106/1 isolates. As expected, all three FCR3/Gambia sub-lines gave 180 bp amplicons, confirming the original source of these parasite isolates. Therefore, these are the authentic FCR3/Gambia sub-lines that ATCC received from Trager in 1981-82, and as expected, the parental line is RESA-positive. Interestingly, the FCB isolate also gave a 180 bp amplicon, indicating that this isolate is RESA-negative, and suggesting that it could be derived from one of the FCR3/Gambia sublines. The origin and diversity of the Chloroquine-sensitive 106/1 line will be compared to other Chloroquine-resistant FCR3/Gambia, ItG2 and FCB isolates in the MR4 collection.

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INVASION PHENOTYPES AND TRANSCRIPT PROFILES IN GAMBIAN *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES

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The specific receptor-ligand interactions involved in the invasion of erythrocytes by the malaria parasite, *Plamodium falciparum*, 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 proteins (EBAs) and reticulocyte binding protein homologues (Rh), cultured adapted parasite lines have been shown to use different erythrocyte receptors to mediate alternative pathways of erythrocyte invasion. However, very few studies have looked at invasion pathways by fresh field isolates and none have reported whether the invasion phenotype is associated with disease severity. We have started a case control study in Gambian children with mild and severe malaria to study the erythrocyte invasion phenotype profiles from fresh field isolates, the associations of these phenotypes with clinical outcome and gene expression profiles. Assays to identify the invasion pathways utilized by parasites make use of enzymes that modify the protein and carbohydrate domains of the receptors. Thus there are neuraminidase-sensitive receptors, trypsin-sensitive receptors and chymotrypsin-sensitive receptors. We are using this approach to look at the phenotypic range shown by field isolates from children with mild or severe malaria. In addition, we are also looking at the expression profiles of five rh genes, three eba genes, four rhoph/clag members and six msp genes from the same field isolates. The results will show whether the mechanism of invasion by the parasite plays an important role in malaria virulence.

(ACMCIP Abstract)

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INVESTIGATION ON THE RELATIONSHIP BETWEEN ERYTHROCYTE BINDING ANTIGEN 175 (EBA 175) GENOTYPES OF *PLASMODIUM FALCIPARUM* AND CLINICAL MALARIA IN A HYPERENDEMIC AREA OF GHANA

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The development of an effective malaria vaccine has become a major public health challenge; identifying and understanding malaria parasite antigens involved in erythrocyte invasion provide major steps in developing primary vaccine candidates.The Erythrocyte Binding Antigen 175 (EBA 175) is a 175 kDalton *Plasmodium falciparum* antigen which plays a major role in erythrocyte recognition by the parasite. It also induces antibodies which inhibit merozoite invasion. cells. EBA 175 has been sequenced from FCR-3 and CAMP strains of *P. falciparum*. The sequences were identical in most parts of the gene, differences were apparent in the 423bp segment in the FCR-3 strain, the F-genotype, and the 342bp segment, the C-genotype. Parasite strains possess either one or the other fragment and never both. The functions and potential effects of this dimorphism remain unclear. The aim of this study is to investigate the relationship between these genotypes and clinical out come of malaria. Polymerase chain reaction (PCR) was used to identify the parasite strains which exhibit these genotypes in cases and controls of malaria in Kassena Nankana, an area endemic with malaria in the Northern region of Ghana. This area has also been earmarked for future vaccine trial. Out of a total of forty - three samples, 67.4% (29/43) were cases and 32.6% (14/43), the controls. The cases had 75% (22/29) of the F genotypes and 24 %(7/29) of the C genotypes, on the other hand the controls had 78% (11/14) of the F genotypes and 21.4 %(3/14) of the C genotypes. The relationship of a mixed infection with F and C and clinical outcome will be discussed. This is however a preliminary study, more samples will be involved to get a true representation of the relationships.

(ACMCIP Abstract)

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COCCIDIOSIS CONTROL IN POULTRY WITH VACCINES OF FIELD ISOLATES AS A MODEL OF EFFICACY, SUSTAINABILITY AND AFFORDABILITY FOR THE CONTROL OF MALARIA

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Coccidiosis is an endemic disease perpetuated by self-infection of chickens that pick up oocysts of *Eimeria* spp. from the litter in crowded commercial barns. Like *Plasmodium* spp., *Eimeria* spp. are obligate intracellular parasites and are similarly known to show "Infection (concomitant) immunity", as reported previously, or "premunition," where a small number of parasites were found to persist in the infection site enabling

the host to be protected from further infection. Coccidiosis, like malaria, requires a constant use of medications for its control. This has led to the continuous emergence of drug resistant coccidia. The development of live coccidiosis vaccines as an alternative was spurred on by the early realization that, in crowded commercial barns, without efficient coccidiosis control there would be no poultry industry. Live vaccines of field isolates have been used for over 50 years and more successfully for over 20 years since the concept of uniform exposure was introduced. Well over 10 billion chickens and turkeys have been vaccinated to date showing efficacy and sustainability of coccidiosis control with live vaccines as declared by the editor of International Hatchery Practice "nowadays coccidial vaccines have virtually made coccidiosis in breeders a thing of the past." The combination of vaccination and medication for the control of coccidiosis in particular is most applicable to the control of malaria. With drug sensitive strains of *Plasmodium* spp. as a live vaccine, the risk of runaway vaccinations can be safeguarded with antimalarials. In any event, use of live vaccines has been in practice for the past few years in the Intermittent Preventative Treatment in infants (IPTi) program with the goal of reducing the incidences of malaria and severe anemia in Africa, as reported previously. Last but not least, affordability, the prime consideration in the poultry industry, is also critical to the success here. Of all the potential vaccine seeds, field isolates which are readily available will bear the least cost. These and the rotating and synergistic uses of vaccination and medication in coccidiosis control can be the added tools for the control of malaria.

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PHASE 1 SAFETY AND IMMUNOGENICITY TRIAL OF BLOOD-STAGE MALARIA VACCINES MSP1$_{42}$-C1/ALHYDROGEL WITH AND WITHOUT THE ADDITION OF CPG 7909 IN US ADULTS

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A Phase 1 clinical trial was conducted in healthy, malaria-naïve, US adults to compare the safety and immunogenicity of recombinant protein blood-stage vaccines, based on *Plasmodium falciparum* MSP1$_{42}$. In order to induce immune responses that cover the major antigenic polymorphisms, the MSP1$_{42}$-FVO and MSP1$_{42}$-3D7 forms of the antigen were mixed (MSP1$_{42}$-C1). To improve the level of antibody response, MSP1$_{42}$-C1 was formulated with Alhydrogel or Alhydrogel plus the novel adjuvant CPG 7909. Responses were compared to MSP1$_{42}$-C1/Alhydrogel alone. Sixty volunteers were given three vaccinations of either 40 or 160 µg protein adsorbed to Alhydrogel ± 500 µg CPG 7909 at 0, 1, and 2 months. Vaccinations were generally well tolerated, with the majority of local and solicited adverse events graded as either mild or moderate in severity. Transient neutropenia developed in 9 volunteers receiving the vaccine with CPG 7909, an abnormality that has been reported previously with CPG 7909 use. No clinical events were associated with this and no serious adverse events occurred during the study. Antibody levels to the FVO and 3D7 form of MSP1$_{42}$ were measured by ELISA pre-vaccination, two weeks after each vaccination, and after a six-month follow-up. The addition of CPG 7909 enhanced anti-MSP1$_{42}$ antibody responses following vaccination by 12-fold to 30-fold two weeks after second immunization ($p < 0.0001$) and 4-fold to 9-fold two weeks after the third immunization ($p < 0.0001$) when compared to MSP1$_{42}$-C1/Alhydrogel alone. A substantial enhancement of specific antibody responses in the CPG 7909 groups was seen following the second vaccination, but no additional enhancement was observed following the third vaccination. Notably, antibody responses were not significantly different in the 40 and 160 µg MSP1$_{42}$-C1/Alhydrogel+CPG 7909 cohorts. In vitro *P. falciparum* growth inhibition assays (GIA) were performed using purified IgG isolated from day 70 sera. Addition of CPG 7909 to the formulation resulted in significantly increased GIA (range 8-32%, $p < 0.0011$) but, biologically relevant inhibition is likely to require much higher levels of anti-MSP1$_{42}$ antibodies. Due to the low level of GIA observed in this trial and the results of the Walter Reed Army Institute of Research/GlaxoSmithKline Biologicals Phase 2 trial of MSP1$_{42}$ formulated with AS02, clinical development of the vaccine formulation MSP1$_{42}$-C1/Alhydrogel+CPG 7909 is being discontinued.

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ANTIBODY-INDUCED PHAGOCYTOSIS OF *PLASMODIUM FALCIPARUM* MEROZOITES BY NEUTROPHIL MEASURED WITH CHEMILUMINESCENCE OXIDATIVE BURSTS AS A RELEVANT FUNCTIONAL ASSAY FOR CORRELATE OF PROTECTION AND MSP VACCINE DEVELOPMENT

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Ongoing morbidity and mortality and widespread multi-drug resistance has accentuated the need for an effective vaccine against *Plasmodium falciparum*. Merozoite surface proteins (MSPs) are relevant vaccine targets, some under field studies such as MSP1. Relevant functional assays are urgently needed to evaluate natural and vaccination-induced Ab responses. A new functional assay is described based on Ab dependent phagocytosis of merozoites (ADPm) by activated polymorphonuclear neutrophils (PMN), using oxidative bursts measured by chemiluminescence in the presence of luminol. The ADPm assay was developed and found highly specific, with negligible backgrounds. One of the main limitations was the short PMN half-life and its variable phagocytic capacity in natural cellular effector populations, however relevant internal positive standards provide good inter-assay reproducibility. In endemic areas of transmission, ADPm activity was significantly correlated with anti-merozoite IgG titers, and in particular, with cytophilic IgG1 and IgG3 subclasses. ADPm was found significantly higher in individuals with higher level of immunity ie holoendemic - compared to mesoendemic - setting (n=228). Importantly, the presence of asymptomatic circulating parasitemia led to significant lower ADPm intensity ($P<0.01$) underlining a detectable dynamic consumption of Abs, contrary to IgG ELISA measures. Most importantly, ADPm was positively associated with age-related natural immunity, and with a reduced risk of clinical malaria episodes in an age-adjusted Poisson regression model. The ADPm assay appears to be a relevant, reproducible functional correlate of antibody-based immune responses for recombinant MSP vaccine candidates, and can be added to the restricted panel of reproducible *P. falciparum* functional assays mostly based on culture inhibition tests. In this line, ADPm assays call for further inter-laboratory assessments.

(ACMCIP Abstract)

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EVALUATION OF IMMUNOGLOBULIN PURIFICATION METHODS AND THEIR EFFECTS ON IGS ANTIBODY SPECIFICITY

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Antibodies are the main effectors against malaria blood-stage parasites. Evaluation of immune sera functional activities from Phase IIa/b vaccine trials could provide invaluable information in the search for immune correlates of protection. Often, improper collection/storage conditions and/or the presence of anti-malarial drugs can cause sera to be toxic, i.e. simulating anti-parasite activity in functional assays. Thus, removal

of any toxic components from serum is essential for measuring the true antibody-mediated activity. To determine a suitable method for immunoglobulin (Ig) purification, Igs isolated from pooled rabbit sera and human plasma/sera by either chromatography or precipitation were evaluated for their quality, quantity and functional activity. Rabbit sera were derived from an immunization with either *P. falciparum* MSP1-42 (FVO allele) or reduced-alkylated MSP1-42 (FVO allele). Malaria naïve and immune human plasma/sera were obtained from individuals living in the USA and in a malaria endemic region of western Kenya, respectively. Igs were purified using: protein G sepharose, protein A/G sepharose, SEP-EASE (PEG) and Caprylic Acid-Ammonium Sulfate precipitation. Igs were analyzed for purity by SDS-PAGE, for quality by ELISAs (antibody specificity as determined by changes in titer, affinity and isotype distribution) and for functional activity by pLDH Growth Inhibition Assay (GIA). We will show that not all Ig purification methods are equal relative to the tested parameters. Consequently, critical consideration of Ig purification methods is required to avoid selecting non-representative populations of recovered Ig which could influence interpretations of vaccine efficacy or the search for immune correlates.

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PLANS FOR CLINICAL TRIALS OF A METABOLICALLY ACTIVE, NON-REPLICATING *PLASMODIUM FALCIPARUM* SPOROZOITE VACCINE

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Data generated over the past three and a half decades demonstrate that the only reliable way to consistently generate sterile protection in humans against *Plasmodium falciparum* (Pf) is via immunization by the bites of mosquitoes infected with radiation-attenuated Pf sporozoites. However, until recently there had been no practical way to generate adequate quantities of aseptic, purified sporozoites for parenteral injection, and for this reason the development of an attenuated sporozoite vaccine for human use had been deemed untenable. The biotechnology company Sanaria, Inc., with support from multiple funders, has spent the past 3 years developing methods to overcome these obstacles. Based on this achievement, the Bill and Melinda Gates Foundation has awarded a major grant to support a partnership between Sanaria and the PATH Malaria Vaccine Initiative for the manufacture and clinical testing of Sanaria's radiation attenuated Pf sporozoite vaccine candidate. The first, open-label, dose-escalation Phase 1/2a clinical trial, to be conducted in healthy malaria-naïve adults by the Malaria Program of the Naval Medical Research Center and the Center for Vaccine Development - University of Maryland School of Medicine is scheduled to begin in 2008. Based upon demonstration of adequate safety and protective efficacy, the plan is to move rapidly into additional Phase 2a U.S. trials evaluating number of doses, route of administration, and heterologous strain challenge, as well as concurrent Phase 1 trials in Africa. We will present an update on our progress towards the "first-in humans" clinical trial as well as our strategy for moving safely as rapidly as possible into the most vulnerable target populations: infants, young children, and pregnant women living in malaria-endemic areas.

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DEVELOPMENT AND OPTIMIZATION OF A *PLASMODIUM FALCIPARUM* INHIBITION OF SPOROZOITE INVASION (ISI) ASSAY

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Despite major vaccine development efforts to stem the tide of malaria-related illness and death, this parasitic infection remains one of the deadliest and most costly diseases in the world, each year leading to 300-500 million new infections, 1.5-3 million deaths and the loss of as much as 2% of gross national product of the most affected areas, such as sub-Saharan Africa. Though many candidate vaccines have been evaluated in clinical trials, only a small number have shown measurable protection against experimental or natural challenge, and even for these partially protective vaccines, immunological correlates of protection have remained elusive. There is a growing consensus that traditional assays, such as the measurement of antibody titers via ELISA, or of antigen-induced T cell secretion of IFN-γ via ELISpot assay, may not be adequate, and that efforts should focus on functional assays that assess the impact of immunological responses on parasite development. One example is the inhibition of sporozoite invasion (ISI) assay, developed to test the ability of antibodies to block *Plasmodium* sporozoite invasion of hepatocytes. The ISI assay is performed by adding a test serum to *P. falciparum* sporozoites and adding this mixture to cultured human hepatocytes. These hepatocytes are then fixed to a slide, stained with primary and secondary antibodies, and viewed under the microscope to count sporozoites that have successfully invaded. Unfortunately, there are several flaws in this procedure, including the loss of host cell/parasite material during extensive washing and antibody labeling steps, and the time-intense and subjective nature of differentiating between invaded and externally bound sporozoites under the microscope. To improve the accuracy of the assay, we have developed a quantitative real-time PCR (qRT-PCR) method to detect *P. falciparum* sporozoites that have invaded cultured HepG2 human liver cells, similar to qRT-PCR methods previously developed to detect *P. yoelii* parasites in the livers of mice, and *P. berghei* parasites in *in vitro* hepatocyte cultures. We have designed primer and probe sequences specific for 18S rRNA expressed in both the mosquito (sporozoite) and blood stage of the *P. falciparum* life cycle to facilitate detection of newly invaded sporozoites and parasites in the late liver stage of development. Results of this assay will be presented.

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PRE-CLINICAL EVALUATION OF SAFETY AND IMMUNOGENICITY OF *PLASMODIUM FALCIPARUM* LSA1/AS01B WHEN ADMINISTERED SEPARATELY OR CONCURRENTLY WITH RTS,S/AS01B IN RHESUS PRIMATES

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Vaccination of humans with attenuated sporozoites, via multiple bites with irradiated mosquitoes, provides protection against malaria. This suggests that targeting pre-erythrocytic stage parasites for malaria vaccine development can provide sterile immunity. The objectives of this study were to: 1) Pre-clinically evaluate safety and immunogenicity of a new recombinant pre-erythrocytic antigen, liver stage antigen 1 (LSA1) in non human primates; and 2) Investigate the potential for immune interference between LSA1 and the leading malaria vaccine candidate RTS,S by

comparing the immune responses after single antigen vaccination to responses after simultaneous administration of both antigens at separate sites. Using a rhesus monkey model, we found that LSA1 formulated with GSK proprietary adjuvant system AS01B (LSA1/AS01B) was safe and immunogenic, inducing high levels of antigen-specific antibody and $CD4^+$ T cell responses as monitored by the production of IL-2 and IFN-γ using intracellular cytokine staining. RTS,S/AS01B vaccination was well tolerated and demonstrated robust antibody and moderate $CD4^+$ T cell responses to circumsporozoite protein (CSP) and HBsAg. Positive $CD8^+$ T cell responses to HBsAg were detected whereas the responses to CSP and LSA1 were negligible. For both LSA1/AS01B and RTS,S/AS01B, no statistically significant differences were observed between individual and concurrent administration in the kinetics, magnitude or duration of antibody and T cell responses. Our results reveal that both pre-erythrocytic antigens were safe and immunogenic when administered separately or simultaneously to rhesus monkeys, and that no significant immune cross interference occurred with concurrent administration.

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DETECTING ANTIBODY FINE SPECIFICITIES TO *PLASMODIUM FALCIPARUM* MSP1 SUBUNITS BY PARTICLE BASED FLOW CYTOMETRY IN NAÏVE AND MALARIA EXPERIENCED POPULATIONS VACCINATED WITH FMP1/AS02A

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High-throughput serological analysis of clinical trial samples is of critical importance to malaria vaccine development. Previous studies have shown that some MSP1 fragment-specific antibodies are associated with parasite growth inhibitory activities, lowered parasite densities, and improved clinical outcomes, indicating that antibody fine specificities may correlate with protection. Analyzing antibody responses by ELISA is limited to measuring one antigen specificity at a time, making the analysis of sera from a large sample population time consuming and costly. Furthermore, for some studies, low sample volumes preclude a complete evaluation of responses to all related homologous and heterologous antigen-specific responses. As an alternative, different antigens can be coupled to carboxylated microspheres that bear different fluorescent signatures, which allow small volume samples to be assayed concurrently against several antigens in a multiplexed format by flow cytometry. We will report results comparing ELISA and multiplexed flow cytometric measurements of MSP1 antibody fine specificities against MSP1-42; MSP1-33, MSP1-19, and EGF-like domains 1, and -2 of the *Plasmodium falciparum* 3D7 strain. The sera used for this analysis was from volunteers vaccinated with FMP1/AS02A, the 3D7 allele of MSP1-42, from various trials, i.e. malaria-naïve U.S. adults, and malaria-experienced Kenyan adults and children, and from non-vaccinated malaria experienced Kenyan adults. We will also report results from multiplexed flow cytometric testing of all possible subunits of MSP1, from the FVO, 3D7, and CAMP strains. Defining, more finely, strain-specific antibody responses, may aid in establishing antibody correlates of protection, and possibly direct the future of malaria vaccine development.

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MINIATURIZED HIGH THROUGHPUT PLDH-BASED *PLASMODIUM FALCIPARUM* GROWTH INHIBITION ASSAY FOR LOW VOLUME SAMPLES

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To date, no immune correlates exist for blood stage specific immunity against *Plasmodium falciparum* malaria parasites. Growth-/invasion inhibition assays (GIA/IIA) using sera from Phase IIa/b trials may provide a correlation with protective immunity. A major obstacle is the availability of sufficient volumes of serum to perform these assays. Current methods require as much as 250 µL serum for the 96 well plate format (for triplicates), and minimally require 0.5-1.0 mL of serum for IgG purification approaches. We have developed a miniaturized, high-throughput approach to measure growth inhibitory activities (GIA) from low volume samples, i.e. pediatric trials. Parasite cultures are grown in 10-20 µL total volume in the presence or absence of immune sera or purified immunoglobulins and are assessed for growth inhibitory activities by measuring parasitic lactate dehydrogenase (pLDH). Test samples used in this study were rabbit immune sera, malaria exposed immune sera from western Kenya, and US vaccinated volunteer-immune sera. To determine optimal assay conditions, we explored various brands of 384 well plates, starting parasitemias, assay hematocrits and final assay volumes using the 3D7, FVO and Camp/FUP strain parasites. We observed overall low variability in the assay (< 10% CV) and good correlations with results from the 96 well plate format ($r^2 = 0.863$). We conclude that within the parameters established, as little as 5 µL of serum can reproducibly quantitate parasite growth inhibitory activities. This culture method can be expanded beyond the pLDH assay to other microtiter plate techniques commonly used to determine growth-/invasion inhibition.

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HELMINTHIASIS AS A CONFOUNDING FACTOR IN HIV AND MALARIA VACCINE TRIALS

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Experiments using mice have shown that helminthiasis diminishes the efficacy of HIV and malaria vaccines that elicit a cell-mediated immune response (although more than just Th1/Th2 antagonism could possibly be involved). In light hereof, some researchers will probably try to control for the likely influence of certain kinds of helminthic infection in HIV and malaria vaccine trials in humans by screening fecal specimens for the presence or absence of worm eggs. Logical though this approach may seem, it does not take recently-acquired knowledge concerning the immunology of helminthiasis into account. It has been found that where soil-transmitted helminths are hyperendemic, there can be strong worm-associated, Th2-polarized immune activation in apparently or actually egg-negative individuals. This will likely often be important in downregulating cell-mediated immune responses to vaccines against various non-helminthic diseases (including tuberculosis). In theory, diagnostically patent (low to medium-intensity) and even severe intestinal helminthiasis will frequently play a less significant immunoregulatory role in this regard, which is contrary to what might be assumed. The rationale behind this postulation will be explained in detail. It will, furthermore, be pointed out that in attempting to assess the influence of helminthiasis in HIV and malaria vaccine trials, it is vital that the results of qualitative and quantitative fecal examinations be accompanied by consideration of immunological parameters (including immune responses to worm antigens).

(ACMCIP Abstract)

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EXPRESSION OF MSP3-MSP1 CHIMERIC PROTEIN AND EVALUATION OF ITS IMMUNOGENICITY USING HUMAN COMPATIBLE ADJUVANTS

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Malaria antigens expressed on the surface of blood stage merozoite are the potential vaccine candidates given their role in invasion of erythrocyte.

19 kDa C-terminal region of Merozoite surface protein 1 was selected for its role in erythrocyte invasion and C- terminal merozoite surface protein 3 for its role in induction of cytophilic antibodies, are among the leading vaccine candidate antigens. It has been suggested that a promising vaccine candidate would be a combination of antigens or its chimera, rather than a single antigen. Thus in the present study, a chimeric protein PfDGP19 was constructed by genetically coupling C-terminal conserved region of merozoite surface protein 3 (DG210 region) with C-terminal 19 kDa fragment of merozoite surface protein 1. The PfDGP19 was produced in *E.coli* and was purified by two step procedure involving affinity and ion exchange chromatography to a final product yield of 50.0 mg/L. The final product was homogeneous, monomeric and >98.0% pure, and had low permissible level of endotoxin content. Western blot analyses using polyclonal mice antibodies against antigenic components of MSP3 and MSP1 recognized the chimeric protein. Conformation specific MSP-1_{19} monoclonal antibodies also interacted with the recombinant protein and were reduction sensitive, indicating that the cysteine rich 19 kDa fragment in the fusion protein retained its critical conformational epitopes, formed by two EGF like domains. Majority of the human immune sera tested recognized the chimeric protein. The fusion protein was found to be highly immunogenic in mice as well as in rabbits, and induced both anti-MSP1_{19} and anti-MSP-3(DG210 region) antibodies.

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OWNERSHIP AND USAGE OF ITNS IN NIGER AFTER DISTRIBUTION DURING A NATIONWIDE INTEGRATED CAMPAIGN

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Mass distribution of free insecticide-treated nets (ITNs) is an important strategy to decrease the burden of malaria in endemic countries. In December 2005 and March 2006, Niger distributed polio vaccine and ITNs to children <5 years in a nationwide integrated campaign. Our objective was to determine the post-campaign ownership and usage of ITNs in Niger during rainy (high malaria transmission) season, with a focus on pregnant women and children <5. We conducted a community-based, cross-sectional nationwide survey during peak malaria transmission season using a personal digital assistant (PDA) based questionnaire. We selected 156 villages using probability proportional to size sampling, and mapped each village using Global Positioning System (GPS) equipped PDAs. We randomly selected 16 households per village for questionnaire administration (total 2,450 households). Analyses were weighted by population. Results: Nationally, 75.5% of households had one or more children <5 and were thus eligible to receive an ITN; 73.4% of these (63.0% of all households) received an ITN. Of households that received one or more ITNs, 97.7% retained at least one. In households with children <5, ITN ownership increased from 4.2% to 74.6%, with ownership increasing most among the poorest (equity ratio increased from 0.17 to 0.81). The night prior to the survey, 89.2% of ITNs were hung; 55.5% of children <5 and 42.8% of pregnant women had slept under an ITN. Twenty-two percent of children <5 and 26.0% of pregnant women lived in households that owned at least one ITN, but did not sleep under it. In conclusion, the Niger integrated campaign dramatically increased ITN ownership and usage and reduced inequities in net ownership. ITNs were retained and used by high risk groups. Further distribution and education on net use are necessary to reach Roll Back Malaria Partnership target levels for ownership and usage of ITNs.

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DICHLORODIPHENYLTRICHLOROETHANE (DDT) FOR INDOOR RESIDUAL SPRAYING IN AFRICA: HOW CAN IT BE USED FOR MALARIA CONTROL?

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In 2006, the World Health Organization issued a statement promoting the use of indoor residual spraying (IRS) with dichlorodiphenyltrichloroethane (DDT) for malaria control and other organizations concurred. Although the Stockholm Convention of 2001 targeted DDT as 1 of 12 persistent organic pollutants for phaseout and eventual elimination, it allowed a provision for its continued indoor use for vector control. A low-cost insecticide, DDT remains effective against malaria vectors and many African countries are considering its application. We reviewed the DDT controversy addressing: prior use in Africa; *Anopheles* resistance; recommendations for effective deployment; and training, monitoring and research needs for sustainable implementation. Few African countries have implemented effective IRS programs with DDT and other insecticides. In this region, DDT use decreased in the late 1990s to less than 50 tons per year, but increased to an annual average of 300 tons between 2000 and 2002. In endemic regions where DDT use decreased, the consumption of synthetic pyrethroids and other insecticides increased. IRS is a very cost-effective intervention. Programs with DDT were found slightly cheaper than programs using other insecticides. It is likely that IRS with DDT will lead to decreased sensitivity and resistance in malaria vectors. *Anopheles* resistance to DDT was, however, not detected in South Africa during its application from 1946 to 1995. This is probably because strict measures prevented its use in agriculture. Each country should assess if IRS is a viable strategy after considering program goals, current interventions, local transmission patterns, and existing operational and monitoring capacities. To ensure sustainability in IRS activities, DDT alternatives and deployment methods must be the focus of the research agenda.

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EXPRESSION OF WARP, A PUTATIVE TARGET FOR TRANSMISSION BLOCKING VACCINES, DURING *PLASMODIUM GALLINACEUM* SEXUAL DEVELOPMENT

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During the life cycle of malaria parasites, one of the most crucial stages is the midgut invasion in the invertebrate host, when the number of invading parasites steadily decreases and reaches the lowest count throughout its cycle. Paradoxically, the elements and mechanisms involved in such significant bottleneck are yet poorly understood. One of the micronemal proteins believed to play a part on the invasion is the von Willebrand Factor A Related Protein (WARP), a secreted, strongly conserved protein that has already been shown as a promising target for inhibiting oocysts development. The goals of this study are to determine how and when WARP is expressed as zygotes transform into ookinetes and correlate its expression pattern to its putative function. New findings on elements involved in midgut invasion mechanisms may develop into novel transmission blocking strategies based on effector molecules capable of disturbing parasite development. The vWA domain sequence was produced as a recombinant protein using an expression prone plasmid, purified by affinity chromatography by using Ni resin, and the protein was used to immunize rabbits for the production of polyclonal monoespecific antibodies. Confocal microscopy was carried out using the polyclonal antibodies to detect WARP in cultured *Plasmodium gallinaceum*

sexual stages. WARP can be detected from the early stages of ookinete development up to the mature palmate-shaped forms. It presents an intracellular granular distribution with focal concentration towards the apical end in mature ookinetes, corroborating its micronemal localization. Currently, experiments involving confocal imaging of infected midguts and expression profiling through Real Time RT-PCR are also underway.

(ACMCIP Abstract)

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DEVELOPMENT OF *PLASMODIUM FALCIPARUM* IN CULEX MOSQUITOES

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Culex mosquitoes are extremely prevalent across the malarious world serving as important vectors for a number of infectious diseases and are primary vectors of avian Plasmodia, yet they are known to be totally refractory to human Plasmodia species. This is especially fascinating because many species, such as *Culex quinquefasciatus*, are highly anthropophilic and consequently, have repeatedly been exposed to human Plasmodia. However, despite extensive and prolonged exposure, human malaria parasites have never adapted to exploit Culex mosquitoes. Thus, there is something unique about the Culex physiology that prevents the successful development of human Plasmodia, but to date the exact processes remain unknown. Here, we compare the developmental success of *Plasmodium falciparum* in two African mosquitoes (*Cx. quinquefasciatus* and *An. gambiae*) in key events: fertilization, midgut invasion, oocyst maturation, sporozoite invasion and accumulation in salivary glands. We trace the successful development of parasites in both mosquito species, identifying any potential barriers to transmission. We investigate whether a single critical barrier is responsible for refractoriness in *Cx. quinquefasciatus* and whether the lifecycle of *P. falciparum* can be successfully completed when this barrier is bypassed or whether multiple barriers to transmission exist.

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COMMUNITY-BASED SURVEILLANCE OF MALARIA VECTORS IN DAR ES SALAAM, TANZANIA

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The Dar es Salaam City Council has initiated a pilot phase Urban Malaria Control Program that delegates responsibility for routine mosquito surveillance and control to community members. Exhaustive weekly surveillance of larval habitat abundance and occupancy in an area of 55 km2 with a population of 614,000 people is reported to municipal level by community-owned resource persons (CORPs).All reported potential breeding habitats with larvae within three selected intervention wards are treated with larvicides Bacillus sphaericus (Bs) and B.thuringiensis var. israelensis (Bti) followed by close monitoring of any changes. Additional CORPs carry out monthly human landing catch to estimate sporozoite prevalence, vector biting rate and entomological inoculation rate (EIR) in 268 sites across the same area. Independent spot checks of randomly chosen population clusters by municipal inspectors are used to evaluate the coverage, sensitivity, specificity and accuracy with which habitats are identified and characterised by CORP teams. Here we assess the impact of this new larviciding and complementary surveillance system on malaria transmission and identify area for improving overall effectiveness.

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COST-EFFECTIVENESS OF ADDING BEDNET DISTRIBUTION FOR MALARIA PREVENTION TO ANTENATAL CLINICS

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Insecticide-treated bednets (ITNs) reduce low birthweight (LBW) deliveries among pregnant women in sub-Saharan Africa. ITN distribution in Kenyan antenatal clinics was highly effective, with 84% of women using them during pregnancy and 91% of infants protected during their first year of life. However, to date there has been no economic evaluation of ITN distribution at the antenatal clinic. In Kinshasa, Democratic Republic of Congo, we distributed ITNs free of charge in 31 antenatal clinics to 17,893 pregnant women who participated in an existing prevention of mother to child transmission of HIV (PMTCT) program. Our aim was to estimate programmatic costs, benefits, and the cost-effectiveness of adding ITN distribution to existing antenatal care from the health provider perspective. We employed decision analysis using data derived from the program. Adherence with ITN use was measured at one clinic using questionnaires administered to 360 randomly-chosen women. Costs (reported in 2005 U.S. dollars [$]) included purchase, transportation, storage, and distribution of the nets during antenatal clinic visits, and were derived from clinic and program records. Other required parameters were derived from the peer-reviewed literature. Outcomes modeled include LBW deliveries and infant deaths averted, and life-years (LY) saved. Probabilistic sensitivity analyses were conducted using Monte Carlo simulation. Adding ITN distribution to existing antenatal clinics offering PMTCT services would be expected to avert 251 low birth weight deliveries and 224 infant deaths at an additional cost of US $9.43 per pregnant woman (US$1.43 for distribution and $8.00 for purchase and transportation). The incremental cost-effectiveness was US$357 per LBW delivery averted, US $224 per infant death averted, and US $8.92 per LY saved. The greatest benefit was observed among women in their 1st-2nd pregnancies, with benefits coming at substantial cost for those in their 5th or later pregnancy. Bednet distribution for malaria prevention may be a cost-effective addition to existing antenatal clinics.

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COMPARISON OF A SIMPLE CHELEX PROCEDURE AGAINST STANDARD SALT PROTOCOL FOR DNA EXTRACTION FROM WILD *ANOPHELES* SPP.

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Salt extraction methods are commonly employed for mosquito DNA extractions. Relatively costly reagents and shipment requirements pose limitations for use in the developing world. Therefore, we compared DNA quality from a simple chelex procedure against established salt extraction protocol. We morphologically identified 48 mosquitoes before homogenizing their head and thorax sections in 20µl of deinoized water. Of the homogenate, 10µl was subjected to chelex or salt DNA extraction. Extracts were amplified using PCR targeting the mitochondrial ND4 to check DNA quality, a PCR for identification of *Anopheles gambiae* sibling species, and a nested PCR for detection of *Plasmodium* DNA. We found sensitivity and specificity of the chelex approach to be 92% and 82% on ND4, and 92% and 78% for sibling species identification PCR, with

similar *Plasmodium* detection. Our results show that the chelex method is comparable to existing salt extraction and can be substituted as a cost-saving alternative.

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MULTIPLE INSECTICIDE RESISTANCE MECHANISMS IN *ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS* FROM BENIN (WEST AFRICA) AND OPERATIONAL CHALLENGE FOR MALARIA VECTOR CONTROL

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Because free-Insecticide Treated Net distribution is planned in Benin (West Africa) during the next few years, we investigated the type, frequency and distribution of insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes in four localities selected on the basis of contrasting agricultural practices, use of insecticides and environment. Bioassays with WHO diagnostic test kits were carried out using pyrethroid, carbamate, organophosphate and organochlorine insecticides. *Anopheles gambiae* mosquitoes were identified to species and to M or S molecular forms using PCR techniques. Molecular and biochemical assays were carried out to identify *kdr* and *Ace.1* mutations in individual mosquitoes and to detect any increase in the activity of enzymes typically involved in insecticide metabolism (oxidase, esterase and Glutathion-S-transférases). WHO diagnostic tests showed high frequency of resistance in *An. gambiae* and *Cx. quinquefasciatus* to permethrin and DDT in 3 areas. This was consistent with the presence of target site insensitivity due to *kdr* mutation and to increased metabolism through enzymatic activity. *Kdr* was expressed in both M and S forms. However, less than 1% of *An. gambiae* or *Cx quiqnuefasciatus* showed the presence of the $Ace.1^R$ mutation. Carbamate/OP resistance was present at higher frequency in *Culex* than in *An. gambiae*. Dieldrin resistance was present in both species at all four localities. A higher frequency of pyrethroid-resistance was found in *An. gambiae* mosquitoes collected in urban areas compared to those collected in rice growing areas. The expansion of vegetable growing within urban areas probably contributed to selection pressure on mosquitoes. The detection of multiple resistance mechanisms in both *An. gambiae* and *Cx. quinquefasciatus* in Benin may represent a threat for the efficacy of ITNs and other forms of vector control such as indoor residual spraying in the future.

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ANNOTATION AND EXPRESSION PROFILING OF PRESUMPTIVE APOPTOSIS REGULATORY GENES IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

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Apoptosis has been extensively studied in *Drosophila* both by biochemical and genetic approaches, but there is a lack of knowledge about the molecular mechanisms of apoptosis regulation in other insects. In mosquitoes, apoptosis has been shown to occur during malaria infection in the midgut of anopheline species and during arbovirus infection in midgut and salivary glands in culicine and aedine species, suggesting that apoptosis may play a role in mosquito innate immunity. Using the available genome sequence of *Aedes aegypti*, we mined for presumptive apoptotic regulators by performing BLAST searches and overlapping EST analysis, using well-characterized *Drosophila* proteins and predicted proteins from *Anopheles gambiae* as querries. Using this bioinformatics approach we found homologs corresponding to ten caspases, three inhibitor of apoptosis (IAP) proteins, and four other proteins that have been shown to regulate caspases in *Drosophila*. We analyzed expression of these genes by real-time RT-PCR in *Ae. aegypti* larvae, pupae and adults. For some of the candidate genes we found restricted expression while others were expressed in all stages and tissues analyzed. This study represents a necessary first step in elucidating the molecular mechanisms of apoptosis regulation in *Ae. aegypti*.

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PREVALENCE OF INSECTICIDE RESISTANCE IN POPULATIONS OF THE DENGUE VECTOR *AEDES AEGYPTI* IN THAILAND

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Dengue fever and dengue haemorrhagic fever are a major cause of mortality and morbidity and are a serious public health and socioeconomic problem in Southeast Asian countries such as Thailand. Currently, control of the vector mosquito *Aedes aegypti* is the only method of reducing disease transmission but in many countries reports of insecticide resistance to the limited number of insecticides available for vector control may impact on control programmes in the near future. A survey of six *Ae. aegypti* populations collected from Thailand found high levels of resistance to DDT throughout the country and pyrethroid resistance in geographical pockets. Determining the molecular basis of this resistance will facilitate vector control programmes. Two mutations were identified in the sodium channel gene, encoding the target site of DDT and pyrethroids and a simple assay which uses a combination of a hot ligation and colorimetry has been developed to detect these mutations in individual *Ae. aegypti* mosquitoes. We have used this assay to determine the frequency of these mutations in field populations of *Ae. aegypti* from Thailand. The presence of other resistance mechanisms is also being investigated.

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RNA INTERFERENCE OF THE INSULIN RECEPTOR IN *CULEX PIPIENS* ARRESTS OVARIAN DEVELOPMENT AND SIMULATE DIAPAUSE

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An essential characteristic of *Culex* diapause is the arrest in development of the primary ovarian follicles. To test involvement of the *Culex* insulin signaling pathway in ovarian development during diapause, the C. pipiens Insulin receptor (InR) was cloned based on the homologous gene of *Caenorhabditis elegans* daf-2, which regulates worm dauer formation equivalent to diapause. The dsRNA of *Culex* InR (dsInR) was injected into non-diapausing adults of C. pipiens. Ovaries were dissected at 10 days after dsRNA injection. Among untreated diapausing mosquitoes, 92.3% (n:26) contained primary follicles arrested at Christophers' stage I. Among nondiapausing mosquitoes that were either untreated or injected with β-galactosidase (dsβ-gal), 9.7% (n=31) or 13.3% (n=30), respectively, were in Christophers' stage I. But, RNAi against InR caused 82.1% (n=28) of the nondiapausing recipients to arrest development at Christophers' stage I. This result thus simulates diapause and suggests a potential for InR in regulating the overwintering adult diapause in *C. pipiens*.

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TRANSLATIONAL REGULATION OF EARLY TRYPSIN SYNTHESIS BY TARGET OF RAPAMYCIN IN THE MOSQUITO *AEDES AEGYPTI*

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Blood feeding by mosquitoes is required for mosquito reproduction and disease transmission. Interruption of blood meal digestion could therefore be an important target for vector control. Blood meal digestion occurs in two phases; an early phase that is translationally-regulated, and a late phase that is transcriptionally-regulated. Early trypsin is an early phase serine endoprotease which is transcribed before feeding but only translated after feeding. It is known that ingestion of free amino acids is sufficient to induce translation of early trypsin in the midgut. Using a combination of biochemical and molecular genetic approaches, we demonstrate that the cellular kinase Target of Rapamycin (TOR) is activated by the presence of free amino acids in the midgut. Consistent with this observation, ingestion of amino acids, or a meal containing the blood proteins albumin or γ-globulin, results in the phosphorylation of at least two TOR targets in midgut, the translational regulator p70S6 kinase, and the translational repressor 4E-Binding Protein (4E-BP). We found that culturing unfed midguts with amino acids *in vitro* also results in phosphorylation p70S6K and 4E-BP, and importantly, this phosphorylation can be reduced by inclusion of rapamycin, a specific TOR inhibitor. Lastly, phosphorylation of p70S6K and 4E-BP, as well as, early trypsin translation, are reduced by injection of rapamycin or TOR-directed RNAi into adult mosquitoes. These results directly link TOR activation and early trypsin protein synthesis to amino acid signaling in the midgut epithelial cells. A molecular understanding of TOR signaling in the mosquito midgut may provide novel strategies to disrupt blood meal digestion.

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FUNCTIONAL CHARACTERIZATION OF THE *AEDES AEGYPTI* CARBOXYPEPTIDASE GENE FAMILY

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To gain a detailed understanding of mosquito digestive physiology, we undertook a comprehensive molecular study of digestive carboxypeptidases in *Aedes aegypti*, an insect vector for Dengue and yellow fever viruses. Using degenerate PCR primers based on conserved sequences in known *Drosophila melanogaster* and *Anopheles gambiae* carboxypeptidase genes, we initially isolated full-length coding sequences corresponding to ten *Ae. aegypti* carboxypeptidase genes from whole body RNA. Based on sequence analysis, seven of the genes were found to be members of the carboxypeptidase A gene family (AaCPA-I to AaCPA-VII), whereas, three belonged to the carboxypeptidase B gene family (AaCPB-I to AaCPB-III). Subsequent database mining of the *Ae. aegypti* genome using VectorBase tools identified nine additional carboxypeptidase genes, of which five belonged to the carboxypeptidase A family (AaCPA-VIII to AaCPA-XII) and four were carboxypeptidase B genes (AaCPB-IV to AaCPB-VII). Interestingly, all seven of the *Ae. aegypti* carboxypeptidase B genes are contained within a single 120 kb genome contig, suggesting they arose from recent gene duplication events. In order to characterize the expression pattern of the 19 *Ae. aegypti* carboxypeptidase genes, we used real-time RT-PCR to quantitate transcript levels in the midguts of blood fed mosquitoes. These data revealed that 11 carboxypeptidase genes were induced 5-50 fold in the midgut in response to feeding, while the other eight were expressed in non-midgut tissues. The peak time of expression for the midgut carboxypeptidase genes ranged from 3-24 hours post-feeding, demonstrating that peak gene expression occurs throughout digestion. Phylogenetic analysis using distance methods of 25 mosquito carboxypeptidases from four different species, suggested that most of the sequence divergence in the carboxypeptidase gene family occurred prior to the separation of *Aedes* and *Anopheles* mosquito lineages. Current efforts are aimed at identifying upstream signaling pathways that control coordinate expression of the CPA and CPB carboxypeptidase genes in *Ae. aegypti*.

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ADULT *AEDES AEGYPTI* MOSQUITOES CAN SYNTHESIZE UREA USING AN AMPHIBIAN URIC ACID DEGRADATION PATHWAY

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Blood feeding mosquitoes must be able to deal with the potentially life-threatening overload of nitrogen that makes up a disproportionate amount of the nutrients in the blood meal. To better understand how the female mosquito is able to overcome this metabolic challenge and avoid ammonia toxicity, we investigated nitrogen metabolizing pathways in *Aedes aegypti* mosquitoes using a variety of biochemical and molecular approaches. For these studies, female mosquitoes were fed a sucrose solution containing either $^{15}NH_4Cl$, [5-^{15}N]-Gln, [^{15}N]-Pro, allantoin, allantoin-UL-$^{15}N_4$ 50%, or allantoic acid. At 24 hours post-feeding, the feces were collected and analyzed by mass spectrometry. The quantification of unlabeled and labeled urea was performed by a multiple-reaction monitoring scan at a series of different neutral losses. Specific enzyme inhibitors confirmed that female mosquitoes can incorporate ^{15}N from $^{15}NH_4Cl$ into [5-^{15}N]-Gln and use the ^{15}N of the amide group of glutamine to produce labeled uric acid. More importantly, we found that the $^{15}N_2$-uric acid that is not directly excreted in the feces, is metabolized to singly-labeled ^{15}N-urea as nitrogenous waste. Although an amphibian-like uric acid degradation pathway was not thought to exist in mosquitoes, we found that *Ae. aegypti* mosquitoes express all three genes in this pathway, namely, urate oxidase (UO), allantoinase (ALN), and allantoicase (ALLC). We confirmed the functional relevance of these genes by showing that feeding mosquitoes ^{15}N-labeled allantoin led to the production of ^{15}N-urea, and moreover, that feeding unlabeled allantoin or allantoic acid, significantly increased urea production. Lastly, knockdown of UO expression by RNA interference in adult mosquitoes demonstrated that this pathway is indeed active in NH_4Cl and blood-fed females based on a significant increase in uric acid levels in whole body extracts. These unexpected findings could lead to novel metabolism-based strategies for mosquito control.

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TRANSGENIC EXPRESSION OF A VARIANT BEE VENOM PHOSPHOLIPASE A_2 IN *AEDES FLUVIATILIS* MOSQUITOES TOWARDS *PLASMODIUM GALLINACEUM* DEVELOPMENT

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The genetic manipulation of malaria vectors has been appointed as one alternative for disease control. The PLA_2 protein isolated from the bee venom, when added to an infectious blood meal and offered to mosquitoes inhibited *Plasmodium gallinaceum* and *P. falciparum* development, although impaired mosquito fitness when expressed by transgenic mosquitoes, as reported previously. To overcome this problem, we performed two point mutations on the PLA_2 coding sequence in order to inactivate the enzyme ($mPLA_2$). The mosquito *Aedes fluviatilis* is an important avian malaria experimental vector. Recently, we generated four *Ae. fluviatilis* transgenic lines expressing this antiparasitic gene, $mPLA_2$, driven by the *Anopheles gambiae* peritrophic matrix protein 1 promoter (AgPer1). The objective of this work was to evaluate the $mPLA_2$ expression by transgenic mosquitoes towards the development of *P. gallinaceum*. By Southern blotting, we confirmed that this gene has been integrated into the *Ae. fluviatilis* genome. This gene was expressed only in

transgenic female midguts, as showed by RT-PCR (500bp). There was no modification on gene expression before or after the blood meal, similarly to the endogenous AgPer1 mRNA. By confocal microscopy, it was possible to localize the $mPLA_2$ protein throughout the midgut of all transgenic mosquito lines. In 13 experiments to test the ability of $mPLA_2$-transgenic mosquitoes to block *Plasmodium*, the number of oocysts was significantly reduced (17.5 to 68.5%), when compared to non-transgenic mosquitoes. In conclusion the *P. gallinaceum* oocyst development was significantly impaired in *Ae. fluviatilis* transgenic mosquitoes expressing the $mPLA_2$ gene. Transgenic mosquito fitness studies are underway.

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ANTIBODY RESPONSE AGAINST SALIVA ANTIGENS FROM MALARIA AND ARBOVIRUS VECTORS IN TRAVELERS IN TROPICAL AFRICA

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Exposure to vectors of infectious diseases has been associated with antibody responses against salivary antigens of arthropods among people living in endemic areas. This immune response has been proposed as a surrogate marker of exposure to vectors appropriate for evaluating the protective efficacy of antivectorial devices. The existence and potential use of such antibody responses in travelers briefly exposed to malaria or arbovirus vectors in tropical areas has never been investigated. The IgM and IgG antibody responses of 88 French soldiers against the saliva of *Anopheles gambiae* and *Aedes aegypti* were evaluated before and after a four-month journey in tropical Africa. Immune responses against anopheles and aedes saliva increased significantly in 41% and 15% of the individuals, respectively, and appeared to be specific to the mosquito genus. A proteomic and immunoproteomic analysis of anopheles and aedes saliva allowed for the identification of some antigens that were recognized by most of the exposed individuals. These results suggest that antibody responses to the saliva of mosquitoes could be considered as surrogate markers of exposure of travelers to mosquito vectors that transmit arthropod borne infections.

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IDENTIFICATION OF CDNAS ASSOCIATED WITH MEIOTIC DRIVE IN *AEDES AEGYPTI*

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The meiotic drive gene in *Aedes aegypti* is tightly linked with sex determination and causes highly male biased sex ratios. Because meiotic drive violates Mendelian rules for independent segregation, it has the potential to drive novel genes into feral populations. The T37 strain carries the meiotic drive gene on chromosome 1, while the RED strain is sensitive to the driver. We prepared cDNA libraries from testes from both T37 and RED strains. Randomly selected clones from each library were end sequenced and searched against the VectorBase database using BLAST. A total of 682 sequences in the T37 strain and 845 sequences in the RED strain were generated from 864 random clones per library, respectively. About 41-48% of the sequences represent conserved hypothetical and hypothetical genes. The unique sequences in each library were searched for Gene Ontology terms using Amigo and Pfam and analyzed across the two strains. We observed strain-specific differences in cDNA expression profiles and identified several genes for future consideration as candidates for the meiotic drive gene in *Ae. aegypti*.

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REMOBILIZATION OF *PIGGYBAC* IN *ANOPHELES GAMBIAE*

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An approach being considered to control the spread of malaria is to genetically modify mosquitoes that are vectors for this disease with transgenes that will inhibit the development of P*lasmodium*. Transposable elements have been suggested as possible genetic agents that can be used for the spreading of these transgenes through mosquito populations. We are testing the ability of *piggyBac*, a common insect transposable element gene vector, to serve as a genetic drive agent in *Anopheles gambiae*, the major malaria vector in Africa. *piggyBac* is a broad host range gene vector that has been successfully used to transform *An. gambiae.* We are performing experiments that will allow us to test the remobilization, replication and spreading potential of *piggyBac* elements in *An. gambiae.* We have constructed a conditionally inducible autonomous *piggyBac* element that contains an autofluorescent protein gene as genetic marker. A mixture of DNA containing a *piggyBac* transposase-expressing helper plasmid, the conditional autonomous gene vector, and a non autonomous control plasmid was injected into *An. gambiae* eggs. By adding tetracycline to the larval environment, transposase expression was induced and element remobilization was stimulated. Transposable element remobilization was assessed using an ALFP-like procedure that permitted copy number and element position to be assessed. Results from individual insects and from the results of pools of 10-100 individuals were used to assess the rate of jumping and the frequency of the element. Results of these experiments will be discussed in terms of *piggyBac*'s potential to serve as a gene drive.

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FIRST REPORT OF *ACE.1* MUTATION IN *ANOPHELES ARABIENSIS* POPULATIONS FROM BURKINA FASO (WEST AFRICA)

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The *ace.1* mutation, conferring resistance to organophosphates and carbamates, formerly observed in both *Anopheles gambiae* S and M molecular forms has been detected for the first time in *An. arabiensis* from Burkina Faso. The detection of this mutation raises the question of evolutionary process that may explain the cause of its emergence in this member of *An. gambiae* complex. If confirmed on a large-scale survey, the result will be important in epidemiological level as organophosphates and carbamates had been proposed to be used as alternative insecticides in vector control programs.

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THE ROLE OF THE INSULIN SIGNALING CASCADE AND THE TGF-β SIGNALING PATHWAY IN ANTI-PARASITE DEFENSE IN *ANOPHELES GAMBIAE*

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Given the re-emergence of malaria, malaria is responsible for over 300 million illnesses and one million deaths per year. Malaria is a disease caused by parasites of the genus *Plasmodium* and is transmitted by female *Anopheles* mosquitoes. In order to combat this devastating

disease, new control measures are must be designed. One strategy is focused on the development of transgenic mosquitoes that are resistant to parasite development. At this time we need to improve our understanding of how to best control malaria, we must first understand natural transmission or why only a small percentage of mosquitoes in the field actually transmit the parasite. We have previously shown in the laboratory that the insulin signaling cascade (ISC) and the transforming growth factor (TGF)-β signaling pathway regulate malaria parasite development in *Anopheles* mosquitoes, including the African malaria vector *An. gambiae*. In most organisms, insulin signaling is initiated by the binding of insulin-like peptides (ILPs) to an insulin receptor which can activate two signaling cascades: one that is characterized by sequential activation of phosphoinositide 3-kinase and Akt/Protein Kinase B and the other by activation of the kinases Ras, Raf, MEK (a mitogen-activated or extracellular signal-regulated protein kinase), and ERK (an extracellular signal-regulated protein kinase). In mammals and insects, the TGF-βs signal through binding of heteromeric receptors, which activates a canonical Smad signaling pathway. In addition to the Smad pathway, however, TGF-βs can signal via alternative pathways, including those involving Ras, ERK, mitogen-activated protein kinase (MAPKs), and c-Jun N-terminal kinases. Preliminary data from our lab indicate that human TGF-β1 activates Smad signaling as well as a variety of alternative signaling kinases in *Anopheles* cells. We predict that natural variation in ISC- and TGF-β-related genes would be expected to affect parasite development in mosquitoes in endemic areas. In support of this hypothesis, we have identified naturally occurring mutations in *An. gambiae* genes encoding an insulin-like peptide, a MEK ortholog, two TGF-β receptor proteins, and a MAPK that is known in mammals for its roles in both ISC and TGF-β signaling. We discuss these mutations with respect to their predicted effects on anti-parasite defenses in *An. gambiae*.

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THE STAT PATHWAY LIMITS *PLASMODIUM* INFECTION IN *ANOPHELES GAMBIAE*

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Anopheles gambiae is the major vector for the human malaria parasite *Plasmodium falciparum* transmission. It mounts powerful antiparasitic responses that cause marked parasite loss during mosquito midgut invasion. Mosquitoes respond systemically to *Plasmodium* infection by activating genes that, in vertebrates, are known to be regulated by the STAT pathways such as nitric oxide synthase (NOS) and suppressor of cytokine signaling (SOCS). Two members of the STAT family (AgSTAT-A and AgSTAT-B) are present in *An. gambiae* and both transcription factors participate in the transcriptional activation of NOS and SOCS in response to bacterial or plasmodial infections. AgSTAT-B has six introns and maps to Chromosome X, while AgSTAT-A is intron-less and located in Chromosome 3, suggesting that STAT-A arose from a retro-transposition even. AgSTAT-A regulates the basal expression of AgSTAT-B and silencing of AgSTAT-B significantly increases the number of oocysts that develop following *P. berghei* or *P. falciparum* infection. Silencing of AgSOCS, a suppressor of the STAT pathway, has the opposite effect and reduces *P. berghei* infection. These data indicate that the STAT pathway plays a central role in limiting *Plasmodium* infection in *An. gambiae*.

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OLFACTORY RESPONSIVENESS TO THE HOST ATTRACTANT CARBON DIOXIDE IN DIAPAUSING AND NONDIAPAUSING ADULTS OF *CULEX PIPIENS*

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In the northern United States, *Culex pipiens* (L.), a major avian vector of West Nile virus, spends a good portion of the year in a state of developmental arrest (diapause). Short daylength and low temperature received by fourth instar larvae and early pupae dictate the entry into diapause as adults. A number of physiological and behavioral changes occur at this time; ovarian follicular development is suppressed, diapause-destined females increase sugar feeding, blood feeding ceases due to a lack of host-seeking response, their fat body hypertrophies, and they seek well-protected sites for hibernation. Whether or not diapause-destined females will take a blood meal prior to entering hibernation has been a great debate in the literature, and is of significance to their role, if any, in maintaining arboviruses through the winter. Although diapausing females placed in close proximity to a host can be enticed to take a blood meal, this bypasses the host-seeking step necessary under natural conditions. It may be that diapausing females do not detect certain host attractants such as carbon dioxide (CO_2), or they do not respond to such stimuli. We report differences in olfactory responsiveness to the host attractant carbon dioxide (CO_2) in diapausing and nondiapausing *Cx. pipiens*. We have also cloned and sequenced putative chemosensory receptors from *Cx. pipiens*, and have determined differences in expression patterns by real-time PCR.

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MONITORING INSECTICIDE-TREATED NETS (ITN) EFFICACY AND INSECTICIDE RESISTANCE IN MALARIA VECTORS IN TANZANIA

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Increasing coverage with insecticide-treated nets for malaria control and widespread use of insecticides in agriculture may select for insecticide resistance in malaria vectors. There is concern that resistance will interfere with the effectiveness of vector control strategies. A study was conducted to measure pyrethroid susceptibility in populations of malaria vectors and nuisance-biting mosquitoes in Tanzania and to test the biological efficacy of current insecticide formulations used for net treatment. *Anopheles gambiae* Giles s.l., *An. funestus* Giles s.l. and *Culex quinquefasciatus* Say were collected during three national surveys and two insecticide-treated net (ITN) studies in Tanzania. Knockdown effect and mortality were measured in standard WHO susceptibility tests and ball-frame bio-efficacy tests. Test results from 1999 and 2004 were compared to determine trends in resistance development. *Anopheles gambiae* s.l. and *An. funestus* s.l. were highly susceptible to permethrin (range 87-100%) and deltamethrin (consistently 100%) in WHO tests in 1999 and 2004, while *Culex quinquefasciatus* susceptibility to these pyrethroids was much lower (range 7-100% and 0-84%, respectively). Efficacy of pyrethroid-treated nets was similarly high against *An. gambiae s.l.* and *An. funestus s.l.* (range 82-100%) while efficacy against *Cx. quinquefasciatus* was considerably lower (range 2-100%). There was no indication of development of resistance in populations of *An. gambiae s.l.* or *An. funestus s.l.* where ITNs have been extensively used; however, susceptibility of nuisance-biting *Cx. quinquefasciatus* mosquitoes declined in some areas between 1999 and 2004. The sustained pyrethroid susceptibility of malaria vectors in Tanzania is encouraging for successful malaria control with ITNs. Continued monitoring is essential to ensure early resistance detection, particularly in areas with heavy agricultural or public health use of insecticides where resistance is likely to develop. Widespread low susceptibility of nuisance-biting *Culex* mosquitoes to ITNs raises concern for user acceptance of nets.

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HABITAT-BASED LARVAL INTERVENTIONS: A NEW PERSPECTIVE FOR MALARIA CONTROL

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The interest in environmental management of larval habitats has recently been rekindled as the burden and transmission of malaria continues to deteriorate, particularly in tropical Africa. Conventional larval intervention programmes are typically "all-out" campaigns that require targeting all aquatic habitats within a short period of time when larvae are present. Clearly, such a strategy is beyond the capacity of most local communities and governments in Africa. Ecological surveys of anopheline larval habitats, however, show substantial heterogeneities in adult production, emphasizing the need for targeted management programmes. Here, we propose a framework for habitat-based larval interventions by examining issues hampering our current understanding of the heterogeneity of mosquito productivity and underpinning biological processes. We emphasize that productivity should be defined at the habitat level to develop a prioritized ranking for targeted habitat management. The heterogeneity of habitat productivity should be examined by adoption of landscape approaches which explicitly deal with spatial processes governing mosquito oviposition and host-seeking activities. A cost-effective analysis is also included to illustrate the advantages of source reduction over insecticide application tactics. Our proposed habitat-based interventions offer a new perspective that relates mosquito oviposition processes to the abundance and dispersion of aquatic habitats in assessment of environmental management programmes. We conclude with an appeal for an increase of vigorous investigations to reveal the mechanisms regulating mosquito production for evidence based larval interventions.

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DYNAMICS OF INTERPLAY BETWEEN *AEDES AEGYPTI* SALIVA PROTEINS AND HOST IMMUNE RESPONSES

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The diverse repertoire of pharmacologically active molecules in mosquito saliva is essential for successful blood feeding and pathogen transmission by circumventing host defenses such as hemostasis and immunity. Of the several *Aedes aegypti* saliva proteins, only sialokinin has been reported to modulate host immunity. Understanding the immunomodulatory properties of saliva may lead to better approaches to control pathogen transmission by mosquitoes. This study examines immunomodulatory properties of previously uncharacterized saliva components. We quantitatively analyzed *Ae. aegypti* salivary gland gene expression upon blood feeding. Six of those genes (30kDa salivary allergen, 34kDa protein, antigen-5, P1A3, P1E4, and P5F9) were cloned into DNA vaccination vector pUMVC-1. Each DNA vaccination construct was injected into the ears of naïve mice to study mouse cytokine responses in skin using quantitative Real Time PCR. At 3 hour post-injection of DNA vaccination clones, T_H1 cytokines were observed to be down regulated, while T_H2 cytokines were up regulated. Antigen-5 and 34kDa protein expressing constructs up-regulated host IL-10 gene expression by 12 and 13 fold, respectively. Interferon-γ, known to contribute to anti-viral responses, was significantly down-regulated by P1E4 and P5F9. Similarly, cytokine responses in the draining lymph node (auricular) are now being analyzed. Down-regulation of anti-viral cytokines by P1E4, P5F9 and polarization from a T_H1 to T_H2 response by antigen-5 and 34kDa protein could provide pathogens a significant advantage for establishment in the host during the early stage of infection. Understanding the dynamics of host responses to mosquito saliva proteins could lead to novel immune based strategies to block pathogen transmission.

(ACMCIP Abstract)

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DIVERSITY OF CULICINE MOSQUITOES (DIPTERA: CULICIDAE) IN AN AFRICAN RICE AGROSYSTEM, MWEA-KENYA

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Development of irrigation projects has often been associated with increased risk of mosquito-borne diseases by creating ideal larval habitats for vector mosquitoes. However, whereas several studies have demonstrated the relationship between malaria vectors and irrigation, little work has been done on culicine mosquitoes despite their significant nuisance densities in these areas and their potential in transmission of filariasis and arboviruses. This study examined the diversity of culicine mosquito fauna and their larval habitats at two sites in Mwea Kenya over a 12-month period. The habitat types present at each site within a 200 m radius around each study village, including randomly selected rice paddies and canals, were sampled fortnightly to examine the relationship between vegetation cover, water depth, turbidity and culicine larval densities. Ten culicine species belonging to 4 genera were identified with 73.1% of the total collection comprising of *Culex duttoni* and *Cx. quinquefasciatus*. Other species collected included *Cx. annulioris, Cx. poicilipes, Cx. cinereus, Cx. tigripes, Cx. trifilatus, Aedes spp, Coquilettidia fuscopennata* and *Ficalbia splendens*. Murinduko village was more diverse than Kiamachiri in terms of species richness (10 versus 7 species) and larval habitat diversity (11 versus 8 habitat types). Paddies, canals and rain pools were the most diverse habitats in terms of species richness while ditches, rock pools and tree holes were the least diverse. Principal component and correlation analyses revealed a strong association between three *Culex* species and the measured habitat characteristics. *Cx. poicilipes* was strongly associated with floating vegetation, *Cx. annulioris* with clean water containing emergent vegetation, while *Cx. quinquefasciatus* was associated with turbid water. Seasonal changes in larval density in water reservoirs, pool and ditch habitats were closely associated with rainfall. These findings provide important information on larval habitat preference for different culicine species, useful in designing and implementation of larval control operations.

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POTENTIAL INCRIMINATION OF *ANOPHELES (NYSSORHYNCHUS) ALBIMANUS AND AN. (KERTESIA) NEIVAI* AS MALARIA VECTORS IN THE PACIFIC REGION OF COLOMBIA

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Malaria is an important public health problem in Colombia, especially in rural areas, where conditions are appropriate for transmission. Species in the subgenus *Nyssorhynchus* are important vectors; among them, *Anopheles albimanus* is recognized as a primary vector and is distributed along the Atlantic and Pacific Coasts and on San Andres Island. Several studies have demonstrated the importance of *An. albimanus* in the Pacific Coast of Colombia where it is the predominant species; however, its role as vector in the Atlantic Coast is not clear. Recent studies by our group detected *An. albimanus* with low levels of parasite infection. Therefore, in our study we explored the potential participation of species coexisting with

An. albimanus in malaria transmission in four localities of the Colombian Pacific Coast and three of the Atlantic Coast. From 12,105 anophelines collected, 6,355 were from the Pacific Coast, and corresponded to 80% *An. albimanus*, 8.5% *An. aquasalis*, and six other species which were present at <1% and the rest too damaged to identify. From the Atlantic Coast we identified 5,760 individuals: 47% *An. albimanus*, 30% *An. triannulatus*, 3% *An. aquasalis,* and seven other species at 2% or less. In both coasts, species present at lower percentages included several species incriminated as vectors in neighboring countries: *An. albitarsis* s.l., *An. neomaculipalpus*, *An. nuneztovari, An. punctimacula*, *An. pseudopunctipennis*, and *An. darlingi*. The study of infectivity analyzing 7,572 specimens, detected one *An. albimanus* infected by *P. vivax* VK247, confirmed by ELISA-PCR, and two *An. neivai* infected by *P. falciparum*, not confirmed by PCR; the three specimens infected came from the Pacific Coast. The difference in the vector status of *An. albimanus* in the two regions could be due to different factors: genetic, ecological and/or behavioral. The extreme geographic differences in Colombia, which could drive population differentiation, may also contribute to differences in *An. albimanus* parasite transmission in these two areas.

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EFFICACY OF CDC LIGHT TRAP SAMPLING TO MONITOR THE HOST-SEEKING BEHAVIOR OF *ANOPHELES ARABIENSIS* IN SOUTHERN ZAMBIA

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Anopheles arabiensis is the primary vector responsible for *Plasmodium falciparum* transmission in Macha, Zambia. The comparative performance of CDC light traps and human landing catches in determining the seasonal human biting and sporozoite rates of this vector was assessed in two village areas. Furthermore, the impact of insecticide treated nets (ITNs) on the effectiveness of CDC traps was examined. In a comparison of sleeping huts within individual households, entering behavior did not differ between houses equipped with ITNs or untreated bed nets. The lack of an apparent repellant effect of treated nets on *An. arabiensis* demonstrates the continued utility of CDC light traps to monitor this vector after bed nets have been distributed throughout a village area.

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HUMAN TGF-β1 REGULATION OF THE ANTI-MALARIAL RESPONSE IN MOSQUITOES

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Malaria is caused by infection with intraerythrocytic protozoa in the genus *Plasmodium* that are transmitted by *Anopheles* mosquitoes. Previously, we have demonstrated that *Anopheles* mosquitoes regulate malaria parasite infection through the induction of *Anopheles* NO synthase (*NOS*) expression and synthesis of inflammatory levels of nitric oxide (NO). This anti-parasite response is regulated in part by human TGF-β1, a cytokine ingested with the blood meal. In mammalian cells, ERK, JNK and p38 MAPK signaling proteins regulate TGF-β-induced target gene expression. Here, we demonstrate that human TGF-β1 regulates *NOS* expression in mosquito cells through ERK, JNK and p38 MAPK signaling pathways. In mosquito cell lines and in the midgut epithelium *in vivo*, human TGF-β1 dose-dependently induced ERK phosphorylation, while JNK and p38 phosphorylation were reduced. The MEK inhibitor PD98059 abolished TGF-β induced ERK phosphorylation *in vitro* and *in vivo*. Further, inhibition of ERK phosphorylation increased TGF-β induced *NOS* expression and decreased parasite development. These results suggest that ERK phosphorylation negatively regulates the human TGF-β1 induced anti-malarial response. Parallel studies with p38 MAPK and JNK are underway. Taken together, our data demonstrate that crosstalk between human TGF-β1 and mosquito cells is mediated through MAPK signaling pathways to regulate mosquito innate immunity.

(ACMCIP Abstract)

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LOCATION, LOCATION, LOCATION -- DISTANCES OF BREEDING WATER TO HUMAN HABITATIONS ARE AN IMPORTANT INDICATOR FOR DESIGNING TARGETED INTERVENTIONS OF HABITAT MANAGEMENT

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The role of larval control of anopheline mosquitoes for malaria management remains controversial. Traditionally, larval campaigns were indiscriminately treated as many aquatic habitats as possible, with little consideration of cost-effectiveness. In contrast, targeted intervention strategies promise an increase in effectiveness and sustenance. Here, we introduce an agent-based model by using object-oriented programming techniques to investigate the interrelationship between flight capability of mosquitoes and the impact of two control scenarios. Three georeferenced objects were specified, i.e. mosquitoes, houses and aquatic habitats in a grid-based landscape. Life table approaches were adopted to simulate demographic processes of mosquitoes. Host-seeking and oviposition searches were assumed to conduct random flight by exploring the neighbouring grids. Three flight capabilities, i.e. 1, 3 and 5 grid-length/day were investigated. Surprisingly, the difference in flight search alone could account for considerably abundances of mosquitoes in the area. For mosquitoes of limited flight capabilities, only waters near human houses were oviposited. Targeted removals of aquatic habitats near houses, i.e. those located in the average distance between original habitats and houses in the area, can achieve substantial advantages over the control measures which randomly eliminated the same number of aquatic habitats. Conclusively, our results suggest that distances of breeding water to blood meal sources is an important indicator for designing targeted larval interventions.

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THE ROLE OF FLIGHT TONE CHARACTERISTICS ON MATE SELECTION OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

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Insufficient effort has been placed on understanding the mating biology of medically important mosquito vectors, despite the potential benefit of manipulating vector mating systems for disease control programs. We addressed one aspect of the mating system on mosquito fitness. We propose to test the hypothesis that wing beat frequency is correlated with *Aedes aegypti* fecundity and fitness. Further, we explore the behavioral implications of these flight tone differences and their influence on mating success in male and female individuals. Male fitness is of special interest because female choice may occur in this mating system. Large and small cohorts of both male and female *Ae. aegypti* were tethered and recorded using a particle velocity microphone. Our results suggest a difference between flight tone generated by males and females representing different fitness traits. We propose that these findings will inform onging control programs that utilize a transgenic mosquito strategy.

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COMPARATIVE RESPONSE OF MOSQUITOES TO INSECTICIDAL TOXIC BAITS

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Plant-derived carbohydrates and honeydew contribute to the survival of male mosquitoes and enhance survival of female mosquitoes. Since mosquitoes characteristically feed on these sources just after emergence and intermittently through their lives, toxic baits incorporating carbohydrates provide an opportunity for localized control. In the present study, we evaluated a range of insecticides as potential toxicants for use with toxic baits for mosquitoes. Nine insecticides from 5 classes (pyrethroids, phenylpyroles, pyrroles, nicotinoids, macrocyclic lactones) were selected for comparison and commercially formulations used to facilitate incorporation of insecticides into aqueous sucrose solutions. Mortality was evaluated at 1, 4 and 24 hr over a range of doses in laboratory assays against male and female *Culex quinquefasciatus*, *Anopheles quadrimaculatus* and *Ochlerotatus taeniorhynchus.* Using probit analysis, doses eliciting male mortality (LC_{50} and LC_{90} values) were lower for all orally-ingested insecticides compared to females. For all species, the most effective active ingredients at 24 hours were fipronil and imidacloprid, followed by the pyrethroids, bifenthrin, deltamethrin and permethrin. Deltamethrin was the most toxic of the pyrethroids tested. For all insecticides tested, *Cx. quinquefasciatus* was the least susceptible with *Anopheles quadrimaculatus* the most susceptible. Mortality was more rapid with pyrethroids than with nicotinoids or macrocyclic lactones. Sucrose as a phagostimulant significantly enhanced mortality to toxicants ($P < 0.05$) and mortality was further increased using a non-nutritive phagostimulant (sorbose) ($P < 0.05$). Screened field cage trials using dye markers in toxic bait solutions demonstrated high mortality of marked individuals. In conclusion, several pesticides (such as fipronil and imidacloprid) from different classes of compounds have potential for use in development of toxic bait systems for mosquitoes.

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SPATIAL EXPLORATION OF HUMAN WNV DISEASE INCIDENCE IN THE NORTHEASTERN UNITED STATES

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Disease caused by West Nile virus has affected urban areas in the northeastern US since 1999. We use seven years of county based human WNV disease surveillance data to describe the epidemic and identify predictors of disease incidence. States were selected where WNV disease was reported for more than one year and where *Culex pipiens* is the primary enzootic vector. Trend surface analysis was used to quantify the spread of first case from Queens, NY. Regression analyses were used to identify predictors of WNV disease incidence. Percent urban or forested land based on classified maps and census based population and income data were included as covariates in the models at the county level. Incidence was dichotomized at the median (0.75 cases /100,000) to calculate odds ratios. Income and distance to queens were used to control for income based surveillance bias and spatial autocorrelation, respectively. A total of 977 cases occurred over the 565 million residents in the 207 counties of CT, DE, MA, MD, NJ, NY, PA, and RI. Trend surface analysis of the time to first case indicates WNV moved 98.5km/year in a southwesterly direction from Queens, NY until 2004 ($p<0.01$, $R^2= 0.16$). By 2005, no new counties reported cases. We found a significant trend of decreased incidence with increasing forestation ($p< 0.001$, $R^2= 0.20$). More urban counties (<38.9% forest cover) are 4.7 times more likely (95% CI: 1.6-13.8, p= 0.01) to have high WNV incidence than more rural counties (>69.6% forest cover). Counties with 38.9% to 56.6% forest cover are 2.92 times more likely (95% CI: 1.0-8.2, p=0.04) to have high WNV incidence than counties with greater than 69.6% forest cover. There is no significant difference between counties with 56.6 to 69.6% forested cover and those with greater than 69.6% forest cover (OR: 0.9, 95% CI: 0.4-2.1, p=0.74). Our results indicate urbanization is a significant predictor of WNV cases while controlling for population density, income, and spatial autocorrelation. This finding is supported by the ecology of the vector species involved in this region.

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REGIONAL COMPARISONS OF THE HOST FEEDING PATTERNS OF MAJOR VECTORS OF WEST NILE AND EASTERN EQUINE ENCEPHALITIS VIRUSES IN THE U.S.

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Understanding the blood feeding behavior of local populations of mosquitoes is essential to assessing their vectorial capacity. To characterize the host-feeding patterns of regional populations of mosquito vectors and to evaluate their contribution to enzootic and epidemic transmission of WN and EEE viruses, we collected blood-fed mosquitoes at virus foci in Connecticut, New York, Texas, and California, by using CO_2-baited CDC light traps, mosquito resting shelter traps, and CDC gravid traps over a five-year period (2002 to 2006). We identified the sources of vertebrate blood by nucleotide sequencing of PCR products of the mitochondrial *cytochrome b* gene. Our analyses revealed that *Culex pipiens*, *Cx. restuans*, *Culiseta melanura*, and *Cs. morsitans* acquire blood meals mostly from birds, whereas *Cx. quinquefasciatus* and *Cx. salinarius* feed on both avian and mammalian hosts. *Culex pipiens* complex mosquitoes showed regional differences in their blood feeding behavior; *Cx. pipiens* in northeastern US is principally ornithophilic, whereas its southern counterpart, *Cx quinquefasciatus* in Texas and southern California indiscriminately obtain blood meals from both avian and mammalian hosts with noticeable regional differences. We found that a number of reservoir competent passerine birds such as American robin, gray catbird, house sparrow, house finch and wood thrush frequently served as the blood meal source for host seeking mosquitoes. Results will be discussed and interpreted in conjunction with concurrent avian and mosquito surveillance activities for WN and EEE viruses.

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RISK FACTORS FOR HOUSE-ENTRY BY MALARIA VECTORS IN A RURAL TOWN AND SATELLITE VILLAGES IN THE GAMBIA

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In the pre-intervention year of a randomized controlled trial investigating the protective effects of house screening against malaria-transmitting vectors, a multi-factorial risk factor analysis study was used to identify factors that influence mosquito house entry. Mosquitoes were sampled using CDC light traps in 976 houses, each on one night, in Farafenni town and surrounding villages during the malaria-transmission season in The Gambia. Catches from individual houses were both (a) left unadjusted and (b) adjusted relative to the number of mosquitoes caught in four sentinel houses that were operated nightly throughout the period, to allow for night-to-night variation. Houses were characterized by location, architecture, human occupancy and their mosquito control activities, and the number and type of domestic animals within the compound. The key findings of this study will be presented along with a brief description of the intervention trial design.

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EVALUATION OF THE RELATIONSHIPS BETWEEN HUMAN ACTIVITIES AND CONTAINER BREEDING *AEDES* IN URBAN WEST AFRICA USING A GEOGRAPHIC SAMPLING STRATEGY

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Yellow fever (YF) is endemic in sub-Saharan Africa, where an estimated 200,000 cases and 30,000 deaths occur annually. Transmission of YF virus to humans in urban areas is primarily by *Aedes aegypti* mosquitoes. *Ae. aegypti* and other YF vectors develop in containers close to human habitations. Conditions such as crowding and poverty often contribute to increased mosquito breeding because of refuse, such as old tires, plastic containers, earthen jars, and bottles, being strewn about next to homes and schools. This study addressed potential ecological and human behavioral factors that might promote *Aedes* container breeding. It was conducted during the rainy season in N'Zérékoré, a town of approximately 300,000 people in Guinea, West Africa, using a geographic sampling strategy conceived and implemented by Keating *et al*. in 2003. Satellite imagery, normalized difference vegetation indices (NDVI), a questionnaire on human behavior, and household evaluations for larval and pupal development sites were used to assess ecological and man-made structures conducive to mosquito breeding. Because larval collections may not give a true indication of adult survivorship and subsequent potential for virus transmission, pupal indices were also used to assess the presence and population density of mosquitoes. A total of 368 households were visited in NDVI-derived and classified urban, sub-urban, and transitional urban/rural areas. Approximately 1600 man-made and natural containers were checked in household evaluations including plastic bottles, clay water storage pots, abandoned tires, banana trees, and snail shells. Immature mosquitoes were found and collected from 93 of the 1600 containers (6%), and 71 of the 368 households visited (19%). Fifty-seven percent (1340/1003) of larvae collected were *Aedes* sp.; sixty-one percent of reared pupae were identified as *Ae. aegypti* (46/75). On the ground housing density values and NDVI are compared as ecological indicators of urban, sub-urban, and transitional urban/rural classifications. Further results will be discussed. The approach used in this study may help guide public health agencies to define specific human behavioral and environmental risk factors for *Aedes*-borne pathogens and develop focused mosquito prevention strategies.

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CHARACTERIZING SPECIES DIVERSITY OF VECTORS IMPLICATED IN NON-TRADITIONAL EEE TRANSMISSION IN TENNESSEE

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Epizoodemic outbreaks of Eastern Equine encephalitis (EEE) have occurred sporadically throughout southeastern United States however, the enzootic cycle for EEE transmission is not well understood in inland sites. We report the first characterization of such an outbreak in Tennessee. To better understand the dynamics of disease transmission, the Vector-Borne Disease Branch of the Tennessee Department of Health conducted a survey of the mosquito fauna at a hardwood swamp in western Tennessee that was at the epicenter of a multi-equine outbreak in fall of 2005. The study was carried out over a twelve-week period from June to September 2006. Collections were made at least once a week using both CO_2-baited CDC light traps and vacuum collections from resting boxes at three study sites located around the swamp. Over forty thousand mosquitoes were collected and identified to the species level. Potential epizoodemic vectors include: *Anopheles quadrimaculatus* (65%), *Culex erraticus* (13%), *Coquillettidia perturbans* (5%), *Culex pipiens* (5%), *Uranotaenia sapphirina* (3%), *Aedes vexans* (1%), *Anopheles punctipennis* (1%) and *Cx. territans* (1%). We report that the primary enzootic vector implicated in EEE transmission, *Culiseta melanura,* was found in low numbers in our study, accounting for less than one percent of the total adult mosquito population collected. Furthermore, we report that the seasonality of mosquito species varied over the course of the summer. Potential vectors such as *Cx. erraticus*, however, were found in high abundance throughout. Finally, we detail the use of a multiplex real-time PCR protocol for virus detection for West Nile, Flanders and EEE viruses on the samples collected. This study is the first description of arboviral surveillance, mosquito diversity and population dynamics at an enzootic EEE focus in Tennessee.

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PARTICIPATORY MAPPING OF TARGET AREAS TO ENABLE OPERATIONAL LARVAL SOURCE MANAGEMENT TO SUPPRESS MALARIA VECTOR MOSQUITOES IN DAR ES SALAAM, TANZANIA

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Half of the population of Africa will soon live in towns and cities where it can be protected from malaria by controlling aquatic stages of mosquitoes. Rigorous but affordable and scaleable methods for mapping and managing mosquito habitats are required to enable effective larval control in urban Africa. A simple community-based mapping procedure that requires no electronic devices in the field was developed to facilitate routine larval surveillance in Dar es Salaam, Tanzania. The mapping procedure included (1) community-based development of sketch maps and (2) ground truthing of sketch maps through expert teams using laminated aerial photographs in the field which were later digitized and analysed using Geographical Information Systems (GIS). Three urban wards of Dar es Salaam were comprehensively mapped, covering an area of 16.8 km². Over thirty percent of these were not included in the first round of community-based sketch mapping, mostly because they were areas that do not exist on local government residential lists. The use of aerial photographs and basic GIS allowed rapid identification and inclusion of these key areas, as well as more equal distribution of the workload of malaria control field staff. In conclusion, the procedure developed enables complete coverage of targeted areas with larval control through comprehensive spatial coverage with community-derived sketch maps. The procedure is practical, affordable, and requires minimal technical skills. This approach can be readily integrated into malaria vector control programmes, scaled up to towns and cities all over Tanzania and adapted to urban settings elsewhere in Africa.

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UNDERSTANDING THE SPATIAL AND TEMPORAL DISTRIBUTION OF POTENTIAL MOSQUITO VECTORS OF RIFT VALLEY FEVER IN THE U.S.

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Rift Valley fever (RVF) is a mosquito-borne zoonotic hemorrhagic viral disease confined primarily to sub-Saharan Africa. In RVF endemic regions human and livestock populations suffer prominent health and economic impacts during RVF outbreaks. RVF virus is listed as an overlap Select Agent by both the CDC and APHIS and no approved human or animal vaccines exist for use in the U.S. If introduced into the U.S. RVF could spread rapidly via mosquitoes but could also be transmitted by contact with infected vertebrate tissues or aerosols, potentially affecting humans, wild ungulates, and U.S. livestock industries on a large scale. Several U.S. mosquito species have been identified as competent RVF vectors in the lab. Although the general ranges of these species in the U.S. are known, we describe work underway to better understand environmental factors driving the natural heterogeneity of their spatial and temporal distribution. Using GIS, we are comparing historical relationships between long-term mosquito population surveillance data and satellite climate and environmental data to develop models of mosquito distribution, timing, and abundance in the U.S. We show how both coarse and fine scale population-environment models can be integrated into programs designed to reduce the risk of introduction of RVF into the U.S. and programs designed to detect and contain RVF should it be introduced.

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ISOLATIONS OF JAMESTOWN CANYON VIRUS (BUNYAVIRIDAE: ORTHOBUNYAVIRUS) FROM FIELD-COLLECTED MOSQUITOES (DIPTERA: CULICIDAE) IN CONNECTICUT, USA: A TEN-YEAR ANALYSIS, 1997-2006

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Jamestown Canyon virus (JCV) (Bunyaviridae: *Orthobunyavirus*) is a mosquito-borne zoonosis belonging to the California serogroup. It has a has a wide geographic distribution occurring throughout much of temperate North America, and in humans causes mild febrile illness with acute central nervous system infection including meningitis and encephalitis, and frequent respiratory system involvement. White-tailed deer, *Odocoileus virginianus* are the principal amplification hosts and boreal *Aedes* and *Ochlerotatus* mosquitoes are the primary vectors. A 10 year study was undertaken in Connecticut to identify potential mosquito vectors, quantify seasonal prevalence rates of infection and define the geographic distribution of JCV in the state as a function of land use and white-tailed deer populations which have increased substantially over this period. JCV was isolated from 22 mosquito species. *Ochlerotatus canadensis, Oc. cantator, Anopheles punctipennis, Coquillettidia perturbans* and *Oc. abserratus* were incriminated as the most likely vectors based on yearly isolation frequencies and the spatial geographic distribution of infected mosquitoes. Frequent virus isolations were also made from *Aedes cinereus, Ae. vexans,* and *Oc. sticticus* and new isolation records were established for *An. walkeri, Culex restuans, Culiseta melanura, Cs. morsitans, Oc. sticticus, Oc. taeniorhynchus,* and *Psorophora ferox*. JCV is widely distributed throughout Connecticut and consistently circulates in a diverse array of mosquito vectors. Virus activity occurs from June through September and closely parallels mosquito abundance, with peak infection rates extending from mid-June through mid-July. Infection rates in mosquitoes are consistent from year to year and overall virus activity is directly related to local mosquito abundance. Infected mosquitoes are equally distributed throughout the state, irrespective of land use, and infection rates are not directly associated with the abundance of white-tailed deer possibly due to their saturation throughout the region.

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THE USE OF EMPIRICAL MODE DECOMPOSITION FOR THE STUDY OF THE TRANSMISSION OF DENGUE HEMORRHAGIC FEVER IN THAILAND

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This paper has a two-fold purpose: 1) to present in relatively simple language the nature of Empirical Mode Decomposition (EMD) analysis and 2) use EMD analysis to reassess the previously published conclusions which shows the existence of a spatial-temporal traveling wave in the incidence of DHF [dengue haemorrhagic fever] in Thailand. We demonstrate the use of EMD, which subdivides time-series data into what are called Intrinsic Mode Functions. The methodology appears to be ideally suited for circumstances where one need not assume linearity and stationarity. EMD analysis is particularly useful for analyzing a wide range of oscillating time series data, such as disease transmission time series data. We apply EMD analysis to previously piblished data, but go further than they in the analysis of EMD outcomes. Our results call for certain qualifications of their conclusions. We find that while developing a clear and comprehensive study design, they overstated their results about the existence of spatial-temporal traveling waves of DHF. The supposition that the disease travels in waves, if adopted, implies that new strains of the dengue viruses are being created in a particular place. We find no evidence that that phenomenon exists. More data on herd immunity and the prevalence of secondary infections would go a long way toward helping better understand the susceptibility to DHF among the Thai people.

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A NEW CLIMATE BASED MODEL FOR FORECASTING WEST NILE MOSQUITO VECTOR POPULATION ABUNDANCE AND HUMAN RISK

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Although daily weather and seasonal to inter-annual climatic variability influence mosquito vector biology and risk of vector-borne disease, this information is not readily employed in disease control programs. The reasons for this disconnect between climatic information and vector management are: (1) accurate relationships between climate and infectious disease are most likely dependent upon local scale parameters that have not been related to regional climate data and (2) interaction among climatologists, entomologists, public health and vector control professionals has not been integrated at the level at which information can be developed, validated, and readily incorporated into mosquito management plans. We developed a unique model that describes Culex mosquito population dynamics in the northeastern USA. This model incorporates temperature dependent development and longevity effects, rainfall and moisture indices, egg laying rate and photoperiod. We surveyed mosquito control personnel regarding their perception of the most practical and useful models and gathered data for these climate based parameters from natural larval and adult mosquito populations in the field over several years. Results of forecasting mosquito populations with the model will be discussed in detail.

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GIARDIA DOG GENOTYPES IN URBAN SETTINGS OF PERU AND THE UNITED STATES: ZOONOTIC TRANSMISSION POTENTIAL?

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Giardia lamblia (syn. *G. duodenalis*, *G. intestinalis*) is a protozoan parasite affecting people in developing and developed countries. To investigate the molecular epidemiology of *G. lamblia* in humans and dogs, studies were conducted in Lima, Peru and Tucson, Arizona. The possibility of zoonotic transmission in Peru was assessed by collecting samples from dogs in San Juan de Miraflores and comparing the genotype of the infection to that of family members. Fecal samples were collected weekly during a three-month period in 2002 and 2003. In Tucson, Arizona, dog fecal samples were collected from the local Humane Society. *Giardia* positive samples were detected using either microscopy or a direct immunofluorescent assay. Positive samples were purified by sedimentation and chromosomal DNA was isolated using the QIAmp DNA Stool Kit. For the Peruvian canine samples, a nested PCR targeting the SSU rRNA gene was used; for the canine samples collected from Tucson, an external PCR targeting the GDH gene was used; for the human samples, a nested PCR targeting the TPI gene was used. Sequences were compared to known *G. lamblia* DNA through the NCBI database and matched to their relevant genotypes by alignment with ClustalW. Genotype data was obtained for 115 Peruvians; all had AII, B or mixed AII and B genotypes. No dog genotypes were detected in humans. From the Peruvian study genotypes were determined for 49 dogs, 29 D, 18 C and 2 mixed genotypes were detected. There was also one dog with a B (human) genotype but this dog also had a D genotype. In the Tucson study, 431 fecal samples were collected from the Human Society; of those samples, 116 were positive for *G. lamblia*. Genotype data was determined for 46; there were 29 C, 13 D, and 4 mixed C and D genotypes. No human genotypes were found. The fact that only one human genotype of *Giardia* was detected in a canine sample on one occasion and no dog genotypes were found in humans lends support to the idea that in these urban settings dogs are not reservoirs for human *Giardia* and zoonotic transmission in not likely occurring.

(ACMCIP Abstract)

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MOLECULAR, BIOCHEMICAL AND PATHOLOGIC CHARACTERIZATION OF *ACANTHAMOEBA CASTELLANI* IRON- SUPEROXIDE DISMUTASE (FE-SOD)

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Acanthamoeba castellanii is a pathogenic protozoa causing acanthamoebic keratitis in human. Superoxide dismutases (SODs) from various parasitic protozoa have been considered as virulence factors since it protects parasites from intra- and extracellular oxidative death by virtue of their ability to dismutate O_2-. In order to figure out the pathogenicity of *A. castellani* in infection and Fe-SOD involved in the pathogenecity, molecular, biochemical and immunological characterization of *A. castellanii* Fe-SOD (AcFe-SOD) was investigated. The Fe-SOD gene was isolated from *in vitro* cultured *A. castellanii* though RT-PCR using mRNA and 3'-5' RACE PCR. Southern blot was done to analysis the DNA structure of AcFe-SOD gene. The ORF region of AcFe-SOD gene was cloned, sequenced, expressed in *Escherichia coli*. To examine biochemical characterization of Fe-SOD, the enzyme activity of AcFe-SOD under oxidative stress caused by H_2O_2, paraquat and pyrogallol, and the biochemical features based on pH and temperature stability were performed. To clarify the molecular pathogenecity of AcFe-SOD involved in infection, *Achanthamoeba* with suppressed Fe-SOD via RNA interference (RNAi) was compounded. The sensitivity of active oxygen derivative of Fe-SOD (-) and wild type *Acanthamoeba* was examined. A 791 bp-full length AcFe-SOD gene was composed with 5 exons and 4 introns and a single copy gene. By SDS-PAGE, a clear 25kDa product was observed. The enzyme activity of Fe-SOD was the highest level in the reaction treated with pyrogallol. The decrease of Fe-SOD signal in siRNA-transfected *Acanthamoeba* was observed, while wild type *Acanthamoeba* exhibited no decrease in the expression of Fe-SOD mRNA. In conclusion, AcFe-SOD was cloned and expressed. The molecular and biochemical characterization of AcFe-SOD revealed a similarity between a recombinant and a nature Fe-SOD protein. We demonstrated that Fe-SOD expression in *A. castellanii* cells significantly inhibited by transfection with siRNA.

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USE OF MULTIPLEX REAL-TIME PCR TO IMPROVE THE DETECTION OF *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM PARVUM* IN HUMAN FAECAL SAMPLES

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Giardia lamblia and *Cryptosporidium parvum* are the two most commonly detected protozoan parasites causing diarrhoea in humans. This study compared a multiplex real-time PCR with microscopy and the ImmunoCard STAT! ® (Meridian, Bioscience, Inc.) lateral flow immunoassay. Examinations were performed on 112 faecal samples submitted for routine screening to Queensland Health laboratories. The multiplex real-time PCR was positive in 7 of 7 faecal samples shown to contain *G. lamblia* by microscopy and was positive in 1 faecal sample in which *G. lamblia* antigen was detected, but no *G. lamblia* was demonstrated by microscopic examination. The PCR assay showed agreement in 6 of 7 faecal samples that screened positive for *C. parvum* by microscopy and 1 additional positive in which *C. parvum* antigen was detected but no *C. parvum* oocysts were found on microscopy. This study demonstrates that multiplex real-time PCR assay provides a sensitive and specific alternative to microscopy and lateral flow immunoassay for the detection of these important pathogens.

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ANTIPARASITIC EVALUATION OF HYBRIDS OF BENZIMIDAZOLE DERIVATIVES AND 2-AMINO-5-NITROTHIAZOLE

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Gastrointestinal infections are among the major problems of public health; specially, in underdeveloped countries. Amoebiasis caused by the protozoan parasite *Entamoeba* histolytica causes up to 100000 deaths per annum. *Giardia intestinalis* is a protozoan parasite that causes extensive mobility worldwide. Hymenolepiasis is an illness caused by the cestode *Hymenolepis nana*. Metronidazole and Nitazoxanide have been used in the treatment of the protozoan infections and Albendazole have been used in the treatment of hymenolepisis. However, clinical

resistance has been reported, especially to Metronidazole. As a part of our search for new antiparasitic agents, five hybrids of two antiparasitic agents, 2-amino-5-nitrothiazole with benzimidazole derivatives, were designed and synthesized. The antiparasitic micromolar spectrum showed by these molecules included antiprotozoal activity (*Giardia intestinalis* and *Entamoeba histolytica*) and anthelminthic activity (*Hymenolepis nana).* Results indicate that some compounds tested were as active as Metronidazole. Compound 2-methyl-*N*-(5-nitro-1,3-thiazol-2-yl)-1*H*-benzimidazole-5-carboxamide showed the same efficacy than Albendazole against *Hymenolepis nana*.

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SYNTHESIS AND ANTIPROTOZOAL ACTIVITY OF NOVEL 1-METHYLBENZIMIDAZOLE DERIVATIVES

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As part of our research program aimed to determine the structural requirements that benzimidazole derivatives most have for antiparasitic activity, we have build a data base through the years that now we use in a systematic Quantitative Structure Activity Relationships study in three dimensions (3D-QSAR), using Comparative Molecular Similarity Index Analysis (CoMSIA) to design new 1-methyl-1H-benzimidazole derivatives substituted at position 2 with ethoxycarbonyl, aminocarbonyl, *N*-methylaminocarbonyl and *N*,*N*-dimethylaminocarbonyl groups. These compounds were tested *in vitro* against *Giardia intestinalis and Trichomonas vaginalis*, in order to validate the computational model. Most of the synthesized compounds resulted to be new structures; some of them were highly potent against *T. vaginalis*, especially those with a chlorine atom at position 6.

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INACTIVATION EFFECT AND MOLECULAR DOCKING STUDIES OF BENZIMIDAZOLE DERIVATIVES AGAINST TRIOSEPHOSPHATE ISOMERASE FROM *ENTAMOEBA HISTOLYTICA* AND CORRELATION WITH *IN VITRO* ACTIVITY

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Parasitic infections, such as Entamoebosis, are still major threats against public health, especially in developing countries. One of the areas of the utmost importance in pharmaceutical research is the structure-based drug design. Triosephosphate isomerase (TIM) has been proposed as an antiparasitic target for drug design. As part of our research project aimed to find new antiparasitic agents, we tested a set of 96 compounds, previously synthesized in our laboratories, against TIM from *Entamoeba histolytica* (EhTIM). From this set we found that five compounds, benzimidazole derivatives, partially inactivated this enzyme at 250 μM concentration. Based on these results, we studied the possible binding sites by molecular docking, using AutoDock 3.0.5. Thus, we found that the compounds were perfectly docked in the interface of the EhTIM dimer. This interface is formed by an aromatic cluster. In that sense; we hypothesize that aromatic cluster plays a key role in the inactivation effect caused by these benzimidazole derivatives. In addition, these compounds were tested *in vitro* against *E. histolytica*. In this assay, compounds were active at 1.38-0.026 μM concentration. These results suggest that the mechanism of action is not necessarily the inactivation of the EhTIM. However, the results obtained from both studies, molecular docking and inactivation, are important for designing new compounds structure-based, and specifically, pointed to EhTIM as target.

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ANTIPROTOZOAL ACTIVITY OF NOVEL BENZIMIDAZOLE DERIVATIVES

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Four novel benzimidazole derivatives: methyl 2-{[2-oxo-2-(1,3-thiazol-2-ylamino)ethyl]thio}-1*H*-benzimidazole-5-carboxylate (1); methyl 2-({2-[(5-nitro-1,3-thiazol-2-yl)amino]-2-oxoethyl}thio)-1*H*-benzimidazole-5-carboxylate (2); 2-mercapto-*N*-1,3-thiazol-2-yl-1*H*-benzimidazole-5-carboxamide (3) and 2-mercapto-*N*-(5-nitro-1,3-thiazol-2-yl)-1*H*-benzimidazole-5-carboxamide (4), were synthesized and tested *in vitro* against the protozoa *Giardia intestinalis* and *Trichomonas vaginalis*. These compounds were more active than metronidazole against *G. intestinalis* and more active than albendazole agaisnt *T. vaginalis*. Compounds 3 and 4, which have the 1,3-thiazole group at position 5 of the benzimidazole ring, were more active against *G. intestinalis*. On the other hand, compounds 1 and 2, with the 1,3-thiazole moiety at position 2 were more active against *T. vaginalis*. In both assays nitro compounds were more active than their analogues without the nitro substituent.

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PROTEOMICAL EVALUATION OF NOVEL GIARDICIDAL BENZIMIDAZOLE DERIVATIVES

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As part of our efforts to find new antiparasitic compounds, and to know more about the mechanism of action, two giardicidal compounds: 2-(methylthio)-*N*-(1,3-thiazol-2-yl)-1*H*-benzimidazole-5-carboxamide (CMC-20), a hybrid nitroheterocyclic-benzimidazole and a 1-methylbenzimidazole derivative (RSD-8), designed to find out the importance of the hydrogen at position 1, were synthesized and tested *in vitro* against *Giardia intestinalis*. In the present study, a proteomic assay was carried out on *G. intestinalis* trophozoites treated with compounds CMC-20 and RSD-8, as well as MTZ. Essentially, following drug treatment, the trophozoites were submitted to thaw-freeze cycles, centrifuged and the supernatant used for the proteomic analysis. First dimension electrophoresis was carried out in 18 cm immobilized pH 4-7 gradient strips. For double dimension electrophoresis, samples were placed and separated onto 18 x 22 cm 12.5% SDS-PAGE gels (2-D gels) and proteins were visualized by colloidal Coomasie. Approximately 500 spots were resolved in each treated and untreated samples, and some of them were excised for mass spectrometry analysis (MALDI TOF-TOF). PD-Quest analysis of 2-D gels revealed an increase or decrease in the expression of some proteins that could be identified. Tubulin β chain, axoneme associated protein and thioredoxin-peroxidase increased in metronidazole treated simples compared to control samples. CMC20 and RSD-8 significantly reduced the expression of giardins, arginine deiminase and UPL-1. Giardins and the axoneme-associated protein are a group of cytoskeletal proteins, that together

with tubulins are the main components of ventral disk and of ventral disc and flagella, these variations on protein expression correlated with observations performed by Scanning Electronic Microscopy (SEM) of the parasite. These approaches (proteomic and SEM analysis) could be useful for evaluating the action of drugs, or potential drugs, and also they can be, as a first glance, a good chance for knowing what kind of proteins are involved.

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MONITORING MICROARRAY-BASED GENE EXPRESSION PROFILE CHANGES IN *VACCINIA* VIRUS

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We developed a *Vaccinia*-specific microarray based on complete genomic sequences of ACAMBIS2000 strain *Vaccinia* virus to explore transcriptional gene expression profiles and mechanisms of signaling pathways at different stages of infection. This is the first study in which at least two different regions of all possible genes (early, intermediate, and late) or gene-like sequences and inter-generic regions (a total of 638 sequences including 223 novel genes and 96 inter-generic regions) was fingerprinted and used to design a microarray chip. Additional variants were identified by including unique sequences of WR and other strains of this virus. In this study, the RNA was extracted by TRIZOL method at eight different time points (0, 1, 2, 4, 8, 12, 24, 48 hr) from both *Vaccinia*-infected cells and uninfected Vero81 cells to understand gene expression in ACAMBIS2000 strain. The quality and quantity of RNA was measured by Agilent BioAnalyzer. The fluorescent cDNA probes was made using Amersham Post-Labeling Kit. Hybridization and washing of chips was carried out following manufacturers' protocols; hybridized microarray slides were scanned by Axon Gene Pix 4000B scanner. Although, we had successful hybridization at some time points, efforts are underway to standardize various steps. Our preliminary results suggest a differential expression pattern over a time period of infection. These chips may prove a useful guide to evaluating the effects of therapeutic drugs on *Vaccinia*, and ultimately *Variola* infection, especially so in the absence of human infection by *Variola* or good animal models for it.

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IDENTIFICATION OF NON POLIO ENTEROVIRUSES ISOLATED FROM SELECTED ACUTE FLACCID PARALYSIS (AFP) STOOL SAMPLES IN THE GHANA POLIO REGIONAL REFERENCE LABORATORY: IMPLICATION FOR CAUSATIVE AGENTS TO AFP OTHER THAN POLIO

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This study wwas undertaken to support the World Health Organization (WHO) effort in the global eradication of Poliomyelitis (polio), which is an enterovirus found worldwide and belong to the family picornaviridae, it has become necessary to identify other agents in the subgroups (coxsackieviruses, echoviruses, and the "newer enteroviruses"), to establish their probable role in AFP. This work is therefore aimed at identifying some of the non polio enteroviruses isolated from acute flaccid paralysis (AFP) cases in the laboratory in 2005. Thirty-six non polio isolated samples were randomly selected and investigated in this study. The samples were re-inoculated on human rhabdomyosarcoma (RD) cells which are highly susceptible to most enteroviruses including polioviruses, echo viruses, coxsackie viruses and other enteroviruses. Two hundred microliter (200µl) of isolate was re-inoculated on 1.0 X 10^5 of cells cultured in sterile tissue culture tubes and incubated at 36°C. After the cultured cells have shown > 75% cytopathic effect some days post-inoculation, the isolates were harvested, frozen and thawed two times and then aliquoted into properly labeled 2ml sterile vials with caps and stored for use. To identify the viruses, microneutralization tests were performed with enterovirus antiserum pools. The results of the neutralization tests indicated, Coxsackie $B_{1\text{-}6}$(n=4, 11.1%), echo 6 (n=6, 16.7%), echo 7 (n=6, 16.7%), echo 11 (n=2, 5.6%), echo 13 (n=1, 2.8%), echo 20 (n=2, 5.6%) and 15 (41.7%) are inferably "newer enteroviruses". Although not significant, it was observed that more males were infected with non polio enteroviruses than females in relation to the sample size. In conclusion, the predominant enteroviral infection rate was between the 12-24 months age group. However, in order to sustain the gains made by the WHO in the global eradication of polio, other non-polio enteroviruses need to be identified for elimination through education on how to practice personal hygiene and also keep the environment clean so as to decrease the rate of infection amongst children, to prevent fatal clinical diseases in future.

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QUANTITATIVE PCR ASSAY FOR THE DETECTION AND DIFFERENTIATION OF MONKEYPOX VIRUS FROM OTHER ORTHOPOXVIRUSES

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Monkeypox disease is caused by the monkeypox virus and was first identified in cynomolgus monkeys in 1958. Monkeypox virus is transmissible to people and it has become the most important Orthopoxvirus infection in human beings since the eradication of smallpox in 1970s. Although it is less severe, the clinical symptoms of monkeypox are similar to that of smallpox and its potential development as an agent of bioterrorism persists. In case of a poxvirus outbreak, there is a need for an accurate and effective method to distinguish monkeypoxvirus from smallpox and other orthopoxviruses. We have developed a hemagglutinin (HA) gene-specific quantitative PCR assay that can discriminate monkeypox viruses from other orthopoxviruses. The real-time PCR probe was designed with fluorescin (6FAM) at the 5' end and minor groove binder (Applied Biosystems) at the 3' end to enhance its specificity and was tested against orthopoxviruses and other virus samples from the ATCC and BEI Resources collections. The real-time quantitative results were compared against cycle threshold (C_T) values calculated from a serially diluted plasmid standard containing an HA gene insert that resulted in standard curves with correlation coefficient values of ≥ 0.98. Using a previously published pan-orthopoxvirus assay, we have accurately quantitated and normalized the concentration of all the samples in our experiment. Among the orthopoxvirus strains that were included in the experiments were; monkeypox strain Zaire, monkeypox strain 7-61 (WRAIR), vaccinia strain MVA, vaccinia strain NYBOH, rabbitpox strain Utrecht, cowpox strain Brighton Red, racoonpox strain Herman, and ectromelia recombinant strain Moscow. Extracted DNA from vero cells, Rickettsia and Adenovirus were also included in the experiment as negative controls. Our data suggests that the monkeypox assay showed greater specificity for monkeypox strains as compared to vaccinia and other virus strains. We believe that, although the HA genes in orthopoxviruses share over 90% homology, we have successfully developed a quantitative PCR assay to differentiate monkeypox from other closely related poxviruses.

(ACMCIP Abstract)

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ELEVATED TESTOSTERONE AND REDUCED 5-HIAA CONCENTRATIONS ARE ASSOCIATED WITH WOUNDING AND HANTAVIRUS INFECTION IN MALE NORWAY RATS

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In rodent reservoir populations, males are more likely to engage in aggression and to be infected with hantaviruses than are females. One mode of hantavirus transmission is via the passage of virus in saliva during wounding. Whether hantaviruses cause physiological changes in their rodent host that increase aggression and virus transmission has not been fully delineated. To assess whether steroid hormones and neurotransmitters contribute to the correlation between aggression and Seoul virus infection, Norway rats were trapped in Baltimore, Maryland and wounding, infection status, steroid hormones, and concentrations of neurotransmitters, including norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenol acetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindole-3-acetic acid (5-HIAA) in select brain regions were examined. Older males and males with high grade wounds were more likely to have anti-Seoul virus IgG and viral RNA in organs than either juveniles or adult males with less severe wounds. Wounded males had higher circulating testosterone, lower hypothalamic 5-HIAA, and lower NE in the amygdala than did males with no wounds. Infected males had higher concentrations of testosterone, corticosterone, NE in the hypothalamus, and DOPAC in the amygdala than did uninfected males, regardless of wounding status. In the present study, wounded males that were infected with Seoul virus had elevated testosterone and reduced 5-HIAA concentrations, suggesting that these neuroendocrine mechanisms may contribute to aggression and the likelihood of transmission of hantaviruses in natural populations of male Norway rats.

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COMMON MARMOSETS (*CALLITHRIX JACCHUS*) AS A NON-HUMAN PRIMATE MODEL FOR EASTERN EQUINE ENCEPHALITIS

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Common marmosets (*Callithrix jacchus*) were evaluated as a non-human primate (NHP) disease model for eastern equine encephalitis (EEE). Cohorts of 3 animals were infected intranasally with 10^6 PFU of either North American (NA) EEEV strain FL93-939 or South American (SA) EEEV strain 436087 and monitored daily for weight loss, fever, anorexia, depression, and neurologic signs. On days 1, 2, 4, 7, 9, and 11 after infection, a complete blood cell analysis, biochemistry panel, and virus titration was performed on blood samples. When animals were euthanized, either at day 16 after infection or when moribund, tissues were collected for virus titration and histopathological analysis. Consistent with previous murine and NHP studies, the NA EEEV-infected animals either died or were euthanized on days 4-5 after infection due to anorexia and depression and the SA EEEV-infected animals appeared healthy and survived. Both cohorts experienced weight loss for the first 4 days after infection; however, only the NA EEEV-infected animals developed fever. Unlike the SA EEEV-infected marmosets, the NA EEEV-infected marmosets developed transient leukopenia during the first two days after infection followed by leukocytosis, and for both cohorts, the biochemistry profile was unremarkable. The SA EEEV-infected animals developed peak viremia titers of 2.8-3.1 $\log_{10}$ PFU/ml either at day 2 or 4 after infection, but there was no detectable viremia in the NA EEEV-infected animals. In contrast, virus was detected in the brain, liver, and muscle of the NA EEEV-infected animals at the time of euthanasia or death, but not in the SA-infected animals when the experiment was terminated 16 days after infection. Similar to the brain lesions described for human EEE, the NA EEEV-infected animals developed mononuclear cell leptomeningitis and moderate encephalitis in the cerebral cortex, with some perivascular hemorrhages. These findings confirm epidemiological evidence that SA EEEV strains are attenuated for humans, and also describe a NHP model that could be useful for vaccine evaluation.

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RECOMBINANT SINDBIS VIRUSES THAT REGULATE APOPTOSIS IN THE C6/36 *AEDES ALBOPICTUS* CELL LINE

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Sindbis virus (SINV), a mosquito-borne virus, belongs to the genus *Alphavirus* in the *Togaviridae* family. SINV induces dramatic apoptosis in most mammalian cell lines, but mosquito cell lines, such as C6/36 cells from *Aedes albopictus*, exhibit only moderate cytopathic effects with persistent SINV infection. In this study, we are using SINV as a vector to express either pro-apoptotic or anti-apoptotic genes in C6/36 cells, in order to study apoptosis regulation in mosquito cells. Expression of the pro-apoptotic genes *Drosophila* Reaper (Rpr) or *Aedes aegypti* Michelob_x (Mx) using SINV caused apoptosis in C6/36 cells. We observed chromosomal DNA condensation and fragmentation typical of apoptosis by Hoechst staining, DNA agarose gel electrophoresis and TUNEL staining after Rpr or Mx recombinant virus infection. We also detected cell membrane blebbing and caspase activation after infection. These apoptotic features were accompanied by high production of virus during the infection in mosquito cells. Expression of the baculovirus caspase inhibitor P35 inhibited actinomycin D-induced caspase activity in SINV-infected C6/36 cells. Based on the data above, these recombinant viruses can be used as tools to study apoptosis and its effects on vector competency in mosquitoes.

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THE CARRIER RATE OF NEWCASTLE DISEASE VIRUS IN PIGEONS IN OWERRI AREA OF IMO STATE, NIGERIA

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The role of domestic pigeons (*Colombia livia domestica*) in the transmission of Newcastle disease was investigated in 3 Local Government Areas (LGAs) - Owerri Municipial - Owerri North - Owerri West LGAs of Imo State, Nigeria between February and May 2004. Isolation of Newcastle Disease Virus (NDV) was attempted from 20 apparently healthy pigeons encountered per L.G.A. Cloacal swabs from pigeons coloured mainly BLACK or WHITE were inoculated into 9-11 day old embryonated hens' eggs via the allantoic route and incubated for 96 hours at 37^{O} C in a humidified incubator. Three (3) of the 60 pigeons tested positive for NDV. Two (2) positive results were recorded for Owerri Municipial council Area, 1 for Owerri North LGA and none for Owerri West LGA. Two (2) positive results were recorded for WHITE pigeons. Only 1 BLACK pigeon showed evidence on NDV. The carrier rate on NDV in pigeons in Owerri area of Imo State was 5%.

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NIPAH OUTBREAK WITH PERSON-TO-PERSON TRANSMISSION IN BANGLADESH, 2007

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We established surveillance for human Nipah outbreaks by identifying clusters of encephalitis cases at 10 hospitals in northwest Bangladesh beginning in February 2006. No Nipah outbreaks were confirmed in 2006. In February 2007 a surveillance physician identified a cluster of encephalitis cases in Haripur Upazila of Thakurgaon District. A field team conducted an investigation. Persons were classified as cases if between January 15 and February 2007 they resided in the affected area, had fever and had IgM antibodies against Nipah virus, or if they died before blood was collected, if they had fever with new onset of seizures or altered mental status. For each case the field team selected three neighborhood controls and collected information on their exposures. Seven persons met the case definition. Five had IgM antibodies to Nipah virus. The median age of cases was 24 years; 5 were males. The most common symptoms included fever (100%) altered mental status (71%) and vomiting (71%). Three (43%) died. The initial case developed illness 13 -18 days before the subsequent cases. All of the subsequent cases had close contact to the initial case during his illness. Throughout the initial case's illness his personal care was provided by family members and friends. Illness was significantly associated with being in the same room with a Nipah case when he had fever and altered mental status (86% versus 9.5%, odds ratio [OR] 57, confidence interval 4.4-744, p<0.001) or was coughing (86% versus 0%, odds ratio [OR] undefined, p=0.04). In conclusion, this outbreak was caused by Nipah virus. The initial case presumably contracted Nipah infection from a spill over from wildlife, and the subsequent infections were transmitted by close personal contact with the initial case. Improving caregiver's understanding of communicable disease and successfully promoting basic steps of infection control could prevent transmission of Nipah virus and other dangerous pathogens.

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GENETIC RELATIONSHIPS OF JAMESTOWN CANYON VIRUSES INFECTING CONNECTICUT MOSQUITOES

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Jamestown Canyon virus- JCV (Bunyaviridae: *Orthobunyavirus*) is a mosquito-borne virus that has been implicated in the etiology of encephalitis and meningitis with human cases documented from southern Canada, Michigan, New York and Connecticut. Despite the recognition of symptomatic cases from northeastern US, little is known about the genetic relationships of JCV variants circulating in this region. Accordingly, we compared the phylogenetic relationships of 56 JCVs isolated from mosquitoes collected in Connecticut over a 40 year period. We were able to distinguish three genetically-distinct clades circulating in Connecticut, based on phylogenetic reconstruction of S segment nucleotide sequences. Viruses representing each clade infected a diverse group of mosquito species collected throughout the state. One of these lineages was detected in Connecticut from 1966-2006 with few mutational changes accumulating over time. Phylogenetic trees generated from portions of the M and L segments yielded different topologies from S segment sequences as 2 of the 3 clades became consolidated into one clade but otherwise, membership into each clade was consistent between all three genomic segments. Together, these results suggest that JCV variants are stably maintained in Connecticut where they infect a wide diversity of mosquito species.

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IMMUNOLOGICAL RESPONSE AND PROVIRAL LOAD AS FACTORS INFLUENCING DISEASE EXPRESSION IN HTLV-1

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The Human T cell Lymphocytotropic Vírus Type 1 (HTLV-1) is the causal agent of the HTLV-1 associated myelopathy (HAM/TSP), adult T cell leukemia (ATLL) and Infective Dermatitis (ID). More recently, it has been shown that children with ID are at high risk to develop HAM/TSP and ATLL. Although the majority of HTLV-1 infected subjects (95%) are considered to be carriers, we have documented a higher frequency of urologic manifestations, erectile dysfunction, poliarthralgia, arthritis and periodontal disease in HTLV-1 infected individuals than in non-infected HTLV-1 controls. Herein we show data on proviral load and cytokine production in four groups of subjects: 1) Patients with HAM/TSP; 2) Patients with ID; 3) HTLV-1 infected subjects whom do not fulfill the criteria for HAM/TSP but have neurogenic bladder; 4) HTLV-1 asymptomatic carriers. Neurogenic bladder was defined as the presence of at least two of the following symptoms (nocturia, urinary loss and urgency) and documentation of abnormalities in the detrusor function by urodynamic study. Cytokines were determined in supernates of unstimulated cultures and proviral load by real time PCR. IFN-γ levels and proviral load in HAM/TSP, ID and in the group with neurogenic bladder were higher (P<0.01) than in HTLV-1 carriers. There was no difference in IFN-γ and proviral load in patients with HAM/TSP, ID and in those with neurogenic bladder. In patients with ID an exaggerated Th1 (IFN-γ and TNF-α) and Th2 (IL-4, IL-5 and IgE levels) was documented. While IL-10 and anti-IL-2 were able to down modulate the immune response in HTLV-1 carriers and in subjects with neurogenic bladder who do not fulfill the criteria for HAM/TSP, IL-10 and anti-IL-2 fail to down modulate immune response in patients with ID and in those with HAM/TSP.The proviral load and high production of IFN-γ and TNF-α were associated with clinical manifestations in HTLV-1 infection, and patients with more advanced forms of disease have an impairment in down modulate IFN-γ and TNF-α production. The documentation of immunological abnormalities and proviral load in patients with neurogenic bladder similar to that observed in HAM/TSP, indicate that these individuals may have an oligossintomatic form of myelopathy.

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REFERENCE AND DEVELOPMENTALLY EXPRESSED GENES OF *CLONORCHIS SINENSIS* QUANTIFIED BY REAL-TIME PCR

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In analyses on gene expression using quantitative real-time PCR (Q-rt-PCR), reference genes are employed to normalize target gene expression. However, expression level of housekeeping genes is dependent on tissues, developmental stages and external stimuli. Validation on stability of reference genes at a given condition is prerequisite to compare and analyze a relative expression between experimental groups.Total RNA was independently extracted from three groups of *Clonorchis sinensis* metacercariae and adults. The Q-rt-PCR was performed using SYBR Green I and the ABI prism 7000. Seven candidate reference genes were retrieved from a *C. sinensis* EST pool. Amount of target mRNA was determined using $2^{-\Delta\Delta Ct}$ method. Reference genes evaluated good for comparison between developmental stages were β-actin, phosphoglycerokinase and calcyphosine, for γ-ray irradiation experiment were α-tubulin,

phosphoglycerokinase, glyceraldehyde-3-phosphate dehydrogenase and β-actin, and for bile-treatment were small nuclear ribonucleoprotein and phosphoglycerokinase. When rt-PCR'd and compared with an average of the three reference genes, 6 genes were identified overexpressed in the adult stage and 10 genes were in the metacercariae. In adults, higher expression of antioxidant and glycolytic enzymes reflected well biological need and defense activities of *C. sinensis* in the final host. Collectively, for quantitative comparison of *C. sinensis* gene expression, most applicable normalization standard is an average of more than 3 reference genes validated at the same experimental settings.

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RELATIONSHIP OF SPECIFIC FIBRINOGEN-RELATED PROTEINS TO ACQUIRED RESISTANCE IN THE SNAIL *BIOMPHALARIA GLABRATA*

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Several digenetic trematode species use the freshwater snail *Biomphalaria glabrata* as an obligatory intermediate host. Previous exposure to trematode parasites may lead to acquired resistance in *B. glabrata*. Previous observations that prior exposure to radiation-attenuated miracidia of *Echinostoma paraensei* yields experimental induction of acquired resistance in *B. glabrata* were confirmed and the involvement of fibrinogen-related proteins (FREPs) as candidate factors in acquired resistance was investigated. Snails were exposed to irradiated *E. paraensei* miracidia, and then challenged 8 days later with normal miracidia. Only 10-30% of snails previously exposed to irradiated miracidia became infected following the challenge compared to 80-90% of snails exposed only to normal miracidia. Quantitative PCR using mRNA from whole snail bodies revealed different transcription profiles of FREPs between first response and acquired resistance. First exposure to miracidia resulted in an upregulation of FREP2, but not 3, 4, or 7. Induction of acquired resistance was associated with increased amounts of transcripts of FREP2, 3, 4, and 7. Preliminary immunoblot analysis of snail hemolymph with antisera raised against recombinant IgSF domains from FREP3 and 4 revealed the presence of FREP3 protein in 66% of the snails exhibiting acquired resistance. Only 11% of the snails that were exposed to normal miracidia and became infected had FREP3-immunoreactive hemolymph proteins. The protein expression of FREP4 was variable between the test groups with no obvious pattern. These mRNA and protein results suggest that FREPs3 and 7 may play a role in *B. glabrata*'s acquired resistance to *E. paraensei*. Results from microarray analysis of snails subjected to experimental induction of acquired resistance will be also presented.

(ACMCIP Abstract)

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RNA SILENCING IN *SCHISTOSOMA MANSONI*

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Schistosoma mansoni is an important human parasite, infecting millions of people worldwide. As with other pathogenic parasites such as *Plasmodium* and trypanosomes, the genome sequencing of *S. mansoni* was initiated with the anticipation that such research may provide new resources to better understand its biology and control the diseases it causes. Our additional interest is in understanding the regulation of gene expression in this parasite, in terms of RNA silencing machinery. Initial indications of our analysis of the *Schistosoma* genome show the presence of several repetitive sequences and sequences associated with transposable elements. Indications of these sequences prompted us to further investigate if there is RNA silencing machinery in the genome and whether it can be used to regulate repetitive and mobile-like elements. To conduct this stage of the analysis, we first used a known sequence of proteins involved in the RNA silencing mechanism in order to identify their orthologs in the genome. The results indicate that *Schistosomas* possess the common set of proteins in the complete RNAi machinery, including Drosha, Dicer, and Argonaute RNA-binding protein. Next, we set out to clone and analyze putative miRNAs, which initial results indicate that the parasite contains miRNAs that are highly conserved between organisms, as well as distinctive ones. Finally, we chose 50 miRNA candidates to confirm the predicted size of the RNA molecule using northern blot analysis. In conclusion, at least part of the RNA silencing machinery exists, and we are currently seeking functional characterization of the silencing process.

(ACMCIP Abstract)

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GENE EXPRESSIONAL CHANGES DURING THE *SCHISTOSOMA JAPONICUM* LIFE CYCLE

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We have utilised online sequence databases to design and construct a 22,575 feature schistosome oligonucleotide microarray. We have used this resource to undertake the largest-scale functional investigation of the schistosome transcriptome performed to date, targeting the Asian species *Schistosoma japonicum* for study. We have identify changes in the transcriptome profiles of *S. japonicum* during the mammalian, free swimming and the molluscan phases of its complex lifecycle. The microarray has allowed the identification of genes associated with many important aspects of schistosome development and differentiation, which may provide insight into new drug / vaccine targets. Microarray data has been correlated with transcriptional fluctuations of known genes to key biological changes in response to the environmental changes schistosomes encounter during the life cycle. Future work will involve the ranking and prioritising of genes of biological relevance that will be expressed and further characterised.

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CHARACTERIZATION OF A P-GLYCOPROTEIN HOMOLOG IN *SCHISTOSOMA MANSONI*

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P-glycoprotein (Pgp), a member of the ATP-binding cassette superfamily of proteins, is an ATP-dependent efflux pump involved in transport of toxins and xenobiotics from cells. In a variety of vertebrate tumor cells, increased expression of Pgp is associated with multidrug resistance. There have also been reports indicating that Pgp may play a role in drug resistance in helminths. Thus, Pgp and other proteins involved in the parasite's excretion of wastes and xenobiotics may provide new targets for antischistosomal agents. Indeed, praziquantel (PZQ), the current drug of choice against schistosomiasis, has recently been shown by others to be a potent inhibitor of mammalian Pgp. Hence, we are examining the relationship between PZQ and Pgp expression in *Schistosoma mansoni*, focusing on SMDR2 (Acc.#L26287), a Pgp homolog from *S. mansoni*. We have monitored expression levels of SMDR2 RNA and anti-Pgp immunoreactivity following exposure to PZQ. Our preliminary studies indicate that exposure of adult male schistosomes to a sublethal concentration (100 nM) of PZQ *in vitro* results in increased levels of SMDR2 RNA. PZQ-dependent changes also appear to be found at the protein level, with a corresponding increase in an anti-Pgp immunoreactive protein following PZQ treatment of adult male parasites. Immunohistochemical labeling showed an increase in prominent anti-Pgp immunoreactivity within the gut and surrounding

the gut. Interestingly, we also find increased levels of SMDR2 RNA and anti-Pgp-immunoreactive protein in adults from an *S. mansoni* isolate with reduced PZQ susceptibility, indicating a possible role for multidrug resistance proteins in development of PZQ resistance. Finally, we are examining the function and pharmacological sensitivity of schistosome Pgp by expressing SMDR2 in CHO cells and using a fluorescence assay (calcein AM) to measure transporter activity. Our results show that SMDR2 functions as a drug transporter, which, like mammalian Pgp, is inhibited by verapamil. This assay will allow us to explore the functional characteristics of this and other schistosome drug/xenobiotic transporters in greater detail.

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TRANSGENESIS OF *SCHISTOSOMA MANSONI* MEDIATED BY MURINE LEUKEMIA VIRUS

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Retroviral transduction of cultured schistosomes offers a potential means to establish transgenic lines of schistosomes and thereby to facilitate the elucidation of gene function and expression. We have been investigating the utility of the Moloney murine leukemia retroviral (MLV) vector pLNHX modified to incorporate EGFP or luciferase reporter genes under the control of endogenous schistosome gene promoters, and virions pseudotyped with vesicular stomatitis virus glycoprotein (VSVG) for transduction of *Schistosoma mansoni*. Schistosomules cultured *in vitro* for one to two weeks after cercarial transformation were exposed to virions of VSVG-pseudotyped murine leukemia virus. Genomic DNA extracted from the transduced schistosomes was analyzed by Southern hybridization and PCR-based techniques to detect integrations of retroviral transgenes into schistosome chromosomes. Southern hybridization analysis using a MLV-specific probe detected the presence of proviral retrovirus in the transduced schistosomes. A PCR-based approach that we term Retrotransposon-Anchored PCR (RAP) recovered fragments of the MLV transgene and flanking schistosome sequences, demonstrating widespread integration of MLV into schistosome chromosomes. Investigation of the transduced schistosomules and extracts of their tissues revealed luciferase reporter transgene expression. These findings indicate the utility of VSVG-pseudotyped MLV virions for transgenesis of *Schistosoma mansoni*, and herald a tractable pathway forward towards germline transgenesis and functional genomics of schistosomes.

(ACMCIP Abstract)

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A CLUSTER-RANDOMISED BOVINE INTERVENTION TRIAL AGAINST *SCHISTOSOMA JAPONICUM* IN THE PEOPLES' REPUBLIC OF CHINA

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Schistosomiasis japonica is a zoonosis of major public health importance in southern China. We undertook a pilot drug intervention study (1998-2003) around the Poyang Lake, Jiangxi Province China, which established proof of principle that bovines, particularly water buffaloes are major reservoirs for human infection in the marshland/lake areas, where one million people are infected. This cluster randomised bovine Intervention trial (2004-2008) aims to be the definitive study to determine the importance of bovines for *Schistosoma japonicum* transmission in Southern China. Specifically, the trial aims are to: (1) Reproduce and validate the results of the pilot drug intervention study on a larger, more rigorous and generalizable scale; (2) Examine the efficacy of bovine chemotherapy on human infection and reinfection rates so as to provide insight into the potential effectiveness of an anti-schistosome vaccine targeting buffaloes; (3) Assess bovine chemotherapy as a plausible schistosomiasis control method, particularly in combination with human treatment; and (4) Integrate the empirical data collected from the study into our previously developed mathematical model of Schistosomiasis japonica. The study involves four matched village pairs in Hunan and Jiangxi Provinces, with a village within each pair randomly selected as intervention (human and bovine praziquantel treatment) or control (human praziquantel treatment only). The primary end point of the trial will be human infection incidences. Here we present the study design, village characteristics and results at baseline. The results of this study will have major implications for the development and deployment of a transmission blocking bovine vaccine against *S. japonicum*. The combination of such a vaccine with other control strategies such as human chemotherapy has the potential to eliminate *S. japonicum* in southern China.

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AN OUTBREAK OF SCHISTOSOMIASIS MANSONICA: IMMUNOLOGICAL STATUS OF ACUTE AND INTESTINAL CASES IN AN ENDEMIC REGION OF BRAZIL

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The present study compares the immunological profile of 19 individuals that were accidentally infected by contact with *S. mansoni* contaminated water with 21 intestinal patients resident in endemic areas of the state of Minas Gerais, Brazil. Stools from the accidentally infected individuals were collected and examined for egg deposition by qualitative (Hoffman) or quantitative (Kato-Katz) method. All the patients that had stools positive for schistosome eggs some days after the infection, reported symptoms as fever, diarrhea, abdominal pain, lost of appetite, headache, wasting and urticaria, did not report past diagnosis of schistosomiasis or contact with suspect water before this outbreak were identified as an acute case.They were submitted to physical and ultrasound (US) examinations. Proliferative responses of PBMC, levels of cytokines in supernatants and intracellular cytokine staining were measured after SEA or SWAP stimulus. Models of multiple linear regressions that included all the variables that were significant in 0.30 levels in univariate analyses and variables whose effects are reported as important in literature were tested to estimate how each variable predicts the cytokine levels while adjusting for the other possible predictors. The individuals examined were between 5 - 50 years old and the infection ranged from 12 to 1812 eggs/g of faeces. The specific *S. mansoni* antigen responsiveness was higher in acute cases. There were no significant differences between SEA or SWAP stimulus. Predominance of IFN-γ and TNF-α production was observed in acute cases indicating the presence of a robust Th1 response in acute human infection. After controlling the other variables in our model the variable that was positively associated with IFN-γ in acute cases after SEA stimulation was IL-10 (β=42; IC95%=15.25-69.89), and…. after SWAP stimulation. The variable associated with TNF-α was egg count (β=0.60; IC95%=0.42-0.87).The models tested were fitted, presented normal residuals distributions and were homoskedastics. Our results suggested that in acute phase patients the low levels of IL-10 and IL-5 were not sufficient to establish regulation

of the Th1 response that is characterized by higher levels of both IFN-γ and TNF-α.

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NOVEL DRUGS FOR SCHISTOSOMIASIS: ESTABLISHMENT OF A MEDIUM-THROUGHPUT WHOLE-ORGANISM SCREEN AT UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO

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Praziquantel (PZQ) is the mainstay drug for treatment and control of schistosomiasis, as recommended by the WHO. Given this reliance on one drug and the recent momentum to increase its availability, PZQ-treatment failure that becomes both clinically relevant and widespread is a worrying prospect. Therefore, the University of California at San Francisco Sandler Center (http://www.ucsf.edu/mckerrow/slide.html) and SMDC (http://smdc.ucsf.edu/about/index.htm) have initiated a medium-throughput screen to identify compounds with anti-schistosomal activity, based on the availability of an in-house *Schistosoma mansoni* life-cycle and growing collections of focused compound libraries. This system utilizes newly transformed schistosomula (NTS), harvested in their tens of thousands from the snail vector, and assayed on a weekly basis in a 96-well plate format. The screen was inaugurated with a collection of 1,998 compounds, including drugs approved for human use, some of which are already off-patent. Our intention is to rapidly identify novel anti-schistosomals, for which pharmacokinetic and toxicological data are known, while minimizing the potential for downstream intellectual property conflicts and production costs. Whereas death is the desired phenotype in screens of protozoal parasites, schistosomes display a variety of deleterious phenotypes, which we have categorized into six groups based on visual assessment by at least two independent screening analysts. Primary screen "hits" are verified for activity in secondary screens and, eventually, for bioactivity against adult parasites *in vitro*. The Sandler Center also maintains animal models of the disease with which the most promising compounds to arise are tested. The results generated from each of these screen components will be made public - the ultimate goal being to make the data freely available through a dedicated database hosted by Collaborative Drug Discovery, Inc. (http://www.collaborativedrug.com).

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CYTOKINE EXPRESSION AND IMMUNOGLOBULIN ISOTYPE PRODUCTION IN PRE-PATENT *SCHISTOSOMA MANSONI* INFECTION

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Using a murine model of *Schistosoma mansoni* infection, it has been demonstrated that schistosomes evade and exploit host CD4+ T cell responses during the establishment of infection. These data suggest that modifying interactions between schistosomes and host T cells might provide a novel approach to interfering with parasite establishment. We hypothesize that the phenotype of the CD4+ T cell response to schistosome antigens during the early stages of infection are implicated in the process by which schistosomes evade immune destruction. To gain further insights into this process, we sought to (i) identify the immunodominant antigens recognized by CD4+ T cells and (ii) further characterize the phenotype of the CD4+ T cell response during the first 4 weeks of infection. Analysis of humoral responses identified a pair of antigens known as Sm31/32 as the dominant targets of the B cell response. Anti-Sm31/32 antibodies were predominantly of the IgG1 isotype, suggesting that CD4+ T cell help for this response is primarily of the Th2 type. To evaluate the significance of this response, infected mice were treated with neutralizing anti-IL4 monoclonal antibody during the pre-patent phase of infection. While IL-4 neutralization had little effect on the establishment of schistosome infection, alterations in the CD4+ T cell response were found. Our results suggest IL-4 serves as a stimulus for B cell responses and an inhibitor of IFN-γ production. Interestingly, the latter effect is largely mediated by IL-10 when IL-4 is neutralized, underscoring the apparent importance of IFN-γ regulation during early schistosome infection.

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COMPARATIVE ANALYZE *BIOMPHALARIA AMAZONICA* AND *B. COUSINI* IN RELATION TO OTHER SPECIES OF THE GENUS, USING MORPHOLOGICAL AND MOLECULAR DATA

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In Brazil, there are ten species and one sub-specie of the genus *Biomphalaria*, being *B. glabrata, B. tenagophila* and *B. straminea* intermediate snail hosts of trematode *Schistosoma mansoni*. The species *B. peregrina* and *B. amazonica* proved susceptible under experimental conditions being considered a potential host of the parasite. The morphological identification of the molluscs of the genus *Biomphalaria* is difficulty mainly by similarity and large intraspecific variation observed in morphological characters used in classical identification of these molluscs. Therefore it is very important the correct identification these species. In order increase the knowledge this potential intermediate host of the *S. mansoni* the aim this project will be get the nucleotide sequence of ITS region of the rDNA and partial sequence of 16S region of the mitochondrial rDNA of the populations *Biomphalaria* original of: Amazonas and Mato-Grosso (Brazil), Letícia (Colômbia) and Santa Cruz (Bolívia) and compare with the sequence got paratypes of *B. amazonica* and *B. cousini* of malacological collection of the Oswaldo Cruz Institution for elucidation of the phylogenetic position this two molluscs in relation to the other species of the genus. At moment, the sequence analyze of ITS2 rDNA ribosomal shown that these two species are similarly and the sequences will be used for comparative analyze phylogenetic.

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF *SCHISTOSOMA MANSONI* PKA: A POTENTIAL NEW DRUG TARGET

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Protein kinases represent novel drug targets for the treatment of diseases caused by eukaryotic pathogens such as helminth parasites. We therefore explored the anti-parasite potential of targeting protein kinase A (PKA) enzymes in *Schistosoma mansoni*. Examination of the *Schistosoma mansoni* genomic sequence database (SchistoDB) identified sequences of four putative PKA genes in the *S. mansoni* genome. Using reverse transcriptase-PCR and RACE, transcripts from two distinct PKA genes were identified in adult *S. mansoni* cDNA, one of which is expressed as two distinct splice variants that utilize different exons at the 5′ end. Western blot analysis of adult *S. mansoni* proteins, using a polyclonal antibody directed against conserved sequences of the PKA α catalytic subunit, identified several protein species with expected molecular weights of PKAs. PKA activity was detectable in adult *S. mansoni* lysates at various nanogram concentrations, confirming that *S. mansoni* worms express

active PKAs. Further, schistosome PKA activity was readily inhibited by commercially available PKA inhibitors. Finally, two PKA inhibitors were shown to have schistosomocidal effects on adult worms *in vitro* at various micromolar concentrations within four to eighteen hours. These data suggest that inhibitors of PKA and perhaps other protein kinases have potential as novel chemotherapeutics for the treatment of schistosomiasis and other helminth infections.

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PREPARATION OF NOVEL VACCINE CANDIDATES AGAINST *SCHISTOSOMA MANSONI*

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Most currently available schistosome vaccine candidates show poor vaccine efficacy. In the past, these were usually identified by using immune sera from animals vaccinated with radiation-attenuated (RA) parasites that recognized immunodominant, but not necessarily protective antigens. In the present study our goal is to identify larval secretory and/or surface proteins that are essential for successful parasite invasion and survival and are principal targets of immune elimination. Previously, a series of such larval molecules were identified by mass-spectrometry of 2D gels and/or by microarray analysis from which eight potential vaccine candidates were selected based on their completeness of sequence and homology to other proteins important for the development and survival of the parasite. Four candidates were cloned and expressed, and three were further purified. Purified molecules include a 38kD annexin present in the lung stage, a 42kD membrane serpin present in the larval and adult stages, and a 15kD protein, which is the most abundantly secreted molecule in the lung stage. All three proteins are recognized by sera from *Schistosoma mansoni* infected humans, and the 42kD serpin and the 15kD protein are both recognized by sera from RA vaccinated baboons. Anti-sera raised against the annexin recognized a protein present in an extract of larval stage parasites. These results indicate that the candidate proteins have been exposed to and are recognized by the host immune system. Studies are currently underway to assess their functional significance and vaccine efficacy in animal models.

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INVESTIGATING THE SOURCE OF IL-10 EARLY IN SCHISTOSOMIASIS INFECTION

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Schistosomes are intravascular helminths that affect approximately 200 million people throughout the tropics and subtropics. Immune responses to helminths are typically dominated by a Th2 type response, characterized by production of IL-4 and IL-13 and suppression of Th1 type responses. The paradigm of the immune response to schistosomes is an early Th1 response, followed roughly six weeks later by a Th2 response upon egg deposition. Here we provide evidence that in addition to producing IFN-γ, $CD4^+$ T cells also produce IL-10 during the early stages of infection. As an anti-inflammatory cytokine that aids in the suppression of host immune responses, we hypothesize that IL-10 production may contribute to creating an immuno-modulatory milieu that is permissive for parasite establishment and development. Currently, we are characterizing the IL-10 producing $CD4^+$ T cells further, to determine if they are T helper cells, T_{reg} cells or natural killer T cells. To establish whether T_{reg} cells are an important source of IL-10, C57BL/6 mice were treated with monoclonal antibodies that deplete T_{reg} cells, including S4B6 (αIL-2) and PC61 (αCD25). T_{reg} depletion was confirmed by FACS analysis and cytokine production by remaining $CD4^+$ T cells was investigated by ELISA. Inhibition of IL-2 signaling in infected mice causes a reduction in IL-10 production and reduces the population of $CD4^+CD25^+Foxp3^+$ T cells in the spleen, indicating $CD4^+CD25^+Foxp3^+$ T cells as a potential source of IL-10. Further, in single sex infections, IL-10 was produced by spleen and liver $CD4^+$ T cells in the absence of egg deposition, showing that IL-10 is independent of egg production. The identity and biological relevance of these IL-10-producing $CD4^+$ T cells is currently under further investigation.

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PROTEOMIC ANALYSIS OF EXCRETORY-SECRETORY PROTEINS RELEASED DURING IN VITRO *SCHISTOSOMA MANSONI* MIRACIDIUM-TO-SPOROCYST TRANSFORMATION

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Penetration of a suitable snail intermediate host by miracidia of *Schistosoma mansoni* is followed by dramatic morphological and, presumably, physiological changes, as the free-swimming larva transforms to the parasitic mother sporocyst stage. During this period of transformation, excretory-secretory proteins (ESP) are released into the medium and represent an initial source of antigenic materials presented to the snail host. At present, little is known regarding the composition of this larval ESP, its tissue origin within the miracidium and how these proteins interact with the host immune system. To address these questions, we have initiated a proteomic analysis of *S. mansoni* transformation ESP using 1- and 2-D SDS-PAGE in combination with LC-MS/MS analyses. In addition, a polyclonal antibody to whole ESP was used to immunolocalize ESP within *in vitro* cultured larval stages. MS analysis of peptides from digested 1-D PAGE-separated gel slices revealed the presence of numerous presumptive proteins with significant Mascot scores (>79; $P \leq 0.05$). These included various soluble egg antigens (secretory glycoprotein k5, p40), metabolic enzymes (PEPCK, malate dehydrogenase, fructose phosphate aldolase, TPI), signaling-related proteins (calpain, phosphodiesterase interacting protein, 14-3-3 epsilon, HSP70), an antioxidant thioredoxin peroxidase. a venom-allergen-like protein, an albumin and an albumin precursor. Although representing a subset of total ESP, analysis of selected 2-D PAGE-separated spots confirmed the presence of presumptive HSP70, PEPCK, secretory glycoprotein k5, and p40 egg antigen. Additional peptides revealed by 2-D analysis include SME16 (16-kDa egg antigen), HSP60, enolase, and keratin. The keratin most probably represented a human hair contaminant, while recent evidence (DeMarco et al., 2007) suggests that the albumin also may be a contaminant from mouse serum that had bound to miracidia *in ovo* and retained throughout the larval isolation and cultivation process. The anti-ESP antiserum reacted in Western blot analyses with multiple bands of similar molecular mass in both whole miracidial and ESP samples, Interestingly, confocal immunofluorescence of Triton-permeablized miracidia revealed immunostaining only within ciliated epidermal plates, suggesting that the majority of immunoreactive ESP released during transformation is of plate origin.

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ANTI-TREMATODE PARASITE RESPONSES OF THE SNAIL *BIOMPHALARIA GLABRATA:* ARCHITECTURE OF *FREP* LOCI

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The snail *Biomphalaria glabrata* reacts to infection by digenetic trematodes with increased expression of fibrinogen-related proteins (FREPs). FREPs are hypothesized to function in non-self recognition by binding parasites and precipitating parasite-derived molecules. This response is interpreted

as part of a "best effort response" by the snail aimed at immuno-elimination of parasites, whether it ultimately results in success or failure (in susceptible snails). FREPs are remarkably diverse; this likely increases the non-self recognition repertoire. *FREP* genes are thought to diversify somatically by recombinatorial processes and point mutations. Towards further understanding of the diversification mechanism, we investigated the arrangement of *FREP* gene loci in the genome of *B. glabrata.* Southern analysis indicated that the *B. glabrata*-derived genomic insert of bacterial artificial chromosome (BAC) clone 0125N01 contains multiple FREP genes. Sequencing (subcloning and primer walking) revealed clustering of 4 *FREP* genes within a ~120 kbp region of the *B. glabrata* genome. Surprisingly, 2 *FREPs* displayed identical 5' sequences while the 3' termini differed considerably. This pattern and the clustering of loci concord with the notion that sequence exchanges may contribute to *FREP* diversity. Analysis of intergenic regions of clustered FREP genes for consensus regulatory sequences may provide insights into the regulation of transcription of FREPs.

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THE ACQUISITION OF INVASION INHIBITORY ANTIBODIES AND ANTIBODIES TO ERYTHROCYTE INVASION LIGANDS OF *PLASMODIUM FALCIPARUM*

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Increasing morbidity and mortality of *Plasmodium falciparum* malaria highlights the necessity for the development of control measures against this disease. Progress towards an effective vaccine against blood stage infection has been hindered by a lack of understanding of the key effectors and targets of protective immunity. Antibodies play a key role in blood stage immunity and antibodies that inhibit merozoite invasion of erythrocytes, by targeting functional epitopes of invasion ligands, are thought to be important in acquired and vaccine-induced immunity. However, there is limited knowledge of the acquisition of invasion-inhibitory antibodies and their specific targets. We investigated acquired *P. falciparum* antibodies amongst a cohort of 50 adults and 100 children, and a second cohort of 300 children (<10 y) in a malaria-endemic area of coastal Kenya. Antibody growth inhibition assays were performed using defined parasite clones, complemented by conventional immunoassays to measure antibodies specific for merozoite antigens involved in erythrocyte invasion. We evaluated the effect of age, parasitemia, malaria episodes, and malaria transmission intensity on the acquisition of antibodies. Results demonstrate that invasion-inhibitory antibodies show a different pattern of acquisition to antibodies against merozoite antigens, which has implications for understanding and measuring functional immunity. Inhibitory antibodies can be acquired at an early age but do not show strong evidence of boosting with re-exposure or concurrent infection, often observed with other antibody measures. Antibodies to recombinant antigens were generally poorly predictive of invasion-inhibitory activity, but some antigen-specific antibodies were significantly associated with inhibitory activity. These findings have significant implications for defining the mechanisms and targets of acquired immunity and for identifying correlates of immunity to aid vaccine development.

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TWO LONGITUDINAL COHORT STUDIES INVESTIGATING MECHANISMS OF INNATE AND ACQUIRED IMMUNITY TO MALARIA IN CHILDREN FROM HIGHLY ENDEMIC REGIONS OF PAPUA NEW GUINEA

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We have undertaken two prospective longitudinal cohort studies investigating the association of immunological parameters with altered risk of malaria infection and disease. A longitudinal treatment/time-to-reinfection study of 206 Papua New Guinean (PNG) elementary school children (5-13 years) was undertaken in Madang province over 12 months, to examine immunological risk factors for re-infection and symptomatic mild malaria caused by different Plasmodium species. Age-dependent acquisition of immunity was evident across the cohort. By 9 years of age, children appeared to have acquired almost complete clinical immunity to *Plasmodium vivax*, whereas the acquisition of immunity to *P. falciparum* malaria remained incomplete, suggesting that different mechanisms of immunity may be important for protection against the two species. A longitudinal cohort study of 200 pre-school children (1-4 years) is being conducted over 18 months in the Sepik province of PNG, highly endemic for all four human malaria Plasmodium species. This study combines a powerful longitudinal design and active case detection with PCR for speciation of every infection, enabling the investigation of cross-species protection against infection and symptomatic malaria. The younger aged cohort allows the investigation of immune responses to malaria during the critical period at risk for disease. In both studies, peripheral blood mononuclear cells drawn at baseline and longitudinally were subject to *in vitro* stimulation by malaria parasites. An array of cytokine/chemokine responses were measured and cellular source(s) identified by FACS-based intracellular cytokine staining and surface phenotyping. Prospective analyses are being undertaken to investigate the association of these immune parameters with parasite densities and subsequent risk of malarial infections and morbidity (fever, splenomegaly and anaemia), thus providing insights into immunological risk factors for disease and the acquisition of immunity to malaria in PNG children aged 1-13 years.

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THE RATE OF ACQUISITION OF HUMAN ANTIBODY ISOTYPE PROFILES TO *PLASMODIUM FALCIPARUM* BLOOD STAGE ANTIGENS IN GAMBIAN INFANTS

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Antibodies are a major component of the human immune response against the invasive blood stage form of *Plasmodium falciparum,* the merozoite, helping to prevent the development of clinical disease. Understanding the period during which infants lose their maternally derived antibodies and begin to acquire their own natural immune responses against parasite antigens is very crucial for malaria vaccine development and will inform the time for vaccine administration since children are the target group. This study therefore aimed to investigate the rate of acquisition of antibody isotypes (IgG1, IgG2, IgG3, IgG4, IgM and IgA) to blood stage antigens (AMA1, MSP1-19, MSP2-ch150/9, MSP2-Dd2, MSP3-3D7 and MSP3-K1) in a birth cohort of 53 children from Sukuta in the Gambia. The Sukuta Birth Cohort were followed up from

birth to 18 months with the collection of blood samples at birth, 0 (cord blood), 4 months, 8 or 9 months and 18 months. The indirect ELISA was used to determine antibody isotypes reactivities to blood stage antigens. Our results showed that antigen-specific maternally derived antibodies were isotypes IgG1, IgG2, IgG3 and IgG4 and these were depleted by 4 months in the first year of life. In the infant, acquisition of specific antibody isotypes to these antigens begins from birth with IgM and IgA, whereas IgG1, IgG2 and IgG4 specific for these antigens was generally acquired between 4 and 8 months. IgG3 acquisition against all the blood stage antigens studied occurs after18 months. This is the first study to monitor the acquisition of IgG subclass antibodies against these blood stage antigens. The implication of these findings to malaria erythrocytic stage vaccine development is further discussed.

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MULTIPLEXED MEASUREMENT OF EPSTEIN BARR VIRUS, CYTOMEGALOVIRUS AND *PLASMODIUM FALCIPARUM*-SPECIFIC ANTIBODIES USING THE LUMINEX SYSTEM

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Malaria, and in particular *Plasmodium falciparum*, is thought to modulate human immunity as a means to evade detection and thus persist in asymptomatic carriers. Malaria-induced immune down-regulation has been shown to have significant effects on the control of viral co-infections. To better understand how malaria affects immunity to viral pathogens, longitudinal field studies in children are needed. However, a comprehensive analysis of antibody responses has been limited by the small sample volumes obtained from children. Recent technological advances using the Luminex bead-based suspension array could circumvent this limitation. We developed a Luminex assay to measure IgG levels to several *P. falciparum* (AMA-1, MSP-1, LSA-1, CSP) and viral antigens (EBV -VCA, -EBNA1, -EA, -Zta and CMV whole protein). The assay required less than 2 microL plasma from each sample to measure all antigens, and was found to be specific, using *P. falciparum* unexposed US study participants and EBV or CMV seronegative study participants as negative controls. Furthermore, there was a good correlation with standard ELISA (for all antigens, Spearman's correlation 0.752-0.983, $p < 0.005$) and we could differentiate between responses against *P. falciparum* strains 3D7 and FVO within one sample. Interestingly, in a small cross-section of 20 subjects, CMV-specific IgG was elevated [median 2340 vs. 266 fluorescent units, $p<0.05$, Mann-Whitney test] and EBV EA titers tended to be elevated [median 608 vs. 197 fluorescent units, $p=0.063$] in Kenyan vs. US study participants, suggesting effects of malaria exposure on herpesviruses humoral immunity. We are currently analyzing the data from a cross-section of 300 Kenyan children with divergent exposure to *P. falciparum*. In conclusion, the multiplexed bead-based array is a very promising tool in order to unravel the impact of repeated *P. falciparum* infections on the control of viral infections, in the setting of longitudinal field studies.

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IMMUNOGENICITY OF A MULTI-STAGE, MULTI-ANTIGEN ADENOVIRUS-VECTORED *PLASMODIUM FALCIPARUM* MALARIA VACCINE

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An effective vaccine against malaria is likely to incorporate multiple antigenic targets administered with a delivery platform that induces both T cell and antibody responses against the multiple targets. We are currently evaluating the immunogenicity in humans of an adenovirus-vectored malaria vaccine expressing two *Plasmodium falciparum* antigens, PfCSP (expressed in sporozoite and liver stages) and PfAMA1 (expressed in sporozoite, liver and erythrocytic stages). The vaccine, designated as NMRC-M3V-Ad-PfCA, and developed by the Naval Medical Research Center in collaboration with GenVec Inc. and the US Agency for International Development, is designed to induce both cellular and antibody responses in vaccinees, and to confer protection against sporozoite challenge by the simultaneous induction of immunity to pre-erythrocytic and erythrocytic stages of the parasite. The capacity of the adenovectored vaccine to induce antigen-specific immune responses in humans and the kinetics of these immune responses are being assessed by indirect fluorescent antibody test (IFAT) against sporozoites and blood stage parasites, ELISA against recombinant proteins and/or synthetic peptides derived from the PfCSP and PfAMA1 antigens, cytokine ELIspot assays and intracellular cytokine staining (ICS) assays. Immunogenicity data from various time points during the study will be reported.

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IMMUNOLOGICAL STUDIES OF *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN 1, MSP1-33 AND ITS POTENTIAL INFLUENCE TOWARD MSP1 VACCINE DESIGN

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Fragments of the *Plasmodium falciparum* Merozoite Surface Protein 1 (MSP1) are leading vaccine candidates against erythrocytic malarial parasites. Previous research has shown that the processed MSP1-42 and MSP1-19 fragments have the capacity to produce a protective antibody response. MSP1-33, the N-terminal processed fragment of MSP1-42, has also been shown to have relevance in vaccine development, since anti-MSP1-33 antibodies have been shown to enhance the inhibitory activities of anti-MSP1-42 antibodies. However, little is known of the relevant epitopes on MSP1-33 and their antigenicity and immunogenicity. In this study we investigated the antibody responses elicited during natural *P. falciparum* infections toward the C-terminal MSP1 processed fragments. Of the C-terminal MSP1 antigens tested, MSP1-33 was found to have the lowest antibody recognition after exposure from single or multiple malarial infections. This trend reflects what was previously observed in the vaccine-induced responses. A single infection by parasites carrying one allelic form of MSP1-33 produced anti-MSP1-33 antibodies that were equally reactive with heterologous MSP1-33. The results suggest that although anti-MSP1-33 antibodies are beneficial, active immunizations will be needed to elicit a biologically relevant antibody response in malaria exposed human populations. As MSP1-19 lacks adequate T helper epitopes, the presence of T cell epitopes on MSP1-33 that would provide functional help in

inducing antibody responses was also investigated. To this end, out-bred Swiss Webster mice were vaccinated using three recombinant subunit proteins consisting of truncated segments of MSP1-33 linked to MSP1-19; with MSP1-19 serving as a control. These truncated subunits were also used in rabbit immunizations to test for the induction of parasite inhibitory antibodies. The ability of MSP1-33 specific sub-fragments to broaden the antibody response in out-bred populations and to induce parasite inhibitory antibodies will be presented.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE ON THE DEVELOPMENT OF IMMUNE RESPONSES TO *PLASMODIUM FALCIPARUM* IN MOZAMBICAN CHILDREN

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The aim of the study was to evaluate the impact of Intermittent Preventive Treatment in infants (IPTi) with sulfadoxine-pyrimethamine (SP), given through the Expanded Program of Immunizations, on the development of immune responses to *Plasmodium falciparum* in Manhiça, Mozambique. Antibody and cytokine responses were analyzed at 5, 9, 12 and 24 months of age in 240 children receiving SP or placebo at ages 3, 4, and 9 months. IgM, IgG, IgG1, IgG2, IgG3 and IgG4 antibodies specific for PfMSP-1_{19}, PfAMA-1 and PfEBA-175 were measured by ELISA, and IgG antibodies specific for *P. falciparum* blood stage antigens were assessed by indirect immunofluorescence antibody test (IFAT). The presence of *P. falciparum* infection at 5, 9, 12 and 24 months was determined by PCR. We found no significant differences in IgG IFAT titers between the SP and the placebo groups in either of the time points. Similarly, IgG and IgM responses to recombinant proteins did not significantly differ between children receiving IPTi with SP or placebo, at any of the cross-sectional visits, with the exception of IgG responses to PfAMA-1, that were significantly higher in children who received SP compared to those who received placebo at age 5 months. IgG subclass responses, predominantly of the cytophilic IgG1 and IgG3 isotypes, did not significantly differ between children receiving IPTi with SP or placebo for most of the cross-sectional visits. In some cases, levels of cytophilic IgG1 antibodies to PfAMA and PfMSP-1_{19} were significantly higher in the SP than in the placebo group, and there was an age-related build up of responses. When the antibody analyses were controlled for possible confounders, there was a significant positive association between previous and present malaria episodes and antibody levels; however, antibody results according to treatment did not differ from those obtained in the crude analysis. When children were stratified according to past and present malaria status, IgG and IgG1 to some antigens were significantly higher after IPTi with SP compared to placebo at some time points. Finally, preliminary analysis indicates that levels of cytokines were not significantly different between children receiving SP or placebo. In conclusion, IPTi with SP does not negatively affect the development of immune responses to *P. falciparum* antigens and, in some cases, it appears to be associated with higher antibody levels.

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CYTOKINE GENE AND PROMOTER POLYMORPHISMS IN HUMAN SCHISTOSOMIASIS MANSONI

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The most agreed upon immunologic findings that correlate with resistance in human schistosomiasis are high levels of IgE and low levels of IgG4. Genes that encode cytokines associated with regulation of these isotypes contain gene and promoter polymorphisms linked to producing high and low levels of the cytokines in other systems, but have not been studied in schistosomiasis. Our group studies a cohort of men occupationally exposed to schistosomiasis in western Kenya. Drawing on this cohort, polymorphisms in IL-4 (-590T high IgE; intron dinucleotide repeat associated with severe asthma), IL-13 (-1055T high producer; +130Gln high serum IgE), IL-10 (-1082A/-819T/-592A in which GCC/GCC high producers; GCC/ACC and GCC/ATA intermediate producers; ACC/ACC, ACC/ATA, and ATA/ATA low producer, IFN-γ (+874A high producer), TGF-β 10 T/C and 25 C/G (regulatory cytokine and IgA isotype switching) and TNF-α -308 A/G (role in severe morbidity) have been genotyped and will be analyzed in relation to resistance and susceptibility, as well as severe morbidity. Genotypic influence on schistosome antigen-induced cytokine and anti-schistosome antigen antibody isotype levels are being evaluated. To this point, the literature-based IL-10 genotypic high responders, indeed, do produce higher levels of IL-10 to both phytohemagglutinin and soluble worm antigen preparation. Also, several correlates between antibody isotypes and given cytokine responses have been seen, such as a direct correlation between SWAP induced IFN-γ responses and anti-SWAP IgG3 levels ($p = 0.0037$). Other such relationships will be sought and reported on with subsequent analyses between resistance and susceptibility and morbidity at the genotype, immunophenotype and infection/disease levels.

(ACMCIP Abstract)

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INVESTIGATIONS INTO THE UPTAKE AND ROLE OF IRON IN SCHISTOSOMES

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Iron (Fe) is an essential factor in a wide range of redox reactions that occur in nearly all organisms. Fe-containing proteins catalyse vital reactions associated with oxygen and electron transport, energy transduction, folate metabolism, nucleic acid synthesis and detoxification. In addition to these roles, some invertebrates also use the metal to stabilize extracellular matrices and poly-protein complexes. Fe is essential for growth of many single-celled parasites and pathogens and for many species is a virulence factor. Schistosomes appear to have extensive requirements for the element. The adult parasites live in and feed on host blood, where they process and egest Fe-rich by-products of hemoglobin digestion. Cells of the vitelline follicles of schistosomes express an isoform of the cytoplasmic Fe-storage molecule ferritin and are major store of the element. There are two possible sources of Fe for schistosomes in their vascular niche. The parasites may obtain the element from Fe-carriers in host serum, or by stripping Fe from heme in the gastrodermis. This latter hypothesis seems unlikely, as there are no robust data supporting the presence of heme catalytic pathways in schistosomes or other helminth parasites. Evidence is presented for the likely sources of Fe acquisition in adult schistosomes and the potential role of members of the highly conserved divalent metal transporter family (DMT1 or NRAMP2 families) in Fe transport and acquisition will be discussed. Energy dispersive spectroscopy and mass spectroscopy performed on purified egg-shells of schistosomes revealed elevated levels for Fe in the egg-shell matrix of these parasites.

We hypothesise that a major role for Fe is in stabilization of the egg-shell matrix in a manner to that used by free-living invertebrates. We are currently investigating the effects of Fe depletion on schistosomes as a means to further elucidate the function of this molecule in parasite homeostasis and reproduction and to determine whether there is therapeutic potential in disruption of Fe metabolism in these parasites.

(ACMCIP Abstract)

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BLOOD FLUKE EXPLOITATION OF INNATE-ADAPTIVE IMMUNE INTERACTIONS TO FACILITATE PARASITE DEVELOPMENT

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Paradoxically, *Schistosoma* larvae exploit CD4+ T cells to facilitate their development into egg-producing adult worms. Our recent studies have focused on the mechanism by which CD4+ T cells mediate this effect. While T cell homeostasis mediated by IL-7 and by IL-2 appear to be critical, neither of these cytokines affect parasite development directly. Indeed, no role has been identified for any of the major effector cytokines produced by CD4+ T cells. We now show that conventional antigen-specific activation of CD4+ T cells is not required for schistosome maturation and development. Further, we show that the presence of CD4+ T cells, with or without concomitant TCR activation, alters the immune environment at the site of parasite development in the liver by modulating the expression of genes associated with APC function and the acute phase response. Consistent with a potential role for the acute phase response in schistosome development, we show that acute phase responses are induced by *S. mansoni* in the presence of CD4+ T cells. Finally, we show that the requirement for CD4+ T cells in schistosome development can be bypassed by inducing acute phase responses directly; demonstrating that the role of CD4+ T cells in this process is indirect and that schistosomes exploit the innate immune response to facilitate their own development.

(ACMCIP Abstract)

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ASSOCIATION BETWEEN PRETREATMENT CYTOKINE PRODUCTION AND INTENSITY OF INFECTION AND RESISTANCE TO REINFECTION IN HUMAN SCHISTOSOMIASIS MANSONI

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Evidence suggests that humans can acquire protective immunity to schistosome infection. Our group is currently conducting a 5-year longitudinal study designed to examine the changes in immune responses that correlate with the development of this resistance. Adult males exposed to *Schistosoma mansoni* by washing cars in Lake Victoria near Kisumu, Kenya, are followed over several rounds of infections, cures, and reinfections. Exposure is documented by tallying the number of cars each man washes. We now present baseline data on initial enrollees of this cohort. Upon enrollment, subjects' mean egg count was 721 eggs per gram (range 0-4786). Low egg counts were correlated with high responses to schistosome egg antigen (SEA) by IL-13 (R=-0.45, p=0.0138) and IFN-γ (R=-0.56, p=0.0004) and high responses to soluble worm antigen (SWAP) by IFN-γ (R=-0.40, p=0.0135). Egg count was also negatively associated with responses to PHA by IL-13 (R=-0.44, p=0.0162) and IFN-γ (R=-0.70, p=<0.001). Thus, if lower worm burden is an indicator of resistance to schistosome infection, protection correlated with both Th1- and Th2-type immune responses to both crude antigens and mitogens. Intensity of infection was not associated with IL-10 production in response to any of the studied antigens or mitogens. However, measuring resistance in another manner, high pretreatment IL-10 responses to SEA (R=0.71, p=<0.001), SWAP (R=0.55, p=0.0042) and anti-CD3 (R=0.44, p=0.0275) were associated with increased number of cars washed until *S. mansoni* reinfection following cure of the initial infection. We conjecture this correlation of IL-10 production with resistance could be due to IL-10 down regulation of a mechanism that contributes to susceptibility. Baseline levels of IL-5, IL-13, and IFN-γ did not predict resistance measured by cars washed until reinfection. However, most of these untreated subjects were relatively susceptible at this initial timepoint. We predict subjects will develop resistance upon follow-up and their cytokine production patterns will change and correlate with change in resistant status.

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ROLE OF CD4+ T CELL RESPONSES DURING EARLY SCHISTOSOME INFECTION

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Schistosome infection leads to significant morbidity and mortality, associated primarily with the production of eggs by schistosomes. The pathology caused by egg deposition is mediated by CD4+ T cell responses to egg antigens, which are heavily biased towards a Th2 phenotype. However, CD4+ T cell responses that occur earlier in schistosome infection, before the fifth or sixth week of infection, are also of considerable interest because of their potential ability to kill migrating schistosomula and mediate protection against infection. For this reason, we initiated studies to better characterize the early CD4+ T cell responses to schistosome worm antigens in greater detail. Interestingly, CD4+ T cells from livers of 4 week infected mice expressed predominantly IL-10 mRNA and protein. IL-10$^{-/-}$ mice showed that IL-10 is responsible for regulating IFN-γ levels, but not IL-4, in early infection We also report that disruption of other regulatory mechanisms, such as adenosine 2A receptor signaling, leads to the dysregulation of CD4+ T cell responses to worm antigen, and that this dysregulation is mediated directly at the level of the CD4+ T cells. Our results indicate that early schistosome infection induces a regulatory response in the host, possibly allowing *S.mansoni* worms to evade the immune response and establish a chronic infection. Further, these findings suggest that overcoming regulatory responses in early infection could lead to development of more effective vaccines for preventing schistosome infection.

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DISPARATE IMMUNOREGULATORY POTENTIALS FOR CD4-CD8- α/β AND γ/δ T CELLS FROM CUTANEOUS LEISHMANIASIS PATIENTS

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This study has a goal of understanding the role that CD4$^-$CD8$^-$ (double negative - DN) T cells may have in the development of protective and pathogenic immune responses in human leishmaniasis. We performed a detailed study characterizing the activation state, cytokine profile, and TCR repertoire of this subpopulation in a group of well-defined, cutaneous leishmaniasis patients. The data demonstrate that with respect to Vβ

usage and their activation state, DN T cells express a distinct profile as compared to both CD4 and CD8 T cells from the same patients when analyzed *ex vivo* and after culture, suggesting that different sets of antigens influence the activation of the DN T cells in human cutaneous leishmaniasis. Strikingly, on average, 75% of DN T cells from cutaneous leishmaniasis patients expressed the α/β T cell receptor, with the remainder expressing the γ/δ receptor, while healthy donors displayed the opposite distribution with ~75% of the DN T cells expressing the γ/δ TCR. DN α/β T cells from cutaneous leishmaniasis patients are compatible with previous antigen exposure, recent activation and present a pro-inflammatory cytokine profile (high IFN-γ and TNF-α) while DN γ-δ T cells express a regulatory profile exemplified by IL-10 production. Further studies to determine the antigen specificity and antigen presenting molecule (CD1 vs. MHC I or II) are being carried out. These results demonstrate important differences concerning the populations that make up DN T cells from leishmaniasis patients, providing further insights into their potential immunoregulatory function.

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GBV-C VIREMIA INFLUENCE DTH RESPONSE TO *LEISHMANIA*

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GB virus type C (GBV-C) appears to promote a type 1 immune response and is associated with prolonged survival in HIV-infected people. *Leishmania chagasi* causes a spectrum of illnesses that vary from severe potentially fatal visceral leishmaniasis to self-resolving infection. Individuals with the latter outcome develop a positive DTH response and are protected against symptomatic disease. To determine whether GBV-C viremia might influence the outcome of *Leishmania* infection, we characterized GBV-C status in a cohort of subjects residing in a *L. chagasi* endemic area in Brazil. GBV-C viremia was more prevalent in blood donors from urban than periurban regions of Natal, Brazil (16% and 7.5% respectively, p=0.0028). Evidence of prior GBV-C (anti-E2 antibodies) was detected in 24% and 12% of these groups respectively (p=0.0011). Anti-E2 increased with age (p=0.0121). No difference in GBV-C viremia was found in the DTH+ and VL groups (p=0.269). However, subjects with visceral leishmaniasis were more likely to have anti-E2 response, indicating clearance of viral infection, than DTH+ subjects (p=0.0012). The size of DTH induration was significantly smaller in subjects with E2 antibodies (4.5 mm) compared to those without (7.12 mm) (p=0.002). Furthermore, the size of the Leishmania DTH response was greater in GBV-C viremic subjects (6.8 mm) compared to non-viremic subjects (3.3 mm; p=0.0054). These findings suggest that GBV-C virus may promote a type 1 immune response that could influence the development of a curative type immune response, potentially influencing the outcome of Leishmania infection.

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NEUTROPHILS DOMINATE THE EARLY INFLAMMATORY RESPONSE DURING ACUTE INTRADERMAL INFECTION WITH *LEISHMANIA CHAGASI*

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Leishmania chagasi establishes long-term infection in susceptible mice. Macrophages are the primary cellular hosts for *L. chagasi* in infected mouse livers and spleens. Much less is known about the acute immune response at the dermal site of infection with *L. chagasi*. We used a model of intradermal ear infection of BALB/c mice with *L. chagasi* metacyclic promastigotes to investigate the local response. Infiltrating cells in ear dermal sheets and draining lymph nodes were studied by multi-color flow cytometry. Neutrophils were shown to be the primary cell type recruited to the site of infection during the early inflammatory response, peaking at 6 hours. Neutrophil recruitment was restricted to the infected ears. NK cell recruitment was observed 24-72 hrs after infection and B cell expansion began after 72 hours in the draining lymph nodes. The early neutrophil recruitment was dose dependent and using CFSE-labeled parasites, neutrophils were shown to internalize parasites in the dermal tissue. We investigated whether parasite factors promote neutrophil recruitment using EZ-TAXIScan chemotaxis assay to study chemotactic factors. *L. chagasi*-conditioned media exhibited potent chemotactic activity for human neutrophils. The chemotactic factor was released by log and stationary phase promastigotes, and by axenic amastigotes into extracellular medium. We hypothesize that the first inflammatory cell type at the site of *Leishmania* infection is the neutrophil, attracted by released parasite-derived factors.

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DETERMINATION OF CANINE IMMUNE DEFICITS WHICH PREDISPOSE TO INFECTION WITH *LEISHMANIA INFANTUM*

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Leishmaniasis is a vector-borne disease caused by the protozoa *Leishmania*. Infection with *L. infantum* causes fatal visceral leishmaniasis in dogs globally. After 2000 Leishmaniasis was found to be endemic in foxhounds within United States. Since then numerous foxhounds have tested positive, leading to an average 5% seropositivity in all foxhound hunts. There is concern for spread of *Leishmania* to other dogs and humans, as presence of infected dogs is the primary risk factor for disease in endemic locations. Vector transmission has not been identified in the U.S.; instead we have identified a primary means of transmission to be vertical from bitch to pup. This unique means of infection is likely to predispose foxhounds vs. other breeds to infection and leads to an inability of infected dogs to mount a productive adaptive immune response due to loss of *L. infantum*-specific T and B cells during development. Importantly, vertical transmission may make serology, the most common method of testing for this disease, quite inappropriate as a stand-alone test for the current strain of Leishmaniasis in the U.S. We have developed a highly sensitive and specific real-time PCR based assay to detect *L. infantum* infection in canine blood prior to seroconversion. This assay is based on amplification of parasite kinetoplast DNA. This disease provides a canine model system to analyze cell-mediated immunity to vertically transmitted pathogens and highlights that serology may be grossly underestimating actual disease prevalence of canine Leishmaniasis in this country. Our studies determine whether presence of parasites during gestation alters the ability of the canine immune system to mount a productive immune response to *L. infantum*, predisposing foxhounds to this disease.

(ACMCIP Abstract)

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OLIGOCLONAL EXPANSIONS AMONG SPECIFIC Vβ EXPRESSING T CELLS IN HUMAN CHAGAS DISEASE

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Human infection with *Trypanosoma cruzi* leads to Chagas disease, which affects approximatelly 18 million people in Latin America. It is estimated that over 100 million people are currently at risk of infection. Chagas

disese presents as several different clinical phenotypes ranging from an asymptomatic form to a severe dilated cardiomyopathy that is the major cause of morbidity and mortality associated to this disease. Several groups have demonstrated that T cells play a critical role in cardiac pathology as well as in immunoregulation during chronic disease. However, the mechanisms involved in T cell activation are not completely understood. We have previously shown that $CD4^+$ T cells from chagasic patients preferentially express T cell receptor (TCR) β-chain variable region (Vβ) 5. Moreover, $CD8^+$ T-cells expressing Vβ17 were expanded upon *in vitro* stimulation with parasite-derived antigens. The aim of this work was to determine whether these expansions were composed of oligoclonal *versus* polyclonal T cell responses. Strikingly, CDR3 junctional region sequencing of Vβ5.1 expressing $CD4^+$ T cells revealed oligoclonal expansions composed of a homologous CDR3 with conserved TCR Jβ region usage among different patients with cardiac but not indeterminate Chagas disease, suggesting that these T cells may be activated by a common dominant antigen and may be involved in disease pathology. Although not as striking as seen in $CD4^+$ T cells a CDR3 motif was also present in $V\beta17^+$ $CD8^+$ T cells from chagasic patients. These results provide additional insight into the involvement of specific $CD4^+$ and $CD8^+$ T cells in Chagas disease and will direct future studies designed to determine the antigen(s) involved in the induction of this dominant T cell response.

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COMPARISON OF IMMUNE RESPONSES DURING LEISHMANIASIS THERAPY

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Sodium stibogluconate (SSG) treatment of cutaneous leishmaniasis may work by modulating the host immune response. Immunity to leishmaniasis likely involves development of T cell effectors, T cell memory cells (Tcm) and possibly T regulatory cells (Treg). This clinical trial comparing systemic SSG to a localized skin heat treatment allowed evaluation of the immune response at pre-, on-, and 6 months post-treatment. 54 military patients with cutaneous *Leishmania major* (Lm*)* infection were randomized to single treatment thermotherapy (day 1) using a Thermomed™ device (TM) or 10 days of intravenous SSG. On days 0, 10 and 180, peripheral blood mononuclear cells (PBMC) were obtained. T lymphocyte subsets were analyzed on days 0 and 10. Various flow cytometry panels were used to monitor phenotypic trends at all time points. PBMC were frozen for longitudinal analysis. Lymphocyte proliferation assays (LPA) and cytokine analysis of bulk and CD8 depleted PBMC were obtained for each group. The cohort included 39 evaluable participants with cells at all time points; 20 received SSG and 19 received TM. The median CD4% dropped from 45.5 (day 0) to 44 (day 10), p=0.04 in the SSG arm with no change in the CD4% in the TM arm. There was no change in CD8% in either treatment arm. Soluble Leishmania Antigen (SLA) stimulation of bulk PBMC showed significant drops in both median lymphocyte stimulation index (LSI, 50 to 29, p=0.001) and cytokine production (IFN-γ, TNF-α) comparing pre- to post-treatment, but when CD8 cells were depleted, the LSI was unchanged. Despite unchanged LSI in CD8 depleted cells, significant decreases remained in cytokine production. Significant decreases in $\%CD25^+/CD4^+$ T cells were seen comparing the pre- to post-treatment group, (17.8 ± 1.0 v 12.5 ± 1.1%), p=0.0001.In conclusion, protective immunity to leishmaniasis involves changes in T cell populations. We report changes in T cell number and function in peripheral blood samples at different time points before, on or after treatment. Further studies are underway to assess the role of Tcm and Treg subsets in this cohort.

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MOLECULAR IDENTIFICATION OF THE GREGARINES OF PHLEBOTOMINE SAND FLIES

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Phlebotomine sand flies vector the protozoan parasite *Leishmania*. They are naturally infected with gregarines, an apicomplexan protozoan parasite. The low level of gregarine infection in nature apparently does not affect sand fly populations however, in laboratory colonies the gregarine parasites may build up to levels where reproductive capacity is severely reduced. Ascogregarina chagasi is described from Lutzomyia longipalpis, an important new world *Leishmania* vector. Previous to this study morphological descriptions and the basic life cycle has been investigated but this parasite has not been examined using molecular tools. We carried out a study using the small-subunit (SSU) rDNA sequence for molecular characterization of the parasite and to find the genetic relationship of this isolated gregarines with other closely related gregarines. DNA was extracted from isolated parasites, PCR amplified and cloned. Phylogenetic reconstruction using maximum likelihood analysis and Bayesian interference suggest that this gregarine parasite might be more closely related to neogregarine than to the eugregarine family Lecudinidae where it has been placed on the basis of morphology. We isolated another undescribed gregarine from *Phlebotomus sergenti* that also exhibits sequence similarity to neogregarines. This opens a new controversy in sand fly gregarine systematics in the light of phylogenetic context. More morphological and molecular studies are needed on the sand fly gregarines in order to understand their phylogenetic status.

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GENETICALLY-ATTENUATED *PLASMODIUM BERGHEI* LIVER-STAGES INDUCE STERILE PROTRACTED PROTECTION THAT IS MEDIATED BY MHC CLASS I-DEPENDENT IFN-γ PRODUCING CD8+ T CELLS

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A vaccine against malaria is desperately needed and promising vaccines are being tested in endemic areas. Presently, radiation-attenuated *Plasmodia* sporozoites (γ-spz) constitute the only vaccine that induces sterile and long-lasting protection in malaria-naïve humans and laboratory rodents. However, radiation-attenuated parasites are not without risks, some of which relate to the heterogeneity of the γ-spz population, which could explain occasional break-through infections. Therefore, genetically-attenuated parasites might serve as an alternative and improved vaccine strategy. We have constructed a genetically-attenuated *P. berghei* parasite in which two genes, *UIS3* and *UIS4,* upregulated in infective sporozoites, were deleted, thus creating a double-knock-out *Pbuis3(-)/4(-)* parasite. In this study we evaluated both protective efficacy and the contribution of CD8+ T cells to protection induced by *Pbuis3(-)/4(-)* parasites. Like *Pb*γ-spz, *Pbuis3(-)/4(-)* spz induced sterile and long-lasting protection in C57BL/6 mice. Protection was linked to CD8+ T cells as mice deficient in β_2m were not protected. Analysis of *Pbuis3(-)/4(-)* spz-immune CD8+ T cells revealed the presence of effector/memory phenotypes and robust IFN-γ-producing CD8+ T cells that persisted during protracted protection. On the basis of these observations we propose that the development of the genetically-attenuated *P. falciparum* parasites is warranted for tests in clinical trials as a pre-erythrocytic stage vaccine candidate.

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INFECTION-INDUCED CYTOKINE PRODUCTION INFLUENCES THE SUPPRESSION OF *PLASMODIUM YOELII* PARASITEMIA FOLLOWING PROTECTIVE IMMUNIZATION

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Immunization with a blood-stage malaria vaccine will likely reduce parasite burden and/or disease severity but not prevent infection. Therefore, the combined effects of vaccine-induced and infection-induced immune responses will ultimately determine the outcome of infection and both must be considered when defining correlates of protection. We previously showed that immunization with *Plasmodium yoelii* merozoite surface protein-8 (*Py*MSP-8) induces antibodies against conformational epitopes that protect mice from lethal malaria but do not prevent infection. Using this MSP-based vaccine model, the aim of the current study was to investigate vaccine-induced and infection-induced immune responses that contribute to the clearance of blood-stage parasites in C57BL/6 and BALB/c mice which tend to develop polarized Th1 (IFN-γ, IgG2a) and Th2 (IL-4, IgG1) immune responses, respectively. Analysis of vaccine-induced antibodies in C57BL/6 and BALB/c mice revealed similar high titers of total *Py*MSP-8-specific antibodies. There were slight differences in *Py*MSP-8-specific antibodies considering IgG isotype profile and the recognition of conformational epitopes, but both strains were similarly protected against *P. yoelii* malaria. Passive transfer of sera from immunized C57BL/6 or BALB/c mice to naïve mice similarly delayed the onset of parasitemia in challenged mice, yet all BALB/c mice eventually succumbed to infection. To evaluate the contribution of key cytokines to protection, gene knockout mice were immunized and challenged. Protection was abrogated in IFN-γ deficient mice. In immunologically intact, r*Py*MSP-8 immunized mice, the presence of blood-stage malaria parasites induced the early production of IFN-γ which was followed by an increase in TGF-β. Interestingly, parasite burden was maintained at a low level while IFN-γ levels were elevated. The subsequent increase in TGF-β and decrease in IFN-γ correlated with a rise in parasite replication. Neutralization of TGF-β in r*Py*MSP8-immunized mice led to a significant decrease in mean peak parasitemia. Combined these data suggest that the efficacy of MSP-based vaccines is influenced by the quantity and quality of antibodies elicited by immunization along with the balance of IFN-γ and TGF-β production induced by exposure to blood-stage parasites.

(ACMCIP Abstract)

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FUNCTIONAL ASSOCIATIONS BETWEEN HAPLOTYPES OF NITRIC OXIDE SYNTHASE TYPE 2 (NOS2) PROMOTER VARIANTS (-954G/C AND -1173C/T), PEDIATRIC SEVERE MALARIAL ANEMIA, AND HIGH-DENSITY PARASITEMIA

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Nitric oxide (NO) is an effector molecule released as part of the inflammatory response to *Plasmodium falciparum*. Although the inflammatory profile associated with protection against severe malarial anemia (SMA) is largely undefined, high levels of NO generated from nitric oxide synthase NOS type 2 (NOS2) appear to limit parasitemia. Previous studies showed associations between NOS2 promoter variants (-954G/C and -1173C/T) and malaria disease severity. To further examine the role of NOS2 polymorphisms in conditioning malaria disease outcomes, the relationship between -954G/C and -1173C/T haplotypes, SMA (Hb less than 6.0 g/dL), high-density parasitemia (HDP >10,000 parasites/μL) and systemic NO production was investigated in children with acute malaria (n=567) residing in a holoendemic *P. falciparum* transmission area. Complete hematological and parasitological profiles were determined in all study participants. NOS2-954G/C genotyping was carried out by PCR and Bsa I restriction enzyme digestion, while -1173C/T genotypes were determined by mutagenically-separated PCR. Systemic NO production [(nitrite plus nitrate (NOx)] and creatinine (Cr) levels were quantified in urine samples and concentrations expressed as NOx/Cr ratio. Frequencies of the -954G/-1173C, -954C/-1173C, -954G/-1173T, and -954C/-1173T haplotypes were 86.1%, 11.3%, 1.9% and 0.7%, respectively. Multivariate regression analyses controlling for age, gender, sickle-cell trait, HIV-1, and bacteremia revealed that -954C/-1173C was associated with protection against HDP (OR 0.64, 95% CI 0.40-1.02, P=0.05), while -954C/-1173T was associated with an increased risk of SMA (OR 3.68, 95% CI 0.93-14.58, P=0.06). In addition, carriers of the -954C/-1173C haplotype had higher levels of systemic NO (P<0.05) relative to individuals without this haplotype. Results here demonstrate that variation in the NOS2 promoter is associated with functional changes in systemic NO production and susceptibility to severe childhood malaria.

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PLACENTAL INTERFERON-γ AND CORD BLOOD FERRITIN ARE RELATED TO BIRTH WEIGHT IN AN AREA OF INTENSE MALARIA TRANSMISSION

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Inflammation during placental malaria (PM) is associated with low birth weight (LBW), but the relative contribution of maternal or fetal factors that mediate this effect remain unclear. LBW due to PM primarily affects first pregnancies in areas of stable malaria transmission. Pregnancy outcomes were recorded for 808 women presenting for delivery at a district hospital in an area of intense malaria transmission in northeastern Tanzania. Samples of maternal peripheral, placental and cord blood were obtained. Plasma levels of TNF-α, IFN-γ, IL-10, ferritin and leptin were quantified using Bioplex technology. Plasma factors were analyzed for relationships to birth weight, gravidity and PM. Compared to uninfected women, women with PM (n = 90) had 1.2-4.3-fold higher median levels of TNF-α, ferritin and IL-10 in placental and peripheral blood, and were likelier to have detectable IFN-γ in the placenta (all $P < 0.05$). Placental levels of inflammatory cytokines increased significantly during PM of primigravid and multigravid women but not secundigravid women. PM had little effect on cord cytokine levels. In multivariate analysis, cord ferritin was positively associated ($P < 0.0001$) and placental IFN-γ negatively associated ($P = 0.01$) with birth weight in infected primigravid women. Cord leptin had a strong positive relationship with birth weight in offspring of PM- women but not PM+ women from all gravidity groups ($P = 0.02$ to < 0.0001). In conclusion, placental malaria elicits inflammatory cytokine responses in primigravid and multigravid mothers, but does not significantly modify the cytokine milieu of the fetus. The results confirm that placental IFN-γ is related to malaria-associated LBW, and suggest that ferritin in the fetal compartment may play a previously unrecognized role in preventing LBW due to PM during first pregnancies. Because fetal cells are a source of placental IFN-γ and cord ferritin, the fetal response to PM may determine the risk of LBW.

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COMPLEMENT UTILIZATION IN CHILDREN WITH SEVERE MALARIA ANEMIA

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The complement system plays important roles in both innate and adaptive immunity and complement can be activated and depleted during malaria infection, thus potentially compromising overall immune defenses. Activation of complement also leads to production of potent pro-inflammatory mediators such as C3a and C5a, which may explain the genesis of pro-inflammatory cytokines seen in children with severe malarial anemia (SMA). In a case control study, we compared the levels of complement hemolytic activity (CH50) in cases of SMA and in asymptomatic controls with malaria infections. The CH50 in SMA (16 ± 10 U/mL) were below normal (34-70 U/mL) and were half the levels in controls (34± 8.2 U/mL (*P = 0.001*, paired t test). The levels of C3a were 10 times higher than normal (normal ranges = 257-690) in both the cases (mean = 3489 ± 650 ng/ml) and in controls (3852 ± 555 ng/ml), indicating a high degree of complement activation in both groups. Similar trends were obtained for C4a and C5a. PCR detection of C4 null genes (C4AQ0 and C4BQ0) found 5 homozygous individuals for C4BQ0 (1 case and 4 controls), but no patients expressing the C4AQ0 allele in either group. Collectively, these results indicate: 1) Profound uncompensated utilization of complement in patients with SMA. 2) Equal formation of pro-inflammatory complement fragments in cases and controls, indicating that the pro-inflammatory cytokines commonly seen in children with SMA cannot be accounted for by these anaphylatoxic agents. 3) Complement deficiency observed in SMA does not appear to be associated with genetic defects.

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EFFECTS OF CONCOMITANT *SCHISTOSOMA HAEMATOBIUM* INFECTION ON THE INTRACELLULAR CYTOKINE LEVELS AND T CELL MEMORY POPULATIONS ELICITED BY ACUTE *PLASMODIUM FALCIPARUM* MALARIA INFECTION IN MALIAN CHILDREN

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Polyparasitism is common in the developing world. Patent helminth infections elicit a highly polarized Th2 cytokine response and may alter the expected response to a secondary infection, such as malaria. Interleukin-4 (IL-4) is a critical cytokine in eliciting B and T cell memory responses to malaria antigens. In a prospective trial assessing the effect of underlying *Schistosoma haematobium* on the acquisition and frequency of falciparum malaria, we previously demonstrated that schistosomiasis-infected Malian children, aged 4-8 years, have less malaria, prolonged onset until the first clinical episode, reduced parasitemias during that episode and an altered serologic cytokine milieu during infection. To understand the immunological basis of these observations, immunologic responses at the cellular level were measured using an optimized 10-color flow cytometry protocol. Peripheral blood mononuclear cells (PBMC) derived from six schistosomiasis-positive (SP) and six schistosomiasis-negative (SN) children presenting with symptomatic malaria were stimulated with circumsporozoite protein (CSP) and apical membrane protein 1 (AMA1). The time to first malaria infection was 136 days after enrollment for SP children compared to 8.9 days in SN children aged 4-8 years. Significant increases in IL-4 secreting $CD3^+CD4^+CD8^-$ T cells were noted in PBMC derived from SP children after antigenic stimulation compared to SN children (Chi^2: CSP, P=0.003, AMA1, P=0.009). While 30% (range 24-37%) of IL-4-negative cells ($EMA^-CD3^+CD4^+CD8^-IL4^-IFN\text{-}\gamma^-$) corresponded to the T cell central memory (T_{CM}) subset ($CD45RA^-CD62L^+$), the mean proportion of T_{CM} cells among IL-4-secreting cells ($EMA^-CD3^+CD4^+CD8^-IL4^+$) was 49% (range 31-65%) after stimulation with CSP. Detectable IL-10 and IFN-γ production was noted from $CD4^+$ T cells in SP children as compared to SN children but statistical significance was not achieved. These results support a role for IL-4 in helminth-infected children in mediating protection from the acquisition of falciparum malaria.

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MALARIA AND HELMINTHES CO-INFECTIONS IN CHILDREN AGED 6-17 YEARS IN THE BURMA VALLEY AREA OF ZIMBABWE

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Malaria and helminthes co-infections constitute the most prevalent tropical diseases in sub-Saharan Africa and account for high mortality and morbidity, as well as contributing to the underdevelopment of already disadvantaged populations. It is not clear how helminthes co-infections modulate the immune responses towards the malaria parasite *Plasmodium falciparum* and affect the course of malaria. A cohort of 609 children aged 6-17 attending 3 elementary schools within a 10-mile radius in a commercial farming area of Burma Valley in Zimbabwe's eastern province of Manicaland, were enrolled following the parental consent and the children's assent. JHSPH IRB approved the study. The children were diagnosed for helminthes, malaria and anemia and categorized into 6 groups based on their infection status at baseline survey: Group A (Not infected), Group B (Schistosome infected only), Group C (Malaria infected only), Group D (Malaria and Schistosome infected), E (Malaria, Soil Transmitted Helminthes (STH) and/Schistosome infected) and F (STH and/Schistosome infected). All infected children were treated. The children kept the assigned diagnostic groups throughout the study period. Baseline results indicated an increased prevalence of malaria parasites in children co-infected with helminthes (30.7%) compared to 24.8% in children infected with malaria only. Mean malaria parasite counts in the malaria only group were (2591 rings/ul, 73 gametocytes/ul) compared to 2031 rings/ul and 214 gametocytes/ul in the co-infected groups. There was no difference between the two groups in terms of the prevalence of anemia, which was 68% in both groups. However, children free from helminthes showed greater mean malaria antibody titer compared to co-infected children. This difference declined at 6-months following clearance of helminthes and altogether disappeared at 12-month post-treatment follow-up. In conclusion, helminthes infection is associated with increased prevalence of malaria in this population. A preliminary analysis also revealed increased malaria gametocytes in the peripheral circulation in co-infected children, which may have implications in malaria transmission dynamics. The study suggests that helminthes infections may also delay the development of malaria immunity in children residing in endemic areas regardless of repeated exposure to malaria parasites.

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EARLY IMPACT OF *HAEMOPHILUS INFLUENZAE* TYPE B VACCINE INTRODUCTION INTO THE ROUTINE EXPANDED IMMUNIZATION PROGRAMME IN BAMAKO, MALI

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In Bamako, Mali, surveillance of bacteriologically-confirmed invasive *Haemophilus influenzae* type b (Hib) disease among children admitted to the primary hospital (Hôpital Gabriel Touré, HGT) revealed a high burden of disease. As a result, the Malian government petitioned the Global Alliance for Vaccines and Immunization to support the introduction of Hib conjugate vaccine for infants. We continued surveillance for invasive Hib infection at HGT to determine the impact of Hib vaccine. Children age 0-35 months with fever $\geq 39^{o}$ C or syndromes compatible with invasive bacterial disease and admitted to HGT were eligible for inclusion. Blood and relevant body fluid (e.g. cerebrospinal fluid) were cultured after obtaining informed consent. Bacteria were identified by standard microbiologic techniques. Hib vaccine was introduced into the routine Expanded Program for Immunization in 3 stages: Bamako (July 2005), other urban areas (July 2006) and the rest of the country (June 2007). Data on incidence of invasive Hib disease in Bamako were collected at baseline (July 2002 - June 2005), during the transition (July 2005 - June 2006) and after introduction (intervention, July 2006 - December 2006). During the baseline, transition and intervention periods, 3761 of 4059 (92.6%), 1508 of 1555 (97.0%), and 837 of 884 (94.7%) eligible 0- to 23-month olds were enrolled. The mean annual incidences of invasive Hib disease in the 0- to 11-month old and 12-23 month old age groups during the baseline period were 174.9 and 32.9 per 10^5 children, respectively. These rates were 120.9, and 42.5 per 10^5 children during the transition period. During the intervention period, compared to baseline, there was a significant decrease to 75.9 ($p<0.001$) and 108.3 ($p<0.001$) per 10^5 children. At the peak age of Hib incidence (6- to 7-months of age), the rates per 10^5 population were 376.6 at baseline and 222.7 during transition, which decreased to 108.3 during the intervention period ($p<0.001$). Over the same time period, the mean annual number of hospitalized invasive Hib cases has decreased from 7.4 to 3.5 (53% reduction) in 0-11 month olds, from 2.7 to 0.8 (76% reduction) in 6-7 month olds and from 1.2 to 0.0 (100% reduction, $p<0.0001$) in 12-23 month olds. In conclusion, these findings suggest that programmatic introduction of routine Hib conjugate vaccine for Malian infants has resulted in a dramatic reduction in the incidence of invasive Hib disease.

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ENHANCED MICROBIOLOGY LABORATORY CAPACITY FOR PUBLIC HEALTH MISSIONS: CHALLENGES AND SOLUTIONS IN IMPLEMENTING STATE-OF-THE-ART TECHNOLOGY IN SOUTHEAST ASIA

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Effective public health programs to alleviate infectious disease depend on strong laboratory capacity and capability to identify and characterize endemic and epidemic pathogens. Southeast Asia poses significant challenges in the development of such improved laboratory function because of infrastructure issues and the number of emerging and endemic threats in the region. Thailand has a robust national public health system that offers a model to develop state-of-the-art laboratory capacity for infectious disease surveillance, improve clinical care, and support national needs. Through the Thai MOPH-U.S. CDC (TUC) collaboration, modern microbiology laboratories have been established in the provinces of Sa Kaeo and Nakhon Phanom and incorporated into both the existing clinical system and an active disease surveillance program. Provincial laboratory systems were upgraded for the identification of pathogens in patients with disseminated pneumonia, sepsis, and other severe disease by implementing improved blood collection, creating a specimen and supply transportation network, introducing an automated blood culture system to replace manual blood culture, and using international methods for pathogen identification and characterization. Preliminary results are reported to clinicians within 48 hours of specimen receipt. National reference laboratories provide confirmatory analysis and external quality control. This enhanced laboratory network now processes nearly 12,000 blood cultures annually, over twice the prior level, with a sample contamination rate under 5%. Increasing numbers of unusual microbial pathogens have been identified through this network, including *Brucella abortus*, *Burkholderia pseudomallei*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Streptococcus pneumoniae*, non-typhoidal *Salmonella*, and disseminated mycobacterial infections. Patient care has improved through more rapid pathogen identification and antibiotic susceptibility testing. Emerging infections and outbreaks are also being identified more quickly because of the enhanced local laboratory capacity and capabilities. Information from this network can be exploited to guide evidence-based public health interventions and monitor patterns of infectious disease.

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TRANSMISSION OF BACTERIA RATHER THAN ANTIBIOTIC USE DETERMINES RESISTANCE LEVELS: DATA AND MODELS FROM NORTHERN ECUADOR

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The rise of antibiotic resistance (AR) is a major public health problem occurring on a global scale. The current narrow focus on medical antibiotic overuse ignores the role of broader ecological processes, such as the spread of AR among animals and humans, in observed patterns of resistance. Much can be learned by examining the role such processes play in the development and spread of AR in areas where conditions that promote disease transmission, such as poor sanitation, increase the potential for human-to-human transmission of resistance. The construction of a new road in northern coastal Ecuador provides a valuable natural experiment to study how changes in the social and natural environment, mediated by road construction, affect the epidemiology of AR enterobacteria. Twenty-one villages from this region were randomly selected to provide a broad representation with respect to road access. Bi-annual 15-day visits to each village were conducted over the course of four years, in order to compare villages located along the road to villages with no road access (situated along one of three rivers). Data from the first four visits to each village were pooled and analyzed. An analysis of resistance levels in road versus non-road communities suggests that road communities have an approximately two-fold higher resistance level for several different antibiotics (Ampicillin: OR = 2.5 [1.5, 4.1]; Sulfamethoxasol/ Trimethroprim: OR = 2.1 [0.9, 4.5]; and Tetracycline: OR = 2.3 [1.1, 4.7]). Survey data we collected suggest that patterns of human antibiotic use do not differ between road and non-road communities. To explore why this might be so, we fit and analyzed a dynamic model of AR spread to a more remote village located on a river and a less remote village located on a road using the data on resistance levels, antibiotic use, and rates of transmission of *E. coli* to parameterize the model. Results of simulation analyses of AR levels in these two villages suggest that, even

though antibiotic use is the same in the two villages, the equilibrium level of resistance is approximately 80% higher in the road village. We suggest that this is because resistance level can be at least partially explained by the human-to-human spread of resistance via water, sanitation, and hygiene environmental pathways.

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ANTIBIOTIC USE BEFORE CULTURE REDUCES BACTERIAL YIELD AMONG PATIENTS EVALUATED FOR COMMUNITY-ACQUIRED BACTEREMIA IN THAILAND

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Accurate measures of community-acquired bacteremia in Thailand are needed to guide prevention strategies, yet the impact of antibiotic use on incidence estimates unknown. We established population-based surveillance for community-acquired bacteremia in 2 rural Thai provinces and evaluated pre-culture antibiotic use and its impact on blood culture yield. Blood cultures were requested as clinically indicated from recently hospitalized patients. Culture bottles were processed using a BacT/ALERT 3D incubator that alarms when cultures turn positive. Pathogens were identified by standard methods. Antibiotic use during the 72 hours before culture was recorded. In a subset of patients, serum antimicrobial activity was assessed by serum disc assay. A zone of inhibition >6 mm was considered positive. Pre-culture antibiotic use was common; 36% (4109/11359) of patients had reported use and 28% (of 4431 tested) had serum antimicrobial activity. Patients with pre-culture antibiotic use were less likely to have positive blood cultures (i.e., incubator alarmed) than those without antibiotic use: 33% vs. 35% (p=0.01) by reported antibiotic use and 14% vs. 23% (p<0.001) by serum assay. Pathogens were isolated in 30% of patients with reported antibiotic use compared to 32% in those without reported use (p=0.03) and from 11% with serum antimicrobial activity compared to 20% without activity (p<0.001). Specifically, *Streptococcus pneumoniae* yields were >6-fold lower in patients with vs. without reported antibiotic use (0.08% [3/3770] vs. 0.5% [32/6362], p<0.001). An even greater reduction in *S. pneumoniae* yield was observed for patients with serum antimicrobial activity: 0/1163 with positive assays vs. 15/2743 (0.5%) with negative assays (p=0.01). Antibiotic use before blood culture is common in rural Thailand and may result in large underestimates of the burden of invasive bacterial diseases, such as *S. pneumoniae*. Antibiotic use data should be collected as part of surveillance activities and used to inform and refine diseases incidence estimates.

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IDENTIFICATION OF ANIMAL SOURCES OF HUMAN BARTONELLOSIS IN THAILAND: COMPARISON OF *BARTONELLA* SEQUENCES FROM HUMAN PATIENTS AND RODENT HOSTS

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This study was undertaken to investigate whether rodent-associated *Bartonella* bacteria might be associated with undiagnosed illnesses in humans in Thailand, isolates and PCR products from human patients were compared by DNA sequencing with *Bartonella* strains found in Thai rodents. Blood clots collected from 319 human patients evaluated for febrile illnesses at rural hospitals in Thailand were screened for the presence of *Bartonella* using either a Vero E6 cell-PCR-subculture technique or inoculation into BAPGM liquid medium. Whole blood samples from 538 small mammals of 16 species obtained from 18 Thai provinces were cultured on blood-enriched agar. Co-cultures from 24 human patients with *Bartonella* infection were characterized by DNA sequence analysis of the *glt*A fragment to determine their relatedness to 159 animal isolates. Sequence analysis revealed that 5 human isolates and 1 isolate from *Rattus exulans* clustered with *Bartonella elizabethae*; 3 human sequences were 100% identical and 2 were >99% similar to the rat sequence. Five human sequences that clustered with *Bartonella tribocorum* were related (98.5%-99.1%) to a large group of isolates obtained from *Rattus* rats: *R. norvegicus* (12), *R. rattus* (4), and *R. exulans* (1). Among other human sequences that clustered with *Bartonella* species also found in animals, one was nearly identical (99.7% DNA and 100% protein) to sequences from *R. norvegicus* (1) and *Tupaia glis* (1), one was identical by DNA or protein sequences to a group of 11 isolates obtained from *Bandicota* rats (*B. indica* and *B. savilei*), and one was identical to sequences from *R. rattus* (2) and *B. savilei* (1). The remaining 11 sequences from humans belonged to *B. henselae, B. vinsonii,* or *B. tamiae*, but we did not identify homologous mammalian *Bartonella* sequences. Among sequences obtained from 24 human patients, at least 13 were closely related or identical to homologous sequences identified in small mammals from Thailand. Our findings suggest that rodents and other small mammals are likely reservoirs for a substantial portion of cases of human *Bartonella* infections in Thailand.

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CLINICAL CHARACTERISTICS OF CONFIRMED *BARTONELLA* INFECTIONS AND PREVALENCE OF *BARTONELLA* ANTIBODIES AMONG PATIENTS PRESENTING TO COMMUNITY HOSPITALS IN RURAL THAILAND

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Only one study has shown serologic evidence of *Bartonella* in Thailand and no studies have confirmed active infections in humans. Here we describe the characteristics of the first confirmed *Bartonella* cases and estimate seroprevalence. We prospectively enrolled febrile and non-febrile patients presenting to community hospitals in Chiang Rai and Khon Kaen provinces during February 2002-January 2003. Blood clots were tested for the presence of *Bartonella* species using either a Vero E6 cell-PCR-subculture technique or inoculation into BAPGM liquid medium. Acute and convalescent sera were tested for IgG to *B. henselae, B. quintana, B. elizabethae* and *B. vinsonii* by an indirect immunofluorescent assay. Cases were confirmed by a positive culture or ≥4-fold rise in *Bartonella* IgG. Seroprevalence was estimated using acute sera with seropositivity defined as an antibody titer ≥256. *Bartonella* species were isolated from 24 (7.5%) of 319 patients with cultured blood clots. Among an additional 61 patients with paired sera, 14 had ≥4-fold rise in IgG for a total of 38 (10% of 380) confirmed cases. The median age was 20 years and 63% were male. Presenting symptoms included fever (88%), headache (84%),

lethargy (66%), dizziness (66%), muscle pain (63%), and vomiting (50%). Rash was found in 34%, lymphadenopathy in 22% and conjunctival suffusion in 19%. Elevated alkaline phosphatase was found in 60%, elevated SGOT in 47%, anemia in 44% and thrombocytopenia in 25%. Exposure to rats during the previous 2 weeks was reported by 77%. Among 279 patients with acute sera, 17% were seropositive (1.4% to *B. henselae,* 0% to *B. quintana,* 6.5% to *B. elizabethae,* 0.7% to *B. vinsonii*, and 8.6% to >1 species); 35 (16%) febrile patients and 13 (24%) non-febrile patients were positive. Our findings identify *Bartonella* as an important cause of febrile illness in Thailand and document high rates of seropositivity. Initial prevention recommendations should focus on minimizing rat exposure; these can be augmented by further studies of human risk factors, animal reservoirs, and potential vectors.

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GEOGRAPHIC INFORMATION SYSTEM ANALYSIS OF BARTONELLOSIS IN AN EPIDEMIC SETTING IN CUSCO PERU

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After an outbreak of bartonellosis occurred for the first time in Cusipata, Cusco, Peru (1998-9), we evaluated environmental risk factors for bartonellosis seropositivity by performing a GIS analysis. We recorded the geographic location, the number of occupants of each household, and collected serum samples from all consenting participants. Ig-G antibodies against *Bartonella bacilliformis* were detected using an immunofluorescent assay (IFA). Houses with <50% participation were excluded from the analysis. We defined positive houses as those with at least one positive case by IFA. A supervised classification of the terrain in a 60 m. radius buffer zone around each house was conducted with a five-band IKONOS image. A 90 m. resolution Digital Elevation Model was used for elevation, slope, and direct sunlight calculations. Distance to hills and rivers was also calculated. The association between these factors and bartonellosis seropositivity was evaluated at the household level using both Student T tests and logistic regression, adjusting for clustering with robust standard error estimators. A total of 1,587 people lived in the three study villages, and 514 individuals living within 387 households participated in the study. From these, 405 living in 144 houses (54 positive) were included in the analysis. There were statistically significant differences in the proportion of positive houses between villages. The 60m buffer around positive households had higher slope ($p=0.05$), more agricultural landcover ($p<0.01$) and marginally less paved road landcover ($p=0.08$) than negative households. Only agricultural landcover (OR=1.56, $p<0.01$) remained significantly associated with household seropositivity in the multiple logistic regression analysis. We concluded that in an epidemic setting, the amount of agricultural field around a house and the terrain slope are probable risk factors for the presence of bartonellosis in a household. How this environmental factors influence vector populations and the presence of disease is still unknown and requires further investigation.

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AMINO ACID TRANSPORT IN SCHISTOSOMES: CHARACTERIZATION OF THE PERMEASE HEAVY CHAIN SPRM1HC

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Schistosomes are human parasitic flatworms that constitute an important public health problem globally. Adult parasites live in the bloodstream where they import nutrients such as amino acids across their body surface (the tegument). One amino acid transporter, Schistosome Permease 1 light chain, SPRM1lc - a member of the glycoprotein associated family of transporters (gpaAT), has been characterized in schistosomes. Only a single member of the SLC3 family of glycoproteins that associate with gpaATs is found following extensive searching of the genomes of *Schistosoma mansoni* and *S. japonicum*. In this study, we characterize this schistosome permease heavy chain (SPRM1hc) gene and protein. The 72kDa gene product is predicted to possess a single transmembrane domain, a $(\beta\alpha)_8$ (TIM barrel) conformation and a catalytic triad. *Xenopus* oocytes functionally expressing SPRM1hc with SPRM1lc import phenylalanine, arginine, lysine, alanine, glutamine, histidine, tryptophan and leucine. Biochemical characterization demonstrates that in *Xenopus* extracts and in schistosome extracts SPRM1hc is associated into a high molecular weight complex with SPRM1lc that is disrupted by reducing agents. Quantitative real-time PCR and Western analysis demonstrate that SPRM1hc is expressed in each schistosome life stage examined (eggs, cercariae, schistosomula, adult males and females). SPRM1hc is widely distributed throughout adult male and female worms as determined by immunolocalization. Consistent with the hypothesis that SPRM1hc functions to facilitate nutrient uptake from host blood, immunogold electron microscopy confirms that the protein is distributed on the host-interactive tegumental membranes. We propose that surface-exposed, host interactive, nutrient transporting proteins like the SPRM1 heterodimer are promising vaccine candidates.

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GENERATION OF AN IGG ANTI-GLYCAN MONOCLONAL ANTIBODY, F2D2.2, THAT REACTS WITH A FUCOSE-CONTAINING EPITOPE OF SCHISTOSOMES AND CROSS-REACTS WITH KEYHOLE LIMPET HEMOCYANIN

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Glycans of *Schistosoma mansoni* glycoconjugates constitute some of the most immunogenic molecules of the parasite. IgM and IgG antibodies to schistosome glycans are generated during the course of *S. mansoni* infection in humans and animals. Thus, schistosome glycans are potential molecular targets for the development of vaccines and serodiagnostics as well as the study of the immunoregulatory events associated with chronic schistosomiasis. We have been generating monoclonal antibodies to schistosome glycans to facilitate the identification and characterization of immunogenic glycans from the parasites. We now report on an IgG monoclonal antibody, F2D2.2, that recognizes a fucose-containing epitope of the parasite and cross-reacts with keyhole limpet hemocyanin. The monoclonal antibody was generated from a hybridoma developed from the splenocytes of Swiss Webster mice infected for 10 wk with *S. mansoni*. F2D2.2, which is an IgG3, reacts strongly with extracts of *S. mansoni* eggs and cercariae as assessed by ELISA and Western blot. Reactivity to the larval extracts is completely sensitive to treatment with bovine kidney α-fucosidase. F2D2.2 reacts poorly with extracts of adult *S. mansoni* but does not react with extracts of adult *S. japonicum* and *S. haematobium*. Interestingly, F2D2.2 cross-reacts with keyhole limpet hemocyanin, a protein known to share cross-reactive glycan epitopes with schistosomes. The glycan epitope recognized by F2D2.2 is currently being purified for structural characterization and synthesis to allow the evaluation of its antigenic potential.

(ACMCIP Abstract)

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PILOT SCALE EXPRESSION AND PURIFICATION OF *SCHISTOSOMA JAPONICUM* PARAMYOSIN

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This study was undertaken to develop a pilot scale expression and purification process for full-length recombinant *Schistosoma japonicum* paramyosin (rSj97) and to assess its efficacy in animal vaccination trials. Paramyosin is a leading vaccine candidate for both *S. mansoni* and *S. japonicum*. Animal vaccination using either biochemically purified or recombinant paramyosin results in significant protection for both schistosome species. Recently, we demonstrated that humans with high Th2/Th1 cytokine ratios in response to Sj97 had 42% lower intensity of infection after treatment compared to individuals with low ratios. Together, these protection data in animals and humans make a compelling case for developing paramyosin as vaccine candidate for schistosomiasis. Unfortunately, despite considerable effort, pilot scale expression and purification of recombinant paramyosin has not been previously achieved. A pET-based plasmid containing full-length Sj97 was expressed in *E.coli* in an oxygen sparged 10L fed-batch fermentor. Sj97 was extracted from inclusion bodies using 8M urea and purified using a 6 step chromatography sequence. Final purification of rSj97 from truncated forms of Sj97 required unusually strong denaturing conditions (9.5 M urea, 60 deg C) due to the strong α-helical coiled-coil structure of the protein. The 10L fed-batch fermentation yielded 700-800 gr of wet cell paste. Purified rSj97 consisted of a single band of expected MW on SDS-PAGE and the amino acid sequence was confirmed by MS based protein sequencing. The yield of rSj97 was 150 mg per 500 gr of wet cell paste. Endotoxin levels were below detection (< 0.03 EU/mg) as determined by a colorimetric LAL assay. Structural assessment by circular dichroism, residual *E. coli* protein contamination, lyophilization-stability assessment and protection studies in mice challenged with both Philippine and Chinese strain *S. japonicum* will be presented. In conclusion, we have developed a pilot scale process to express and purify full-length rSj97. This process will facilitate the pre-clinical evaluation of paramyosin as a vaccine candidate for schistosomiasis.

(ACMCIP Abstract)

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MOLECULAR AND EVOLUTIONARY EPIDEMIOLOGY OF *SCHISTOSOMA MANSONI* IN HUMAN HOSTS

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Schistosomes infect 200 million people worldwide and several million people suffer from severe morbidity as a consequence of these parasites. *Schistosoma mansoni* is the primary infectious agent of schistosomiasis in western Kenya where drug treatment, HIV, and hybridization with other schistosomes possibly interact to exert selective pressures on this parasite. Our research program uses molecular and evolutionary techniques to address epidemiological issues related to this parasite in its environment. Because they live within the circulatory system and cannot be easily removed from a living human, only indirect inferences can be made about adult worm populations. Our approach uses microsatellite data from the offspring of human infections and parentage analysis to infer the worm burden, genetic diversity, presence of clonal genotypes, mating systems, and fecundity of these parasites. Parentage analyses were conducted on datasets that were generated from laboratory infections of mice and also those generated from computer simulations. Data were analyzed with Parentage 1.0, which uses Bayesian inference to estimate parental population size, genotypes, fecundity, and relatedness of offspring. The effects of sample size, number of loci, and number of adults present in a host on the precision of the analysis were examined by comparing the results to the known parental populations. Additionally, we examined the mating system of the schistosomes from the mouse model to determine if they form monogamous pairs and if mating success is equal among individuals and pairs. Finally, we compared our findings from simulated and laboratory infections to data collected from human infections in Kenya. Results indicate that parentage analysis is an effective method to infer infrapopulations in humans from offspring data under our investigated parameters. Also, pairs of *S. mansoni* were typically monogamous over a period of three weeks although one instance of mate switching was detected, and fecundity varied greatly among male and female individuals and worm pairs.

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MASSIVE OVER-DIAGNOSIS OF MALARIA IN SUB-SAHARAN AFRICA: TIME TO REVIEW BLANKET TREATMENT OF UNDERFIVES

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Presumptive treatment of children with fever is one of the strategies promoted by WHO to reduce the burden of malaria in endemic areas. Initially, this strategy was meant to be implemented only for underfives in highly endemic areas, but it became the practice in all age groups and most settings. This study was undertaken to estimate and analyse the magnitude of over-diagnosis of malaria in Sub-Saharan Africa. We performed a systematic review for malaria diagnostic studies. Study selection criteria: 1) study conducted in Africa; 2) patients presenting as presumptive malaria, defined either by the health worker, or by the investigator (history of fever or measured elevated temperature); 3) numerator [parasitologically confirmed cases] and denominator (number of presumptive malaria); 4) microscopy done in reference laboratory or Rapid Diagnostic Tests (RDT). Intervention studies, those aimed at evaluating the incidence of malaria episodes, and studies of severe malaria were excluded. We extracted the following data: parasite prevalence, total number of patients, age group, year, season, country and setting, clinical inclusion criteria. We included 25 studies conducted between 1989 and 2005, half originating from East Africa. 11 studies included only children aged <5. 10 studies were conducted in rural areas, 8 in urban areas, one in both. Median prevalence rate (PR) of documented parasitaemia among presumptive malaria cases was 29% (range 1-75%). Median PR in underfives was 42% (5-70%) vs 34% (13-59%) in older age group. Median PR was 39% (1-75%) in rural vs 16% (2-64%) in urban settings, and 39% (1-75%) in the rainy vs 7% (2-60%) in the dry season. Median PR was 48% (5-75%) in the years 1989-1999 vs 23% (1-59%) from 2000 onwards. In conclusion, the current strategy of blanket treatment of all fever cases in malaria-endemic areas with antimalarials needs to be reviewed, for all settings and age groups. Accurate diagnosis, e.g. with RDTs, is required. This would insure appropriate care of non malaria fevers and rationale use of ACT.

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MALARIA RAPID DIAGNOSTIC TEST USE AND PERFORMANCE BY FACILITY-BASED HEALTH WORKERS IN WESTERN KENYA

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Malaria rapid diagnostic tests (RDTs) based on *Plasmodium falciparum*-specific histidine-rich protein antigen detection are relatively inexpensive, easy to use, and could potentially improve malaria diagnosis. Under ideal conditions, RDTs are highly sensitive and specific when compared to reference microscopy. However, few studies have examined RDT performance when used by health workers (HWs) at the health-facility (HF) level in routine practice. We conducted a HF-based trial of RDTs in low and high malaria transmission areas in western Kenya. Intervention HFs (n=30) received RDTs (Paracheck Pf cassette, Orchid Diagnostics, India) for routine patient management; comparison HFs (n=30) used clinical guidelines with or without microscopy for patient management. In intervention HFs, HWs were trained on RDT use and received a supervisory visit by the study team. HF surveys were conducted to collect RDT and HF microscopy results obtained during routine management of patients ≥ 5 years old who attended the HF on the day of the survey. Study staff also collected a blood film for reference microscopy (gold standard) and an RDT on every patient ≥ 5 years old. Overall 1827 patients were enrolled; 11% had malaria parasitemia by reference microscopy. At the time of the survey, intervention arm HFs had been using RDTs for a median of 32 days (interquartile range 28-34). Sensitivity and specificity of RDTs used by HWs were 85% and 90%, respectively, for any malaria parasitemia, and 100% and 89%, respectively, for malaria parasitemia >5000 parasites/μL. RDT results from HWs were similar to those obtained by study staff. HF microscopy had poor test characteristics (sensitivity 57% and specificity 75%) compared to reference microscopy. Reported fever was sensitive (84%), but not specific (42%) compared to reference microscopy. In conclusion, RDTs used by HWs at HFs in routine practice were highly sensitive and specific in detecting malaria parasitemia in older children and adults and performed better than HF microscopy. Widespread implementation of RDTs at HFs may improve malaria diagnosis. To ensure continued good performance of RDTs, RDT scale-up should build in laboratory quality assurance systems, including strengthening of microscopy for reference at selected facilities.

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INTRODUCTION OF MALARIA RAPID DIAGNOSTIC TESTS, NEW GUIDELINES, AND ARTEMETHER-LUMEFANTRINE IN KENYA: A CLUSTER RANDOMIZED TRIAL

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Use of reported fever to diagnose malaria is sensitive but not specific and leads to over-treatment. With the relatively costly artemether-lumefantrine (AL) as its new first-line treatment for uncomplicated malaria, Kenya has considered using malaria rapid diagnostic tests (RDTs) to improve diagnosis and decrease over-treatment with AL for older children and adults. We studied health worker (HW) adherence to RDT results; and whether RDTs improve AL use and reduce over-treatment. In 60 health facilities (HFs), after HWs were trained on AL use, we conducted a baseline HF survey to assess the case management of outpatients ≥ 5 years old. Consultations were observed, patients were re-examined, and blood films were collected and read at a reference laboratory. We then randomized HFs to an intervention arm (provision of RDTs and AL, guidelines on AL use and malaria management, training on RDT use, refresher training on guidelines, and a supervisory visit to reinforce correct AL use; n=30 HFs) or comparison arm (same as intervention, except RDTs not provided; n=30 HFs). We conducted a follow-up survey 5 weeks later. Of 1729 patients enrolled (intervention arm baseline n=556; intervention follow-up n=384; comparison baseline n=471; comparison follow-up n=318), 11% had malaria parasitemia by reference microscopy. About 50% of patients were seen in HFs with routine microscopy. In the intervention arm follow-up, 35% of febrile patients were tested with an RDT. HWs used RDT results when prescribing AL: 88% of RDT-positive and 8% of RDT-negative patients were treated with AL, respectively. However, 36% of RDT-negative patients were treated with a second-line or non-recommended antimalarial. AL use in parasitemic patients did not change significantly in either arm (intervention: 45% to 39%, p=0.82; comparison: 5% to 34%, p=0.10). Similarly, over-treatment (AL use in non-parasitemic patients) did not change (intervention: 14% to 9%, p=0.49; comparison: 8% to 11%, p=0.63). In conclusion, RDT use in febrile patients was low. HWs used RDT results to prescribe AL, but not other antimalarials. The provision of RDTs with extra training and supervision did not improve AL use in patients ≥ 5 years old with suspected malaria. Overall, parasitemia was low and HWs rarely used AL, resulting in little over-treatment. The utility of RDTs is heavily dependent on HW practices and the success of interventions to improve HW practices.

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COMPARISON OF MICROSCOPY, HRP2- AND PLDH-BASED RAPID DIAGNOSTIC TESTS FOR MALARIA AT SITES OF VARYING TRANSMISSION INTENSITY IN UGANDA

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In Africa, malaria is generally diagnosed on clinical grounds alone, though presumptive treatment results in significant overuse of antimalarials. Microscopy, the standard for malaria diagnosis, remains unavailable to the majority of African patients. Malaria rapid diagnostic tests (RDTs) may offer a practical and reliable alternative for case management, but optimal strategies for different epidemiological settings have not been defined. We compared the diagnostic accuracy of expert microscopy and two RDTs in 1000 patients of all ages at each of seven sites of varying transmission intensity across Uganda, using as the gold standard expert microscopy corrected for presence or absence of *Plasmodium falciparum* by PCR based on ribosomal RNA. Overall, HRP2 showed superior sensitivity across sites and age groups, while pLDH and expert microscopy showed higher specificity. At the site with lowest transmission, the negative predictive value (NPV) was excellent for all three tests, but the positive predictive value (PPV) was significantly better with microscopy and pLDH. At the remaining six sites with medium and high transmission, PPV was excellent for all three tests (≥87.3%), though slightly higher for microscopy and pLDH than for HRP2. HRP2 showed excellent NPV in patients of all ages at all sites (≥96.5%). In contrast, as transmission intensity increased, the NPV for both microscopy and pLDH declined significantly, especially for younger

patients. For pLDH, in children under 5 years, NPV ranged from a peak of 98.9% at a low-transmission site, to just 49.0% at a high-transmission site; in patients aged 5 years and older, the NPV fell from 98.1% to 69.3%. The NPV of expert microscopy was very similar to that of pLDH across all sites and age ranges. In conclusion, considering diagnosis based on PCR as a gold standard, microscopy or pLDH are likely to provide the best diagnostic utility at sites with low transmission, while HRP2 is recommended for medium- and high-transmission areas.

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EVALUATION OF THE NEW MALARIA RAPID DIAGNOSTIC TEST *FIRST RESPONSE*® PF/PV, WHEN USED AS A SCREENING TOOL FOR MALARIA DURING PREGNANCY IN CENTRAL INDIA

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We evaluated a new pLDH malaria rapid diagnostic test (First Response Malaria Pf/Pv Antigen Strips by Premier Medical Corporation Ltd, India) as a potential screening tool for *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) infection in pregnant women attending for antenatal care. Two 6 week cross-sectional surveys were conducted in 3 districts in Central India, one in the dry hot season (March-April) and one post monsoon (October-November), the later representing the peak prevalence of *P. vivax* and *P. falciparum*, respectively. Overall, 1812 pregnant women (815 in the dry season and 997 in the post-monsoon survey) attending routine antenatal care were screened for malaria with the RDT and conventional light microscopy, irrespective of clinical symptoms. Based on microscopy, the prevalence of Pf and Pv was 0.8% and 1.2% in the dry season and 5.7% and 0.7% post-monsoon season, respectively, with marked variations of Pf prevalence between the 3 sites in the latter survey (0%, 2.7% and 18.1%). The overall geometric mean parasite densities (GMPDs) of Pf (n=62) and Pv (n=16) infections were 1380 (95% CI 795-2395) and 908 (95% CI 449-1838), respectively. The GMPDs for *P. vivax* were significantly lower in the dry season survey than the post monsoon survey (422 vs 2434). Using microscopy as the gold standard, the overall sensitivity and specificity of the RDT test were 95.2% and 99.6% for Pf and 68.8% and 99.4% for Pv, respectively. The positive and negative predictive values were 90.8% and 99.8% for Pf, respectively, and 52.4% and 99.7% for Pv. Four out of 7 *P. falciparum* infections with densities <250 parasites/ul were detected by RDT, but this was 1 of 5 for Pv. Confirmation using PCR is ongoing. The First Response Pf/Pv antigen strips test was easy to learn and is a potential alternative to microscopy where the facilities for microscopy are poor or non existent. It was highly sensitive and specific in the screening of mainly asymptomatic pregnant women for *P. falciparum* infection, but was less accurate as screening test for low density *P. vivax* infections.

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CHALLENGES IN ROUTINE IMPLEMENTATION AND QUALITY CONTROL OF RAPID DIAGNOSTIC TESTS FOR MALARIA - RUFIJI DISTRICT, TANZANIA

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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 study settings. Current WHO guidelines for quality control of RDTs provide detailed instructions on pre-field testing, but offer little guidance for quality assurance once RDTs are deployed in health facilities. From September 2006 to April 2007, we introduced HRP2-based RDTs (Paracheck Pf) for suspected malaria cases in 9 health facilities with existing microscopy services in Rufiji District, Tanzania. Rufiji District is a rural setting with holoendemic malaria transmission. Thick blood smears were collected for all patients tested with RDTs and stained and read by laboratory personnel in each facility. Thick smears were subsequently reviewed by a reference microscopist to determine RDT sensitivity and specificity. In all 9 health facilities there were significant problems with the quality of staining and microscopy. Intensive refresher training did not result in substantial improvements in the quality of slide preparation. Sensitivity and specificity of RDTs were difficult to assess given the poor quality of routine blood smear staining. Mean operational sensitivity of RDTs based upon reference microscopy was 64.8%, but varied greatly by health facility, range 18.8-85.9%. Sensitivity of RDTs increased with increasing parasite density. Specificity remained high at 87.8% despite relatively poor slide quality. Institution of quality control of RDTs based on poor quality blood smear staining may impede reliable measurement of sensitivity and specificity and undermine confidence in the new diagnostic. Reliable staining and microscopy for quality control must be a prerequisite to the introduction of RDTs.

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MODELLING COSTS AND BENEFITS OF RDTS FOR THE DETECTION OF *PLASMODIUM FALCIPARUM* IN UGANDA

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With the increasing variety of RDTs on the market, policy makers must identify the most appropriate test, and circumstances where presumptive treatment remains the preferred strategy. This choice is likely to vary widely not only in response to test characteristics but also to characteristics of the population where RDTs are to be deployed. An economic model was developed as a decision aid, adaptable to different scenarios of ACT and RDT costs, and test accuracies. The model also enables the user to vary estimates for other factors, such as the potential harm of treatment, including risk of adverse events and drug resistance, and the probability that clinicians will adhere to test results. In a recent trial the accuracy of two RDTs (detecting either pLDH or HRP2 antigens) was evaluated in 7 sites across Uganda. The data on costs and accuracies were entered into the model to illustrate its use and results. Output was then obtained at increasing levels of comprehensiveness, starting with direct expenditure on diagnostics and treatment alone, and then introducing patient health outcomes, compromised adherence with test results, and the broader societal costs associated with overprescription of antimalarials. Results suggest that given current RDT and ACT prices, use of the HRP2 RDT would be justifiable across most prevalences and age groups. This however depends to a great extent on whether factors such as the harm associated with use of antimalarials and the probability clinicians adhere to results is included in the analysis. Excluding the harm of treatment, presumptive treatment is justified for younger children, and the benefit in the use of RDTs for older patients is also limited. Results also indicate to the need to ensure that clinicians adhere to negative test result if RDTs are to remain an efficient use of resources. Results are expected to vary widely by location and over time as prices and effectiveness of RDTs and ACTs change, therefore the model was designed for easy incorporation of local and up to date parameter estimates for identification to support local decision making.

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SAFETY AND INFECTIVITY OF TETRAVALENT CHIMERIC LIVE ATTENUATED DENGUE VACCINE IN DIFFERENT AGE POPULATIONS IN ENDEMIC AND NON-ENDEMIC AREAS

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A vaccine to protect against severe dengue (DEN) disease is sorely needed. The safety and viremia of a tetravalent live attenuated DEN vaccine containing 5 $\log_{10}$ $TCID_{50}$ of chimeric yellow fever (YF)/DEN1,2,3,4 viruses (ChimeriVax™-DEN 1-4: DV) was tested in 3 randomized controlled blind-observer phase 1 trials in the US, the Philippines (Ph) and Mexico (Mx). Subjects received 1 dose of DV or either placebo (US), Typhim Vi®(Ph), or Stamaril® YF vaccine (Mx) on Day0. Viremia was tested by qRT-PCR and dengue plaque assays in serum collected every 2 days from Day0-20 (US) or on Day0,7,14 (Ph, Mx). Clinical and biological safety was followed to Day 28. 318 adults [18-45 years] and children [2-17 years] were included (adults only in the US). In the US and Mx_non-endemic areas_97% and 92% were dengue-naïve at baseline. In Ph_dengue endemic area_only 20% were dengue and JE naïve. There was no vaccine-related SAE and no dengue like syndrome such as those seen with empirically live attenuated dengue vaccine candidates. More US adults had systemic reactions with DV (79%) than with placebo (58%). Among biological safety parameters, only white blood cell counts showed relevant changes: transient decreases were seen around Day8 in dengue vaccinees. Systemic reactogenicity of DV was comparable to that of YF and Typhoid fever vaccines. Regarding vaccine infectivity, low levels of viremia (<40 pfu/mL) after vaccination were detected by qRT-PCR in 82% of US vaccinees, mainly on D8-14. Four subjects were positive by plaque assay (max 1.6 $\log_{10}$ PFU/mL). In Mx, viremia was more frequent among adults (all were positive), than younger subjects (33% of 2-5 year-olds were positive) and was more frequent than in Ph (25% of adults were positive by PCR). Overall safety and viremia did not differ significantly with flavivirus immune status or among different age groups. These data confirm the satisfactory safety profile of ChimeriVax tetravalent dengue vaccine in American and Asian adult and pediatric populations.

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TETRAVALENT DENGUE VACCINE BASED ON COMPLEX ADENOVIRUS VECTORS PROTECTS RHESUS MONKEYS AGAINST CHALLENGE FROM ALL FOUR DENGUE SEROTYPES

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Two bivalent vaccine constructs were created using replication defective complex adenovirus vectors, one expressing pre-membrane and envelope genes of dengue-1 and dengue-2, and the other of dengue-3 and dengue-4. The two constructs were mixed in equal proportions to formulate a tetravalent dengue vaccine. Two groups of 24 rhesus macaques were vaccinated by intramuscular injection of a total of 1E10 infectious units of dengue vaccine construct or a control vaccine, on days 1 and 57. Groups of 3 vaccinated and 3 control animals each were challenged on day 85 (about one month after the last dose) with dengue virus type-1, -2, -3 or -4. Remaining vaccinated and control animals were challenged similarly six months after the last immunization (day 253). Plaque reduction neutralization tests (PRNT) showed that on day 85, 12 vaccinated animals had $PRNT_{50}$ reciprocal geometric mean titers (GMT) of 622, 249, 1213 and 613 for dengue-1, -2, -3 and -4, respectively. Animals in each challenge group had mean viremia days of 0, 0.33, 0 and 1 for dengue-1, -2, -3 and -4 respectively compared to 5.66, 4.66, 5.66 and 6.66 days for the control animals. The other group of 12 animals which were challenged 6 months after final vaccination, retained $PRNT_{50}$ GMT of 574, 191, 126 and 275 for dengue-1, -2, -3 and -4 respectively, at the time of challenge. When challenged on day 253, significant protection from viremia was demonstrated once again for dengue-1, -2, -3 and -4 with mean viremia days of 0, 0.33, 0 and 2.33 respectively compared to 5.66, 4.66, 5.33 and 5.0 days for the corresponding control groups. Thus, this adenovirus-vectored tetravalent dengue vaccine consisting of two bivalent components elicited appreciable neutralizing antibody titers against all 4 dengue serotypes, and afforded significant protection from challenge when tested 1 month after vaccination or 6 months after vaccination.

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IMPROVED IMMUNOGENICITY AND PROTECTION OF TETRAVALENT DENGUE VACCINES USING A PRIME-BOOST STRATEGY IN NON-HUMAN PRIMATES

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Deployment of live-attenuated dengue vaccines has been hindered by potentially unacceptable levels of reactogenicity and the requirement for a long time interval between priming and effective boosting. To attempt to address these issues, we evaluated an alternative prime-boost vaccination strategy in rhesus macaques by priming with a tetravalent purified-inactivated vaccine (TPIV) or a tetravalent DNA vaccine (TDNA) followed by boosting with a tetravalent live-attenuated vaccine (TLAV). In this study, groups of rhesus macaques (N=4) were primed with either 2 doses of TDNA, one dose of TPIV, or one dose of TLAV, followed by boosting with TLAV. Antibody responses measured by ELISA demonstrated tetravalent immune responses and high titers of dengue-specific IgG in all groups, which were maintained until the day of challenge. Low-titered virus-neutralizing antibodies (Nab) were demonstrated against DEN-1, DEN-3 and DEN-4 after priming in all vaccine groups. Nab against DEN-2 were highest in animals that received the TLAV (GMT=1216) followed by groups that received TPIV (GMT=347) and TDNA (GMT=126). Nab titers peaked one month after the TLAV booster and then declined over time in all groups. The most persistent tetravalent Nab titers were observed with TPIV/TLAV. Six months after the booster vaccination, all vaccinated animals and an unvaccinated control group were challenged with live, non-attenuated DEN-3 virus. Serum viremia was measured for 10 days after the challenge to evaluate protection. Complete protection against viremia was observed in the TLAV/TLAV group and the TPIV/TLAV group. Three of four animals in the TDNA/TDNA/TLAV group exhibited 1 to 3 days of viremia (mean 1.25 days) compared with unvaccinated controls, which had 4.75 mean days of viremia. Measurement of virus Nab titers 14 days after challenge indicated general boosting of the antibody titers in all vaccinated animals. Our results demonstrate that priming with TPIV resulted in increased vaccine immunogenicity and protective efficacy compared with priming with TDNA, and did not prevent effective boosting with TLAV. Such a strategy might be used to improve the immunogenicity and perhaps reduce the reactogenicity seen in human clinical trials with some live-attenuated dengue vaccines.