used for malaria prophylaxis, and the largest study of the PK of MQ. The results from modelling the residual unexplained variability indicated that subjects in the double-blinded cohort (group A) may have been more compliant in taking MQ than those in the open-label cohort (group B).

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POPULATION PHARMACOKINETICS OF TAFENOQUINE DURING MALARIA CHEMOSUPPRESSION IN AUSTRALIAN SOLDIERS

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As part of a Phase III trial comparing the safety, tolerability and effectiveness of tafenoquine and mefloquine for malaria prophylaxis, the population pharmacokinetics (PK) of tafenoquine were studied in Australian soldiers. The subjects (478 M, 14 F) who were on peace-keeping duties in East Timor in 2000, received a loading dose of 600 mg tafenoquine over 3 consecutive days (200 mg base each day) followed by weekly administration of 200 mg tafenoquine for 6 months. The mean age was 25.4 years (range 18-47), and mean body weight was 80.9 kg (range 50-135). For estimating the population PK of tafenoquine, blood samples were collected from each soldier on 4 occasions on day 2 and at weeks 4, 8, and 16 of the prophylactic phase. Plasma tafenoquine concentrations were determined by LC-MS/MS. Population PK modelling was performed using NONMEM. A linear, one-compartment model best described the data. Robustness of the final model was confirmed in NONMEM by bootstrapping (n=200) with replacement and noting that all final model parameter values were close to the centre of the respective 95% percentile bootstrap confidence intervals. The population estimates of the first-order absorption rate constant (K_a), clearance (CL/F) and volume of distribution (V/F) were 0.243 h^{-1}, 0.056 l $h^{-1}kg^{-1}$ and 23.7 l kg^{-1}, respectively. The intersubject variability in CL/F and V/F (coefficient of variation, CV%) were not excessive at 18.1%, and 22.4%, respectively, which reflects the uniformity of the subjects and the carefully controlled conditions under which the study was conducted. The interoccasion variability in CL/F was 18.3%. The mean elimination half-life of tafenoquine was 12.7 days. While age and sex explained a small but significant amount of the variability about CL/F and V/F, respectively, both covariates were correlated with weight and were not considered further in the structural model development. There was a positive, linear association between weight and both CL/F and V/F; heavier subjects tended to have greater CL/F and V/F.

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IMPACT OF HOST IMMUNITY IN CLEARANCE OF DRUG-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA

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In malaria endemic areas children often recover after chemotherapy of drug-resistant *Plasmodium falciparum* malaria. This implies a synergy between drug treatment and acquired immunity. We have examined this hypothesis in Chamwino village, situated in an area of moderately intense transmission of *P. falciparum* in Tanzania. 205 uncomplicated malaria patients below five years of age were randomly allocated to treatment with either sulfadoxine-pyrimethamine (SP), amodiaquine (AQ) or co-artem (CoA) and followed for 28 days. Adequate clinical and parasitological response (ACPR) was seen in 73% and parasitological or clinical treatment failures (TF) in 27% of all children. TF was higher in children receiving SP (68%) and AQ (38%) and less for CoA (< 2%). The prevalence (OR: 5.0; 95% CI: 2.5-9.9, p<0.0001) and levels (95% CI for differences in median: 0.0-6.9; p<0.0001) of GLURP-specific IgG were higher on day 0 in children with ACPR than in children with TF after adjustment for the effect of age. Similar differences were seen on day 14, and when the SP and AQ groups were considered separately. Finally, levels of GLURP-specific IgG correlated inversely with parasite density on day 0 (p=0.006). We did not observe any statistically significant differences between children with ACPR and TF in the prevalence or levels of IgG with specificity for a range of other *P. falciaparum* antigens (AMA-1, CSP, MSP-1, MSP-3, three PfEMP1-antigens and VSA). Our findings suggest that GLURP-specific IgG antibodies contribute to clearance of drug-treated infections and support the hypothesis that acquired immunity enhances the clinical efficacy of drug therapy. Possible implications for drug-trials and -policy will be discussed.

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CARDIAC EFFECTS OF ARTEMETHER-LUMEFANTRINE AND ATESUNATE PLUS AMODIAQUINE DURING TREATMENT OF ACUTE UNCOMPLICATED MALARIA IN NIGERIAN CHILDREN

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Because of reports of cardiotoxicity associated with the use of halofantrine and the structural similarity between lumefantine and halofantrine, concerns have been raised about possible cardiotoxicity resulting from the use of artemether-lumefantrine. The cardiac effects of artemether-lumefantrine (AL) and artesunate plus amodiaquine (ASAQ) were assessed in 38 children aged 6months to 10years with symptomatic acute uncomplicated falciparum malaria by EKG and clinical monitoring over 28 days. AL was administered as a 6-dose regimen (5-<15kg=1 tab; 15-<25kg=2tabs, 25_35kg=3tabs) twice daily for three days while ASAQ was (artesunate=4mg/kg and amodiaquine=10mg/kg) administered daily for three days. Patients were seen daily from day 0 to 7 and then on days 14, 21 and 28. Response of the infection to both drugs was prompt with 28-day cure rates of 95% (19/20) and 94.4% (17/18) for AL and ASAQ respectively. There were no significant PR interval changes (PR >180ms and PR change >25% from baseline) through out the study period. At baseline, the mean QTc was 391.4ms (range 388.3ms - 404ms) and 414.5ms (382.9ms - 413.4ms) for children treated with AL and ASAQ respectively. 3 patients treated with AL and 2 who received ASAQ had QTc intervals >440msec at enrollment. The mean absolute changes in QTc intervals for AL on days 1,2, 3, 4, 7, 14 and 28 when compared with baseline were -17.3ms (-4.7%), -13.4ms (-3.3%), -12.6ms 9-3.0%), -12.1ms (-2.9%), -21.9ms (-54%), -26.2ms (-6.3%) and -23.9ms (-5.8%). Mean absolute changes for ASAQ at days 1, 2, 3, 4, 7, 14 and 28 when compared with baseline were -8.5ms (-2.2%), 22ms (5.6%), 2.5ms (0.6%), 1.1ms (0.3%), 4.6ms (1.2%), -1.4ms (-0.4%) and 3ms (0.8%). All changes in QTc intervals were not statistically significant for the both drugs. The two patients treated with ASAQ who had QTc prolongation at baseline recorded one further increase in QTc prolongation each on day 1 and day 2. One of the two recorded QTc interval prolongations from 445ms on day 0 to 465 at day 1 (20ms, 4.5% absolute changes) while the other recorded changes from 446ms to 478ms (32ms, 7.2% absolute changes). One of the 3 patients treated with AL subsequently recorded further QTc prolongation from 442ms to 448ms at D6 (6ms, 1.4% absolute changes). In no patient was a clinically significant QTc interval prolongation recorded through out the study.

In conclusion, both AL and ASAQ showed good cardiovascular tolerance.

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FLOW CYTOMETRY ASSAY FOR COUNTING PARASITEMIA IN RODENT BLOOD: DEVELOPMENT PROCESS AND VALIDATION

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Rapid and reliable identification of rodent red cells parasites based on the principles of flow cytometry (FCM) have replaced the traditional blood smear in our laboratory. The automated flow cytometric detection has been developed and validated with simple a diagnostic procedure in infected erythrocytes of rats, using a nucleic acid-binding fluorescent dye, YOYO-1. High levels of reticulocytes were detected in the course of the infective duration, ranging from 2.0-51.2%, and were digested by RNase. The counts of lymphocytes, white blood cells, uninfected and infected red cells were obtained with high precision. In addition, cells are divided into different populations according to their physical or chemical properties. Normality can be assessed with a high degree of accuracy, rapidly and reproducibly. In this study, the FCM greatly simplified and accelerated parasite detection, with sensitivity of 96.3%, specificity 98.9% and accuracy 102.9%. Overall, the parasite counts by flow cytometric measurement correlated well with the parasitemia measured by microscopic assay (regression coefficient = 0.97). The detection limit was 0.024% and the quantitative limitation was 0.074% parasitemia. This finding suggested that the high quantitative limit is due to background signals in the blood and may interfere with the performance of the FCM. Further improvement, by eliminating this interference, will make the FCM one of the most promising tests for malaria diagnosis in rats.

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MALARIA AND INTESTINAL HELMINTHIASIS IN SCHOOL CHILDREN OF KUMBA URBAN AREA, CAMEROON

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Malaria and intestinal helminthiasis are parasitic diseases causing high morbidity and mortality in most tropical parts of the world, where climatic conditions and sanitation practices favour their prevalence. These infections do co-exist and have different effects on infected individuals.

This was a cross sectional study done in four schools in the kumba urban area to assess the level of endemicity of malaria and helminth infections in school children and to determine how these infections relate to each other. Two hundred and forty three randomly selected pupils aged four and fifteen years of both sex were recruited for the study. All two hundred and forty three pupils had malaria parasites in their blood. The geometric parasite load was 1282 parasites per µl of blood. Only 17 pupils were anaemic (PCV<30%). The helminth infections showed a 38.3% prevalence, with a geometric mean parasite load of 687 eggs per gram of faeces. Co-infections were recorded in 38.3% of the pupils. There was no significant correlation between the helminth and malaria parasite densities (r =0.04, P =0.7337). In conclusion, both malaria and helminth parasites do co-exist without clinical symptoms of infection in school children of the Kumba Urban Area.

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CLINICAL EXPERIENCE WITH REAL-TIME PCR IN THE DIAGNOSIS OF MALARIA

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From April 2005 through May 2006 we performed real-time PCR (using the LightCycler™) on 46 blood specimens submitted to the Clinical Microbiology laboratory for malaria diagnosis. Our PCR method permits detection of *Plasmodium* and species identification by melting curve analysis in one assay requiring one hour of analytical time. Both the PCR assay and examination of thick and thin blood films were negative with 29 specimens. Both methods were positive for a species of *Plasmodium* in 14 specimens. In eight of these cases, both the blood films and the PCR were positive for the same *Plasmodium* species. There were six instances where, even though the blood films were positive, the PCR was particularly useful in correcting or clarifying the findings on the blood smears. For example, PCR permitted identifications of *P. malariae* and *P. ovale* when the microscopy suggested *P. falciparum* and when there were too few parasites present to adequately determine a species, respectively. In another instance, a relapsing case of malaria was verified by PCR as due to *P. vivax* when the microscopy suggested *P. falciparum.* In three cases, PCR was positive but blood films were negative. Two of these cases were related to patients taking antimalarials prior to collection of their blood. In an additional case, a blood film identification of *Babesia* was consistent with a negative PCR for *Plasmodium*. Our experience with the real-time PCR malaria assay indicates that it has clinical utility in a reference Clinical Microbiology laboratory located in and serving patients from the United States.

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RISK FACTORS FOR THROMBOCYTOPENIA AND ANAEMIA IN PATIENTS TREATED FOR ACUTE UNCOMPLICATED FALCIPARUM MALARIA

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Malaria a major cause of morbidity and mortality causes changes in haematological parameters. Aim:To correlate platelet count, leucocyte count, haematocrit with other risk factors. Methods and design: All randomized controlled clinical trials of acute uncomplicated falciparum malaria between the period of 2000 to 2003 were pooled. Treatment included chlorproguanil-dapson, Coartem, malarone and amodiaquine-sulphadoxine-pyrimethamine. Hematological parameters (hemoglobin, leucocyte count, and platelets counts) were determined using coulter counter, and these were correlated with age, sex, drug, temperature and parasite density. Statistical analysis was done using SAS version 9.1. Results:A total of 695 patients were included from 4 clinical trials. The mean±sd age of the patients was 51.7±33.8months. The mean platelet count was 150,876 ±75,743/mm^3, mean leucocyte count 8357±10611/mm^3 and mean haematocrit 30.1± 5.3%. Anemia occurred in 43.8% of the patients. Under 5 years old had a significantly lower hematocrit 28.4(4.8)% compared with 32.8(4.8)%, p=<0.001 at presentation but not significantly different by day 14 and 28; 27.8±5.5 versus 31.1±5.1, p=0.47 and 30.4±4.9 versus 29.7±4.9%, p=0.89. No difference between both sexes. Thrombocytopenia was found in 59.3%, of whom 1.9% had severe thrombocytopenia, platelet <50,000/mm^3, More than 50% of patients with thromocytopenia had recovered by day 28. Leucocytosis was more frequently seen than leucopenia, 9.5% vesus 3.0%. Baseline platelet was related to day 14 and day 28 platelet count; r=0.6, p<0.0001 and r=0.2, p=0.0015 and haematocrit on day 28, r=0.12, p=0.00197. Platelet showed no correlation with temperature, parasite density, haematocrit or leucocyte and treatment outcome. Hematocrit correlated with age, r = 0.4, p<0.0001; but not with parasite density r=-0.01, p=0.73 or temperature.Leucocyte showed no correlation with age or parasite density. Treatment was also related to the changes in hematological parameters. Conclusion: Changes in hematological parameters are related to age and drug treatment in malaria

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PHARMACOKINETICS AND BIOEQUIVALENCE OF TWO FORMULATIONS OF ARTESUNATE FOLLOWING SINGLE AND MULTIPLE INTRAVENOUS INJECTIONS IN RATS

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The aims of this investigation were to assess the steady-state pharmacokinetic parameters of artesunate (AS) and its active metabolite, dihydroartemisinin (DHA), in either 5% $NaHCO_3$ or 0.3M PBS formulations following single and multiple intravenous administrations. Series blood sampling during 72 hours was performed by using an automated blood sampler; drug concentrations were analyzed by LC-MS/MS with positive ion electrospray ionization using multiple reaction monitoring. The 90% CI of the difference between the two intravenous formulations was contained within 80/125% of the geometric mean of the PK parameters after single and multiple doses of AS in all single and multiple administrations and for DHA in only multiple doses studies. The equivalent result exhibited bioequivalence was fulfilled in two-period studies with the parent compound (AS) (82.3-117.7 for all parameters) and was partially satisfied for DHA in rats following the single or multiple intravenous administrations. The 0.3M PBS formulation of AS was considered to be bioequivalent to the 5% $NaHCO_3$ formulation at steady-state according to the total drug exposure, in terms of both parent drug (AS) and active metabolite (DHA), including single and multiple administrations in rats treated in two-sequence and two-period studies.

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TOLERABILITY OF IM ARTESUNATE

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Intravenous artesunate (AS) is being developed by our institute as an FDA approved product for the treatment of severe malaria. Intramuscular (IM) injection of AS represents a potentially important additional route of administration in patients in which venous access is difficult to obtain. We evaluated muscle biopsies from beagles following IM injection with varied doses of AS for evidence of local toxicity at 1 and 8 days post injection. Twelve beagles were given IM AS at 2.5, 5, or 10 milligrams per kilogram after reconstitution with phosphate buffer solution (PBS) into the left gluteal muscle. A second IM injection of PBS was given into the right gluteal. The dogs were divided into two cohorts with half euthanized at 1 day (cohort 1) and the remaining at 8 days (cohort 2) post injection. Complete blood count (CBC) and creatine kinase (CK) labs were assessed at baseline in the first cohort and prior to euthanasia in both cohorts. Each animal's muscle biopsies were evaluated by a pathologist blinded to treatment side and dose. Scores were then assigned based on type of inflammation and severity. Minimal manifestations of distress were noted at injection. Biopsies from cohort 1 showed myocyte necrosis, hemorrhage, and acute inflammation. A majority of the biopsies from cohort 1 had more extensive necrosis and more severe inflammation on the treated side than the control. Biopsies from cohort 2 showed necrosis, mineralization, fibrosis, and chronic inflammation. Differences in inflammation between the treated and control sides were not as evident as in cohort 1. Analyzed en masse, the average inflammation scores from the treated sides were higher than the control side (p=0.03). No statistically significant increase in inflammation was observed with increasing dose. Baseline and day 1 CBC data were comparable in cohort 1. Initial CK values from cohort 1 were normal, but increased above the reference range at one day post injection. Cohort 2 had normal CK values at 8 days post injection. In conclusion, IM injection with AS was well tolerated in this study. Changes consistent with acute muscle necrosis were evident acutely, but were less so after 8 days. These findings indicate that IM injection of AS is not more toxic than predicted. Further safety and efficacy studies using this route of administration are needed since AS represents a potentially life-saving treatment in severe malaria patients when IV access is not available.

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PHARMACOKINETICS OF CHLORPROGUANIL (CPG), DAPSONE (DDS), ARTESUNATE (ART) AND THEIR MAJOR METABOLITES IN PATIENTS DURING TREATMENT OF ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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As part of a Phase II ART dose finding trial for treatment of acute uncomplicated *Plasmodium falciparum* malaria, the pharmacokinetics (PK) of CPG, DDS and ART and their major metabolites chlorcycloguanil (CCG), monoacetyldapsone (MADDS) and dihydroartemisinin (DHA) were assessed in adult patients (N=115) in Malawi and The Gambia. Patients were randomized to CGP/DDS alone or CPG/DDS with 1, 2, or 4 mg/kg ART once daily for 3 days. For PK analysis, multiple blood samples were collected over a 24 h period post first dose. The AUC(0-t) and Cmax of both ART and DHA increased with increasing dose in a greater than proportional manner. This may be due in part to the longer time that ART and DHA were measurable at the higher doses. ART geometric mean AUC(0-∞) of 64.6, 151, and 400 ng.h/mL and Cmax of 48.9, 106 and 224 ng/mL and DHA AUC(0-∞) and Cmax of 538, 1445, and 3837 ng.h/mL and 228, 581 and 1414 ng/mL were observed at 1, 2, and 4 mg/kg ART doses, respectively. The terminal $t_{1/2}$ values for ART and DHA were similar across doses (ca. 0.5 h and 0.9 h). CPG AUC(0-24) and Cmax were similar across the 4 cohorts (geo. mean AUC and Cmax range 1110-1158 ng.h/mL and 81.2-83.5 ng/mL). CCG AUC(0-24) and Cmax without ART were 265 ng.h/mL and 260 ng/mL, whereas AUC(0-24) was approximately 6-17% higher and Cmax 0-16% higher with ART dosing. DDS AUC(0-24) and Cmax values were similar across cohorts (geo. mean AUC and Cmax range 41.7-43.9 ug.h/mL and 2.44-2.61 ug/mL). Without ART, the AUC(0-24) and Cmax for MADDS were 11.4 ug.h/mL and 0.678 ug/mL. With increasing ART doses, MADDS AUC increased on average 13, 31, and 47% and Cmax increased 8, 23 and 45%. In conclusion, greater than proportional increases in ART and DHA PK were observed with increasing ART dose. CPG and DDS rate and extent of absorption were not significantly affected by co-administration with ART. For CCG and MADDS metabolites, increases in Cmax and AUC with ART dosing were observed. A study in healthy volunteers is under way to further characterize the effect of ART and CPG/DDS on the PK of each other.

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ANTI-PLASMODIAL AND ANTIOXIDANT ACTIVITIES OF CONSTITUENTS OF THE SEED SHELLS OF *SYMPHONIA GLOBULIFERA* LINN F.

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A xanthone derivative, named gaboxanthone (1), has been isolated from the seed shells of *Symphonia globulifera*, together with known compounds, symphonin (2), globuliferin (3), guttiferone A (4), sistosterol, oleanolic acid and methyl citrate. The structure of the compound was assigned as 5,10-dihydroxy-8,9-dimethoxy-2,2-dimethyl-12-(3-methylbut-2-enyl) pyrano [3,2-b]xanthen-6(2H)-one, by means of spectroscopic analysis. The anti-plasmodial and antioxidant activities of the phenolic

compounds were evaluated, respectively, in culture against W2 strain of *Plasmodium falciparum* and using the free radical scavenging activity of the DPPH radical, respectively. Compounds 1-4 were found to be active against the Plasmodium parasites (IC50 of 3.53, 1.29, 3.86 and 3.17 μM, respectively). Guttiferone A (4) showed a potent free radical scavenging activity compared to the well-known antioxidant caffeic acid.

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ARTESUNATE: EMBRYOLETHAL EFFECTS IN CYNOMOLGUS MONKEYS

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Artesunate caused embryo death and malformations (heart defects and bent and shortened long bones) in rats and rabbits with a critical period in rats of Days 10-14 postcoitum (pc). DHA, artemether and arteether caused the same effects when administered on Day 10 pc. We have found that artesunate acts in rats by killing primitive embryonic erythroblasts. To determine whether similar embryotoxicity would occur in a primate species, an embryofetal development study of artesunate was conducted in cynomolgus monkeys with dosing on days 20-50 pc at 0, 4, 12 and 30 mg/kg/day (N=15, 15, 11 and 9, respectively). Fetuses were examined on day 100 pc. Maternal effects consisted of mild decreases in food consumption and a 10% decrease in hematocrit at 30 mg/kg/day, and decreases in reticulocyte counts to <0.2% of blood cells in 4 animals at 30 mg/kg/day and in 1 animal at 12 mg/kg/day (control mean = 1.4%). Among 9 embryos in the 30 mg/kg/day group, 6 died between days 30 and 40 pc and the remaining 3 were removed and saved for histological examination. Among 11 embryos in the 12 mg/kg/day group, there were 6 treatment-related deaths between days 30 and 45 pc, 1 non-treatment related death between before day 30 pc, and 4 that survived to day 100 pc. There were no treatment-related malformations. Among the 4 surviving fetuses in the 12 mg/kg/day group, there was a statistically significant 7% decrease in ulnar length compared with control. Histological examination of live embryos from the 30 mg/kg/day group fixed on days 26, 32 and 36 pc revealed that the blood vessels had a marked reduction in erythroblasts, similar to what had been seen in rats treated on day 10 or 11 pc. The plasma AUC and Cmax values for artesunate and DHA at 12 mg/kg/day on day 33 pc were below clinical levels. In summary, treatment of pregnant cynomolgus monkeys with artesunate starting on Day 20 pc caused embryo death after more than 12 days of treatment at 12 and 30 mg/kg/day. There were no developmental effects at 4 mg/kg/day. Work is continuing to understand the clinical relevance of these findings.

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ANTIMALARIAL PYRONARIDINE TARGETS HEMATIN

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Plasmodium falciparum has become resistant to almost all current antimalarials, except for the artemisinins. Thus there is an urgent need to find new chemotherapeutic drugs that are both efficacious and affordable in affected developing countries. Pyronaridine, 2-methoxy-7-chloro-10[3′,5′-bis(pyrrolidinyl-1-methyl-)4′-hydroxy-phenyl]amino-benzyl-(b)1,5-naphthyridine, a Mannich base schizontocidal developed in China and structurally related to the aminoacridine drug, quinacrine, is undergoing clinical trials in combination with artesunate. We show that pyronaridine targets hematin, as demonstrated by its ability to inhibit *in vitro* β-hematin formation (at a concentration equal to that of chloroquine), to form a complex with hematin with a stoichiometry of 1:2, to inhibit glutathione-dependent degradation of hematin and to enhance hematin-induced human red cell lysis (but surprisingly at 1/100 of chloroquine concentration). Demonstration that pyronaridine exerted this mechanism of action *in situ* by conducting growth studies of *P. falciparum* K1 strain in culture with pyronaridine in the presence of antimalarials that inhibit β-hematin formation (chloroquine, mefloquine, quinine), and also concanamycin A, a macrolide antibiotic inhibitor of vacuolar ATPase derived from *Streptomyces* sp., showed additive or very mild antagonistic inhibitory effect.

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ANTIMALARIAL ACTIVITY OF SUBSTITUTED 1,7-DIAMINOISOQUINOLINES

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The chalcone class of compounds appears to possess antimalarial activity by a mechanism distinct from those employed by other antimalarial agents. We have developed a pharmacophore from the structure-activity relations of the chalcones, and with it determined that 6-benzylated 2,4,6-triaminoquinazolines should also mediate antimalarial effects by this novel mechanism. Such triaminoquinazolines are already known to be potent antimalarials by their inhibition of dihydrofolate reductase. Using the pharmacophore, and published data describing the structural requirements for inhibition of dihydrofolate reductase, we sought to reduce the folate antagonism, whilst retaining the antimalarial activity via the chalcone-mechanism, in the triaminoquinazolines. 7-Benzylated 1,7-diaminoisoquinolines emerged as a class in which these goals were potentially met. Such compounds are not described in the literature, so therefore have not previously been assessed as antimalarial agents. We will describe their preparation, which required seven synthetic steps, and the determination of their *in vitro* efficacy against the W2 and D6 strains of *Plasmodium falciparum*. The emerging *in vitro* SAR of these potent inhibitors will be discussed, along the results of our studies to determine the mechanism by which this novel class of compounds is mediating its effect.

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DEVELOPMENT OF NEW ANTI-MALARIAL DRUGS DERIVED FROM CHLOROQUINE

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Aminoquinolines such as chloroquine (CQ), mefloquine, and tafenoquine are effective antimalarial drugs. NMR studies and molecular dynamics calculations have shown that the aromatic ring of CQ participates in - stacking with host heme protoporphyrin IX in the digestive vacuole of the parasites. Additionally, depending on the local pH of the heme environment, the quinolyl nitrogen may be coordinated to the iron center. A subtle change in the -basicity of both the quinoline ring and the quinolyl nitrogen has been achieved through the synthesis of CQ analogs in which the 4-amino group is replaced by an ether or sulfide group. A series of quinolines exhibiting diethylamino-derived side chains of varying length has been prepared to systematically study drug-heme affinities. Also, chloroquine analogs exhibiting a side chain with two terminal amino functions have been prepared. Since it has been established that the terminal amino function present in the side chain of chloroquine (CQ) is essential for trapping high concentrations of the drug in the food

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vacuole of the parasite, these analogs should in theory effectively promote higher accumulation of the drug in the acidic digestive vacuoles of the parasite relative to chloroquine. The synthesis and antimalarial activity of a series of chloroquine derivatives including fluorescent and radiolabeled aminoquinolines that are useful probes for studying cellular uptake, distribution and degradation in CQ sensitive and resistant strains will be discussed.

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EVALUATION OF *IN-VITRO* ANTIMALARIAL ACTIVITY OF *MOMORDICA CHARANTIA* AND *GOSSYPIUM BARBADENSE* AGAINST FIELD ISOLATES OF *P. FALCIPARUM* IN SOUTHWESTERN NIGERIA

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Malaria is a major public health problem worldwide. It is particularly grave in Africa South of the Sahara where children and pregnant women are at greatest risk from the disease. Chemotherapy is the most reliable form of control of malaria. Common antimalarial drugs have been rendered ineffective due to the development and spread of resistance by the parasite. An urgent need therefore arises to develop alternative effective antimalarial agents from various sources particularly natural products. Two plants used traditionally as antimicrobial agents namely *Gossypium barbadense* (GB) and *Momordica charantia* (MC) were investigated for antimalarial activity *in-vitro*. Crude methanolic extracts and flavonoid fraction of the two plants were used for the study. The extracts were dissolved in DMSO while chloroquine (CQ) and quinine (QN) which served as reference standards were dissolved in water. Twenty-six isolates of wild parasites were obtained directly from *P. falciparum* infected children (aged 9.4 $\pm$ 5.8 years) for schizont inhibition assay using the standard technique of Rieckmann et al (1978). Twelve isolates successfully cultured to schizonts were used for the analysis. The data was analysed using dose-response curve of graph pad prism software. The mean 50% inhibitory concentration (IC_{50}) were 0.46 ±0.13, 0.025 ± 0.013, 6.08 ± 13.17 and 10.55 ± 13.5 µg/ml for chloroquine, quinine, cold and hot extracts of GB while it was 16.31 ± 28.61 and 4.44 ± 5.03 for flavonoid and crude extracts of MC respectively. Only 9 of 11 (82%) isolates were sensitive to CQ while all the isolates were sensitive to QN. 75% of the isolates were sensitive to both MC flavonoid (MC1) and crude extract (MC2) while 67% and 75% of the isolates were sensitive to GB1 (cold extract) and GB2 (hot extract) respectively. The 2 isolates that were resistant to CQ were sensitive to QN and extracts of MC while one was resistant to both GB1 and GB2. Hematological parameters indicated that the extracts are not toxic. MC and GB extracts inhibited schizont maturation in a manner comparable with CQ and QN and could be potent against CQ resistant parasites.

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CONFIRMATION OF EMERGENCE OF MUTATIONS ASSOCIATED WITH MALARONE® RESISTANCE IN UNEXPOSED *PLASMODIUM FALCIPARUM* ISOLATES FROM NIGERIA

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In vitro and *in vivo* resistance of *Plasmodium falciparum* to atovaquone-proguanil hydrochloride combination (Malarone®) has been associated to two point mutations in the parasite cytochrome b (*cytb*) gene (Tyr268Ser and Tyr268Asn). However, little is known about the prevalence of codon-268 mutations in natural populations of *P. falciparum* without previous exposure to the drug in Africa. The prevalence codon-268 mutations in the *cytb* gene of *P. falciparum* isolates from Nigeria was assessed. Genotyping of *cytb* gene in isolates of *P. falciparum* was performed by PCR-Restriction Fragment Length Polymorphism and confirmed by sequencing. One hundred and eighteen (118) samples obtained from Nigerian children were successfully analyzed for detection of *cytb* (Tyr268Ser or Tyr268Asn) mutations. No case of Ser268 was detected in *cytb* gene of all isolates of *P. falciparum*. However, the mutant *cytb* Asn268 allele was detected in 5 out of 118 (4.2%) unexposed *P. falciparum* isolates. In addition, one out of these 5 mutant isolates showed an additional *cytb* mutation leading to a Pro266Thr substitution inside the ubiquinone reduction site. Overall, we report for the first time the presence of *cytb* Tyr268Asn mutation in an unexposed population of *P. falciparum* of Africa. The emergence in Nigerian isolates of *P. falciparum* of *cytb* Tyr268Asn mutation is a matter of serious concern. Continuous monitoring of Malarone® resistant *P. falciparum* in Africa is warranted for the rational use of this new antimalarial drug especially among non-immune travellers.

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FIXATION OF *P. FALCIPARUM* SP RESISTANT MUTATIONS IN AN AREA WITH LOW GENETIC DIVERSITY

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Sulfadoxine-pyrimethamine (SP) is an anti-folate drug commonly used to treat *Plasmodium falciparum* infections. Mutations in the genes encoding dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) have been linked to SP resistance *in vivo*. Recent studies have used microsatellite markers surrounding *dhfr* to investigate the origin of SP resistant alleles in *P. falciparum* in Southeast Asia and Africa. To date, there have not been any studies using markers surrounding *dhfr* and *dhps* to understand the origins of drug resistance in South America. SP resistant parasites are common in the Amazon basin; however, the drug is still effective in the Pacific coast. Thus, a concern is that SP resistance could be dispersed by gene flow. The importance of gene flow, however, will be affected by whether SP resistance has a single or multiple independent origins. We utilize neutral markers around the genes *dhfr* and *dhps*, and on chromosomes 2 and 3 to elucidate SP origins in Venezuela. We utilized 97 samples from Bolivar state, an area where SP is ineffective. We genotyped the samples for mutations at *dhfr* codons 50, 51, 59, 108, and 164 and *dhps* codons 436, 437, 540, 581, and 613 by pyrosequencing. Samples were assayed for 26 microsatellite loci that span 700 kb around *dhfr*, 23 loci that span 698 kb around *dhps*, 4 loci on chromosome 2, and 3 loci on chromosome 3. Genotyping revealed two genotypes present in the samples: *dhfr* 50R/51I/108N *dhps* 437G/540E/581G (90.7%) and *dhfr* 51I/108N *dhps* 437G/581G (9.3%). By using the markers on chromosomes 2 and 3, we are able to demonstrate selection on *dhfr* and *dhps* due to drug pressure as well as clonal population structure. We conclude that there is a single origin for *dhfr* resistant alleles in our sample; however, the *dhfr* lineage found in Venezuela is different from those reported in Africa, demonstrating multiple origins of SP resistant alleles around the world. This study confirms that local ecology and evolutionary history are important factors when studying the origin and spread of drug resistance.

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VARIATION IN *PLASMODIUM FALCIPARUM* SUSCEPTIBILITY TO THE CINCHONA ALKALOIDS IS DETERMINED BY POLYMORPHISMS IN PFCRT

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The *Cinchona* alkaloids are a series of quinolines that exist as stereoisomer pairs, such as (-)-quinine and (+)-quinidine. Their mechanism of action remains poorly defined but involves inhibition of hematin detoxication within the digestive vacuole (DV). Within each stereoisomer pair, the (+)-isomer is more active *in vitro* against field isolates than the (-)-isomer. Our earlier studies showed that a K76I mutation in PfCRT (*P. falciparum* Chloroquine Resistance Transporter) imparted a stereospecific effect on quinine and quinidine sensitivity. Taken together, these data suggest that PfCRT may serve as a *Cinchona* alkaloid receptor. In order to test this hypothesis, we measured the *in vitro* sensitivity of three control parasite lines of diverse genetic background and an isogenic panel of seven lines each containing a single point mutation in PfCRT against eight (-)/(+)-paired *Cinchona* stereoisomers: quinine and quinidine; cinchonidine and cinchonine; hydroquinine and hydroquinidine; 9-epiquinine and 9-epiquinidine. In control strains and most mutants, sensitivity to the alkaloids was variable, but the potency order within stereoisomer pairs remained constant. However, the potency differences against K76I were either reversed or diminished, indicative of a drug-receptor interaction. A possible explanation is that PfCRT forms an efflux channel in the DV membrane, and hydrophobic interaction of residues 72 and/or 76 with (-)- and (+)-alkaloids is stereospecifically determined. The high hydrophobicity of residue 76I determines enhanced interaction with the hydrophobic side of the (-)-stereoisomers. These data provide evidence that PfCRT binding is a critical component in the molecular action of quinine and other *Cinchona* alkaloids.

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PYROSEQUENCING-A HIGH-THROUGHPUT METHOD FOR DETECTING SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN THE DIHYDROFOLATE REDUCTASE AND DIHYDROPTEROATE SYNTHETASE GENES OF *PLASMODIUM FALCIPARUM*

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A pyrosequencing protocol was developed as a rapid and reliable screening method to identify the mutations of *dhfr* and *dhps* genes of *Plasmodium falciparum* that are associated with antifolate resistance. The accuracy and specificity of this method was tested using six laboratory cultured *P. falciparum* isolates harboring known single nucleotide polymorphisms (SNPs) in the genes *dhfr* (codons 50, 51, 59, 108 and 164) and *dhps* (codons 436, 437, 581 and 613). The equivalent amount of DNA to two to four genomes was the lower threshold for detection of all the SNPs tested by pyrosequencing. Also, this method was highly specific to *P. falciparum* as it did not detect DNA from the other species of human malaria parasites. We also mixed wild-type and mutant type parasite DNAs in various proportions and determined how pyrosequencing, restriction fragment length polymorphism (RFLP), and direct conventional sequencing (for *dhfr*) compared with each other in detecting different SNPs in the mixture. In general, pyrosequencing and RFLP showed comparable sensitivities in detecting most of the SNPs in *dhfr* except for the 164L mutation. This target required at least twice the amount of DNA for detection of the mutation by pyroseqencing as compared to RFLP. For detecting SNPs in *dhps*, pyrosequencing was slightly more sensitive than RFLP. In general, conventional direct sequencing was less sensitive than RFLP and pyrosequencing. Pyrosequencing gave unambiguous results as compared to RFLP in differentiating mixed infections; restriction enzymes did not completely cut the target DNA for each reaction. Overall pyrosequencing was faster, cheaper, and simpler than either RFLP or direct sequencing. Thus, pyrosequencing is a useful and reliable method that can be used in a high-throughput format for molecular surveillance of drug resistance to antimalarial drugs.

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PLASMODIUM FALCIPARUM CRT AND *DHFR/DHPS* ALLELES AND EFFICACY OF CHLOROQUINE PLUS SULFADOXINE/PYRIMETHAMINE FOR FALCIPARUM MALARIA MORE THAN ONE DECADE AFTER TREATMENT POLICY IMPLEMENTATION AT MALO ISLAND, SANMA PROVINCE OF THE REPUBLIC OF VANUATU

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Chloroquine (CQ)-resistant *Plasmodium falciparum* was first described in Vanuatu in the early 1980s. In 1991, the Ministry of Health developed new treatment guidelines for uncomplicated falciparum malaria consisting of combination CQ + sulfadoxine/pyrimethamine (SP). During 2005, we conducted cross-sectional malariometric surveys in 45 villages on Malo Island, measured malaria incidence and established the efficacy of CQ for *P. vivax* and CQ + SP for *P. falciparum*. Among 4,063 adults and children screened, the prevalence of parasitemia was 232 (6%). The ratio of *P. falciparum* to *P. vivax* was 1. Twenty percent of persons with patent parasitemia were febrile at the time of screening. Two hundred and three volunteers without parasitemia at initial screening were evaluated weekly for development of malaria over 13 weeks. After 2,400 person weeks of follow-up, the incidence density of malaria was 1.3 cases per person year, *P. vivax* predominating. Among 57 individuals participating in the clinical trial, the 28 day CQ *P. vivax* cure rate was 100%. The 28 day CQ + SP *P. falciparum* cure rate was 97%. The single treatment failure, confirmed by merozoite surface protein (*msp-2*) genotyping, was classified as a day 28 late parasitological treatment failure (LPTF). All *P. falciparum* isolates carried the Thr-76 *pfcrt* mutant allele and the double Asn-108 + Arg-59 *dhfr* mutant alleles. *Dhps* mutant alleles were not present. The long standing Ministry of Health recommended CQ +SP combination remains efficacious in this population, and although the chloroquine resistant *pfcrt* allele is highly prevalent, mutant alleles in the *dhfr* and *dhps* genes do not occur to the extent required to confer resistance.

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LONGITUDINAL STUDY OF SULFADOXINE PYRIMETHAMINE AND CHLOROQUINE EFFICACY IN BURKINA FASO FROM 2001 TO 2003

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Malaria is a public health concern in Sub Saharan African and in Burkina Faso; the main strategy is based on rapid detection of the cases and their management with efficient antimalarial. Throughout Africa, resistance to CQ and SP is widespread with increasing rate of childhood mortality

and morbidity. There is a need of monitoring antimalarial efficacy in our setting. The patients who presented to the clinic with signs suggestive of malaria are screened. The patients who presented malaria and who fulfilled the inclusion criteria are followed up for 14 and 28 days according to WHO guidelines for assess drug efficacy and their results classified based on WHO 2002 protocol. New infections and recrudescences were distinguish using MSP 1 and MSP 2 in 2002 and 2003 and the drug resistance markers by genotyping using the Nested PCR. During the study, from 2001 to 2003, 1090 patients have been screened for chloroquine studies, 933 were enrolled and 829 finished the study. For SP study, 157 patients were enrolled and 143 finished the study. The mean CQ-failure was 24,9% with 26,1% in 2001, 20,4% in 2002 and 30% in 2003 and the SP one was 4.3% in 2003. The mean parasitological resistance was 33% with 26,1% in 2001, 25,4% in 2002 and 45,3% in 2003 and 4.3% for SP. Hematological recovery was better for SP than CQ. CQ is no more useful for treatment of uncomplicated falciparum malaria in Burkina Faso while resistance to SP is increasing. New alternatives treatment are clearly needed.

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MINIMUM GENETIC DIVERSITY IN RESISTANT STRAINS OF *PLASMODIUM FALCIPARUM* FROM KENYA

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Resistance of *Plasmodium falciparum* to antifolates such as Fansidar (sulfadoxine-pyrimethamine, SP) is due to point mutations in the gene that encodes dihydrofolate reductase (*dhfr*). Patients infected with a parasite carrying three mutations in *dhfr* (N51I/C59R/S108N) are at elevated risk of failing SP treatment. Studies of the extended haplotype encompassing *dhfr* suggest that a single triple-mutant allele of *dhfr* emerged in Asia and spread to Africa. However, it is unclear whether this "Asian" strain replaced triple-mutants that had previously evolved in Africa, or simply invaded a population devoid of triple-mutants. To investigate this question, we analyzed 42 blood samples collected in Kilifi, Kenya, between 1993 and 1995, the period immediately following first use of SP. We genotyped each sample at *dhfr* and at five microsatellite loci near *dhfr*. There were 14 wild-type, 19 double-mutants (11 N51I/S108N; 8 C59R/S108N), and 9 triple-mutants. All of the triple-mutants had the same haplotype, and it matched the haplotype of the Asian strain from previous studies. The wild-type parasites had a variety of haplotypes, none related to the triple-mutant haplotype. Each double-mutant (N51I/S108N or C59R/S108N) had a single haplotype. Both of the double-mutant haplotypes shared some alleles with wild-type samples and with each other, but neither shared any alleles with the triple-mutant haplotype. These results suggest that the double-mutant dhfr alleles did not give rise to a triple-mutant allele. Prior to the arrival of the Asian strain, either there were no parasites in Kilifi that carried a triple-mutant allele of *dhfr*, or any local triple-mutant strain(s) disappeared prior to 1993. To explore the history of sulfadoxine-pyrimethamine resistance further, we analyzed *P. falciparum* DNA extracted from archived microscope slides. These slides were collected in Kilifi in 1988, well before any use of SP in the area. Preliminary results suggest that the Asian triple-mutant was already in Kilifi at this time, but that there may have been novel triple mutants, as well.

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PRESENT SITUATION OF ARTEMISININ-BASED COMBINATION THERAPIES (ACT) EFFICACY AND SAFETY IN MULTIDRUG-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA IN CÔTE D'IVOIRE

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Malaria treatment in Côte d'Ivoire has been in process of change since January 2005. Preceding the change, these studies aimed to determine which ACT therapy is more effective to treat multidrug-resistant *Plasmodium falciparum*. The trials were open-label, randomized studies comparing Sulfamethoxypyrazine-pyrimethamine-artesunate (Coarinate®) *versus* Artemether-lumefantrine (Coartem®), Artesunate-amodiaquine (Amonate®) *versus* Artemether-lumefantrine (Coartesiane®) and sulfalene-pyrimethamine-amodiaquine (Dualkin®) *versus* amodiaquine-artesunate (Arsucam®) for uncomplicated malaria treatment with 28 days of follow up. Parasitic genotyping was use to distinguish recrudescent from newly acquired infections. A total of 619 patients were randomly assigned to receive one of the assigned treatment for each study. All patients under sulfamethoxypirazine-pyrimethamine-artesunate demonstrated adequate clinical and parasitoligical response against 97.4% for artemether-lumefantrine with a rate of late clinical failure (LCF) (2.6%). 98.2% and 97.4% patients presented adequate clinical and parasitological response respectively for artesunate-amodiaquine and artemether - lumefantrine. The rate of failure was 1.7% with 0.9 % of LCF and 0.9% of late parasitological for artesunate-amodiaquine against 2.6% essentially LCF for artemether-lumefantrine. Adequate clinical and parasitological response was 100% in artesunate/amodiaquine arm against 99.1% in sulfalene/pyrimethamine/amodiaquine arm with LCF (0.9%). Both regimens were very tolerated with no serious adverse events observed attributable to either combination. Overall, these studies confirm that these artemisinin based combination therapies remain highly effective. During a consensus Workshop, the Ministry of public Health agreed on the combination of artesunate-amodiaquine, artemether-lumefantrine respectively as the first and second line drug for uncomplicated malaria treatment in Côte d'Ivoire. When implemented, ACT efficacy should be monitored in sentinel sites representing different areas of the country.

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A CENTURY AFTER MIAN MIR: WHAT REALLY HAPPENED DURING THE ORIGINAL MILITARY MALARIA CONTROL PROJECT?

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Shortly following the discovery of mosquito transmission of malaria by Ross, a field trial to reduce malaria in soldiers at Mian Mir cantonment in what is now Pakistan was initiated. Drainage of standing water using unskilled local labor was thought to be capable of killing sufficient mosquito larvae to interrupt malaria transmission. When this proved to be mistaken, suppressive quinine and discontinuation of irrigation canals was added. Since malaria in the Punjab is highly seasonal and subject to epidemics, the data obtained from 1901-09 was equivocal at best. Arguments that became increasingly personal continued over whether mosquito control to stop malaria was impractical or had never been pursued with sufficient vigor to be definitive. The original monthly malaria morbidity data was prospectively collected and published for both British and Indian soldiers by geographic military establishment for

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>50y. These historical records were collected and analyzed to determine whether mosquito control at Mian Mir actually had a discernable effect on transmission. Malaria incidence at Mian Mir was compared to a similar post in Delhi 300 miles away: correlation coefficients (r^2) were 0.45 between British and Indian soldiers at Mian Mir and 0.27 between British soldiers at Mian Mir and Delhi. The ratio of Mian Mir/Delhi cases appeared to fluctuate randomly in the period from 1872 to 1920 except for a visible anomaly during 1903-1910 that could not be explained on the basis of differential rainfall. The false dichotomy of mosquito control versus antimalarial drugs has been extended into the present day. Long term malaria epidemiology gathered from historical records can inform present day questions of malaria control, hopefully while minimizing dogmatic solutions to complex problems.

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EPIDEMIOLOGY OF MALARIA, DENGUE AND OTHER ARTHROPODE-BORNE VIRAL INFECTION IN A RURAL COHORT IN WESTERN BRAZILIAN AMAZON (GRANADA, ACRE, BRAZIL)

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Here we describe the baseline prevalence of malaria and the seroprevalence of arthropode-borne virus infections among 466 participants in an ongoing cohort study of risk factors for malaria and arthorpode-borne virus infections that started in 2004 in rural Amazonia. The study population comprises subjects aged <1-90 years living in 113 households in an agricultural settlement in the malaria-endemic Western Amazon Basin of Brazil. Most (72.2%) subjects reported one or more past malaria episodes, and 15.6% of them had been previously hospitalized during a malaria attack, but only 3.6% of the subjects aged five years or more harbored malaria parasites (10 of them *Plasmodium vivax* and 4 *P. falciparum*), as detected by microscopy. Molecular studies detected *P. falciparum* and *P.vivax* infection in 24 subjects that were symptomless when examined (8 by *P. vivax*, 14 by *P.falciparum* and 2 by both Plasmodia). Antibodies to arboviruses belonging to the genera *Alphavirus*, *Orthobunyavirus* and *Flavivirus* were detected by hemagglutination-inhibition tests in 42.6% of the subjects aged five years of more, with a higher seropositivity rate among males (49.2%) than females (36.2%). Since 98.9% of the study subjects had been previously immunized against yellow fever, the presence of cross-reactive antibodies to dengue and other *Flavivirus* cannot be ruled out, but at least 12 subjects (3.3%) with IgM antibodies to dengue virus detected by ELISA had a putatively recent exposure to this virus. Analysis of risk factors for malaria and dengue fever are in progress, and includes epidemiological, parasitological, immunological and genetic variables.

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MALARIA BURDEN IN CHILDREN LIVING IN A MALARIA STABLE TRANSMISSION AREA: RESULTS FROM 5 YEARS OF SURVEILLANCE DURING THE TRANSMISSION SEASON

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Malaria remains the leading cause of morbidity and mortality in under 5 children living in endemic countries. In most of the endemic regions, in absence of microscopes and rapid diagnosis test, the presence of fever is the major criteria on which is based the decision to treat for malaria episode. We investigated the part of the fever attributable to ***P. falciparum*** positive parasitaemia at different threshold; as well as the incidence of clinical malaria episodes. A longitudinal follow up was also carried out every year during the transmission season. The children were visited at home twice a week to record their temperature and obtain a blood slide if the T°≥37.5°C. All the children with fever received a curative dose with an effective antimalarial drug. The severe malaria cases identified were referred to appropriate level of care. The data from the five years surveillance were pooled. We compared the attributable fraction of the Fever to the presence of the positive parasitaemia (Attributable Fraction of the Risk, AFR) in different age groups and at different parasitamia threshold. At any age group, the AFR is maximum when any density of parasitamia is considered; and is decreasing progressively with the increasing threshold of parasite density (the threshold of 5000 trophozoites/µl and 10000 trophozoites/µl were considered). Thus in age group 0-1 year, the AFR was decreasing respectively from 48.4% to 14.9% and 5.8%. In age group 1-3 years, respectively from 79.9% to 57.5% and 40.8%. In age group 4-5 years, respectively from 76.5% to 51.5% and 34.4%. In age group 5-10 years, respectively 42.8% to 12.4% and 7.9%)

The incidence of clinical malaria was inversely correlate with the age (4.3% in 0-1 year, 4.0% at 1-3 year, 3.0% at 4-5 years and 1.5% at 5-10 years). This results also showed that within the same threshold, the AFR was higher in children age 1-3 years. In conclusion, our findings confirm that children below 5 years are bearing a high burden of malaria in endemic countries. A malaria case definition need to be developed

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NATURALLY ACQUIRED HUMORAL AND CELLULAR IMMUNE RESPONSES TO *P. VIVAX* MEROZOITE SURFACE PROTEIN 9 (PVMSP9) IN INDIVIDUALS FROM RONDÔNIA STATE-BRAZIL EXPOSED TO MALARIA INFECTIONS

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Merozoite Surface Protein-9 of *Plasmodium vivax* is highly conserved and has orthologs in several malaria parasite species including the *P. falciparum* surface antigen known as ABRA. In a cross-sectional study carried out in Rondônia state, Brazil, we evaluated the antibody and T cell reactivity to PvMSP-9 in individuals naturally exposed to malaria infections living in Ribeirinha (N=188), a native riverine community and Colina, a transmigrant community living in a rural area close to Porto

Velho (N=122). The reactivity of sera samples were evaluated by ELISA against recombinant proteins comprising the N-terminus (PvMSP-9-Nt), the first and second block of tandem repeats (PvMSP9-RI/RII) and the second block of repeats (PvMSP9-RII) of the PvMSP9 and the cellular responses for IFN-γ and IL-4 by ELISPOT using PvMSP-9 derived synthetic peptides. Our results show that the cytokine profiles were different between the two communities, while IFN-γ and IL-4 production predominates in the native population (Ribeirinha), IFN-γ production predominates in the transmigrant population (Colina). The antibody response was also distinct between both communities, the frequency of IgG positive individuals to PvMSP9-RI/RII and PvMSP9-Nt in Ribeirinha (70,9% and 48,3%) was higher when compared with Colina (43,0% and 15,7%) respectively and no difference was observed in the frequency of positives to PvMSP9-RII (42,4% Ribeirinha and 39,7% Colina, respectively). Interestingly, in both communities the IgG subclasses detected were mainly IgG2 for PvMSP9-RII in Colina (66,7%) and Ribeirinha (67,1%) and IgG1 for PvMSP9-Nt in Ribeirinha (63,1%). The antibodies reactivity indexes were always higher in the native population (Ribeirinha), and were directly correlated with age and time of exposure. Our data shows for the first time that cellular and antibody responses against PvMSP9 are induced in individuals exposed to malaria infections and variation in exposure seems to account for the differences in cytokine and antibody levels of naturally induced immune responses in the studied population.

(ACMCIP Abstract)

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COMMUNITY CONCEPTS OF MALARIA-RELATED ILLNESS WITH AND WITHOUT CONVULSIONS IN SOUTHERN GHANA

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Malaria, both with or without convulsions, is a serious hardship for people living in endemic areas, especially in sub-Saharan Africa. Community references to malaria, however, may encompass other conditions, which was collectively designated malaria-related illness (MRI). Inasmuch as the presence or absence of convulsions reportedly affects timely help-seeking for malaria, a local comparison of these conditions is needed to inform malaria control. Vignette-based EMIC interviews (insider-perspective interviews) for MRI with convulsions (convulsion positive, MRI-CP) and without convulsions (convulsion negative, MRI-CN) were developed to study relevant features of MRI-related experience, meaning and behaviour in two rural communities in Ghana. These semi-structured interviews elicited both qualitative narrative and categorical codes for quantitative analysis. Interviews with 201 respondents were conducted. The conditions depicted in the vignettes were well recognized by respondents and named with various local terms. Both presentations were considered serious, but MRI-CP was more frequently regarded potentially fatal than MRI-CN. More than 90.0% of respondents in both groups acknowledged the need to seek outside help. However, significantly more respondents advised appropriate help-seeking within 24 (p=0.01) and 48 (p=0.01) hours for MRI-CP. Over 50.0% of respondents responding to questions about MRI-CP identified MRI-CN as a cause of convulsions. Local comparison of MRI-CP and MRI-CN based on vignettes found a similar profile of reported categories of perceived causes, patterns of distress, help-seeking and preventive measures for both presentations. This differs from previous findings in sub-Saharan Africa, which assert communities regard the two conditions to be unrelated. The perceived relationships should be acknowledged in formulating strategies to control malaria through timely help-seeking and treatment to reduce childhood mortality.

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DYNAMICS OF *PLASMODIUM FALCIPARUM* MSP-1_{19} GENETIC DIVERSITY AT A MALARIA VACCINE-TESTING SITE IN MALI

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The prospect of an effective malaria vaccine is threatened by extensive genetic diversity in vaccine antigens. It is important to measure the baseline dynamics of vaccine antigen polymorphisms in populations where vaccines will be tested to distinguish natural fluctuations in allele frequencies from the effects of vaccination in malaria vaccine efficacy trials. To understand the natural variation in MSP-1_{19} genetic diversity at a malaria vaccine-testing site in Bandiagara, Mali, Pyrosequencing was used to genotype MSP-1_{19} from 1369 samples collected from 100 children who participated in a malaria incidence study at this site from 1999-2001. The prevalences of 14 MSP-1_{19} haplotypes were compared over three malaria transmission seasons and in three age groups (≤5 years, 6-10 years, ≥11 years). While temporal variation in the frequency of individual MSP-1_{19} haplotypes was observed, the haplotypes corresponding to the FVO (QKSNGL) and FUP (EKSNGL) strains predominated during three consecutive years and in all age groups with overall prevalences of 46% (95% CI=44-49%) and 36% (95% CI=34-39%), respectively. The 3D7 haplotype (ETSSRL), on which a vaccine tested at this site is based, had a lower overall prevalence of 16% (95% CI=14-18%). Multiplicity of infection based on MSP-1_{19} was higher at the beginning of the transmission season and in the oldest children (≥11 years old). In a multivariable model adjusting for time, age, and longitudinal measurements, three MSP-1_{19} haplotypes had a decreased odds of being present in a symptomatic infection versus an asymptomatic infection (ETSSRL: OR=0.67, 95% CI=0.49-0.91, p=0.011; QKNNGL: OR=0.48, 95%CI=0.35-0.68, p<0.0001; and EKSNGF: OR=0.37, 95%CI=0.13-1.09, p=0.07). If immunity elicited by an MSP-1-based vaccine is allele-specific, a vaccine based on one or both of the predominant strains may have better initial efficacy in this geographic location. Analysis of the within-host dynamics of MSP-1_{19} diversity may provide insight into which amino acids are important in determining cross-reactivity between haplotypes.

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THE STUDY OF *AN. MINIMUS* DENSITY GREY EVALUATION MODEL

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This study was undertaken to establish *Anopheles minimus* density evaluation model depending on climate and remote sensing ecological indexes. There are 27 townships of 10 counties in Yunnan province have been chosen as the study fields with *An. minimus* being the main transmission vector of malaria. The successive surveillance data of climate, environmental, remote sensing ecological data and *An. Minimus* density data have been collected from 1984 to 1993. Data of 15 townships in 1984-1993 has been chosen as the model establishing data. There are 18 indexes of climate, environmental, remote sensing NDVI etc., which have been chosen as the initial evaluation indexes of vector density. *An. Minimus* density has been chosen as the main factor, and the grey correlation analysis has been done to choose principle evaluation indexes depending on certain grey threshold. Weights of indexes have been given depending on grey correlation order and *E* has been formed based on addition method. The relationship of *E* and vector density has been

studied to establish vector density fitness evaluation model. The vector density of another 12 townships has been evaluated depending on *An. Minimus* density grey evaluation model. The grey correlation analysis has been done to analyze the correlation of *An. Minimus* evaluation density and actual density standard value. An. Minimus density of Jinghong, Mengla, Menglian, Simao, Yuanjiang, Zhengyuan counties in 1994, 1999, 2000 have been evaluated depending on *this* model. *An.* minimus density evaluation indexes have been chosen depending on grey correlation degree 0.70. grey correlation order of which is as following: Dry season average temperature > Dry season temperature$_{min}$ > wet season temperature$_{min}$ > wet season NDVI > Wet season average temperature > the ratio of paddy field of infield> dry season temperature$_{max}$ > Wet season temperature$_{max}$. The correct rate of model is 92.0%. $e'_{0.5}$ = 18%,average different is: 21%. *An. minimus* density of frontier area is increasing, but *An. minimus* density of Yuanjiang county decreased. Depending on climate, environmental and ecological surveillance, the Anopheles Minimus density can be evaluated and forecasted.

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STATUS OF URBAN MALARIA IN COLOMBIA

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A reduction of 45% in the incidence of urban malaria is part of the Millennium Development Goals for Colombia. However, the knowledge of the burden and epidemiological characteristics of urban malaria needed for the design and implementation of effective and rational control strategies in the country is limited. This study was undertaken to determine the distribution and epidemiological patterns of urban malaria transmission in Colombia. Information about the epidemiological and entomological characteristics of malaria transmission and deployed control strategies in urban areas in Colombia was collected through literature search and structured interviews to malaria control managers in endemic areas. There was not a standard definition of urban area. Taking into account the information collected and using a definition of urban area that includes urbanicity criteria, essential for the understanding of urban malaria epidemiology, we could document malaria transmission in 9 cities. Malaria in these cities was classified in 3 epidemiological scenarios: periurban malaria in small (Puerto Carreño and Guapi) and big cities (Quibdó, Istmina, Caucasia, Tumaco, Buenaventura and Villavicencio), and introduced epidemic malaria (Riohacha). In conclusion, the transmission of urban malaria in Colombia is mainly periurban. The discrimination of the cases reported in cities into autochthonous and imported is necessary to prioritize areas at risk within the towns, and evaluate control activities. These study provides a baseline to identify research needs and to formulate a national urban malaria control policy in Colombia.

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SEROLOGY OF *PLASMODIUM FALCIPARUM* MSP-1 COMPLEX PROTEINS IN RESIDENTS OF AN AREA OF HOLOENDEMIC MALARIA IN WESTERN KENYA

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Merozoite surface protein 1 (MSP-1) is a large precursor protein that undergoes processing into four non-covalently bound subunits (83kd, 42kd, 38kd, 30kd) that is essential for merozoite invasion into erythrocytes. Although most immunological work has focused on the C terminal end (19kd and 42kd fragments) there is only limited data on the immunogenic properties of all MSP-1 and accessory proteins - MSP-6 and MSP-7. In this study, we investigated the antibody responses to two major allelic forms (3D7 [d] and FCB-1[f]) of the MSP-1 complex (30kd, 38kd, 42kd, and 83kd), MSP-6 and MSP-7 using plasma obtained from children and adults residing in a malaria holoendemic part of Western Kenya. The antibody responses were compared among three age groups: children (4-15 yr), adults (16-45 yr), and older adults (46-65 yr). The antibody prevalence was high among all the age groups with an overall prevalence: d30 - 81.1%, f30 - 87.1%, d38 - 87.1%, f38 - 87.8%, d42 - 100%, f42 - 99%, d83 - 100%, f83 - 100%, M6 - 81%, M7 - 97%. Antibody levels (antibody units) were highest with 42kd (d42 - 22870 ± 1952, f42 - 17911 ± 1773) followed by 83kd (d83 - 9498 ± 1014, f83 - 9441 ± 1176), MSP-7 (7290 ± 1120), MSP-6 (3823 ± 680), 30kd (d30 - 2956 ± 452, f30 3675 ± 265), and 38kd (d38 - 2314 ± 192, f38 2581 ± 253). Significant differences in the antibody levels between the allelic forms (3D7 [d] versus FCB-1 [f]) were noted with only the 30kd ($t = -5.02$, $p<0.001$) and 42kd ($t=3.61$, $p<0.001$). The proteins elicited strong IgG1 and IgG3 subclass responses. Whilst the 83kd protein showed bias towards IgG3 (IgG1/IgG3 ratio: 0.72 ± 0.25) other antigens demonstrated mixed IgG1/IgG3 responses with a predominance of IgG1. These findings show that both dimorphic forms of the MSP-1 complex proteins elicit strong antibody responses even among young children in a holoendemic area. Further studies are in progress to determine which of these proteins elicit protective antibody responses. These findings will be relevant for malaria vaccine development.

(ACMCIP Abstract)

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COMPARATIVE ANALYSIS OF CYTOMEGALOVIRUS-SPECIFIC IFN-Γ RESPONSE PROFILES BETWEEN CYNOMOLGUS, INDIAN AND CHINESE RHESUS NONHUMAN PRIMATES

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Rhesus monkeys (*Macaca mulatta*) of either Indian or Chinese origin, as well as Cynomolgus monkeys (*Macaca fascicularis*), have been used for the evaluation of candidate malaria vaccines. To investigate potential immunological differences between the species, we evaluated their cellular responses to rhesus cytomegalovirus (CMV) using IFN-γ ELIspot and multiparameter flow cytometry as readouts. CMV infection is reported to be endemic in wild nonhuman primate (NHP) populations. Herein, we analyzed IFN-γ responses against a lysate of rhesus CMV-infected MRC-5 cell line, for 86 monkeys bred in captivity. 100% Cynomolgus (N=26), 97% Indian rhesus (N=30), and 83% Chinese rhesus (N=30) mounted positive anti-CMV IFN-γ ELIspot responses (net SFC>50/million, ratio of CMV/medium >2). The average magnitude of IFN-γ SFCs in Chinese rhesus monkeys (365 SFCs/million) was lower than that of either Indian rhesus (1220/million, $p<0.05$) or Cynomolgus (995 SFCs/million, $p=0.1$) monkeys. In all species, the CMV-specific intracellular IFN-γ was produced by CD3+ and CD4+ T cells, but not by CD8+ T cells. The frequencies of CMV-specific IFN-γ+ cells from CD3+ and CD4+ subsets were also lower in the Chinese rhesus as compared with the Indian rhesus and Cynomolgus, although these differences were not statistically significant. Interestingly, the three species showed comparable frequency of CD3+ lymphocytes but different percentages of CD4+, CD8+, and CD4+CD8+ double positive subsets. The ratio of CD4+/CD8+ T cells was similar between Chinese and Indian rhesus (1.77 and 1.81) as was the frequency of CD4+CD8+ double positive subset (4.2 vs. 4.9), whereas the Cynomolgus had a lower CD4+/CD8+ ratio (1.4) but higher frequency of CD4+CD8+ T cells (9.4). Overall, these results demonstrate that CMV-specific cellular immune responses are highly prevalent in colony-bred NHPs, but that differences in the cellular response profile do exist amongst NHP species. It is possible that

such differences may affect vaccine-induced T cell-dependent protective immunity.

(ACMCIP Abstract)

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TH1/TH2 BALANCE IN WEST AFRICAN PRIMI/ SECONDIGRAVID MALARIA

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In endemic areas, it has been reported that primi and secondigravid are more susceptible to malaria infection. To verify this hypothesis, we investigated the dendritic cell profile and cytokine production in peripheral, cord and placental blood of primi and secondigravid malaria positives. Results showed a significant decrease of plasmacytoid dendritic cells: 8,3 ± 3% versus 20,8 ± 3% in peripheral blood; 17,1 ± 3% versus 24,5 ± 2% in placental blood and 10,8 ± 2% versus 20,2 ± 2% in cord blood. Myeloid dendritic cells did not show any significative difference between the two groups. However cytokine production showed a significant increase of IFN-γ and IL-10. For exemple, IFN-γ production was 273,1 ± 56 pg/mL versus 103,8 ± 1 pg/mL in peripheral blood; 263,9 ± 6 pg/mL versus 93,37 ± 18 pg/mL in placental blood and 201,7 ± 3 pg/mL versus 88,3 ± 16 pg/mL in cord blood. That of IL-10 was 101,9 ± 2 pg/mL versus 16,4 ± 4 pg/mL in peripheral blood; 107,2 ± 2 pg/mL versus 10,3 ± 1,7 pg/mL in placental blood and 34,5 ± 5 pg/mL versus 16,5 ± 4 pg/mL in cord blood. These data suggest that not only plasmocytoid dendritic cells may induce a Th1 immune response in malaria infection, but in addition with elevated production of IFN-γ and IL-10, as part of a carefully regulated cytokine network, they may also play an important role in the controle of malaria infection in primi and secondigravid.

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ANTIBODIES LEVEL IN SYMPTOMATIC FULANI AND DOGON WITH *P. FALCIPARUM* INFECTION, LIVING IN MALI, WEST AFRICA

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Difference in malaria susceptibily between Fulani and Dogon two neighboring tribes living in sympatry, with different genetic background is well established know. But the mechanism that protects the former is unknown so far. The study was carried out in four villages of Koro area; they are located approximately 850 Km North-East part of Bamako, the capital city of Mali. Sera have been extracted from blood on filter paper using PBS - stam, Tween 20, NaN_3 20 %, and BSA 0.5 % as described elsewhere. We compared Antibodies production in symptomatic subject belonging to the two tribes (n = 69; with 34 Fulani and 35 Dogon). For that we use indirect ELISA for antibodies detection as describe elsewhere. There was no difference according to the age group. The geometric mean of specific IgG, IgM, and IgE using F32 Antigen were 10.7 µg/ml in Fulani, 3.7 µg/ml in Dogon; 0.7 µg/ml in Fulani, 0.5 µg /ml in Dogon, and 163.6 pg/ml in Fulani, 126.04 pg / ml in Dogon respectively (p-value < 0.05). Total IgG1 level was 78.1 µg/ml in Fulani and 40.1 µg / ml in Dogon ($p < 0.05$). The level of tIgG3 was 294.03 ng / ml, 153.3 ng / ml in Fulani and Dogon respectively ($p < 0.05$). Antibodies production was statistically much higher in symptomatic Fulani than in symptomatic Dogon. The protection seen in Fulani might be du to their high antibodies level.

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A FUNCTIONAL POLYMORPHISM IN THE MACROPHAGE MIGRATION INHIBITORY FACTOR PROMOTER (MIF -173 G/C) INCREASES SUSCEPTIBILITY TO HIGH DENSITY PARASITEMIA IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine that regulates both innate and adaptive immune responses to bacterial and parasitic infections. Recent studies have implicated MIF in the pathogenesis of malarial anemia in mice; however, the role of MIF in human malaria remains largely undefined. Functional polymorphisms in the MIF promoter have been shown to influence susceptibility to several chronic inflammatory diseases in Caucasians. To investigate the role of polymorphic variability in the MIF promoter in conditioning severe malaria disease outcomes, we examined the relationship between a polymorphism at MIF -173G/C and susceptibility to high density parasitemia (HDP, 10,000 parasites/µL or greater) and severe malarial anemia (SMA, hemoglobin<6.0 g/dL) in infants and young children (aged 1-36 mos; n=477) residing in an area of western Kenya with holoendemic *Plasmodium falciparum* transmission. In a multivariate model, controlling for age, gender, HIV-1 status, and sickle-cell status, the MIF -173CC genotype was associated with an increased risk of HDP compared to MIF -173GG [Odds ratio (95% confidence interval) = 1.9 (1.1-3.5); $p<0.05$]. There was no significant association between the MIF -173 polymorphism and susceptibility to SMA. Additional investigations revealed that the MIF -173 polymorphism was associated with functional changes in baseline MIF production, with GC ($p<0.05$) and CC ($p=0.32$) individuals having higher circulating MIF levels relative to those with homozygous G alleles. Moreover, stimulation of cultured peripheral blood mononuclear cells (PBMC) with malarial pigment (hemozoin) caused increased MIF production in individuals homozygous for the G alleles and decreased MIF production in heterozygous individuals. Taken together, results presented here illustrate that variation at MIF -173 is associated with functional changes in peripheral blood MIF production and susceptibility to HDP in children with acute malaria.

(ACMCIP Abstract)

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MONOCYTE-ACQUIRED HEMOZOIN SUPPRESSES RANTES IN CHILDREN WITH *P. FALCIPARUM*-INDUCED MALARIAL ANEMIA THROUGH AN IL-10-DEPENDENT MECHANISM

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Malarial anemia (MA) is a leading cause of morbidity and mortality among children in sub-Saharan Africa. Although the molecular mechanisms that govern MA pathogenesis are largely undefined, release of inflammatory mediators by monocytes/macrophages appears to modulate disease outcomes. Regulated on activation and normal, T-cell expressed (RANTES) stimulates innate immunity in protozoan, viral, and bacterial infections. Furthermore, RANTES production is regulated by inflammatory cytokines, such as IFN-γ, TNF-α, and IL-10. We have previously shown that RANTES is suppressed in children with acute malaria. Previous studies also show that hemozoin (Hz) acquisition by monocytes is associated with cytokine dysregulation. Since the host-pathogen interactions that mediate RANTES suppression are unknown, we examined the role of pigment-containing monocytes (PCM) and cytokines (TNF-α, IFN-γ, and IL-10) in regulating RANTES in circulation and in cultured peripheral blood mononuclear cells (PBMC) from Kenyan children (aged 3-31 mos, n=82) with MA (Hb<11.0 g/dL). PCM were determined by examination of peripheral blood smears, while chemokine and cytokine concentrations were determined by ELISA. Plasma and PBMC RANTES production was higher in the no PCM (0%) relative to the low (10% or less) and high (greater than 10%) PCM groups ($P<0.05$ for both). Circulating RANTES was also inversely correlated with absolute PCM numbers ($r=-0.346$, $P<0.01$). Plasma IL-10 and TNF-α increased in low PCM, but were suppressed in the high PCM group, while IFN-γ progressively increased with elevated PCM. Circulating RANTES and IL-10 were significantly inversely correlated ($r=-0.246$, $P<0.05$), while there was a non-significant relationship between RANTES and IFN-γ and TNF-α. Additional investigations in cultured PBMC revealed that rhIL-10 decreased RANTES in children with and without PCM, while neutralizing IL-10 antibodies increased RANTES only in the PCM(+) group. Taken together, these results demonstrate that Hz acquisition by monocytes suppresses RANTES through an IL-10-dependent mechanism.

(ACMCIP Abstract)

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THE REQUIREMENT FOR ΓΔ T CELLS VERSUS NK CELLS IN IMMUNITY TO PLASMODIUM CHABAUDI MALARIA

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Acute blood-stage *Plasmodium chabaudi* infections in B-cell deficient, $J_H^{-/-}$, mice are suppressed by cell-mediated immunity (CMI) thought to be dependent upon γδ T cells regulated by $CD4^+$ T cells. However, the mechanisms by which γδ T cells function to destroy blood-stage parasites during murine malaria remain unknown. To determine the effects of γδ T cell depletion on the cytokine response to infection, sera from $J_H^{-/-}$ mice treated with anti-TCRγδ antibody or hamster immunoglobulin at 4 and 8 days post-infection were assayed for inflammatory cytokines by bead array analysis. Mice treated with anti- TCRγδ antibodies in contrast to controls were unable to suppress their parasitemia and were depleted of γδ T cells as determined by cytofluorometric analysis. Cytokine analysis indicated significant decreases in the serum levels of IFNγ, IL-10 and MCP-1 in γδ T cell-depleted mice compared to controls on both test days. The time-course of parasitemia in both MCP-1 and CCR2 knockout mice was identical to controls, indicating that Gr-1+ monocytes do not play an essential role in parasite clearance. Identical depletion studies done in C57BL/6 mice yielded similar cytokine profiles, suggesting that the slight but significant delay in the clearance of parasites from the blood of γδ T cell-depleted mice in all likelihood, was due to the suppression of the early CMI response to the parasites which were eventually cleared by antibody. Although NK cells have been proposed to function early in the immune response to *P. chabaudi*, NK depletion from both $J_H^{-/-}$ mice and C57BL/6 failed to prolong parasitemia or to alter the serum cytokine profiles compared to controls.

(ACMCIP Abstract)

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MSP1 AND HEMOZOIN INDUCE *IN VITRO* MATURATION AND ACTIVATION OF HUMAN MONOCYTES-DERIVED DENDRITIC CELLS

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Dendritic cells (DCs) are antigen presenting cells specialized in antigen uptake, processing, and presentation to T-cells. They mainly derivate from monocytes and trigger TH1/TH2 equilibrium. Their role in the induction of immune response during malaria was recently highlighted. The capacity of malaria antigens to induce or block differentiation of DCs is thus of main importance to develop vaccines. We investigate the *in vitro* effect of MSP1 (a major vaccine candidate) and hemozoin (an heme-derivated complex which can concentrate in the reticuloendothelial system of the host) on monocytes-derived DCs, in healthy patients from Dakar. Purified Peripheral Blood Mononuclear Cells (PBMCs) were isolated from blood of healthy human donors by ficoll centrifugation. PBMCs were differentiated to immature DCs (iDCs) using IL-4 and GM-CSF. iDCs were stimulated with MSP1 and purified Hemozoin separately, incubated for 48h and then analyzed for maturation and costimulation markers (CD86, CD80). Phenotype of the cells was studied by flow cytometry. Mortality of the cells was analyzed using Propidium iodure. Expression of CD80, CD86, IL10 and IL12 was also studied by realtime PCR. MSP1 was used as a recombinant protein and hemozoin was purified from culture supernatant. Results show that in the presence of LPS, CD83, CD80 and CD86 are up-regulation in DCs. MSP1 also induces up-regulation of maturation marker and co-stimulation markers. For hemozoin, microscope examination of the DCs confirms internalization of the pigment after stimulation. This stimulation is followed by an up-regulation of CD83, CD80 and CD86 expression, and a low rate of mortality. Quantification of expression of mRNA encoding for CD83 and CD86 by real time PCR confirms the results obtained by flows cytometry. LPS increases mRNA transcript for IL10 but not for IL12. In an opposite way IL12 expression was found down-regulated by hemozoin. These results show that DCs can be induced and stimulated by MSP1 antigen and that hemozoin, doesn't deregulate this process.

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INDUCIBLE NITRIC OXIDE SYNTHASE 2 GENE PROMOTER VARIANTS AND SEVERE MALARIA IN NORTHERN GHANA

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Nitric oxide is an important mediator in the host defense against ***Plasmodium falciparum*** malaria. It has antiparasitic effects ***in vitro.*** However, its role in clinical disease remains controversial in different malaria endemic areas in Africa. Studies have shown that polymorphism in the ***NOS2*** gene promoter may influence susceptibility to and severity of malaria. In order to understand the role of ***NOS2*** gene promoter polymorphism in the progression of malaria disease, there is the need to generate data on the diversity of ***NOS2*** gene promoter from the varied eco-epidemiological zones of malaria transmission in Africa.

We tested the hypothesis that nitric oxide is critical in the pathogenesis of severe malaria and that single nucleotide polymorphisms (SNPs) in the *NOS2* gene promoter are important risk factors in malaria disease, in a large frequency matched case control study among children with severe malaria, mild malaria and healthy controls aged five years and below from Kassena-Nankana district (KND) in Northern Ghana. The children were matched on age, sex and location. A high throughput genotyping technique-massARRAY/massEXTEND was used to detect SNPs in 792 naturally exposed children with severe malaria and 806 healthy controls. Seven *NOS2* promoter SNPs with potential relevance to clinical manifestation of malaria were selected and tested in this population. A case control analysis of all seven SNPs did not show a significant association with severe malaria in the KND. However, a subgroup analysis of the *NOS2*-1173C/T polymorphism showed that the odds of developing severe malarial anaemia was 37% lower in children with wild-type (-1173C) allele than in those with mutant allele (-1173T). The mutant homozygous T/T of *NOS2*-1173C/T was associated with an increased risk of severe anaemia (odds ratio=11.71, 95% CI= 1.41-539) whilst, the odds of developing severe malarial anaemia was 35% lower in children with the wild type genotype C/C compared to children with C/T or T/T genotype. Our study lacked the power to detect any *NOS2* association with cerebral malaria (n=47). Marker -3025G/T (rs7208420) was monomorphic in the KND. Strong linkage disequilibrium was observed between the promoter SNP markers tested. Haplotype analysis of the promoter gave seven common *NOS2* haplotypes. However, a case control analysis did not show any haplotypic difference between severe malaria cases and healthy controls.

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PFTSSK: CHARACTERISATION OF A NOVEL PLASMODIUM "TESTIS-SPECIFIC" KINASE ORTHOLOGUE OF A HUMAN TSSK

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Sexual development, or gametocytogenesis, is an essential and irreversible component of the life cycle of malaria. However, the molecular mechanisms regulating gametocytogenesis and subsequent gamete and zygote formation in Plasmodium are poorly understood. Protein kinases are thought to have some role in gametocyte differentiation and gamete development and certain kinases have been shown through microarray data to be upregulated in these stages. We are studying a *Plamodium falciparum* orthologue of a human testis-specific serine/threonine kinase (TSSK), which also has orthologues in the mouse malaria species, P. chabaudi, P. yoelii, and P. berghei. The expression of *P. falciparum* TSSK (PfTSSK) was examined by Northern analysis, but levels were below detection limits in asynchronous and synchronous asexual stages. Expression was then assessed in the asexual stages of gametocyte producing (3D7) and non-producing (F12, HB2, FCR3/A2, and LF4F1) strains using quantitative PCR (qPCR). Asexual stages of the strains generally show a higher expression of PfTSSK in early schizont stages compared with expression in the ring stages. Expression in gametocyte producing and non-producing strains varied, but higher expression was always seen in the schizont stages. PfTSSK expression was also examined using antibodies to a conserved sequence in human TSSK (hTSSK) and antibodies specific to PfTSSK. Western blotting identified immunoreactive bands, and localization in the parasite will be analysed using immunofluorescence. This study is the initial characterization of a novel protein kinase identified in the *P. falciparum* genome, which may have a role in the transmission stages.

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INVESTIGATION OF GENETIC DIVERSITY ASSOCIATED WITH VACCINE CANDIDATE ANTIGENS IN *PLASMODIUM FALCIPARUM* REVEALS NOVEL GENOTYPES

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Plasmodium falciparum causes the most virulent form of human malaria and is responsible for 200 to 300 million infections and 1 to 3 million deaths per year. Genetic variability of this pathogen underlies its transmission success and affects the development of effective immune responses. Characterizing the patterns of DNA sequence variation in major *P. falciparum* surface antigens is important to predict the efficacy of immunization strategies. We analyzed fifty samples of *P. falciparum* from endemic areas in Iquitos collected in 2000 and 2006 and characterized the genetic diversity of 10 regions encoding mainly T and B cells epitopes in circumsporozoite protein (CSP-1), merozoite surface protein-1 (MSP-1), apical membrane antigen-1 (AMA-1), liver stage antigen-1 (LSA-1) and sporozoite surface protein-2 (SSP-2). Alleles identified by DNA sequencing were compared with the vaccine strain 3D7 and with three other strains (D6, W2 and 7G8). We found nonsynonymous substitutions in two non-repeat regions of *Pfcsp* that encode T-cell epitopes and have been implicated in a major role permitting the parasite to avoid the human host's immune system. The analysis of the samples collected during year 2000 revealed that 93% of the isolates have a similar genotype to the Brazilian strain 7G8; meanwhile only 67% of isolates from 2006 were similar to 7G8. Comparative sequence analysis of the remaining 33% showed they resemble the Honduras isolate HB3. On the other hand, analysis of the amino acid sequence variation in block 2 and block 17 of PfMSP-1 indicated the presence of two previously reported alleles, K-1 and MAD20. In addition, novel single nucleotide polymorphisms (SNPs) were identified in the DNA sequence encoding domain I of *Pf*AMA-1, producing nonsynonymous substitutions in a T cell epitope. Nonsynonymous substitutions were also found in the region encoding the N-terminal of PfLSA-1. This N-terminal peptide, designated T1, induces IFN-γ production in peripheral blood mononuclear cells (PBMC) in humans. Finally, novel genotypes previously un-described in South America were found in regions of *Pfssp-2* encoding two B cell epitopes. These findings have implications in understanding the impact of genetic variation on immunity and in the formulation of an effective vaccine that can protect against heterologous infection.

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GLOBAL REGULATION OF TRANSCRIPTION IN ASEXUAL BLOOD-STAGE *PLASMODIUM FALCIPARUM*

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The pathogenicity of the malaria parasite *Plasmodium falciparum* results from its asexual intraerythrocytic form, which develops through several distinct morphological stages while replicating in human red blood cells (RBCs). Microarray studies of these stages report dramatic changes in the steady-state mRNA levels of many genes, suggesting that differential gene expression is important for development, but the mechanisms that control it remain poorly understood. It is currently unclear whether differential expression is driven by gene-specific or global regulation. In order to evaluate the role of gene-specific versus bulk regulation of transcription during the asexual RBC life cycle of *P. falciparum*, transcriptional activity was assayed by nuclear run-on. We observed a sharp increase in the total incorporation of radiolabeled nucleotide by late-trophozoite/early-schizont nuclei, indicating a peak in global transcriptional activity during these stages of development. A similar trend was seen among several genes when radiolabeled RNA was hybridized to filters carrying gene-specific probes. Transcription from the antisense strands of genes was detected in tandem with that of their sense strands. These findings suggest that transcription in the *P. falciparum* RBC life cycle is globally regulated and occurs predominantly during a distinct period in the cycle. Our data cast gene- or operon-specific, sense-only transcription -- the mode of regulation in so many other organisms and evident in so few *P. falciparum* genes -- as the exception, not the rule, in the asexual RBC life cycle of malaria. Instead, gene regulation in RBC-stage parasites may combine elements of pre-transcriptional (i.e., chromatin structure), promoter-driven, and post-transcriptional regulation to extract differential expression amid a bulk transcriptional event. Although such a multifaceted scheme complicates the interpretation of steady-state RNA data, characterization of this phenomenon may prove useful for identifying individually regulated genes through their deviation from the global trend.

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MSP-1_{19} HAPLOTYPE DIVERSITY OF MALARIA PARASITES IN CHILDREN VACCINATED WITH A MSP-1_{42} MALARIA VACCINE IN WESTERN KENYA

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Evaluation of malaria vaccines relies on indicators such as clinical outcomes and parasite densities amidst measurable immunological response. Molecular quantification of target alleles or multiplicity of infections can provide complementary data to evaluate vaccine efficacy. Here we present data on MSP-1_{19} haplotypes of malaria parasites in children vaccinated with MSP-1_{42} malaria vaccine in Western Kenya.

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P. FALCIPARUM ANTI-MSP1-19 ANTIBODIES INDUCED BY MSP1-42 AND MSP1-19 BASED VACCINES DIFFERED IN SPECIFICITY AND PARASITE GROWTH INHIBITION IN TERMS OF RECOGNITION OF CONSERVED VERSUS VARIANT EPITOPES

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The C-terminal 42 kDa fragment (MSP1-42) and its smaller 19 kDa subfragment (MSP1-19) of the *P. falciparum* Merozoite Surface Protein, MSP1 are leading candidate malaria vaccines. Since the targets of protective immunity lie within the MSP1-19, we compared the anti-MSP1-19 antibodies induced by vaccination with recombinant MSP1-42 and MSP1-19. The specificities of the antibody responses were analyzed using five recombinant MSP1-19s expressing different naturally occurring variant amino acid residues. We observed dramatic differences in the specificities of the anti-MSP1-19 antibodies induced by the two vaccines. MSP1-42 consistently induced crossreactive antibodies; whereas the antibodies induced by recombinant MSP1-19 were highly variable among animals in terms of recognition of conserved versus variant epitopes. Of the variant residues examined, only a subset was significantly immunogenic as B epitopes. MSP1-42 consistently induced potent growth inhibitory antibodies that recognized conserved epitopes, leading to efficient inhibition of heterologous parasites. In contrast, MSP1-19 induced strong inhibitory antibody responses in only a subset of animals studied. In some of the MSP1-19 immunized animals, inhibition of homologous parasites may be due to recognition of inhibitory epitopes associated with the variant residues of the immunizing parasite strain, and the induction of antibodies to conserved inhibitory epitopes may not be efficiently achieved. These data suggest an advantage of using MSP1-42 over MSP1-19 based vaccines.

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PROCESS DEVELOPMENT AND MANUFACTURING OF A NOVEL MALARIAL ANTIGEN- *PLASMODIUM FALCIPARUM* CHIMERIC PROTEIN 2.9 (PFCP2.9)

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Plasmodium falciparum chimeric protein 2.9 (PfCP2.9), a *Pichia*-derived recombinant fusion protein consisting of AMA1 (domain III) and MSP1-19, is undergoing development and evaluation as a vaccine candidate against *Plasmodium falciparum* malaria. The two domains are linked by a hinge region encoding a Gly-Pro-Gly-Pro repeat, and the PfCP2.9 molecule contains 18 cysteine residues forming 9 intramolecular disulfide bonds. Both AMA1 and MSP1 are merozoite surface antigens that are believed to play a role in the invasion of red blood cells. Their correct conformation is required in order to elicit antibodies that can inhibit invasion of the parasite into red blood cells. A *Pichia pastoris* expression system containing the PfCP2.9 gene that has been constructed was able to produce 1g/L of target protein at a 30 L scale. This target protein was harvested and further purified by multiple column chromatography steps, such as hydrophobic interaction, anion exchange, cation exchange and size exclusion, at a purity greater than 98% with a 30% overall yield. In anticipation of further advancement of this vaccine candidate, an optimization and scale up of the process was conducted at the 150L scale. Critical process parameters for fermentation, such as temperature, pH, methanol feed rate, and dissolved oxygen, were evaluated and optimized, which resulted in an 80% increase of PfCP2.9 titer, compared to that of the run performed at the 30L scale. Downstream process optimization was also performed by fine-tuning process parameters of each unit's operation, which resulted in a 50% yield improvement, compared to that of the runs performed at the 30L scale. But more importantly, the optimization results

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in a much more robust and rapid process than the previous one. At the 150L scale, this optimized process was able to consistently produce 45g purified PfCP2.9 per batch, which is a 13-fold increase over the previous one performed at the 30L scale.

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EXPRESSION AND FUNCTION OF TOLL-LIKE RECEPTORS ON DENDRITIC CELLS FROM RHESUS MACAQUES

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Toll-like receptors (TLRs) are pattern-recognition receptors of the innate immune system that interact with a broad variety of pathogens. Due to their potent role in activation of dendritic cells, ligands for TLRs are attractive candidates for adjuvant formulations. Based on their close relationship to humans, non-human primates such as rhesus macaques have proven to be valuable as animal models for testing vaccines and immunization strategies. However, so far, it is not known if a similar set of TLRs is present on dendritic cells from human and rhesus. Therefore, our aim was to analyze TLR expression and function on rhesus dendritic cells. We used an optimized protocol for the generation of rhesus monocyte-derived dendritic cells and analyzed expression of TLRs by PCR. Moreover, the ability of several different TLR ligands to stimulate dendritic cell maturation and cytokine production was studied using flow cytometry and ELISA, respectively. Our results show that rhesus dendritic cells responded to TLR2, TLR3, TLR4 and TLR5 ligands by upregulation of costimulatory and maturation markers, but not to ligands for TLR7, TLR8 and TLR9. In comparison, human monocyte-derived dendritic cells were shown to be activated by the same TLR ligands. Unlike human monocyte-derived dendritic cells, rhesus dendritic cells produced very low amounts of IL-12p70. Interestingly, high levels of interferon type I production were induced by poly I:C, a ligand for TLR-3. In addition, we will present results of ongoing research on TLR expression and function of other antigen-presenting cells from rhesus macaques, including blood dendritic cell subsets. Thus, whereas murine dendritic cell subsets substantially differ from their human counterparts, we here show that non-human primate dendritic cells share functionality of TLRs with human dendritic cells. Our results indicate that non-human primates will be useful for testing novel antigen delivery systems containing TLR ligands.

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DIFFERENTIAL EVIDENCE OF NATURAL SELECTION ON TWO LEADING SPOROZOITE STAGE MALARIA VACCINE CANDIDATE ANTIGENS

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Experimental malaria vaccines based on two sporozoite stage candidate antigens of *Plasmodium falciparum*, the circumsporozoite protein (CSP) and thrombospondin related adhesive protein (TRAP), have undergone clinical trials of efficacy. The relevance of naturally existing polymorphisms in these molecules remains unknown. Sequence polymorphism in the genes encoding these antigens was studied in a Gambian population (sample of 48 *trap* and 44 *csp* allele sequences) to test for signatures of selection that would result from naturally acquired immunity. Frequency distributions were analyzed and compared with data from another population and with polymorphism and divergence from related species. Polymorphism in TRAP is under strong selection for amino acid sequence diversity, and allele frequencies are under balancing selection within the Gambian *P. falciparum* population. There was no such evidence for CSP, calling into question the idea that most polymorphisms in this gene are under immune selection. There was a non-significant trend for regions known to encode T cell epitopes to have slightly higher indices suggesting balancing selection. Overall, the results predict more allele-specific immunity to TRAP than to CSP, and are relevant to vaccine design and efficacy testing of these candidates.

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INCREASING THE EFFICIENCY OF ANTIGEN EXTRACTION FROM VACCINES FORMULATED IN ALUMINUM HYDROXIDE GEL BY INCLUDING SURFACTANTS IN THE EXTRACTION BUFFER

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Efficient antigen extraction from vaccines formulated on aluminum hydroxide gel (Alhydrogel, LCI Biosector, Denmark) is a critical step for the evaluation of the quality of vaccines following formulation. It has been shown in our laboratory that the efficiency of antigen extraction for vaccines formulated on Alhydrogel decreased significantly with increased storage time. To increase antigen extraction efficiency, the present study determined the effect of different surfactants on antigen recovery from vaccine formulations. The *Plasmodium falciparum* apical membrane antigen 1 (AMA1) formulated on Alhydrogel and stored at 4 °C for three years was used as a model antigen in this study. The AMA1 on Alhydrogel was extracted in the presence or absence of surfactants including sodium dodecyl sulfate (SDS) (30 mM) and cetylpyridinium chloride (20 mM) in the extraction buffer (0.6 M citrate, 0.55 M phosphate, pH 8.5) using our standard antigen extraction protocols. Extracted AMA1 antigen was analyzed by 4-20% Tris-glycine SDS-PAGE followed by silver staining. The gel image was analyzed by the ImageQuant software (Amersham Biosciences). The results showed that SDS or cetylpyridinium chloride increased the extraction efficiency by 70 % or 50 % under non-reducing conditions, respectively. However, AMA1 on Alhydrogel extracted in the presence of SDS gave a considerable amount of aggregation (30 % of total protein) when compared to the AMA1 reference standard (6% of aggregation). The AMA1 on Alhydrogel extracted in the presence of cetylpyridinium chloride gave much lesser aggregation (13% of total protein) and appeared to migrate similarly to the AMA1 reference standard. These results indicate that either SDS or cetylpyridinium chloride used in extraction buffer significantly increases the antigen recovery and may be used for efficient antigen extraction from Alhydrogel of aged vaccines. Cetylpyridinium chloride appears to be superior to SDS by not causing significant protein aggregation.

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THE LIVER STAGE *PLASMODIUM* TRANSCRIPTOME

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Despite monumental efforts to stem the tide of Malaria-related illnesses and deaths, malaria remains one of the deadliest and most costly diseases in the world. Worldwide, there are between 300 and 500 million new infections and 1.5 to 3 million deaths per year. It is estimated that one African child dies due to Malaria every 30 seconds, and that as much as 2% of Africa's Gross National Product (GNP) is lost due to Malaria each year. As p[reviously reported, mice could be fully protected from *Plasmodium berghei* challenge after immunization with radiation-attenuated sporozoites, leading to several studies showing that protection

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could also be attained in human volunteers. Due to the difficult nature and cost of producing an irradiated sporozoite vaccine, focus over the past two decades has shifted to subunit vaccine strategies. Our current studies aim to identify those antigens effective at inducing a protective immune response against the liver stage of infection. The first step will be to identify genes simply expressed during liver stage development. In order to do this we have begun two different approaches. 1 - In our first approach, infective *P. yoelii* sporozoites expressing GFP are isolated from mosquitoes via salivary gland dissections and used to infect CD-1 mice. Between 24 and 48 hours post-infection, the mice are euthanized and their livers removed and sectioned. Hepatocytes infected with liver stage parasites are isolated via Laser capture Microdissection (LCM), after which total RNA is extracted. Dr. Elizabeth Winzeler and colleagues at The Scripps Research Institute will then assess parasite gene expression levels using high-density oligonucleotide arrays. 2 - In our second approach, infective *P. falciparum* sporozoites are isolated from mosquitoes via salivary gland dissections and used to infect HC-04 human hepatocytes. As described above, liver stage material will be isolated via LCM and resulting RNA sent to Scripps for analysis. Here we report the findings from these studies.

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FIRST *ANOPHELES ARABIENSIS* GERMLINE TRANSFORMATION: TOWARD THE DEVELOPMENT OF A TRANSGENIC GENETIC SEXING STRAIN

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The ability to genetically engineer mosquitoes is likely to have major implications for the development and implementation of genetic control systems against mosquito disease vectors such as the Sterile Insect Technique (SIT). In particular, genetically transformed mosquito strains can be created for genetic marking and sexing, two key factors known to influence the effectiveness of SIT programmes. In addition, the removal of biting females before releasing sterile males in the field will be of critical importance as they contribute to disease transmission and reduce the efficiency of the release campaign. Parallel to the creation of a conventional genetic sexing strain (Y-translocation of a resistance marker), our group is undertaking a transgenic approach to the development of an ***A. arabiensis*** genetic sexing strain (GSS). The sex separation strategy under investigation relies on the sex-specific properties of the ***A. gambiae*** ß2tubulin gene regulatory regions. It is hoped this approach will achieve the high sex separation efficiency (above 99%) and strain stability required for safe and efficient male-only SIT releases. We report here the successful development of transgenic ***A. arabiensis*** lines using the pPB[DsRed]ß2EGFP construct. Wild-type ***A. arabiensis*** embryos were injected with a mixture of pPB[DsRed]ß2EGFP and helper plasmid phsp-pBac (700 and 300 ng/µl respectively) following an appropriate protocol. Injections generated several transgenic sexing lines. The effectiveness of the transgenic-based sex-separation procedure, the stability of transgenic mosquito GSS under various (mass-)rearing regimes, as well as the viability and reproductive competitiveness of transgenic sterile males are being assessed.

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CLONING AND CHARACTERIZATION OF TWO NOVEL CARBONIC ANHYDRASES FROM THE LARVAL *ANOPHELES GAMBIAE* MIDGUT

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Mosquito larvae display a unique characteristic that sets them apart from most other organisms. The anterior portion of the midgut generates a luminal pH as high as 10.5, one of the highest known in any biological system. The mechanisms for midgut alkalinization are largely unknown; however strong evidence suggests a role for the enzyme carbonic anhydrase (CA). Multiple CAs have been cloned from, and immunolocalized to, the *Anopheles gambiae* midgut and it has been shown that inhibition of carbonic anhydrase in mosquito larvae blocks alkalinization. CA catalyzes the reversible conversion of CO_2 to HCO_3^- and can generate HCO_3^- (bicarbonate) within midgut epithelial cells. Bicarbonate can be deprotonated to carbonate with a pKa above 10.0. Together with a strong cation such as potassium, carbonate could serve as the buffer in the anterior gut lumen. We report here the cloning and characterization of two new CAs from *Anopheles gambiae* gut cDNA collections, a putative secreted CA and a putative cytoplasmic CA. Results show that the CA transcripts exhibit different expression patterns throughout the regions of the gut as determined by quantitative real time PCR (qrtPCR), *in situ* hybridization, and microarray analysis. We are also using RNA interference (RNAi) to investigate the role of the CAs in midgut alkalization. To establish the validity of using this method, we used an *A. gambiae* cell line, Ag55, to demonstrate the presence of AgAGO2, a protein likely to participate in RISC formation in these cells. We then treated the cells with long dsRNA to the putative cytoplasmic CA or dsRNA from GFP as a negative control and monitored the expression of the endogenous CA over four days using qrtPCR. Results demonstrated that treating the cells with CA dsRNA produced a dramatic reduction in CA mRNA levels after 24 hours that remained robust for at least four days post treatment.

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COORDINATE REGULATION OF RNA INTERFERENCE COMPONENTS IN *AEDES AEGYPTI*

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RNA interference (RNAi) is a component of innate anti-viral immunity in metazoans; understanding its mechanisms is an important requisite to defining vector competence for a given arbovirus/vector pair. Natural fluctuations in the RNAi component transcripts were assessed during infection with one of four viruses representing three arbovirus families. We looked at Dicer-2 (Dcr-2), Argonaute-2 (Ago-2), and Tudor SN (TSN). In midguts, Dcr-2 and Ago-2 transcript levels were concomitantly induced at 1 dpi during dengue serotype 2 (DENV2) infection, but not later in the course of infection. *Aedes aegypti* infection with DENV2 represents a natural vector/virus pair, in contrast to the three other viruses, TR339-eGFP and MRE16-eGFP, both Sindbis viruses, or La Crosse virus. TSN transcripts follow a more cyclical pattern of suppression and induction that varied depending on the arbovirus; significant changes were not restricted to the natural vector/virus pair. To confirm the requirement of Dcr-2 and TSN in anti-viral immunity, transcripts were silenced by dsRNA injection prior to infection with a weak SINV, TR339-eGFP. A transient increase in viral dissemination and viral titers was seen, indicating the requirement for Dcr-2 and TSN to limit arboviral replication and dissemination. Additional data suggests coordinate transcriptional regulation of RNAi components in *A. aegypti*

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THE REGULATORY REGION OF *ANOPHELES GAMBIAE* VITELLOGENIN CAN DRIVE TISSUE-, STAGE- AND SEX-SPECIFIC EXPRESSION OF GFP IN TRANSFORMED *ANOPHELES STEPHENSI*

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Genetically-modified mosquitoes are proposed as a strategy to control mosquito-transmitted diseases. Transgenic systems that permit the introduction of exogenous genes into mosquitoes are one of the preconditions to implementation of this strategy. The promoters of mosquito vitellogenin-encoding genes have proved to be good candidates for controlling tissue-, stage- and sex-specific expression of exogenous genes. However, transgenesis of *Anopheles gambiae*, the principal vector of malaria in sub-Saharan Africa, is not routine. We therefore investigated whether transformation of *An. stephensi*, a vector of malaria in southern Asia, with heterologous promoters could be used to validate the function of *An. gambiae* control sequences. A genomic DNA fragment of 1.7 kilobases in length of the putative regulatory region of the *An. gambiae* Vitellogenin gene, *VgT1*, was cloned into the transfomation vector pBac[3xP3-DsRedafm]. The coding sequence of EGFP served as the marker to assay the function of the promoter. A total of 1062 *An. stephensi* embryos were microinjected with the construct pBac [3xP3DsRed-AgVgEGFP], and of these, 224 larvae hatched and 141 adults emerged. Two G_1 families with specific red fluorescence in the eyes of larvae were identified from 67 families. Southern blot analyses of the transgenic lines showed that GFP coding sequences were integrated into the genome of the mosquitoes. The two families showed specific GFP expression in fat body tissues of transgenic blood-fed females, but not in transgenic males, non-blood-fed transgenic females and non-transgenic blood-fed females. RT-PCR analyses showed that the expression level of GFP reached a peak at 24 hours post blood meal. The data support the conclusion that the promoter of *An. gambiae* can drive GFP-expressed in a tissue-, stage- and sex-specific manner in *An. stephensi*, and therefore is a good candidate for controlling the expression of exogenous genes in malaria vector mosquitoes.

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MOLECULAR IDENTIFICATION AND PHYLOGENY OF THE MACULATUS GROUP OF *ANOPHELES* MOSQUITOES (DIPTERA: CULICIDAE) BASED ON NUCLEAR AND MITOCHONDRIAL DNA SEQUENCES

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The Maculatus Group of *Anopheles* mosquitoes (Diptera: Culicidae) comprises eight known species, including important malaria vectors in Southeast Asia. The sequences of the second internal transcribed spacer (ITS2) and third domain (D3) of ribosomal DNA, and cytochrome oxidase subunit II (COII) of mitochondrial DNA were obtained for five species of the group from China, as *An. maculatus*, *An. willmori*, *An. pseudowillmori*, *An. sawadwongporni* and *An. dravidicus*. The variation within taxon is much smaller than that between taxa. A diagnostic PCR assay for distinguishing the five members was developed basd on the interspecific ITS2 variation. The phylogenetic relationships for the group were estimated on the ITS2 and D3 data. The Maculatus Group appears monophyletic with *An. pseudowillmori* at a basal position, the Sawadwongporni Subgroup and the Maculatus Subgroup form sister clades. Our data concludes that *An. dispar* and *An. greeni* belong to the Maculatus Subgroup, and *An. willmori* is not closer to either of the subgroups.

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SEQUENCING THE WEST NILE ENCEPHALITIS MOSQUITO GENOME: CULEX PIPIENS QUINQUEFASCIATUS

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Mosquito species belonging to the Culex genus are important vectors of several human pathogens including West Nile encephalitis and lymphatic filariases (120 million people affected in 80 countries). Since its appearance in the United States in 1999 the West Nile virus has spread rapidly with human disease cases reported in 45 of the 48 contiguous states by 2003. A genome sequencing project of the Southern house mosquito (Culex pipiens quinquefasciatus) was undertaken with funding from the National Institute for Allergy and Infectious Diseases (National Institute of Allergy and Infectious Diseases) of the National Institutes of Health, with the majority of sequencing performed by the Broad Institute and The Institute for Genomic Research. This resulted in over 7 million reads for approximately 8X coverage of the genome (540 Mb in size). Preliminary manual annotation of this genome is underway at the University of California as a part of the National Institute of Allergy and Infectious Diseases-funded VectorBase bioinformatics resource center (http://www.vectorbase.org/). Preliminary annotation results and genome architectural features will be presented.

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APPROACHES FOR DISCOVERING INNATE IMMUNE RESPONSE ELEMENTS IN *AEDES AEGYPTI* BASED ON MICROARRAY DATA

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Successful mosquito immune responses depend on a complex network of processes in order to defend the host from a foreign invader. The distinct, but not mutually exclusive, responses of phagocytosis and melanization, in concert with antimicrobial peptides, previously have been implicated in the clearing of pathogens from the hemolymph of infected mosquitoes. To study these various hemolymph responses, spotted microarrays were designed from an immune-activated hemocyte EST project. Sequences from 11,952 ESTs were assembled into 2686 clusters, 979 of which encode completely unknown products. From this, 1978 60-mer oligonucleotides (594 unknowns) were synthesized and spotted on glass slides. Female *Aedes aeygpti* mosquitoes were inoculated with either *Escherichia coli* or *Micrococcus luteus*, in order to elicit a phagocytic or melanotic response, respectively, and hemolymph was collected for RNA isolation at 1, 8, and 24 hours post-inoculation. Purified total RNA was reverse transcribed without amplification and differentially labeled for hybridization and comparison with labeled RNA from control females. Data from at least 3 biological replicates were normalized and analyzed using multiple statistical methods. Using expression profile clustering methods, we are discovering novel gene products with putative roles in the response to invading pathogens. Further, *in silico* exploration of these gene products reveals several new candidate effectors of mosquito innate immunity. Specific examples include non-coding RNAs, novel leucine-rich repeat peptides, and putatively excreted products.

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COUNTRYWIDE DISTRIBUTION AND CHROMOSOMAL POLYMORPHISM OF *ANOPHELES GAMBIAE* MOLECULAR FORMS IN BURKINA FASO

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A countrywide survey of *Anopheles gambiae s.l.* taxa was carried out in Burkina Faso during the peak of the rainy season. Samples of 20 half-gravid females were collected from each of 300 georeferenced villages, chosen according to a stratified random sampling protocol across an area spanning LAT 9°45'N-14°40'N and LONG 5°30'W-1°45'E. Individual mosquitoes (n=4,362) were scored both for their karyotype and rDNA pattern defining the chromosomal / species / molecular form status. Overall, the molecular form S of *An. gambiae s.s.* was the most abundant (48%), followed by the M-form (29%), and *An. arabiensis* (23%). The distribution of the two molecular forms followed a clear geographic pattern: the M-form dominated in the northern arid areas; the S-form was most abundant in the more humid southern regions. This transition was rather abrupt at c. 12°30'N latitude. *An. arabiensis* was most prevalent in the central plateau_between 11°30'N and 13°30'N_decreasing in abundance when moving centrifugally away from this region. Contingency analysis of presence/absence of each pair of taxa in any village, showed that the M-form and *An. arabiensis* co-occurred significantly more than expected, whereas the M- and S-forms were significantly less associated than expected (P<<0.0001 in both cases); for *An. arabiensis* and the S-form, support for statistically lower association was less (P=0.035). Chromosomal analysis of each molecular form revealed divergent patterns of polymorphism characterized by contrasting inversion frequencies. No chromosomal arrangement was exclusive of either molecular form. With the exception of inversion 2R*b* in the S-form, all inversions showed variable clinal changes in frequency with latitude. Our results support the view that molecular forms of *An. gambiae* represent diverging taxonomic units defining an incipient speciation process, whose chromosomal polymorphism modulate ecological niche partitioning in relation to eco-geographical clines at the macro-geographic scale.

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DISTRIBUTION OF *CULEX PIPIENS* COMPLEX IN MEXICO CITY AND THE POTENTIAL OF WEST NILE

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West Nile virus (WNV) was initially isolated in America from species of *Culex* mosquitoes and birds in New York City area in 1999. Subsequently, the virus spread in the United States and many human cases were reported. *Culex* mosquitoes are considered to be the most important vectors of WNV, although this virus has been isolated or detected in more than 20 species of mosquitoes in the United States. Because the importance of this vectors the aim of the present research was to investigate the *Culex* species distribution in Mexico. This studies were focus in Mexico City and the surrounding states to know the potential to spread WNV in this city. WNV has been detected in equines and birds in Monterrey, Yucatan among others. A total of 42 sites were sampled from 2004 to 2005 for mosquito larva stage principally. *Culex quiquefaciatus* was the dominant larva collected along the year. Vertical stratification may be influenced by humidity, temperature, light, and possibly by availability of hosts. The maximum mosquito density was during the temporal rain. *Culex pipiens*, species that preferentially feeds on birds is an important and competent vector of WNV in both the Old and New Worlds was also collected in Mexico city. The likely importance of this species in the natural history of WNV in the northeastern United States prompted us to evaluate the breeding sites of *Cx. pipiens* and associated species to the potential of future epidemics of WNV in Mexico. The molecular and morphological studies showed that the species *Culex quiquefaciatus*, *Culex pipiens* and their hybrids are circulating in Mexico City.

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POPULATIONS OF THE *ANOPHELES GAMBIAE* M-FORM CAN HAVE TYPICAL SAVANNA-FORM INVERSION KARYOTYPES AT THE NORTHWESTERN LIMITS OF ITS GEOGRAPHICAL RANGE

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Scanty data are available on the relative frequencies of ***Anopheles gambiae*** molecular forms and on their chromosomal inversion polymorphism patterns at the western extremes of their geographical range.

At the end of the 2005 malaria transmission season (Oct-Nov), we conducted a cross-sectional survey by pyrethrum-spray and hand aspirator catches at 8 sites covering a west-to-east transect from the coastal region of The Gambia to eastern Senegal. Molecular identifications showed the presence of *An. melas* (N=144) in the 3 sites within 200 km from the coast, and of *An. gambiae* s.s. (N=259) and *An. arabiensis* (N=668) at all sampling sites. A cline was observed in the relative frequencies of *An. gambiae* molecular forms: the S-form i) was reported for the first time in the "coastal" sites; ii) was absent in samples from central Gambian areas, characterised by extensive rice cultivation; and, iii) predominated in eastern Senegal. The M-form was present at all the sampling sites and largely predominated over S in the 5 Gambian sites. Throughout the study area, both molecular forms showed high frequencies of inverted arrangements 2Rb, 2Rd and 2La, typical of the SAVANNA chromosomal form. S-populations from Senegal were characterised by the presence of the 2Rj arrangement, mostly associated with 2Rb and/or 2Rd. Rare carriers of the typical MOPTI-specific polymorphism (2Rbc/u/+) and BISSAU-specific karyotypes (2Rd/d_2L+/a) were recorded in the M-form only. Unexpectedly, individuals characterised by the most typical SAVANNA karyotype (2Rb/b_2La/a) were identified mostly as M-form. The absence of the S-form in the intensely rice cultivated Gambian areas and its predominance in Guinea-savannah eastern localities, suggests a greater ability of the M-populations to adapt to the rice ecosystem, even in the absence of the inversion set-up typical of the MOPTI chromosomal form (2Rbc/u), which is usually found in comparable ecosystems of Mali and Burkina Faso. Collections along the same transect are planned in 2006 to confirm these preliminary data.

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SNP ESTABLISHMENT AND ANALYSIS OF MICROSATELLITE DETECTABLE GENETIC STRUCTURE ACROSS ANOPHELES. GAMBIAE POPULATIONS IN AND AROUND NORTH-WESTERN LAKE VICTORIA

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Malaria remains one of the leading killers in Africa. Control through drug administration and insecticide spraying has proven inadequate, so there is a need for an effective control strategy. Genetic manipulation of vectors to block disease transmission is being investigated and large scale use of insecticide treated nets has been considered. *Anopheles gambiae*, the main vector has complex population structure that is still poorly understood. The study was aimed at verification in natural populations of several Vectorbase predicted A. gambiae genome candidate SNPs. It was also designed to confirm previously detected microsatellite based structure and allow an empirical SNP - microsatellite comparison in an actual natural population analysis. Studies of genetic structure are vital to the success of any vector-targeted control measure. Four Islands (from 20-50 km apart) and two surrounding mainland populations (96 km apart) were studied. Samples of indoor resting adult mosquitoes collected over two consecutive years were genotyped at marker loci distributed broadly throughout the genome and analyzed for general population structure and for marker comparisons. Ne estimates showed island populations to consist of smaller demes compared to the main-land ones. Most populations were significantly differentiated geographically, and from one year to the other. Average geographic pair-wise FST ranged from 0.014-0.105, and several pairs of populations had Ne m < 3. SNPs confirmed the previously observed microsatellite population structure though at lesser resolution. The only SNP grouping with AMOVA significance was that clustering the four islands and closest mainland population from the farthest mainland population whereas microsatellite resolved 3 groupings comprising an all mainland population cluster; an intermediate islands population cluster; and the distant island population cluster. The microsatellite grouping was more reflective of current fine-scale population topographical structure. SNPs population grouping on the other hand was possibly reflective of the demographic past. This study shows that the relative advantages of SNPs over microsatellites are context specific. The islands are significantly genetically differentiated from the two mainland populations and from some of each other. This appears to be the product of separation across water, dynamics of small populations and local adaptation.

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COMPARATIVE FIELD EVALUATION OF BIFENTHRIN AND PERMETHRIN AS BARRIER TREATMENTS FOR MILITARY TENTS AGAINST MOSQUITOES IN QUEENSLAND, AUSTRALIA

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A study to compare the effectiveness of barrier treatment of military tents with Bifenthrin and Permethrin in preventing entry of mosquitoes was conducted at Wide Bay Training Area, Queensland, Australia. Five military tents were erected at a site in bushland at Mosquito Creek, about 2 km from the ocean. Two tents were sprayed with Bifenthrin (Bistar 80SC, 0.1%mix, 12.5ml/l), 2 with Permethrin (Perigen Defence, 0.12%, 24ml/l) and another left untreated as a control. Carbon dioxide baited traps were placed inside each tent 0, 2, 4, 6 and 8 weeks after treatment, and a single trap placed in forest 50m from the tents.

The predominant mosquito species collected during the study was *Ochlerotatus vigilax* (80% of collection). Protection against mosquitoes entering the tents was initially 78.6% for bifenthrin treated tents and 84.3% for permethrin. At 4 weeks, protection was 68.6% for bifenthrin and 50.7% for permethrin. After 6 weeks, less than 34% protection was provided. There was no statistical difference between the protection provided by either chemical treatment. The study shows that barrier tent treatments provide a reasonable increase in preventing the entry of mosquitoes for at least 4 weeks.

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AN OVERVIEW OF VECTORBASE.ORG, A BIOINFORMATIC RESOURCE FOR INVERTEBRATE VECTORS OF HUMAN PATHOGENS

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VectorBase is an online National Institute of Allergy and Infectious Diseases Bioinformatic Resource Center specific to invertebrate vectors of human pathogens. Currently, VectorBase is focused on An. gambiae, Ae. aegypti, Cx. pipiens, and Ix. scapularis, with further organisms to be incorporated upon completion of genomic sequencing. While primarily focused on genome analysis and display, VectorBase takes various data types and consolidates them into a single resource that effectively relates previously discrete sets of information and presents them in a unified, consistent fashion at the VectorBase website, http://www.vectorbase.org. Resources available to researchers include data browsers, analysis and data mining/search tools, and raw data sets, seamlessly integrated for user convenience. Moreover, VectorBase aims to be a more valuable resource than a simple data repository through community involvement. Through the VectorBase website, users are encouraged to submit gene names, annotations, corrections, and gene-associated literature and controlled vocabulary terms. These data are curator reviewed and promptly integrated and displayed within the VectorBase website with due credit. Through the combination of powerful high throughput in silico analysis and user experimental contributions and expertise, VectorBase will continue to grow as a resource for the vector community.

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DYNAMICS OF IMMATURE STAGES OF *ANOPHELES ARABIENSIS* AND OTHER MOSQUITO SPECIES (DIPTERA: CULICIDAE) IN RELATION TO RICE GROWTH STAGE IN A RICE AGRO-ECOSYSTEM IN MWEA, KENYA

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Studies were conducted to determine changes in species composition and densities of immature stages of *Anopheles arabiensis* in relation to rice growth cycle in order to generate data for developing larval control strategies in rice ecosystems. Experimental rice paddies (6.3m x 3.15m) exposed to natural colonization of mosquitoes were sampled weekly for 2 rice growing cycles between February 2003 and March 2004. Overall, 21,325 *Anopheles* larvae were collected of which 91.9% were 1st and 2nd instars and 8.1% were 3rd and 4th instars. *An. arabiensis* was the predominant species (84.1%) with other species, *An. pharoensis* (13.5%), *An. funestus* (2.1%), *An. coustani* (0.3%) and *An. maculipalpis* (0.1%) accounting for only a small proportion of the anophelines collected. *Culex quinquefasciatus* (65.7%) was the predominant species among the non-anopheline species. Others species collected included: *C. annulioris* (9.9%), *C. poicilipes* (7.3%), *C. tigripes* (7.2%), *C. duttoni* (0.6%), *Aedes*

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aegypti (5.3%), *Ae. cumminsii* (3.5%) and *Ae. vittatus* (0.7%). The densities of the major anopheline species were closely related to rice stage and condition of the rice field. *An. arabiensis,* the predominant species, was most abundant over a 3-week period after transplanting. Low densities of larvae were collected during the late vegetative, reproductive and ripening phases of rice. Culicine and aedine species densities were significantly higher during the post-harvest period. Our results suggest that the transplanting stage is favorable for the development of immature stages of *An. arabiensis* and provides a narrow window for targeted larval intervention in rice. The data provide fundamental information about the productivity of immature stages of *An. arabiensis* at different stages of rice development cycle that could be utilized for developing targeted larval control strategies for rice ecosystems. The results further stress the need for rational water management in rice irrigation in order to reduce the effects of unplanned rice cultivation thus minimizing the period paddies are under irrigation.

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PREDATION LIMITATION OF INVASIVE MOSQUITOES

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Invasive species of container mosquitoes recently spread in the USA, such as *Aedes albopictus* and *Aedes japonicus*, may increase the potential for arbovirus transmission to human hosts. On the other hand, selective predation on invasive mosquito vectors may limit the risk of their proliferation and spread. We tested experimentally whether selective predation on larval *A. albopictus* accounted for its stable co-existence with the native mosquito and inferior competitor Aedes triseriatus in Florida treeholes and tires. At all ratios of these two prey species, the two native predators *Corethrella appendiculata* and *Toxorhynchites rutilus* preferentially consumed *A. albopictus*. When equal numbers of the two prey species were reared together in conditions of variable basal resources (leaves) and predation, high levels of both factors promoted their co-existence. The two predator species, which may co-occur in treeholes and discarded tires, may have different functional roles, *C. appendiculata* regulating prey diversity and *T. rutilus* regulating prey abundance. In experimental tires maintained outdoors in a Florida hammock, resident *C. appendiculata* strongly reduced the colonization success of *A. albopictus*. Because invasive vectors may lack anti-predator defenses, they may be more vulnerable than native prey species to predation in newly colonized habitats.

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IDENTIFICATION OF DIFFERENTIALLY REGULATED MOSQUITO PROTEINS - POTENTIAL TARGETS AGAINST FLAVIVIRUSES

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Modulation of vertebrate host immunity by arthropod saliva, favoring vector-borne infections, has been well described. However, in most cases little is known about specific interactions between pathogens and their vector hosts. When infected with the pathogen, the levels and/or composition of secreted saliva in ticks and mosquitoes are often altered. The flaviviruses, dengue virus and West Nile virus are among the several emerging and resurging vector-borne infectious diseases which are transmitted by mosquitoes. In order to identify proteins that are differentially expressed in *Aedes albopictus* cells and *Ae. aegypti* mosquito tissues during infection with dengue virus or West Nile virus versus non-infected cells and tissues, two dimensional differential fluorescence gel electrophoresis was used. We have identified a number of proteins that are differentially expressed during infection with dengue virus or West Nile virus. Although many proteins were identified as differentially expressed during infection with both viruses, many of these protein targets may be virus-specific. We group differentially expressed proteins into one of two categories: antiviral vector proteins and viral induced vector proteins that assist replication or transmission. Our aim is to identify similarities and differences in the regulation of vector proteins and therefore identify potential targets to reduce infection and transmission of theses viruses. Currently, we are using RNA interference of specific target genes to assess the role of viral-induced mosquito proteins in the replication and transmission of dengue and West Nile viruses.

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GENETIC POPULATION STRUCTURE IN THE MALARIA VECTOR ANOPHELES MARAJOARA IN NORTHEASTERN SOUTH AMERICA

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Anopheles (Nyssorhynchus) marajoara (Diptera: Culicidae) is an important regional malaria vector in eastern Amazonian Brazil. A population expansion in *A. marajoara* was estimated to have occurred in NE Amazonian Brazil during the Pleistocene using sequences from the mtDNA *COI* gene. Using 8 polymorphic microsatellite loci, we assessed the population structure of this species from 8 localities in Amazonian Brazil plus one in Trinidad. On the basis of microsatellite allele frequencies we detected three subdivisions: Central (Manaus and Santarém), Northern (Boa Vista and Trinidad), and Eastern (Marajo Island and four localities around the city of Macapá in Amapá state). AMOVA detected significant differentiation among the three groups and within populations, with most variance (78%) within populations, but also a considerable amount (20%) among the three groups. In a UPGMA tree, these three subdivisions were each supported at 100%. The high degree of significant differentiation between the two Northern populations and all others (F_{ST} = 0.198-0.401) combined with the AMOVA, STRUCTURE and UPGMA results and those from an earlier mtDNA sequence study, lend support to the presence of a genetically differentiated type, *A. albitarsis* E, from Boa Vista and Trinidad, with at least partial barriers to gene flow with *A. marajoara*. Isolation by distance was inferred as one likely model to explain levels of differentiation. Results from MSVAR provide good evidence that all *A. marajoara* populations have undergone an historical population reduction except GA (Eastern cluster), which showed modest population growth.

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TRANSCRIPTOME ANALYSIS OF ARMIGERES SUBALBATUS-*BRUGIA MALAYI* INTERACTIONS

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Armigeres subalbatus is a natural vector of the filarial worm *Brugia pahangi*, but it rapidly and proficiently kills *Brugia malayi* microfilariae by melanotic encapsulation. Because *B. malayi* and *B. pahangi* are morphologically and biologically similar this mosquito-parasite system serves as a valuable model for determining the resistance mechanisms in mosquito vectors. We have initiated transcriptome profiling studies in *Ar. subalbatus* to clarify molecular mechanisms involved in *B. malayi* refractoriness. These initial studies assess the transcriptional response of *Ar. subalbatus* to *B. malayi* or *B. pahangi* at 24 hrs after an infective blood meal. cDNA libraries from adult, female mosquitoes in the following groups: naïve 5-7, and 14-21 days post emergence, *Dirofilaria immitis* inoculated (24 and 48 hr post inoculation), *B. malayi* exposed via blood

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feeding (24, 48, 72 hr post infection), bacteria inoculated (24, 48, 72 hr post inoculation with *Esherichia. coli* and *Micrococcus luteus*), and a library generated from hemocytes (1, 3, 6, 12, and 24 hours post bacteria inoculation) were used to generate 8023 EST clusters from which 60-mer oligonucleotides were synthesized that represent 6128 unique EST clusters. When hybridizing labeled hemolymph RNA to the array, there was a 27% increase in the number of significantly up- or down-regulated genes as compared to arrays hybridized with labeled RNA from whole bodies. We have demonstrated that these immune responses are extremely tissue specific, and when conducting studies of this nature it is possible to "wash out" the desired response by using too general of an RNA source. Finally, the study reveals some of the molecular components of anti-filarial worm immune responses, including a number of unknown and conserved unknowns, cytoskeletal and structural components, and stress and immune responsive factors.

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THE DISTRIBUTION OF HATCHING TIME IN *ANOPHELES GAMBIAE*

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Knowledge of the ecological differences between the molecular forms of *Anopheles gambiae* and their sibling species, *An. arabiensis* might lead to understanding their unique contribution to disease transmission and to better vector control as well as to understanding the evolutionary forces that have separated them. The distributions of hatching time of eggs of wild *An. gambiae* and *An.arabiensis* females were compared in different water types. Early and late hatchers of the S molecular form were compared with respect to their total protein content, sex ratio, development success, developmental time and adult body size. Overall, the distribution of hatching time was strongly skewed to the right, with 89% of the eggs hatching during the second and third day post viposition, 10% hatching during the next four days and the remaining 1% hatching over the subsequent week. Slight, but significant differences were found between species and between the molecular forms in all water types. Differences in hatching time distribution were also found among water types (in each species and molecular form), suggesting that the eggs change their hatching time in response to chemical factors in the water. Early hatchers were similar to late hatchers except that they developed faster and produced smaller adults than late hatchers. Differences in hatching time and speed of development among eggs of the same batch may be adaptive if catastrophic events such as larval site desiccation are not rare and the site's quality is unpredictable. The egg is not passive and its hatching time depends on water factors. Differences in hatching time between species and molecular forms were slight, probably reflecting that conditions in their larval sites are rather similar.

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ECOLOGY, GENETICS, AND TRANSMISSION OF *PLASMODIUM FALCIPARUM* BY *ANOPHELES ARABIENSIS* IN MACHA, ZAMBIA

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Entomological aspects of malaria transmission in the Southern Province of Zambia are very poorly understood. Hyperendemic transmission of *Plasmodium falciparum* is maintained by secondary vectors *An. arabiensis* Patton and *An. funestus* Giles s.s. in the apparent absence of *An. gambiae* s.s. Giles. Unlike the highly anthropophilic *An. gambiae* s.s. and *An. funestus* s.s., *An. arabiensis* exhibits exophilic and zoophagic behaviors, complicating its role in malaria transmission. Therefore, our objectives were to characterize the blood feeding behavior, seasonality and intensity of transmission, and population structure of *An. arabiensis* in this novel study area to better understand the vectorial components that contribute to and drive local malaria transmission by this species. Mosquitoes were collected monthly between November and May 2004-2005 and 2005-2006 by human landing catch and pyrethrum spray catch in the villages of Chidakwa and Lupata, and once in the early, middle, and late rainy season from 20 additional villages throughout the Macha catchment region. Mosquitoes were analyzed individually by PCR to obtain species confirmation, blood host identification, *Plasmodiu*m infection status, and microsatellite allelic profile. Monthly mosquito population density plotted against rainfall revealed a peak in *An. arabiensis* activity late in the rainy season following peak rainfall. Monthly and seasonal entomological inoculation rates were estimated from both human landing catch and pyrethrum spray catch data. Blood meal identification and foraging ratio analysis demonstrated a human host feeding preference by *An. arabiensis* consistent with its status as a primary malaria vector, however as expected this species also fed opportunistically on cattle, dogs, and chickens. Finally, preliminary microsatellite analysis of *An. arabiensis* populations elucidated minimal structuring among populations at the regional scale studied, with further comparison to populations from throughout Zambia pending. Together these data represent some of the first entomological studies performed in Zambia aimed at examining vectorial components of malaria transmission by *An. arabiensis*.

(ACMCIP Abstract)

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IDENTIFICATION AND DISTRIBUTION OF THE MOLECULAR FORMS OF *ANOPHELES GAMBIAE SENSU STRICTU* CAPTURED RESTING OUTDOORS IN VARIOUS HABITATS OF KASSENA NANKANA DISTRICT (KND) IN THE UPPER EAST REGION OF GHANA

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Anopheles gambiae s.s. is one of the leading vectors of Malaria in Africa, and there are Mopti (M) and Savanna (S) forms of this species. This study is to find out the molecular forms of *An. gambiae s.s.* in the study area and also to find out the correlation between the molecular forms (M and S) and their preferred resting places outside the dwelling rooms of humans. The forms were identified using the Polymerase Chain Reaction (PCR). The PCR-identified *An. gambiae s.s.* mosquitoes were further differentiated into M and S molecular forms using *Hha I* enzyme digestion of PCR products. A total of 304 mosquitoes were identified by PCR as *Anopheles gambiae s.s.* comprising 294 (96.7%) M forms and 10 (3.3%) S forms. In the habitat distribution, out of those captured from outdoor shed, 123 (94.6%) were M forms and 7 (5.4%) S forms. For animal pen 45 (95.7%) were M forms and 2 (4.3%) S forms. For hencoop 93 (98.9%) were M forms and 1 (1.1%) S forms. The store room and the surrounding bushes recorded only M forms which were 12 (100%) and 21 (100%) respectively. Results showed that M forms were adapted more to the wet season and S forms to the dry season. Seasonal variation affected the abundance of mosquitoes but the form of the mosquitoes whether M or S did not correlate with the type of habitat preferred. Showing that, the preferred habitats (resting behaviour) depended on easy access to host.

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FLUCTUATING VERSUS CONSTANT TEMPERATURES: IMPACT ON DEVELOPMENT AND SURVIVAL OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* AND IMPLICATIONS FOR DISEASE MODELING

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Many models that explore the impact of climate on mosquitoes and disease transmission are based on development and survival data from laboratory experiments at constant temperatures. However, this is unrealistic given the fact that mosquitoes in nature may experience a range of temperatures (i.e. cold night temperatures and hot temperatures during the day). Fluctuating temperatures may cause a *rate summation* effect and additional physiological effects that will alter survival and development of disease vectors. Therefore, we investigated the impact of fluctuating temperatures on *Ae. aegypti* and *Ae. albopictus*, two species that are involved in the transmission of arboviruses, most notably dengue virus. We show how development rate, survival and adult size are affected and will demonstrate how these results may alter the outcome of predictive models.

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BITING BEHAVIOR OF ANOPHELES DARLINGI (ROOT) IN THE SOUTHERN AMERINDIAN REGION OF SURINAME

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A study was made of the biting behaviour of *Anopheles darlingi*, the main malaria vector, and other anophelines, in the Amerindian village Kwamalasamutu (South Suriname). In 2005 this isolated village reported 166 malaria cases which is 2.3 % of the national total for that year. From January until April 2006 a total of 1440 man hours of indoor and peridomestic human bait collecting of mosquitoes, resulted in the catching of 377 mosquitoes. Only 5% of these, 19 specimens, were *An. darlingi*, giving a biting index of 0.158 ± 0.311 per night over this period, with a peak of 1.125 in January. Over 42% of the biting occurred indoor and 94.7 % of the biting occurred in the second half of the night, with a single peak between 4.00 and 6.00 hours (63% of total biting). This is not consistent with what was previously reported along the east border of Suriname. Other investigators had noted a single peak in biting activity around 23.00 hours. Previously published reports for neighbouring countries report peaks at both dawn and dusk for *Anopheles darlingi*. This biting behaviour is important for the planning of preventive measures for malaria transmission, because the local people have a habit of rising early in the morning, many of them leaving for fishing, hunting and agricultural activities at dawn.

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SPATIAL AND TEMPORAL PATTERNS IN THE RECOVERY OF *AEDES AEGYPTI* POPULATIONS AFTER INSECTICIDE TREATMENT

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Given that tools for dengue emergency control are limited, continuous evaluation of the effectiveness of insecticide applications in the field is of utmost importance. Such studies will provide a sound basis for defining spraying schemes for public health authorities in dengue affected countries. In this paper, we address the following research questions: how do different space spraying strategies affect *Aedes aegypti* populations in both space and time? More specifically, how well are these mosquitoes killed, and how quick do their populations recover and from where? Field trials were carried out with ULV sprayers in Kamphaeng Phet province, Thailand, with a Pyrethrin mixture that was applied (i) indoor only, (ii) indoor plus outdoor, (iii) indoor with a doubled spraying time only and (iv) indoor with doubled spraying time plus outdoor. We found that within seven days *Ae. aegypti* populations recovered to approximately 50% of their original numbers. Including the outdoor area and doubling the time sprayed per room only had a significant impact on mosquito numbers one day after spraying. Two and seven days after spraying these effects were no longer detected. By investigating the spatial arrangement of *Ae. aegypti* numbers, we found that during the first two days after spraying immigration from untreated areas extended approximately 15m into the sprayed area, whereas after seven days this effect extended up to 50m. Results will be discussed in relation to ongoing dengue control efforts in Thailand.

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FOCAL POPULATION GENETICS AND DENGUE VECTOR COMPETENCE OF *AEDES AEGYPTI* IN TRINIDAD, WEST INDIES

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Aedes aegypti, the primary vector of dengue virus is unique in its ability to undergo its entire lifecycle closely linked to or within human dwellings. Although globally distributed, *Ae. aegypti* is documented as having a relatively short dispersal distance, thus promoting genetic substructure and variability in vector competence among locally adjacent populations. Here we present results from preliminary investigations of spatial and temporal variation in genetic structure and vector competence in *Ae. aegypti* populations in Trinidad, West Indies. Single Nucleotide Polymorphisms and microsatellite markers were used to examine genetic differentiation between populations collected immediately adjacent to human dwellings, and at a limited distance away from the dwellings, as well as between dwellings, using a simple ovitrap grid system. Vector competence was studied by challenging mosquitoes with a DEN-2 JAM1409 infected bloodmeal and assaying for viral dissemination by RT-PCR. Significant differences in genetic substructure were observed within and among populations and vector competence varied both geographically and temporally.

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BITING PATTERN OF A DENGUE VECTORS, *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN URBAN AND RURAL GRADIENT IN CHIANG MAI PROVINCE, NORTHERN THAILAND

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It is known that in Thailand, primary and secondary vectors of Dengue fever are *Aedes aegypti* (L) and *Ae. albopictus* (Skuse) respectively. *A.e aegypti* is an urban vector that became more widely distributed in large communities along the major transportation routes. Little is known about dengue vector biology in northern Thailand. This study subjected to know a biting pattern of *Ae. aegypti* and *Ae. albopictus* in Chiang Mai province the second largest city where DHF has been reported every year. The mosquitoes were collected in difference seasons dry season (March- April 2004) and wet season (June- August 2004) in two localities, Chiang mai

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city (urban site) and PMD village (rural site). All aedine mosquito both of male and female were collected by aspiration three days a week. There were three collection sites per location, one pair of collector were assigned to collect mosquito every 20 minutes with 10 minutes rest and they were rotated every hrs to another collection site. Collecting time was started from 7.00 hr to 18.00 hr each day. The results showed diurnally biting peak of *Ae. aegypti* collected from urban location. During the rainy season the biting peak appeared in late afternoon (15.00hr.) and it was opposite in the dry season (8.00 hr). In rural location the pattern of biting rate of *Ae. aegypti* was also bimodal with consistent peak at 9.00, 12.00 hr. both of dry and wet seasons. Male *Ae. aegypti* was also collected, the collection peak was identical. *Ae albopictus* was collected mainly in rural locality with bimodal peak during rainy season, 10.00 and 16.00 hr. there was no clear peak in dry season. Understanding the vector biology is advantage to appropriate planning for vector control cost effectiveness.

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COMPARATIVE RESPONSES OF MOSQUITO VECTORS OF WEST NILE VIRUS TO LIGHT TRAPS AUGMENTED WITH CHEMICAL ATTRACTANT AND TO HUMAN HOSTS

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Scientists in the USA seek to develop Global Information Technology (GIS, GPS, remote sensing)-based systems that can be used to deploy sentinel traps for mosquito vectors and for the implementation and evaluation of mosquito control. Achieving this objective requires the development of methods for unbiased estimation of adult mosquito density. Unbiased estimators will enable the identification and analysis of natural mosquito dispersion parameters and the development of GIT-based models for forecasting mosquito activity and distribution. In North America, the CDC light trap (augmented with attractant CO_2 gas) is used to determine the species composition and abundance of vector populations, as well as the geographic distribution and virus infection rate in these populations. But we do not know the relationship between mosquito capture rates by CDC traps and the numbers of mosquitoes attacking the human population. In the present study, the landing rates (LR) of *Anopheles quadrimaculatus, Culex nigripalpus, Cx. quinquefasciatus*, *Ochlerotatus triseriatus* and *Aedes albopictus* on human hosts were compared with capture rates of the same species by CDC traps (with CO_2). We found significant associations ($P \leq 0.05$) among the day-to-day responses to LR and CDC by *An quadrimaculatus* and *Cx. quinquefasciatus*, and among the hour-to-hour (over 24 hours) responses of all species except *Oc. triseriatus*. CDC traps typically underestimate LR by 40-125%, depending on the mosquito species and time of day, but improved precision (R^2 =0.61-0.70) in these estimates is achieved by the identification/removal of outlier responses and the fit of log-transformed LR data for each species to linear or polynomial regression models. Temporal variations in the capture rate of mosquitoes by LR and CDC suggest that each method samples separate components of the mosquito population and/or differentially stimulates competing response patterns in individual female mosquitoes.

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DEVELOPMENT AND CHARACTERIZATION OF A PANEL OF SINDBIS VIRUS-BASED TRANSDUCING SYSTEMS EXPRESSING DIFFERENT FLUORESCENT PROTEINS AS MARKERS OF INFECTION IN *AEDES AEGYPTI* MOSQUITOES

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Alphavirus transducing systems (ATS) have been used in our lab to express heterologous proteins such as green fluorescent protein through the insertion of a second subgenomic promoter upstream of the virus structural proteins. Linking expression of fluorescent proteins with virus particles is an important tool for rapid detection of infected cells without the need to fix and stain tissues. We have constructed Sindbis virus-based ATS's to express a panel of fluorescent proteins that allow the detection of infection patterns of multiple viruses in individual mosquitoes. Female *Aedes aegypti* mosquitoes can be given an infectious oral bloodmeal containing two separate fluorescent protein-expressing viruses. The pattern of infection for each virus may be followed in dissected tissues using a fluorescent microscope or in live mosquitoes using the IVIS II imaging system.

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OVIPOSITION ACTIVITY PATTERNS AND WEST NILE VIRUS INFECTION RATES FOR MEMBERS OF THE *CULEX PIPIENS* COMPLEX AT DIFFERENT HABITAT-TYPES WITHIN THE HYBRID ZONE, SHELBY COUNTY, TN, 2002 (DIPTERA: CULICIDAE)

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Oviposition activity and West Nile virus (WNV) infection rates were assessed for members of the *Culex pipiens* complex from July through December, 2002, using gravid traps placed at four ecologically different sites in the southern portion of the hybrid zone in Shelby County, Tennessee. Molecular assays identified three members of the *Cx. pipiens* complex: *Cx. pipiens pipiens* Linnaeus, *Cx. p. quinquefasciatus* Say, and *Cx. p. pipiens-Cx. p. quinquefasciatus* hybrids (hybrids). The *Culex pipiens* complex accounted for 87.4% of mosquitoes collected in gravid traps. All 285 WNV positive mosquitoes were *Culex* mosquitoes and 277 (97%) were *Cx. pipiens* complex mosquitoes. Infection rates among members of the *Cx. pipiens* complex were not significantly different. Infection rates were significantly higher at two urban sites than at a rural site, and WNV was not detected at a forested site. At urban sites, abundances of members of the *Cx. pipiens* complex corresponded to a simple latitude model of the hybrid zone. *Culex p. quinquefasciatus* was most abundant (46.4%), followed by hybrids (34.1%), then *Cx. p. pipiens* (19.5%). The relative abundances at a rural site were reversed with *Cx. p. pipiens* (48.4%) being most abundant. This demonstrates that spatial habitat variation may profoundly influence the distribution of members of the *Cx. pipiens* complex within the hybrid zone. Members of the *Cx. pipiens* complex did not display different oviposition patterns. However, oviposition patterns at urban and rural sites were significantly different. At urban sites, oviposition activity of *Cx. pipiens* complex mosquitoes was bimodal with an evening peak associated with sunset and a morning peak associated with sunrise. At the rural site, the evening peak was pronounced and the morning peak weak and similar to night time activity.

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THE INFLUENCE OF HOUSE CONSTRUCTION ON THE INDOOR ABUNDANCE OF MOSQUITOES: A PRELIMINARY STUDY

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We examined the potential effects of different house construction features on the indoor abundance of culicine mosquitoes in Trinidad and the Dominican Republic using xenomonitoring surveys. To assess these effects, a survey was taken of different homes in both countries alongside concurrent indoor resting mosquito collections to determine which features may be correlated with a greater abundance. Between June 2002 and April 2003 data were collected from 104 homes in Trinidad (TT) and 121 homes in the Dominican Republic (DR). In Trinidad 61 (58.65%) of the homes were located in urban areas and 43 (41.35%) were located in rural villages whereas in the DR 40 (33.06%) were located in the rural areas and 81 (66.94%) in the urban area. Overall, a total of 1,630 mosquitoes were collected in Trinidad, of which 77% were *Culex quinquefasciatus*, whereas 459 mosquitoes were collected from the DR, of which 46% were

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Cx. quinquefasciatus. It was found that in Trinidad and the Dominican Republic the mean number of *Cx. quinquefasciatus* mosquitoes was greater in cement homes than in either wood or other poorer quality homes (TT cement 17.43 others 14.43, DR cement 4.24 others 3.41). In Trinidad it was found that homes that had painted interiors were significantly less likely to have a high abundance of mosquitoes resting indoors compared to homes without painted interiors (OR.34 CI.13-.88). Likewise, having a painted exterior was only slightly not significant in Trinidad as having a protective benefit (OR.42 CI.17-1.03). Conversely, the benefit of having a painted interior or exterior was not seen in the Dominican Republic where they were instead determined to be predictors for a high abundance of indoor resting mosquitoes (interior OR 3.13 CI 1.41-6.92, exterior OR 1.97 CI.91-4.26).Reduced adult abundance in Trinidad was correlated with homes being built on stilts, that is, more than four people sleeping in the home, and having a painted interior. In the Dominican Republic, reductions were correlated with homes where residents slept under a bed net and with people who lived in a rural location. Changes in construction patterns in the Caribbean region could help prevent human-mosquito contact potentially reducing the transmission of certain vector-borne diseases in the population

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DISTINGUISHING DISEASE SPREADING ANOPHELES SIBLING SPECIES IN PAPUA NEW GUINEA USING DNA-BASED ASSAYS

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Three closely related species: *Anopheles punctulatus* (AP), *A. farauti* (AF), and *A. koliensis* (AK) are vectors of malaria and filariasis in Papua New Guinea (PNG). Standard morphological characteristics (proboscis shape/coloration, wing patterns) used to classify these mosquito species are not reliable, or do not differentiate the seven morphologically indistinguishable AF sibling species. DNA sequence polymorphism in the internal transcribed spacer 2 (ITS2) of ribosomal RNA genes appears to provide a basis for developing molecular diagnostic assays to distinguish members of the AP species complex. Here we evaluate ITS2 polymorphism in mosquitoes captured in Madang (MP) and East Sepik (ESP) provinces of PNG by DNA sequence analysis and a sequence-specific, post-PCR multiplex ligase detection reaction/fluorescent microsphere assay (LDR-FMA). Mosquitoes were collected across a range of breeding habitats where the AP sibling species may or may not co-exist; ITS2 PCR amplification was performed using leg segments excised from individual insects. Our studies observed that species-specific ITS2 DNA sequence polymorphisms, where LDR-FMA probes were positioned to hybridize, were stable in mosquitoes captured at the two collection sites. We did, however, observe new sequence polymorphisms in flanking regions. PCR products from mosquitoes identified as a particular species by morphology were then analyzed by LDR-FMA. For mosquitoes collected in MP sites we observed LDR-FMA results concordant with morphological assessment for 94.4% (334/354) of the mosquitoes overall, including: 98.9% (182/184) of specimens identified as AF (including AF1 and AF4), 85.3% (64/75) of insects identified as AP, and 92.6% (88/95) of insects identified as AK. Comparisons between morphology and LDR-FMA were notably less concordant in mosquitoes collected in ESP where AP and AK were difficult to distinguish. Additionally, whereas AF1 breeds in saline puddles within 1-2 km of the sea coast, this species was observed in villages 25 km from the coast where breeding occurs in freshwater puddles. By improving identification of human disease vectors in PNG, our results contribute to an important first step in developing strategies to identify species most responsible for transmission of microbial pathogens.

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IMPLICATIONS OF HYBRIDIZATION, FEEDING BEHAVIOR AND PARITY RATES OF *CULEX PIPIENS* ON WEST NILE VIRUS ACTIVITY AT A STABLE ENZOOTIC STUDY SITE

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We examined various factors in *Culex pipiens* that influenced enzootic WNV activity in Delaware. Collections of mosquitoes were made at five locations that previously showed elevated epizootic activity in 2003 and 2004. We performed longitudinal comparisons of these five sites in 2005, one of which showed continued enzootic WNV activity based on virus-positive mosquitoes and sentinel chicken antibody seroconversions. The *Cx. pipiens* populations sampled from this site were analyzed using 8 microsatellite DNA markers. Preliminary data indicate hybridization of *Cx. pipiens* form *pipiens* and *Cx. pipiens* form *molestus* may be seasonally related. Blood-meal preference for *Cx. pipiens* hybrids was performed using a PCR-HDA protocol for blooded females collected in the field and by carrying out mosquito choice tests in the laboratory. Parity rates over the mosquito season (June to October) were also examined to determine the age structure of the population. Parous females increased from 52% in early summer to 98.6% (n=30) in late summer indicating an older population later in the season. Other comparisons included mosquito species composition and abundance at all five sites.

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AN AUTOMATED GIS/REMOTELY SENSED EARLY WARNING SYSTEM TO DETECT ELEVATED POPULATIONS OF VECTORS OF RIFT VALLEY FEVER, A MOSQUITO-BORNE EMERGING VIRUS THREAT

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Mosquito transmitted infectious diseases, like eastern equine encephalitis (EEE), Rift Valley fever (RVF), and West Nile virus (WNV), pose an international threat to animal and human health. An introduction of RVF into the U.S. would severely impact wild ungulate populations and the beef and dairy industries, and cause significantly more human illness than WNV. If not rapidly contained with an integrated vaccine and mosquito control strategy RVF would spread by various *Culex* species mosquitoes as rapidly as WNV, and potentially become established in a cryptic *Aedes* mosquito-transovarial enzootic cycle; however, there is no system in place for detecting the spatial and temporal conditions suitable for a RVF outbreak. In Africa remotely sensed environmental data have been used to predict conditions preceding production of large populations of mosquito vectors and thus the earliest stages in a RVF epizootic. We are developing a similar GIS/remotely sensed early warning system for RVF vectors in the U.S. Using satellite data and mosquito surveillance data, the GIS predicts disease transmission patterns based on the quantitative relationship between mosquito activity and patterns of local and global climate, and identifies early warning parameters associated with elevated populations of potential RVF vectors. Linkages between climate and mosquito densities are evaluated with spatial and temporal statistics, generating risk maps to inform control strategies. Mosquito prediction information will be disseminated throughout the U.S., granting several months warning before conditions are suitable for elevated mosquito populations, permitting implementation of control strategies in time to lessen or prevent animal and human disease.

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ENVIRONMENTAL ABUNDANCE OF *ANOPHELES* (DIPTERA: CULICIDAE) LARVAL HABITATS ON LAND COVER CHANGE SITES IN KARIMA VILLAGE, MWEA RICE SCHEME, KENYA

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A study was carried out at Karima Village in the Mwea Rice Scheme Kenya, to assess the impact of rice husbandry and associated land cover change for mosquito larval abundance. A multi-temporal, land use land cover (LULC) classification dataset incorporating distributions of *An. arabensis* aquatic larval habitats was produced in ERDAS *Imagine* (V8.7) using the combined images from IKONOS at 4m spatial resolution from 2005 and Landsat Thematic Mapper ™classification data at 30m spatial resolution from 1988 for Karima. Of 207 larval habitats sampled the majority were either canals (53.4%) or paddies (45.9%) while only one habitat was classified as a seep (0.5%). The proportion of habitats that were poorly drained was 55.1% compared to 44.9% for the habitats that were well drained. A LULC base map was generated in Arc View 9.1®. A grid incorporating each rice paddy was overlaid over the LULC maps stratifying each cell based on levels of irrigation. Paddies/grid cells were classified as 1) well-irrigated and 2) poorly-irrigated. Early stages of rice growth show peak larval production, during the early part of the cropping cycle (rainy season). Total LULC change for Karima over 16 years was 59.8%. Of those areas in which change was detected, the LULC change for Karima was 4.30% for rice field to built environment, 8.74% fallow to built environment,7.19 % rice field to fallow, 19.03 % rice field to fallow, 5.52% fallow to rice field, 8.35% built environment to rice field. Of 207 aquatic habitats in Karima, 54.1 (n= 112) were located in LULC change sites and 45.9 (n =95) were located in LULC non-change sites. LULC maps derived from IKONOS and TM data in GIS can be used to investigate the relationship between rice cultivation practices and higher anopheline larval habitat abundance and distribution.

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TESTING THE EFFICACY OF A NOVEL STICKY TRAP IN COLLECTING *AEDES* ADULTS IN A DENGUE-ENDEMIC AREA IN THAILAND

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The development of new methodologies to collect *Aedes aegypti* and *Ae. albopictus* adults for Dengue surveillance and of new entomological indicators to evaluate Dengue transmission potential is considered to be a priority for the implementation of prevention and control strategies. Here we present the results of a 5-week trial carried out in Kamphaeng Phet Province (Central Thailand, Oct-Nov 2005), where all four dengue virus serotypes are endemic and *Ae. aegypti* and *Ae. albopictus* are syntopic: the results of adult mosquito collections carried out by a new model of sticky trap (ST), recently designed by our group, with those obtained by Modified CDC Backpack aspirator (CDC-BA) are compared. Twenty houses were selected from an area that was subdivided in 150 x 150 m grid cells for the CDC-BA collections and, in a similar way, 20 for the ST collections, in order to get a minimum distance between a backpack and trap house of ≥100 m. Each selected ST house hosted 3 sticky traps indoor and 3 outdoor. CDC-BA collections were carried out twice a week for ~10 minutes indoor and ~10 minutes outdoor (depending on house size), and STs were serviced twice a week by changing the adhesive surfaces and replacing the water. Mosquitoes were counted and identified in the field by species and gender. During the trial, a total of 19,011 adult mosquitoes was collected of which 61% in STs and 39% with CDC-BAs: 90.3% were *Culex* spp., 8% *Ae. aegypti*, 0.8% *Ae. albopictus*, 0.8% *Armigeres* spp. (0.1%) *Anopheles* spp. A total of 977 *Ae. aegypti* females (664 by STs and 313 by CDC-BAs), 538 *Ae. aegypti* males (64 by STs and 474 by CDC-BAs) and 154 *Ae. albopictus* females (149 by STs and 5 by CDC-BAs) were collected. Interestingly, a larger proportion of females was collected outdoor with STs (58,3% in the case of *Ae. aegypti* and 83,2% in the case of *Ae. albopictus*), while the opposite occurred in collections by CDC-BAs (7,3% of *Ae. aegypti* and 20% of *Ae. albopictus*). The results of the trial will be commented in view of the possible application of the ST as a tool for monitoring container-breeding mosquitoes and for studying endophilic/exophilic behaviours, as well as a possible surveillance method for Dengue outbreaks.

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THE POTENTIAL SIGNIFICANCE OF "INDIRECT TRANSOVARIAL/TRANSSTADIAL TRANSMISSION" AND INFECTED MALE AEDES MCINTOSHI MOSQUITOES IN THE ENDEMIC MAINTENANCE AND AMPLIFICATION OF RIFT VALLEY FEVER VIRUS

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Viral maintenance between human disease outbreaks and viral amplification prior to outbreaks are key issues in arboviral epidemiology. Earlier we reported immunocytochemical evidence of infected mosquito ova and associated pathology in *Aedes mcintoshi* infected with Rift Valley fever virus (RVFV) and in Culex tarsalis infected with Western equine encephalitis virus (WEEV). Deposition of such ova has potentially very important epidemiological implications since these ova could be ingested by mosquito larvae of the same or different mosquito species as well as by other organisms associated with the larval habitat. We offer the term "indirect transovarial/transtadial transmission (TOT)" for the potential infected ovum deposition-larval ingestion scenario to contrast with the "direct" TOT which involves hatching of an infected egg with subsequent persistence of the virus into the adult stage. Based on histological study, we identify a possible route of ovarian infection from the hemocoel, probable routes of virus egress from infected eggs, and probable routes of infection once virus is ingested by a mosquito larva. The potential impact of indirect TOT versus direct TOT on rates of virus transmission and amplification are discussed. We also report the results of an immunocytochemical study of male Aedes mcintoshi and consider the possible significance of male infection in the epidemiology of RVF virus and relative to indirect TOT.

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GENETIC DIVERSITY OF *GIARDIA LAMBLIA* CYST WALL PROTEIN 1 GENE FROM KOREA ISOLATES AND CHARACTERIZATION OF RECOMBINANT PROTEIN EXPRESSED IN *E. COLI*

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Giardia lamblia is a flagellated protozoan parasite of medical significance because it is a major cause of waterborne enteric disease worldwide. In 2004's survey of national parasitic diseases done by Korea government, the positive rate of *G. lamblia* was shown as 0.76% (221/28,924). At the present, the microscopic examination method has a lot limitation to diagnosis *G. lamblia*. In order to develop the effective control methods against *G. lamlia*, we have considered the development of diagnostic methods based on antigen and antibody detection. We firstly investigated

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genetic diversity of the candidate, cyst wall protein-1 gene and found that at least two types of *G. lamlia* are prevalent in Korea. In this study, we expressed the dominant Korea isolate of CWP-1 gene excluding the signal peptides in *E. coli* SG13009. A 25kDa recombinant protein was well expressed by inducing with 0.5mM IPTG and purified by using Ni-NTA agarose affinity chromatography. This recombinant protein was reacted with patient sera, indicating that it has host immunity. We immunized BALB/c mice three times with this purified CWP-1 and developed monoclonal antibody for the detection of the cysts from patients stool. The purified monoclonal antibody obtained from ascite fluid of the injected mice showed strong reaction with recombinant CWP-1 in ELISA. Therefore we would like to make rapid antibody diagnostic kit with the recombinant CWP-1 and antigen diagnostic kit with monoclonal antibody for the control of giardiasis in Korea.

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*EH*GEF2, A NEW DBL-RHOGEF FROM *ENTAMOEBA HISTOLYTICA* CONTAINING *ARMADILLO*-LIKE REPEATS: OVERALL CHARACTERIZATION AND ITS POSSIBLE PARTICIPATION IN ERYTHROPHAGOCYTOSIS, PROLIFERATION AND CHEMOTAXIS

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Entamoeba histolytica (*Eh*) is a protozoan parasite that kills more than 50,000 people a year. Amoebic movement is accompanied by changes in cell morphology. In eukaryotic cells, this phenomenon is regulated by proteins belong to the Rho subfamily of small GTP-binding proteins. Activation of these GTPases is under the direct control of guanine nucleotide exchange factors (GEFs), the Dbl family proteins. Here, we present the molecular, biochemical and cellular characterization of a novel Dbl family RhoGEF, termed *Eh*GEF2, from *E. histolytica* genome database. The *Eh*GEF2 gene is single copy, as revealed by Southern hybridization and encoded a protein containing 732 amino acids. It contains in order from N- terminal to C- terminal: 5 Armadillo-like repeats, a highly conserved Dbl Homology (DH) domain and a poorly conserved Plekcstrin Homology (PH) domain. The Northern blot analysis indicated that the mRNA is expressed in trophozoites cultured axenically. Recombinant *Eh*GEF2 catalysed guanine nucleotide exchange of *Eh*RacA, *Eh*RacB, *Eh*RacC, *Eh*RacD, *Eh*RacG, *Eh*RacH and *Eh*Cdc42, but not for *Eh*Rho1, as determined by GDP release, methylanthraniloyl (mant)-GTP binding, suggesting that *Eh*GEF2 protein could be involved in diverse mechanisms related to actin cytoskeleton activation in trophozoite. Deletion of the C-terminal end and/or the N-terminal end of *Eh*GEF2 had significant effect on the GEF catalytic activity towards *Eh*RacG *in vitro*. By immunofluorescence, the *Eh*GEF2 protein was localized underneath the plasma membrane in *Eh*GEF2 HSV-tagged transfected cells. *Eh*GEF2 protein could be involved in erythrophagocytosis, proliferation and chemotaxis as was suggest by expression of a dominant negative mutant.

(ACMCIP Abstract)

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CHANGES IN BACTERIAL PROFILE DURING AMEBIASIS

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In amebiasis patients, trophozoites of *Entamoeba histolytica* live in the colon region of the human intestine in close association with resident microbial flora. Little is known about the changes in the flora that occur due to invasiveness of amebiasis like amebic liver abscess condition. Fecal samples from 35 amebic liver abscess patients; 19 healthy, *E. histolytica* negative and 11 subjects that are positive for *E. histolytica* but remained asymptomatic were tested extensively for the anaerobic and aerobic bacterial genera using genus/ species specific primers. Pus samples from amebic liver abscess patients were also tested for the bacterial presence. Statistically significant reduction due to protozoan infection, in the population of beneficial bacterial members like *Lactobacillus, Bacteroides, Bifidobacterium, Clostridium* was observed in amebic liver abscess patients. The most significant results of this study are: 1) Presence of two gut resident anaerobic bacteria viz.; *Bacteroides* and *Peptostreptococcus* in amebic liver abscess pus samples and 2) metronidazole drug resistance genes (nim genes) in fecal and pus samples of amebic liver abscess patients. The demonstration of the anaerobic bacteria in pus sample (so far considered to be sterile) show that there is a need to rethink and reconsider the ameba-bacterium relationship inside the intestine and extraintestinal tissues.

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ELECTROPHORETIC ISOLATION OF ENTAMOEBA HISTOLYTICA AND ENTAMOEBA DISPAR FROM STOOL SAMPLES IN SOUTHERN PART OF IRAN

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Microscopic analysis of the stool samples for identification and differentiation of *Entamoeba histolytica* and *E. dispar* can not be a reliable method. Electrophoretic separation of different isoenzymes of the two species is known to be as a golden standard method to identify the different zymodems of *E. histolytica* and dispar, 23 positive samples from southern part of Iran were collected and isoenzymes of four different enzymes (Malic enzyme, Phosphoglucomutase, Hexokinase and Glucose Phosphate Isomerase) were separated and identified using electrophoretic method. Out of 23 samples, 6 cases of *E. histolytica* and 17 cases of *E. dispar* were identified. Zymodems of *E. histolytica* were II and XIV and *E. dispar* were I, XVI, XVII and XVIII. Both zymodems of *E. histolytica* were isolated from the patients who were symptomatic.

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A NOVEL NESTED MULTIPLEX POLYMERASE CHAIN REACTION (PCR) ASSAY FOR DIFFERENTIAL DETECTION OF *ENTAMOEBA HISTOLYTICA*, *E. MOSHKOVSKII* AND *E. DISPAR* DNA IN STOOL SAMPLES

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Entamoeba histolytica, the pathogenic amoebae and the causative agent of amoebiasis, is indistinguishable in its cyst and trophozoite stages by microscopy from those of *E. moshkovskii*, considered to be primarily a free-living amoeba, and *E. dispar*, a noninvasive amoeba. This leads to confusion in the diagnosis of intestinal amoebiasis. Therefore, there is a need to a develop method for definite identification and differentiation of *E. histolytica* from *E. histolytica* like amoeba such as *E. moshkovskii* and *E. dispar* in stool samples. A novel nested multiplex polymerase chain reaction (PCR) targeting 16S rRNA gene for differential detection of all the three morphologically similar species *E. histolytica*, *E. moshkovskii* and *E. dispar* in stool samples has been developed and evaluated in our laboratory. In the present study a total of 122 stool samples, which included 97 stool samples positive for *E.histolytica* / *E.dispar* / *E.moshkovskii* complex trophozoites/cysts by microscopy and/or culture, and 25 stool samples negative for *E.histolytica* / *E.dispar* / *E.moshkovskii* complex trophozoites/cysts by both microscopy and culture were subjected to nested multiplex PCR study. The nested multiplex PCR evaluated in the study showed that the size of diagnostic fragments of PCR products was clearly different for all the three *Entamoeba* species, the species-specific product size for *E. histolytica*, *E. moshkovskii* and *E. dispar* was

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439,553 and 174bp, respectively. Of 97 stool specimens, only 91 stool specimens were positive for *E. histolytica*, *E. moshkovskii* and *E. dispar* by nested multiplex PCR, thus showing a sensitivity of 94%. All 25 stool samples negative by microscopy and culture for *E.histolytica* / *E.dispar* / *E.moshkovskii* complex trophozoites/cysts were also negative by nested multiplex PCR, thus showing a specificity of 100%. This study represents for the first time the application of nested multiplex PCR for species-specific detection and differentiation of all the three species *E. histolytica*, *E.dispar* and *E.moshkovskii* in stool DNA.

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CRYPTOSPORIDIUM INFECTION OF HUMAN INTESTINAL TISSUES CAUSES INCREASED EXPRESSION OF THE OSTEOPROTEGERIN (A TNF RECEPTOR FAMILY SECRETED DECOY RECEPTOR)

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Cryptosporidium is an intestinal parasite of the phylum apicomplexa. The humans are infected by *C. parvum* and *C. hominis*. In immunocompetent individuals the infection leads to a self-limiting diarrhea, while immunocopromised patients frequently develops chronic diarrhea. Due the intracellular localization of *Cryptosporidium*, epithelial cells appear to play a key role in activating and communicating whit the immune system. The goal of our group is try to understand the biochemical processes that are regulated within infected epithelial cells. In order to determinate which genes could be regulated during early infection we used explants dissected from human ileum tissue to carry out a micoarray study. The explants were cultured and infected ex vivo with *C. parvum* and *C. hominis* for 24 hours. RNA was extracted and analyzed using the Affmetrix GeneChip. Interestlying, statistical analysis showed that the Osteoprotegerin (OPG) was one of the up regulated genes increasing 1.58 folds in *C. parvum* and 2.54 folds in *C. hominis* comparing with uninfected explants. Osteoprotegerin has been recently identified as a member of the TNFR family that functions as soluble decoy receptor. It has two known TNF family ligands: TNF-related apoptosis-inducing ligand and receptor activator of NF-KB ligand. Although OPG is expressed in the intestine and immune cells, the possible physiological role of the up regulated OPG during cryptosporidium infection has not been investigated. The next step in this study was to confirm the microarray result by Real Time PCR. The result showed a 2.2 and 2.6 folds OPG increase for *C. parvum* and *C. hominis* respectively. In order to confirm the induction of the OPG at the protein level we undertook infection of HCT-8 cells with *C. parvum*. The uninfected and uninfected cells were incubated at 37°C during 48 hrs The supernatant was collected at 0 hours 1, 24 and 48 hrs. The OPG concentration was determined by ELISA and the result showed that the OPG was detected after 1 hr and in greater magnitude in the *C. parvum* infected HCT-8 cell culture than in the control. In conclusion our study shows that OPG is increased at mRNA and protein levels in response at cryptosporidium infection suggesting a possible role of the OPG as immunomodulator during the cryptosporidium infection.

(ACMCIP Abstract)

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REAL TIME PCR: A SENSITIVE METHOD FOR DETECTION OF *BABESIA MICROTI* IN BLOOD DONOR SAMPLES

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Human babesiosis, primarily caused by *Babesia microti,* occurs predominantly in the northeastern and upper Midwest USA, with a few cases recently reported in Europe and Japan. In 2004 alone, at least 10 cases of transfusion-transmitted *B. microti* were reported in the USA, making *B. microti* one of the agents most frequently transmitted by blood transfusion. An ongoing seroprevalence study performed in Connecticut identified 1.3% of the blood donor population positive for *B. microti* antibody by IFA (≥ 1:64 considered positive). As part of a related natural history study, 150 donors identified as seropositive by IFA and enrolled in a natural history study were also tested for parasitemia by nested PCR and only 16 (11%) were parasitemic. In an attempt to add sensitivity to the detection of the parasite, we compared nested PCR to real-time PCR (RT-PCR) using selected subjects. We develop a real-time PCR for detection of *B. microti* with primers and probe designed using the Applied Biosystems software Primer Express and 18S ribosomal RNA sequence as a template. We used this technique to re-test multiple sequential samples from 19 donors previously found positive by IFA and enrolled in the study between 2000 and 2004, for a total of 177 samples. Of 177 samples tested with both methods, the RT-PCR revealed 31 (18%) positives versus only 10 (6%) identified by nested PCR. The majority of the newly identified positive samples belonged to donors whose antibody titers fluctuated, but rarely were identified as positive by nested PCR. These data show that the RT-PCR is a more sensitive technique than nested PCR for detection of *B. microti*. Thus, the enhanced sensitivity observed with RT-PCR may allow for the identification of parasitemic donors, even in the absence of a positive IFA. At present, *B. microti* testing has not been implemented in routine blood screening, but donors identified as positive by IFA during these studies are deferred from donating blood for life. However, our data suggest that donors, who were initially deferred from future donation because of a positive IFA titer, may perhaps be re-entered upon a combined negative result by RT-PCR and IFA. In addition, RT-PCR it is less time consuming and the chances for contamination are limited. Lastly, the correlation between newly positive samples detected by RT-PCR and a recurring high antibody titer by IFA may explain the apparent ongoing infection in some donors.

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MIXED PROTOZOAN INFECTION IN CROSS BRED COWS

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Tick and tick born diseases are major problem in India badly affecting the economic condition of farmers and livestock owners. In Punjab, these diseases are more prevalent in summer and rainy season due to increased activity and preponderance of tick vector. This report is the description of two cross bred cows in a well managed dairy farm which suffered from mixed infection of clinical Theileriosis and anaplasmosis. The animals were having history of high fever and anorexia. On clinical observation, all developmental stages of Hyaloma anatolicum anatolicum were found on the body of the animal. Prescapular lymph was enlarged. Temperature was 105.5°F. On blood cell examination, leukocytosis, neutrophilia with corresponding lymphopenia was seen. Parasitology examination revealed presence of typical annular form along with rod and comas and large number of dots at the margin of erythrocytes indicating mix infection of Theileria annulate and Anaplasma marginale. Both the animals were treated with in Berenil -5g/im and oxytetracycline @ 30 mg/kg body weight intramuscularly for five days. One animal died in spite of treatment. On postmortem examination typical punched out ulcers were found in the abomasum of animal. 2nd animal were well responded to treatment. The owner was advised to manage the ticks with the acaricide like Butox @ 2 ml/litre of water to be applied after every 10 days on the body of the animals, floor and shed of the animals. He was also advised to go for regular examination of blood samples of his animals.

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PREVALENCE OF MICROSPORIDIA IN STOOL SAMPLES OF HOSPITAL PATIENTS AND SCHOOL CHILDREN IN THE VHEMBE DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

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Long considered as animal parasites, micosporidia have recently emerged as an important group of human pathogens especially in HIV positive and AIDS patients and other immunocompromised human beings. In South Africa very few studies have reported the prevalence of microsporidial infections and no study has been conducted in the Venda region. In this study we used a PCR assay utilizing a primer pair that amplifies a conserved region of the small-subunit rRNA of all four major microsporidian pathogens, *Encephalitozoon cuniculi*, *Encephalitozoon hellem*, *Enterocytozoon bieneusi*, and *Septata intestinalis* followed by restriction endonuclease digestion by *PstI* to determine the prevalence of microsporidia in stool samples collected from hospital patients and primary school children. The restriction digest indicated that only *E. bieneusi* was present in the stool samples and was found in 33 (12.9%) of 255 samples from the hospitals and in 3 (4.5%) of 67 samples from primary school children. *E. bieneusi* was found in 9 (20.5%) of the 44 HIV positive patients and in 24 (11.5%) of the 211 HIV negative individuals. To our knowledge, this is the first report of *E. bieneusi* in the Venda region of South Africa showing the increase importance of these parasites which seem to be a major cause of diarrhea in HIV infected individuals in the Vhembe district.

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MOLECULAR CLONING AND CHARACTERIZATION OF A MEMBER OF THE *FASCIOLA HEPATICA* FERRITIN-LIKE PROTEIN FAMILY EXPRESSED AT EARLY STAGE OF INFECTION

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Fasciola hepatica is the causative agent of liver fluke disease or fascioliasis. The disease, which primarily infects sheep and cattle, results in a global annual economic loss of approximately 3billion dollars. Recent reports indicate that fascioliasis is also an important emerging pathogen of humans; with approximately 2-5 million people infected worldwide. The TBZ is the most effective drug for controlling *Fasciola*; however, resistance to drug has been reported in sheep infected with *F. hepatica*, suggesting that selection of resistance parasites may eventually compromise the use of this drug. Vaccines represent the most attractive long-term alternative to invert this scenario. We are dedicated to search novel *Fasciola* genes Ags with protective potential. For this a cDNA library from adult worms was constructed. A rabbit antibody against *Fasciola* ES antigens was used for screening of the cDNA library. A cDNA encoding a 18kDa polypeptide was identified. Analysis of its primary structure suggests that this novel antigen is potentially a *Fasciola* / *Paragonimus* cross-reactive antigen that could be involved in iron metabolism. Because during its migration through liver parenchyma *F. hepatica* feed of blood, this molecule may be essential for parasite's metabolism and its survival into the mammalian host. A vaccine directed to block Fe-transporter protein could kill the parasite before establishment in the bile ducts. Therefore, it could constitute an important target for developing of a vaccine against *F. hepatica*. In the present study we reported the molecular cloning and partial characterization of this novel *Fasciola* antigen.

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MAPPING OF B-CELL EPITOPES ON A NOVEL 11.5KDA *FASCIOLA HEPATICA/SCHISTOSOMA MANSONI* CROSS-REACTIVE ANTIGEN BELONGING TO A MEMBER OF THE *F. HEPATICA* SAPOSIN-LIKE PROTEIN FAMILY

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The 11.5-kDa *Fasciola hepatica* protein termed FhSAP-2 is highly immunogenic. By structural analogies with several related proteins, FhSAP-2 was classified as a novel member of the *F. hepatica* saposin-like / NK-lysin protein family. FhSAP-2 induces in rabbits significant levels of protection to a live challenge infection with *F. hepatica* metacercariae. It is also a *Fasciola* / *Schistosoma* cross-reactive antigen. The amino acid sequence of FhSAP-2 as deduced from the sequence of its encoding cDNA was used to synthesize a complete set of 18 overlapping peptides. This was used in an indirect ELISA and a peptide-inhibition ELISA to identify continuous epitopes recognized by a number of antisera against FhSAP-2 or sera from rabbits infected with *F. hepatica* containing anti-FhSAP-2 antibodies. This comprehensive epitope-scanning study revealed the presence of three continuous antigenic regions within the protein sequence that were not sensitive to DTT reduction. The first antigenic region contains the amino acid residues ^{21}SKQPTIDIDLCDICT^{35}. The second antigenic region was identified within the central region of the protein spanning the residues ^{46}ADQTVEEHIG^{55} and the last antigenic region was considered to be in the C-termini region spanning the residues ^{66}RSQDACIEFVQQEVDYIIDH^{85}. Two dominant B-cell epitopes were identified within these antigenic sites, one of them covering the residues ^{21}SKQPTIDIDL^{30} and another one covering the residues ^{76}QQEVDYIIDH^{85}. These dominant B-cell epitopes were defined as *Fasciola* / *Schistosoma* cross-reactive epitopes. Identification of the antibody responses to FhSAP-2 could provide the basis for the development of novel immunity-based prophylactic and diagnostic techniques for the management of fascioliasis.

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IDENTIFICATION OF CD4$^+$ T-CELL EPITOPES FROM *FASCIOLA HEPATICA* 11.5KDA SAPOSIN-LIKE PROTEIN, A VACCINE CANDIDATE

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The protein termed FhSAP-2 is a novel *Fasciola hepatica* antigen expressed at early stage of infection. It exhibits a potent lytic activity against human erythrocytes and PBMC. A previous vaccination study demonstrated that FhSAP-2 induces in rabbits significant levels of protection to a live challenge infection with *F. hepatica* metacercariae. A structural analysis of FhSAP-2 has shown that it contains 6 conserved cysteine residues arranged within 5 amphipathic α-helical domains and 7 hydrophobic residues in strictly conserved positions that might form T-cell epitopes. Consistent with this prediction, our previous immunization study in mice has shown that when FhSAP-2 is injected by subcutaneous route induces high antibody response characterized by high levels of IgG_1, IgG_{2a} and IgG_{2b}. These results suggested that FhSAP-2 possesses functional epitopes inducing both Th1 and Th2 response. In the present study, we mapped the CD4$^+$ T-cell epitopes using a panel of 18-overlapping peptides that encompassed the full-length 303 amino acid of FhSAP-2. The capacity of peptides of inducing Th1 or Th2 response *in vitro* was measured using lymphocyte proliferation, cytokine detection experiments, flow cytometry and IgE or IgG isotyping antibody detection in peptide-sensitized BALB/c mice. The results showed the presence of five continuous T-cell epitopes spanning amino acid residues ^{16}SFDVPSKQPT^{25}, ^{26}IDIDLCDICTNTMDV^{40}, ^{41}IKKMLADQTVEEHIG^{55}, ^{71}CIEFVQQEVD^{80}, ^{81}YIIDHVDQHN^{90}. Peptides

containing amino acid residues 16-25; 41-55 and 81-90 drove the highest levels of IL-4 as well as of IgE and IgG_1 antibodies. Peptides containing amino acid residues 26-40 and 71-80 drove the highest levels of IFNγ and IL-2 as well as the highest levels of IgG_{2a} and IgG_{2b} antibodies. Therefore, it was considered that residues 26-40 and 71-80 are functional Th-1 type T-cell epitope. Th1 epitopes will be useful for the development of effective vaccines which can trigger acquired immunity against *F. hepatica*.

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GENE EXPRESSION PROFILE OF GAMMA-RAY IRRADIATED *CLONORCHIS SINENSIS* METACERCARIAE

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The therapeutic irradiations produce adverse effects destroying normal cells. The DNA repair proteins, such as p53, p21, hRAD50, reached at peak activity 12 hours after gamma-irradiation. The hRAD50 has repair activity on the damaged DNAs. *Clonorchis sinensis* belongs to the lowest animals of the bilateralia in the evolution tree. LD_{50} of *C. sinensis* metacercariae to gamma-irradiation was 16.5 Gy, and 30 Gy did not provoke chromosome aberration to the metacercariae. It was expected that molecular biological analyses on gamma-ray-induced genes of *C. sinensis* metacercariae may provide genetic information identifiable DNA repair proteins. Total RNA was extracted from the gamma-ray irradiated *C. sinensis* metacercaria and reverse transcribed into cDNA. cDNAs were amplified by GeneFishing™ Differentially Expressed Genes (DEG) method using 120 pairs of ACP-primers. The amplicons were electrophorezed in agarose gel and 21 DEGs were selected, which were overexpressed more than 2 times compared to the control group. The DEG cDNA fragments were subcloned by TA-cloning method and sequenced. With the DEG sequences, contigs were searched in the *C. sinensis* EST pool and aligned with the DEG, then extended by DNA-walking. The DEGs extended were 624 - 1,500 bp long and 15 ones were annotated. Functionally 5 DEGs were categorized to energy metabolism group, 5 DEGs to protein processing group, and 3 DEGs to DNA repair group. The DNA repair group comprised of *p58^{IPK}, DNA repair, apoptosis inhibitor* genes. It is suggested that the DNA repair-related genes are overexpressed in response to gamma-irradiation and increase the repair activity toward damaged DNA and enhance cellular functions for survival of the *C. sinensis* metacercariae. Genetic information on the DNA repair of *C. sinensis* can be employed as clues for cloning homologues of the higher animals.

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CIRCULATING CALCIUM-BINDING PROTEINS (MRP-8 AND MRP-14) IN MURINE SCHISTOSOMIASIS

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The pathogenesis in schistosomiasis is due largely to the circumoval granulomatous response. Two calcium-binding proteins (MRP-8 and MRP-14), produced by myeloid cells including monocytes and macrophages, have been implicated in a wide range of inflammatory responses. A number of stimuli (LPS, IFN-γ, TNF-α) are known to induce expression of MRP-8/MRP-14 homodimers and heterodimers. These proteins have been found in hepatic circumoval granulomata in murine schistosomiasis. We measured MRP-8 and MRP-14 homodimers in the sera of CD-1 female mice infected with *Schistosoma mansoni* for 10 weeks post-infection. MRP-8 levels were elevated early in infection (2nd week), coinciding with larval migration, returned to those of uninfected mice during weeks 3 to 7 post-infection; increased again during the 8th week and remained elevated during the next two weeks ($p = 0.007$; ANOVA). MRP-14 levels were substantially increased during the 4th week post-infection ($p = 0.004$; t-test) and remained slightly elevated in infected mice during subsequent weeks of the study ($p = 0.216$; ANOVA). These proteins may have a role in recruitment and differentiation of macrophages and thus formation of circumoval granulomata in murine schistosomiasis.

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PREVALENCE AND INTENSITY OF URINARY TRACT SCHISTOSOMIASIS AND ASSOCIATED BACTERIAL INFECTIONS IN EBONYI LGA OF EBONYI STATE NIGERIA

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Urinary schistosomiasis endemicity in ten rural villages of Ebonyi State was studied and epidemiological and bacteriological data were recorded. Out of the 1284 persons randomly examined, 276 persons (21.5%) were infected with ***Schistosoma haematobium***. Distribution of infections varied significantly among the various villages in the LGA. Prevalence and intensity of infection between different age groups

varied significantly ($P<0.05$).Haematuria increased with intensity of infection. Urinary tract infections were higher in individuals positive for Urinary schistosomiasis than those negative for it. Bacterial infections were more frequent in individuals with haematuria. Bacteria species isolated from urinary schistosomiasis patients include: *Salmonella typhi, Staphylococcus aurues, Streptococcus* spp., *Klebsiella* spp., *Escherichia coli* and *Proteus* spp.

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MOLECULAR DETECTION AND SEQUENCE ANALYSIS OF A NEW HEPATITIS E VIRUS ISOLATE FROM PAKISTAN

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Sporadic and epidemic acute viral hepatitis E in many developing countries were caused by hepatitis E virus (HEV). The HEV genome has been classified into three major genotypes. However, extensive diversity has been noted among HEV isolates from patients with acute hepatitis in China and Taiwan. Some reports indicated that multiple genotypes of HEV isolates could cocirculate in the same area; even distinct genotypes of HEV could exist in the same patient. Pakistan is a highly endemic area for the hepatitis E. So far only two Pakistan HEV isolates Sar-55 (87-Pakistan-A) and Abb-2B (88-Pakistan-2B) have been characterized and the nucleotide sequences identified in these two HEV isolates show only 90 % similarity. In this study, we report the third HEV isolate from Pakistan (87-Pakistan-B). The sequences of 438 bp in the ORF-2 region and 259 bp in the ORF-1-3 region of this new HEV isolate have been obtained. The sequence analysis shows that this new HEV isolate very close to the Sar-55 HEV isolate but different from the Abb-2B HEV isolate. These results indicated that the Sar-55 (87-Pakistan-A) genotype is the main endemic HEV strain in Sargodha area. These data will be useful for HEV epidemiological studies, diagnosis and vaccine development.

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CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO HEPATITIS E VIRUS (HEV) CAPSID PROTEIN AND IDENTIFICATION OF THEIR BINDING AND NEUTRALIZATION ACTIVITIES

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Twenty-seven monoclonal antibodies (Mabs) recognizing the open reading frame 2 (ORF-2) structural protein of the Pakistan strain of

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hepatitis E virus (HEV) were generated by conventional hybridoma technique. Twenty-five were identified as IgG 1 isotype and two were IgG 2b isotype. The concentration of each Mabs was determined using enzyme-linked immunosorbent assay (ELISA) with plates coated with known concentration of goat anti-mouse Ig G1 and IgG 2b. These Mabs were characterized by ELISA, antigen-captured reverse transcriptase-ploymerase chain reaction (AC/RT-PCR), immune electron microscopy (IEM), and *in vitro* neutralization assay. Twenty-seven Mabs were positive (2 weakly positive) by ELISA, suggesting these Mabs recognized the liner epitopes of HEV protein. In AC/RT-PCR, 24 Mabs were positive. Twenty-four Mabs bound to Pakistan, Mexican and Namibia HEV strains in AC/RT-PCR, suggesting these Mabs can recognize the conformational epitopes of HEV. The highest binding ability of Mabs to HEV in AC/RT-PCR was 1: 1,600 dilutions (Mab 7). Thirteen Mabs have been examined by IEM. Nine of them, which were positive by ELISA and AC/RT-PCR, bound and aggregated to Mexican HEV strain. We tested five Mabs that were positive by ELISA, AC/RT/PCR, and IEM by neutralization assay. Only one Mab (Mab 7) showed neutralization activity that inhibited the ability of HEV to attach to Alexander hepatoma cells (PLC-PRF-5). When Mab 7 was diluted to 1: 160, its neutralization activity persisted indicating that Mab 7 might be a potential candidate for further evaluation (in passive protection experiment) in primates. This neutralizing Mab may be useful in immunological experiments and human immunotheropy after modification (humanization)

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RAPID RESPONSE TO A CASE OF MUMPS PREVENTS AN OUTBREAK AT A RESEARCH FACILITY

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Mumps is a disease caused by a paramyxovirus that is transmitted via the respiratory route. In Sep 03, an active case of mumps was discovered in a senior laboratory technician at the Naval Medical Research Center Detachment (Naval Medical Research CenterD) in Lima, Peru. Because mumps was not part of the routine vaccination series in Peru at the time, there was risk of transmission among the staff and potentially the non-human primate (NHP) colony at Naval Medical Research CenterD. An investigation was conducted, interviewing 106 subjects present at the research facility. Each was asked about previous history of mumps, history of vaccination or known immune status and close contact with the active case. Blood specimens for IgG serology were drawn from subjects who had close contact with the index case during the transmission period and had no history of clinical mumps or vaccination. The index case was confirmed clinically by the occupational health physician at Naval Medical Research CenterD. She had right-sided parotitis and acquired the disease from her son, who had been diagnosed with mumps 14 days earlier. She was restricted from work for ten days. It was determined that 81/106 (76%) of the staff had close contact with the index case, mostly at a common breakfast (62/81, 77%). Only 6/81 had MMR (4 American and 2 local staff) and 33 Peruvian staff reported having had mumps. Therefore, 47/81 (58%) of exposed subjects appeared to be susceptible, including two pregnant women. 19% of the potentially susceptible individuals (9/47) did not have immunity to mumps (IgG > 20.0), none were pregnant. All the susceptible, exposed individuals received MMR vaccine. Access to the NHP colony was restricted to those with pre-existing immunity to mumps. There were no secondary cases of mumps. In conclusion, in developing countries where MMR immunization is not required, mumps has the potential to cause severe disease among susceptible adults because of large pools of immunologically naïve individuals. This may not to be sufficient for preventing outbreaks through herd immunity. Immediate and thorough investigation and occupational health response was imperative in preventing secondary cases of mumps among humans and NHP.

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PROTECTION AGAINST HEPATITIS A AND B WITH A COMBINATION VACCINE ADMINISTERED USING AN ACCELERATED ADMINISTRATION SCHEDULE

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The availability of an effective and well-tolerated combined hepatitis A and B vaccine, administered using an accelerated schedule, will provide benefit for travellers who need rapid protection. The objective of this study was to investigate the immune response to the combined hepatitis A and B vaccine, Twinrix® (≥720 EL.U inactivated hepatitis A antigen and 20 µg recombinant hepatitis B surface antigen per mL) with concurrent administration of Havrix® (1440EL.U/mL inactivated hepatitis A antigen) and Engerix-B® (20 µg/mL recombinant HBsAg) using an accelerated schedule. A prospective, open-label, randomized, comparative study of Twinrix® administered at 0, 7, 21-30, days and 12 months and concurrent administration of Havrix®, at 0 and 12 months, and Engerix® at 0, 1, 2, and 12 months, in seronegative healthy adults. Immune responses were similar between the two groups. The anti-hepatitis B seroprotection rate for the combined vaccine was 96.4% (95% CI: 92.7, 98.5) compared to 93.4% (95% CI: 89.0, 96.4) for the monovalent vaccines, one month after completion of the schedules. The anti-hepatitis A seroconversion rate was 100% in both groups. At Day 37, seroconversion rates for anti-hepatitis A were similar in both groups (98.5% and 98.6% for the combined and monovalent vaccine group, respectively), but anti-hepatitis B seroprotection rates were significantly (P<0.001) higher with the combined vaccine (63.2% versus 43.5%). These results are similar to those of an European study, which reported pre-booster seropositivity levels for anti-hepatitis A of 96.2% and 95.0% and for anti-hepatitis B of 94.0% and 91.6% for the combined hepatitis A and B vaccine and monovalent vaccines, respectively. All vaccines studied were well tolerated. In conclusion, taken together, these findings indicate that an accelerated schedule for the combined hepatitis A and B vaccine provides comparable immunogenicity and tolerability to equivalent monovalent vaccines, offering a valuable option to travellers who need rapid protection.

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DEVELOPMENT AND CHARACTERIZATION OF RECOMBINANT ARENAVIRUS PROTEINS AND VIRUS-SPECIFIC MONOCLONAL ANTIBODIES FOR USE IN DIAGNOSTIC AND THERAPEUTIC APPLICATIONS: AN INTEGRATED APPROACH TO PUBLIC HEALTH AND BIODEFENSE

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Lassa virus (LASV), Junin virus (JUNV) and several other members of the *Arenaviridae* are capable of inducing severe hemorrhagic fever syndromes and are classified as Biosafety Level 4 (BSL-4) agents. LASV, the etiologic agent of Lassa fever, is endemic to countries of the Mano River Union (MRU) (Guinea, Sierra Leone and Liberia), where it is estimated that as many as 300,000 cases of Lassa occur per year, resulting in up to 5,000 deaths annually. To date, there are no commercially available Lassa fever diagnostic assays, and none are available for reference laboratories in endemic regions. Toward this end, we are engineering enzyme-linked immunosorbant assay (ELISA)-based recombinant antigen diagnostic methods for LASV and other arenavirus infections. Our prior results

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suggest that antigen-capture and antibody-detection ELISAs serve as the most reliable, sensitive, and specific serologic tests for diagnosing acute LASV infection. However, several opportunities exist for improving these assays. Currently available LASV antigen-capture ELISAs employ a limited set of murine monoclonal antibodies (MAbs) specific for a single arenavirus protein. The inclusion of additional viral protein targets could improve assay sensitivity. As a result, we are developing a panel of arenaviral MAbs, which will be produced in high concentrations using novel *in vitro* methodologies. Prior studies also suggest that recombinant arenavirus proteins are suitable for antibody-capture ELISAs, thus eliminating the need for BSL-4 produced material. Therefore, we expressed the LASV glycoproteins and nucleoprotein in bacterial and mammalian cells. Using these recombinant proteins and MAbs, we are developing antigen- and antibody (IgM, IgG) - detection ELISAs. As several New World arenaviruses, such as Junín and Machupo viruses, also pose public health and bioterrorism threats, we will formulate each of these ELISAs for use as multiagent detection assays. Field-testing of the new assays will be performed in West Africa and Argentina. Additionally, the protective efficacy of MAbs generated by this effort will be evaluated by passive transfer studies in various animal models. Development of virus-specific MAbs and rapid immunodiagnostic assays can improve treatment of arenaviral diseases, facilitate studies to understand their prevalence and natural history, and lead to vaccines for preventing these major causes of morbidity and mortality.

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RISK FACTORS FOR MONKEYPOX ILLNESS DURING AN OUTBREAK IN THE UNITED STATES, 2003

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In 2003, an outbreak of human monkeypox causing a febrile rash illness occurred in the United States that was precipitated by the importation of monkeypox virus-infected animals from Ghana. Infected African rodents were temporarily housed in close proximity to prairie dogs prior to distribution, and virus transmission to the latter occurred at a facility. Numerous infected prairie dogs were sold and purchased as pets, and contact with these animals resulted in 47 confirmed and probable cases of human monkeypox. A case-control study was conducted to evaluate potential risk factors for monkeypox transmission from animals to humans. Participants completed a questionnaire requesting exposure (i.e. type of contact with prairie dog), clinical, and demographic information. Sera were obtained for anti-orthopox IgG and IgM analysis. For this study, cases classified as confirmed or probable based on clinical, epidemiologic and laboratory criteria were combined [n=30] while controls were persons with exposure to infected prairie dogs, but exhibited no symptoms or serologic evidence of infection (n=28). Odds ratios with 95% confidence intervals [CIs], and odds ratios adjusted for smallpox vaccination status, were calculated to examine potential risk factors of disease. Individuals not vaccinated against smallpox were five times more likely to develop disease [95% CI=1.4, 10] than those who were vaccinated against smallpox. When adjusted for smallpox vaccination status, diseased cases were more likely than controls to have had daily contact with an ill animal, cleaned cages and bedding, or tactile contact with the animal (OR=4.0 [95% CI=1.2-13.4], OR=5.3 [95% CI=1.4-20.7], OR=4.0 [95% CI=1.2-13.4], respectively). This study indicates how monkeypox infection might occur and implicates both direct and indirect mechanisms for virus transmission.

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SMALLPOX RESEQUENCING GENECHIP HYBRIDIZATION CAN DETECT HUMAN COWPOX VIRUS

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We developed a set of seven resequencing GeneChips for rapid identification of any potentially unique or genetically engineered strains of smallpox (*Variola*) virus, based on multiple alignments of complete genomic sequences of 24 major and minor strains of this human-pathogenic virus. These chips cover almost 99% of the genome comprising 463 instruction sets (5 to ~29000 bases) designed to detect maximum amount of sequence variations across smallpox genomes. The chips were fabricated using standard photolithography and solid phase DNA synthesis by Affymetrix, Santa Clara, California. At the outset study, we successfully resequenced 14 smallpox strains, and also noticed that chip-based resequencing-by-hybridization was fast and reproducible with a high call rate (>94%) in the 14 genomes characterized. Later, we hybridized an isolate of monkeypox (MPXV-RCG-2003) with these chips. A lower call rate (ranging from 28% to 73% across the seven chips with an average of approximately 63%) was observed, and blast searches and multiple alignments of chip-based resultant nucleotide sequences revealed homology most significantly to monkeypox. In this study, attempts were made to hybridize a human cowpox isolate (CPXV-GER91, GenBank accession number DQ437593) with these chips. As expected, a further reduction in call rate (24% to 71%, average nearly 50%) was obvious. However, our preliminary data analysis of chip-based sequences in turn suggests them to be cowpox virus. Thus the resequencing GeneChips designed to characterize smallpox genomes can also serve to detect close relatives of smallpox and could be used as a rapid screening tool in the future.

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ECOLOGICAL CORRELATES OF BUGGY CREEK VIRUS INFECTION IN CIMICID SWALLOW BUGS *OECIACUS VICARIUS*, SOUTHWESTERN NEBRASKA, 2004

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Buggy Creek virus (BCRV) is an alphavirus within the western equine encephalitis complex that is primarily vectored by the swallow bug (Hemiptera:Cimicidae: *Oeciacus vicarius*), an ectoparasite of the colonially nesting cliff swallow (*Petrochelidon pyrrhonota*) that is also a frequent host for the virus. We investigated ecological correlates of BCRV infection in 100-bug pools at 14 different swallow colony sites in southwestern Nebraska from summer 2004, using plaque assay on Vero cells to identify cytopathic virus and reverse transcription polymerase chain reaction (RT-PCR) to identify non-cytopathic viral RNA. We found 26.7% of swallow bug pools positive for BCRV, with 15.6% showing cytopathic ("infectious") virus and 11.0% non-cytopathic ("noninfectious") viral RNA. The prevalence of cytopathic BCRV increased with cliff swallow colony size in the current year; the percentage of non-cytopathic samples at a site did not vary with colony size in the current year but increased with the previous year's colony size at a site. Active colony sites (those used by swallows) had higher percentages of cytopathic BCRV in bug pools than at inactive colony sites, but the reverse held for noncytopathic viral RNA. Nests that were occupied by birds at some time in the season had more pools with cytopathic BCRV than did inactive nests. Colonies used by birds for the first or second time had less virus in bugs than did sites that had had a longer history of bird use. The percentage of pools with BCRV was affected by whether bugs were clustering at nest entrances or distributed elsewhere on a nest. The prevalence of cytopathic

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samples decreased at inactive colony sites and increased at active sites over the course of the summer, while the reverse pattern held for non-cytopathic samples. Non-cytopathic bug pools seem to reflect infection patterns from a previous year. The results suggest that the birds play an important role in amplification of the virus, and that the spatial foci of BCRV epidemics can be predicted based on characteristics of cliff swallow colonies and the cimicid bugs that are associated with them.

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MULTIPLEX REAL-TIME RT-PCR SHOWS A WEAK RELATIONSHIP BETWEEN PLAQUE GROWTH AND VIRUS CONCENTRATION FOR BUGGY CREEK VIRUS

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A survey of Buggy Creek virus (BCRV) in pools of cimicid swallow bugs (*Oeciacus vicarius*) by conventional reverse transcription-polymerase chain reaction (RT-PCR) found that only some of the positive samples caused cytopathic effects in Vero cells. We therefore developed a new one-step multiplex QRT-PCR assay to study the relationship between viral RNA concentration and extent of plaque formation on Vero cells. Surprisingly, plaque formation was poorly correlated with viral RNA concentration as measured by QRT-PCR. Although non-plaque forming samples tended to have lower viral RNA concentrations, there was extensive variation, with some non-plaque forming pools having high RNA concentrations, while others that formed large numbers of plaques had relatively low RNA concentrations. Thus, screening samples solely by plaque assay would not have detected some samples that contained high levels of BCRV RNA. Why some samples with relatively high concentrations of viral RNA did not form plaques is unclear, but may be related to environmental or metabolic changes affecting the swallow bugs over the previous winter. Our multiplex QRT-PCR assay proved advantageous in that it controlled for sample-to-sample variation, fluctuation in amplification efficiency, and variation in results across days, and allowed inferences to be made about relative viral RNA concentration without use of standard curves.

(ACMCIP Abstract)

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PHYLOGENETIC ANALYSIS OF BUGGY CREEK VIRUS: EVIDENCE FOR MULTIPLE CLADES IN THE WESTERN GREAT PLAINS, U.S.A.

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We present the first detailed phylogenetic analysis of Buggy Creek virus (BCRV), a poorly known alphavirus with transmission cycles involving a cimicid swallow bug (*Oeciacus vicarius*) vector and cliff swallows (*Petrochelidon pyrrhonota*) and house sparrows (*Passer domesticus*) as the principal avian hosts. Nucleotide sequences of a 2075-bp viral envelope glycoprotein-coding region, covering the entire PE2 gene, were determined for 33 BCRV isolates taken from swallow bugs at cliff swallow colonies in Nebraska and Colorado in summer 2001, and compared with the corresponding region of BCRV isolates collected from Oklahoma in the 1980's. We also analyzed isolates of the closely related Fort Morgan virus (FMV) collected from Colorado in the 1970's. Phylogenetic analysis indicated that BCRV falls into the western equine encephalomyelitis-complex of alphaviruses, in agreement with antigenic results and an earlier alphavirus phylogeny based on the E1 coding region. We found four distinct BCRV/FMV clades, one each unique to Nebraska, Colorado, and Oklahoma, and one containing isolates from both Nebraska and Colorado. BCRV isolates within the two clades from Nebraska showed 5.7-6.2% nucleotide divergence and 0.7-1.9% amino acid divergence, and within these clades we found multiple sub-clades. Nebraska sub-clades tended to be confined to one or a few cliff swallow colonies that were close to each other in space, although in some cases near-identical isolates were detected at sites up to 123 km apart. Viral gene flow occurs when cliff swallows move (bugs) between colony sites, and the genetic structure of BCRV may reflect the limited dispersal abilities of its insect vector.

(ACMCIP Abstract)

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A CLINICAL STUDY TO ASSESS THE SAFETY AND IMMUNOGENICITY OF ATTENUATED MEASLES VACCINE ADMINISTERED INTRANASALLY TO HEALTHY ADULTS

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Despite the availability of a safe and effective vaccine for over four decades, measles remains one of the most common infectious disease killers of children in the world. Mucosal administration of currently licensed measles vaccine has been proposed to address issues of needle safety and vaccine uptake. Healthy adult volunteers were randomized to receive live-attenuated monovalent measles virus vaccine (Moraten Berna) via the standard subcutaneous (SQ) or the experimental intranasal (IN) route in a masked fashion. Safety, reactogenicity, immunogenicity, and shedding were assessed. Safety, reactogenicity, and viral shedding were not significantly different in the two study groups. Immunogenicity was markedly lower in the group of volunteers that received vaccine via the IN route. Plaque reduction neutralization (PRN) geometric mean titers (GMT) were 125 (95% confidence interval [CI] 68-228) milli International Units per milliliter (mIU/mL) on day 28 in recipients of IN vaccine versus 645 (95% CI 468-889) mIU/mL in recipients of vaccine SQ; $p<0.001$ by Mann-Whitney Rank Sum. 50 % of measles non-immune individuals mounted titers above the protective threshold of PRN 200 mIU/mL after IN administration versus 100% of volunteers who received the vaccine SQ. In conclusion, intranasal administration of live-attenuated measles vaccine was safe and well tolerated, but failed to mount significant immune responses when compared to subcutaneous administration. It is possible that higher doses or smaller particle size are necessary for successful mucosal measles vaccination and boosting.

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ULTRASTRUCTURAL PATHOLOGY OF THE LUNGS OF SYRIAN HAMSTERS INFECTED WITH ANDES VIRUS

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Andes virus, a cause of hantavirus pulmonary syndrome (HPS) in South America, can infect Syrian hamsters. The animals develop a progressive illness and die within two weeks. In an attempt to understand the pathogenesis of this disease, we inoculated hamsters intramuscularly with 2,000 plaque-forming units of Andes virus (250 LD_{50}) and performed necropsies every 2 days for 2 weeks after inoculation. Tissues were examined for pathological changes by light and electron microscopy (EM). As expected, the most striking pathology was seen in the lungs.

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The first signs of focal pulmonary edema were noticed on day 6 and only by EM examination. By day 8, alveolar edema became diffuse and was accompanied by a fibrin precipitation, type-2 pneumocyte degranulation and accumulation of extracellular surfactant in alveoli. Type-1 alveolar epithelial cell edema was also prominent, and endothelial cells in capillaries developed numerous vesicles and intracytoplasmic granular and filamentous inclusions that reacted with specific anti-hantavirus antibodies. Occasionally, there was focal damage to the capillary walls that led to the escape of circulating red blood cells into alveoli. Apoptotic lymphocytes and polymorphonuclear leukocytes were noted as early as day 4 post inoculation. At day 6 and later, many circulating lymphoblasts were seen in alveolar capillaries, suggesting that cytokines released from activated lymphoblasts might also contribute to the epithelial cell edema and increase the permeability of endothelial cells leading to pulmonary edema. This is the first temporal evaluation of ultrastructural pathology associated with HPS.

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PREVALENCE OF IGG AGAINST SELECTED ARBOVIRUSES AMONG PATIENTS ADMITTED WITH FEBRILE ILLNESSES AT THREE HOSPITALS IN KENYA

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Beginning in November 2002, researchers from the Kenya Medical Research Institute (KEMRI) and the US Army Medical Research Unit - Kenya (USAMRU-K) performed surveillance for arboviral infection among patients admitted with acute febrile illnesses in three district hospitals in Kenya. Serum was sent to KEMRI's arbovirus laboratory and tested for evidence of acute arboviral infection and later tested for presence of IgG against chikungunya, dengue, Rift Valley fever, West Nile virus, and yellow fever viruses. IgG ELISA was performed on 820 patients: 147 from Alupe (Ugandan border), 215 from Isiolo (semi-arid) and 458 from Malindi (coastal). IgG against chikungunya was detected in 58 (7.06%) of patients, dengue in 57 (6.93%), Rift Valley fever in none, West Nile virus in 18 (2.19%), and yellow fever in 10 (1.22%). Not surprisingly, there was considerable variation among the hospitals, with little anti-arboviral IgG being detected in semi-arid Isiolo, while Alupe and Malindi demonstrated similar proportions of IgG positive patients for all tested arboviruses except dengue, which was found almost exclusively in Malindi. In summary, more than 5% of patients tested positive for anti-chikungunya and anti-dengue IgG, while few or no patients tested positive for anti-Rift Valley fever, anti-West Nile virus, or anti-yellow fever IgG. Significant regional differences in antibody rates are noted, as expected given the vastly different ecologies of the districts under study.

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EFFICACY OF DNA EXTRACTION AND REAL TIME PCR FOR DETECTION OF PLASMID *IPAH* OF *SHIGELLA SPP.* IN UNIDENTIFIED LYOPHILIZED STOOL SAMPLES

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Since 2004 the Uzbekistan Scientific Research Institute of Epidemiology, Microbiology and Infectious Diseases (STCU-SRIEMID) project has focused on identifying the causative agents of diarrheal disease in five regions of Uzbekistan. 2500 stool samples collected in remote regions were frozen and transported to SRIEMID. One-hundred-ninety lyophilized stool samples were identified in our reference laboratory, Armed Forces Research Institute of the Medical Sciences Enteric Diseases Department (Bangkok, Thailand), by standard culture methods and real time TaqMan PCR for the presence of *ipaH* gene specific to *Shigella spp.* The comparison of results between conventional bacteriologic methods and PCR revealed a 6.5 fold greater frequency of identifying *Shigella species* by PCR as opposed to culture, 24.2% versus 3.5%, respectively. Thus, freeze-dried stool aliquots are a suitable sample for PCR; DNA is preserved intact. In some cases poor isolation of *Shigella spp.* may be explained by antibiotic administration prior to sampling or improper samples' transportation temperature or media pH. However, molecular methods, like PCR may overcome such obstacles.

(ACMCIP Abstract)

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HIGH RATES OF CARRIAGE OF DRUG-RESISTANT *ENTEROBACTERIACEAE* IN HEALTHY VOLUNTEERS IN HO CHI MINH CITY

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Vietnam has one of highest rates of antimicrobial drug resistance across a broad range of pathogens. We studied the carriage of multidrug-resistant (MDR) Gram-negative microorganisms in healthy volunteers in Ho Chi Minh City. In addition, we studied the prevalence of genes encoding transferable quinolone resistance in these isolates and we characterized a subset of isolates using RAPD. Stool samples were collected from 27 volunteers on day 0, 2, 7, 10. Volunteers were healthy, aged 19-30, and did not use antimicrobial agents in the previous 2 weeks. Samples were inoculated on MacConkey agar with gentamicin (8 µg/ml, MCG) or ceftazidime (2 µg/ml, MCC). A single colony of each morphology present, was identified and tested by disk diffusion (CLSI breakpoints). *Qnr* genes were detected by PCR. *E. coli* from 7 volunteers isolated on day 0, and from 1 volunteer on days 0, 2,7,10, were typed by RAPD. All 27 volunteers carried gentamicin and ceftazidime resistant *E. coli* (EC) or *K. pneumoniae* (KP) in at least 1 stool sample. Of 158 resistant isolates (66 % from MCG, 34 % from MCC), 71 % were EC, 15 % KP and 14 % other. 8/20 (40%) EC and 2/8 (25 %) KP isolated on day 0, were MDR (≥ 3 agents). 9/20 (45%) EC and 4/8 (50%) KP were ESBL+. 18/20 (90%) of EC were ampicillin resistant (R); 17/20 (85%) EC and 5/8 (63%) KP were cotrimoxazole R, 9/20 (45%) EC and 2/8 (25%) KP were nalidixic acid R, 12/20 (60%) EC and 5/8 (63%) KP were gentamicin R. *Qnr*S was detected in 4/20 (20%) of EC and 3/8 (37%) of KP.13 EC isolated from 7 volunteers on day 0 were unique by RAPD. 9 EC isolated from 1 volunteer on days 0, 2, 7, 10 showed 2 different clones carried on all days. In Vietnamese healthy adults there is an extremely high prevalence of EC and KP resistant to most commonly used antibiotics. RAPD suggests carriage of individual strains that have acquired resistance rather than clonal spread. Transfer of resistance genes between commensal and pathogenic bacteria may facilitate further development of drug resistance among pathogenic bacteria.

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IS NALIDIXIC ACID-RESISTANCE LINKED TO CLINICAL VIRULENCE IN *SALMONELLA ENTERICA* SEROTYPE *TYPHI* INFECTIONS?

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Decreased susceptibility of *Salmonella enterica* serotype *Typhi* to fluoroquinolones is emerging as an important problem in south Asian

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countries. Earlier, we reported the association of nalidixic acid-resistant *S. typhi* (NARST) infection with increased duration of illness at presentation and clinical severity among patients with typhoid fever. The aim of the present work was to study the influence of infection with drug-resistant *S. typhi* on fever clearance time. The study group comprised of 60 patients with blood culture-confirmed typhoid fever, treated at a teaching hospital in northern India and followed-up prospectively. Fifty-one patients in whom the fever clearance time was assessable are considered in the analysis. Mean age of patients was 16 ± 9 years. Forty-one (80%) and 18 (35%) patients had NARST or multidrug-resistant *S. typhi* (MDR-ST) infection, respectively. Median fever clearance time was 3 (IQR 2.75 - 5) days in patients with nalidixic acid-susceptible *S. typhi* (NASST) infection and 5 (3.9 - 6.1) days in the NARST group. By Kaplan-Meier method, distributions of fever clearance times in NARST and NASST groups were significantly different (P = 0.028). There was no significant difference in fever clearance distributions between the MDR-ST group and the rest (P = 0.948). In 12 (24%) patients, the initial antibiotic regimen included a fluoroquinolone, of which 8 (67%) had NARST infection. At any given point of time, fever clearance occurred about two times less likely in the NARST group as compared to the NASST group (unadjusted hazard ratio 0.46 [95% CI 0.22 - 0.95]; P = 0.037). In the Cox proportional-hazards regression model, the effect-size of the association between NARST infection and delayed fever clearance remained the same, even after adjusting for potential confounders such as age, duration of fever at presentation, and use of fluoroquinolone in the initial antibiotic regimen (adjusted hazard ratio 0.45 [0.19 - 0.97]; P = 0.042). An independent association between NARST infection and prolonged fever clearance time suggests that NARST might be inherently more virulent than NASST.

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ENTEROINVASIVE *ESCHERICHIA COLI* ISOLATED FROM EGYPTIAN CHILDREN WITH ACUTE GASTROENTERITIS ARE MULTI-DRUG RESISTANT AND ENCODE FOR MULTIPLE VIRULENCE FACTORS

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Enteroinvasive *Escherichia coli* (EIEC) cause *Shigella*-like dysentery as a consequence of invasion and multiplication within colonic epithelial cells. The contribution of EIEC isolates to the diarrheal disease burden in developing countries is relatively unknown. We sought to define the disease burden due to EIEC and to characterize these isolates using microbial techniques. Ten EIEC defined as *E. coli* (by API20E testing) carrying the *ipaH* gene (by PCR) were isolated from Egyptian children (n=340) with diarrhea. EIEC isolates were characterized for their biochemical activities, antibiotic susceptibility, hemolysin production, and adherence and invasion properties. Additional characterization involved plasmid profiling, pulsed field gel electrophoresis, detection of the pAA plasmid and genes *elt, est, stx1, stx2, set, sen,* and *eae* encoding for the heat labile (LT) and stable (ST) enterotoxins, shiga-toxins 1 and 2, shigella enterotoxin 1 and 2, and intimin, respectively. Six of the ten EIEC isolates fermented lactose, four produced lysine decarboxylase, two were motile and seven were β-hemolytic. Eight EIEC isolates were resistant to at least three antibiotic; however all were sensitive to cephalosporins, fluoroquinolones, monobactams and carbepenems. No common HEp-2 adherence pattern was observed. Three isolates were non-invasive; four showed low invasion percentages and three had strong invasion ability as judged by INT407 invasion assays. EIEC isolates were genetically heterogeneous. The most common combination of virulence genes detected was *ipa*H and *sen* (n=5) or *ipa*H alone (n=2). The LT, ST or shiga-toxin genes were not detected. The *ipa*H gene was detected on both chromosomal and plasmid DNA (50%), plasmid DNA alone, (40%) or chromosomal DNA (10%). Although it is likely that the prevalence of EIEC is underestimated because of the obstacles accompanied with its identification, EIEC appears to have the same isolation frequency as *Shigella* spp. in Egypt. There is an urgent need to find a simple assay that unequivocally captures all of the EIEC strains.

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PROTEOMIC ANALYSIS OF *IN VIVO* EXPRESSED AND IMMUNOGENIC PROTEINS OF *VIBRIO CHOLERAE*

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Proteins expressed by *Vibrio cholerae* during human infection may be targets of protective immune responses. Here, we used mass spectrometry to characterize the *V. cholerae* proteins present in greatest abundance in samples of stool and vomitus from cholera patients. We also defined a subset of these proteins that were recognized uniquely by convalescent sera of cholera patients. Stool and vomitus were collected from cholera patients at the International Center for Diarrhoeal Disease Research,B hospital in Dhaka, Bangladesh. Bacterial pellets were recovered from the samples, reduced, alkylated, and fractionated on an SDS-PAGE gel. Gel slices were dried and treated with porcine trypsin (Promega). Peptides were extracted, lyophilized and loaded onto a LC-Q DECA XP Mass Spectrometer. Peptide identifications were made using the SEQUEST program through Bioworks Browser 3.1. To identify immunogenic *V. cholerae* proteins recovered from human samples, we purified IgG from pooled acute and convalescent cholera patient sera with a protein G column (Amersham) and bound the purified IgG to a NHS-activated column (Amersham). Tryptic digests of *V. cholerae* recovered from human vomitus and stool were then bound to the IgG-loaded column, and elutions were analyzed by mass spectrometry. Six paired samples of *V. cholerae* recovered from human stool and vomitus were analyzed. A total of 445 *V. cholerae* proteins were identified in the six stool specimens, including the A and B subunits of cholera toxin, multiple outer membrane proteins, chemotaxis proteins, and flagellar and pilus components. Many *V. cholerae* proteins were common to stool samples from different patients. Less *V. cholerae* proteins were identified in the human vomitus samples; notable among these were the secreted colonization factor, TcpF, and the quorum sensing protein, LuxP. Twenty-five *V. cholerae* proteins recovered from human samples were bound uniquely by convalescent IgG. Among the immunogenic proteins identified were the known immunogens, CtxA and CtxB, as well as a number of novel antigens. Current work is focused on confirming the immunogenicity of the identified proteins by alternate techniques.

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TRANSCUTANEOUS IMMUNIZATION WITH A NEOGLYCOCONJUGATE CONTAINING A *VIBRIO CHOLERAE* HEXASACCHARIDE DERIVED FROM *V. CHOLERAE* O1 OGAWA LIPOPOLYSACCHARIDE BOUND TO A PROTEIN CARRIER

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Anti-*Vibrio cholerae* lipopolysaccharide (LPS) responses are common in humans recovering from cholera, and constitute the primary component of the vibriocidal response, a serum complement-mediated bacteriocidal response strongly correlated with protection against cholera. Parenteral immunization with *V. cholerae* LPS provides protection against cholera

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challenge in both animals and humans. A recent study has demonstrated that parenteral immunization with synthetic *V. cholerae* O1 Ogawa LPS fragments conjugated to a BSA protein carrier (neoglycoconjugate) is both immunogenic and protective in mice. A needle-free alternative to parenteral immunization is transcutaneous immunization (TCI). In order to determine whether immune responses may be elicited against *V. cholerae* LPS using TCI, we immunized mice with a neoglycoconjugate comprised of synthesized hexasaccharide derived from the O-PS component of *V. cholerae* O1 Ogawa lipopolysaccharide bound to BSA. We applied neoglycoconjugate in the presence or absence of immunoadjuvantative cholera toxin (CT). Transcutaneously applied neoglycoconjugate elicited prominent anti-*V. cholerae* LPS IgG responses in the presence of CT, but minimal IgM and IgA responses. CT applied on the skin alone induced both strong IgG and IgA anti-CT serum responses. Despite the induction of prominent serum anti-LPS IgG responses, TCI with neoglycoconjugate did not elicit vibriocidal responses or stool anti-*V. cholerae* IgA responses, irrespective of presence or absence of CT. Passive immunization with sera from mice immunized with neoglycoconjugate and CT provided protection against *V. cholerae* challenge in an infant mouse challenge model; however, this protection was comparable to that seen in mice receiving sera from animals immunized with CT alone. Our results suggest that transcutaneous application of synthetic neoglycoconjugate is safe and immunogenic, resulting in prominent anti-*V. cholerae* serum IgG responses, and warrants further evaluation.

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BROMINATED POLYSTYRENE-HYDANTOIN BEADS FOR LOW-COST, HOUSEHOLD DISINFECTION OF DRINKING WATER

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Enabling consumers to cost effectively and easily treat contaminated water into potable household supplies that are not only clear and safe, but also free of objectionable taste and odor, remains a serious technical challenge in the developing world. Halogen-based disinfection has a long and successful history. Currently, household systems rely on chlorine or iodine for disinfection, however treatment with either halogen usually produces water with a smell or bad taste, as well as often with a high potential for unacceptable toxicological profiles. Another halogen available for water treatment is bromine. Covalent binding of oxidative bromine onto polystyrenehydantoin beads allows for contact biocidal action. We show here that bromination of the polymer provides higher efficacy and improved sensory quality of the product water without generating adverse byproducts. These beads typically hold 18% w/w of bromine atoms that are uniformly distributed throughout the macro-porous bead. When these beads are used in gravity-feed filtration devices, and challenged with poliovirus and Klebsiella terrigena, consistent and sustained reductions are demonstrated at 4 and 6 log levels, respectively, after a single pass, even at both 10C and 40C. Unique household units designed and developed with cyst-removing prefiltration to produce water that meets USEPA purifier standards are used in this study, and disinfection byproduct generation is within limits defined by NSF Standard #61. Passing the water over a small tablet located prior to the bead bed, repopulates the Br binding sites and furthers the purification capacity. This permits use of the device in daily household water needs for years without replacement. Bromine residuals in the product water are low but still provide a degree of protection in the collection reservoir. This combination of a low cost rechargeable bromine-based contact biocide with a durable, user-friendly gravity-feed purifier design requiring minimal maintenance, goes a long way towards meeting the objective of safe household water treatment and storage for third world use.

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ANALYSIS OF THE *BRUGIA MALAYI* RPS12 PROMOTER IN A HOMOLOGOUS TRANSFECTION SYSTEM

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Previous studies of the HSP70 promoter of *Brugia malayi* revealed that this promoter lacked all of the elements that characterize a typical eucaryotic promoter. Instead, the core promoter was mapped to three regions upstream in the start codon, which included a heat shock element, a 6nt domain and a 20 nt domain that included the splice leader addition site. The activity of the latter was not associated with trans splicing, as the transgenic mRNA was not trans spliced. In order to determine if these unique characteristics were a more general property of *B. malayi* promoters, the promoter of the 12 kDa small subunit ribosomal protein (BmRPS12) gene was analyzed in detail. Similar to what was found with the BmHSP70 promoter, the BmRPS12 promoter lacked canonical TATAA, CAAT and *inr* domains. The elements essential for transcription were mapped to five domains. The most striking feature was the region extending from positions -331 to -64, which consisted of 5 1/2 repeats of a 44nt sequence. Sequential deletion analysis revealed that 2 1/2 repeats were necessary for full activity. Constructs containing 1/2 to 1 1/2 repeats exhibited activities that were 50% of those seen with the native promoter, while deletion of all repeats resulted in a loss of 80% of promoter activity. The 44nt repeat sequence consisted of a number of imperfect sub-repeat elements that encoded sequences predicted to bind a GATA like transcription factor. Among the remaining elements essential for transcription, the sequence including the splice leader site was found to be essential for promoter activity, although as in with the BmHSP70 promoter, transgenic mRNAs driven from the BmRPS12 promoter were not trans spliced. This study confirms that the unique features exhibited by the BmHSP70 promoter are not confined to this gene, and further suggest a role for the region including the SL addition site in initiating transcription in *B. malayi*.

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DISRUPTION OF *PLASMODIUM* TRANSCRIPTION FACTOR *HMGB2* IMPAIRS OOCYST FORMATION

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The many coordinated events that occur during malaria parasite proliferation, development, and differentiation, imply a fine regulation of gene expression in response to external stimuli. There is growing evidence that supports a role for transcriptional control of the sexual cycle in malaria parasites. With a relative paucity of canonical eukaryotic transcription factors, malaria parasites may instead encode a few "master regulators" responsible for controlling the important steps of its life cycle. A High Mobility Group nuclear factor (HMGB2) has been biochemically characterized and functions *in vitro* as a genuine transcription factor. The *hmgb2* gene is highly conserved among malaria parasites and shows differential expression during the malaria life cycle with a peak in gametocytes, although the transcript can also be detected in asexual parasites. To study the role of this nuclear factor during the life cycle of *Plasmodium*, we disrupted the *hmgb2* gene in *Plasmodium yoelii*. Knock-out of *hmgb2* (PyHMGB 2 KO) resulted in a total loss of detectable transcription of the gene as assayed by classic and real-time quantitative RT-PCR. The PyHMGB2 KO parasites exhibit a slight slowing of asexual growth when compared to wild type (wt) parasites. As measured by real-time quantitative RT-PCR, the expression levels of markers of gametocyte development differ in PyHMGB2 KO parasites compared to the wt parasites. Moreover, mosquitoes fed on mice infected with PyHMGB2 KO

parasites have a 10-fold decrease in oocyst number. Thus *Plasmodium* HMGB2 is a transcription factor likely to play a crucial role in the sexual cycle of malaria parasites.

(ACMCIP Abstract)

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A DETERMINATE OF SPECIES RANGE AND VIRULENCE IN *PLASMODIUM FALCIPARUM* MALARIA

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Malaria parasites infect a wide range of vertebrates with a high degree of host specificity, so host sharing and cross-species transmission are rare. Therefore only higher primates and New World monkeys can be experimentally infected with the human malarias. The owl monkey, *Aotus nancymai*, is widely used in malaria vaccine research, but only a few *Plasmodium falciparum* lines are virulent in this model. To explore the nature of this species restriction and to identify determinants critical to the use of the *P. falciparum-Aotus* model, we have completed a genetic cross between a *P. falciparum* clone unable to infect *Aotus* and a clone highly virulent to these monkeys. Thirty-three independent recombinant progeny have been isolated and typed with microsatellite markers at 3 cM resolution. Linkage analysis of this cross maps *Aotus* infectivity to a 13kb locus on chromosome four. This locus contains just two genes. Genetic manipulations and immuno-fluorescence studies are offering insights into the gene underlying the virulence/invasion phenotype and will be discussed.

(ACMCIP Abstract)

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RECOMBINATION PATTERNS IN *VAR* GENE REPERTOIRES OF *P. FALCIPARUM*

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In *Plasmodium falciparum*, cytoadherence and immune evasion properties are encoded into a large, diverse protein family named *P. falciparum* erythrocyte membrane 1 (PfEMP1), which is responsible for sequestration of mature stage infected erythrocytes. These PfEMP1 proteins are encoded by approximately 50-60 different *var* genes in the genome. Since PfEMP1 proteins are involved in both antigenic variation and adhesion, understanding genetic diversity in the *var* gene family is important to revealing the mechanisms of malaria pathogenesis. Although the *var* gene family is known to be diverse, little is known about the overall similarity of *var* repertoire between parasite isolates. To investigate the inter-strain diversity of the *var* genes, we have sequenced large fragments or full-length *var* genes from the IT4/25/5 (IT4) strain that is widely used in adhesion studies. We have found immense diversity between and within IT4 and 3D7, a parasite isolate that was sequenced for the malaria genome project. In addition, sequence analyses suggest that *var* genes form separate recombination groups. In contrast to the diverse profile of *var* genes, a small number of genes are well conserved among parasite isolates from around the world. These conserved *var* genes may not be recombining with any of the other *var* genes, suggesting potential recombination constraints in maintaining a particular *var* gene in a parasite's *var* repertoire. These studies aid our understanding of *var* gene evolution and suggest genetic mechanisms for the structural and functional specialization of parasite adhesion ligands involved in malaria pathogenesis.

(ACMCIP Abstract)

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MULTIPLE INDEPENDANT ORIGINS OF ATOVAQUONE-PROGUANIL *FALCIPARUM* RESISTANCE

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Clinically relevant emergence and spread of antimalarial drug resistance is one of the most serious threats to the control of malaria. Atovaquone-proguanil (AP) resistance is determined by mutation at codon 268 in the mitochondrial (mt) *cytochrome b* gene (*pfcytb*). Owing to its high cost, AP is mainly used by travelers for *P. falciparum* malaria treatment and prophylaxis and selective pressure in endemic countries is yet negligible. Nevertheless, highly AP resistant parasites were isolated from a dozen of AP treatment failure observed in the past 3 years in Europe and North America. To determine the origin(s) of AP resistance alleles, we amplified and sequenced the 6-kilobase mt genome from *P. falciparum* isolates with the *pfcytb* Y268S (n=5) or Y268C (n=2) mutation derived from patients which failed to be cured with AP in France. Several polymorphisms were identified. Remarkably, the mt haplotype from each resistant isolate differed, demonstrating that these several 268 mutations occurred independently in natural isolates. We then compared each mt haplotype at the day of treatment failure (Dfail) with the mt haplotype at admission (D0). In all mt genomes, except for the 268 mutation, no difference was observed between D0 and Dfail haplotypes. Furthermore, five nuclear microsatellites studied demonstrated identical D0 and Dfail haplotypes. Finally, to analyze the background of mitochondrial diversity in the three contamination countries of the patients, we determined the mt haplotype of isolates from the Côte d'Ivoire (n=10), Burkina Faso (n=10) and Guinea (n=10). In these wild isolates, no haplotype similar to those obtained from AP treatment failure isolates was identified. Altogether, our observations support multiple independent within-host origins of AP resistance alleles and their subsequent selection during treatment.

(ACMCIP Abstract)

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THE EVOLUTION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN PLASMODIUM

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The purpose of our phylogenetic study of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in *Plasmodium* is two-fold: To infer the mode of evolution of this gene in *Plasmodium*, and to establish divergence times for species within the *Plasmodium* genus. G3PDH is a housekeeping gene, and Southern analysis as well as genome sequence information from several Plasmodium species show that the gene is present as a single copy, contrary to what is observed in other Apicomplexa like *Theileria* and *Toxoplasma*. It is expected that G3PDH will evolve at a neutral rate, unlike previously studied genes such as circumsporozoite (CSP) gene which is subject to immune pressure. In addition, *Plasmodium* ssrRNA genes belong to a multigene family and undergo gene conversion among non-homologous gene copies, which generates misleading data on rates of divergence. We have isolated and sequenced the G3PDH gene from *P. falciparum, P. reichenowi*, *P. vivax*, *P. knowlesi*, *P. coatneyi,*

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P. fragile, P. cynomolgi, P. brasilianum and *P. malariae*. This, in addition to the available sequences from *P. yoelii, P. berghei, P. chabaudi* and *P. gallinaceum* were used to develop a database to carry out our analyses. A comparison of the sequences to the solved crystal structure of the *P. falciparum* G3PDH proteiin, as reported previously, confirms the presence in all species of a Lysine-Glycine insertion motif near the NAD+-binding site common to other members of the Alveolata. Phylogenetic tree construction using Maximum Parsimony and Neighbor-Joining have confirmed previously proposed relationships among Plasmodium species. Ongoing studies include analysis of the patterns of nucleotide and amino acid substitutions as well as molecular clock tests to determine if G3PDH evolves at similar rates in all lineages. The results of this study will enhance our understanding of the relative evolutionary rates of various *Plasmodium* species. In addition, the inference of divergence times within the genus and the rates of evolution at a single copy gene will constitute an important framework for the understanding and determination of the rates of evolution of multigene families like variant antigen genes.

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GENOME-WIDE VARIATION AND IDENTIFICATION OF VACCINE TARGETS IN THE *PLASMODIUM FALCIPARUM* GENOME

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One of the goals of sequencing ***Plasmodium falciparum*** genome, the agent of the most lethal form of malaria, is to discover vaccine and drug targets; however, identifying vaccine targets from a genome having ~60% of genes with unknown functions is an enormous challenge. Since the majority of the known malaria antigens and drug targets are under selection and are highly polymorphic, we hypothesized that a systematic genome-wide search for signatures of selection may lead to potential unknown vaccine and drug targets. Here we surveyed 3539 ***P. falciparum*** genes (~65% of the predicted genes) for polymorphisms and identified 3918 single nucleotide polymorphisms (SNP, ~70% non-synonymous) and 2548 polymorphic microsatellites (MS), providing a high-resolution map (one marker/~4 kb) for mapping parasite traits and studying parasite populations. Search for signatures of selection revealed various genes encoding potential antigens, cell adhesion molecules and drug targets. Further ***in vitro*** protein expression experiment showed some of these proteins were confirmed as novel antigens by infected human sera, which will be evaluated as potential vaccine targets. These data show a highly polymorphic genome, which may present a formidable obstacle for the development of disease control strategies.

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IDENTIFICATION OF GENETIC POLYMORPHISMS WITHIN THE TNFA AND COMPLEMENT PATHWAYS INFLUENCING RESISTANCE TO MALARIA-ASSOCIATED SEVERE ANEMIA IN KENYA

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The relationship between *Plasmodium falciparum* and its human host is a delicate balancing act between the virulence of the parasite and the immune response of the host. Because malaria parasites have coevolved for so long within the human population, considerable evolutionary pressure has been placed on host genes responsible for conferring protection against severe forms of malaria. To identify genetic polymorphisms in genes and pathways involved in conferring protection against infection and severe forms of malaria, we have undertaken a candidate gene approach to examine polymorphisms in genes that regulate the innate immune response. The candidate genes we have examined include TNFα and its two receptors, TNFRSF1A and TNFRSF1B, as well as genes involved in the complement pathway (C6 and C7). We have used the iPlex multiplexing technology from Sequenom to genotype single nucleotide polymorphisms (SNPs) across these gene regions in a cohort of 1100 children from the Asembo Bay region of Western Kenya, an area with intense malaria transmission. Based on the univariate analysis of the genotyping data, we identified a polymorphism within the first intron of TNFRSF1B (rs595254 at +1661) that was determined to be associated with protection against malaria-associated severe anemia (Hb < 6g/dl and the presence of *P. falciparum* malaria) (OR = 0.59, 95%CI = 0.40-0.85, p=0.0034) and overall severe anemia (Hb < 6g/dl) (OR = 0.65, 95%CI = 0.46-0.93, p=0.0140). We also identified a polymorphism just upstream of the C7 transcription start site (rs1376178 at -812) that was determined to be associated with protection against malaria-associated severe anemia (OR = 0.66, 95%CI = 0.49-0.87, p=0.0024) and overall severe anemia (OR = 0.62, 95%CI = 0.47-0.81, p=0.0004). We are currently conducting a more comprehensive multivariate analysis to control for any potential confounders. Identification of these genetic polymorphisms that influence susceptibility of the human host to malaria infection and severe disease outcomes will help us to better understand the immune response to malaria in order to develop novel treatments against severe malaria.

(ACMCIP Abstract)

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QUANTITATIVE DETECTION OF TRANSCRIPTS OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-3 (PVMSP-3) ELEVEN GENE FAMILY MEMBERS BY REAL-TIME PCR

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Plasmodium vivax merozoite surface protein-3 (PvMSP-3) antigens are important blood-stage malaria vaccine candidates. So far, three members (α, β, and γ) of this gene family have been characterized and reported in the literature. Here we present eight new family members, confirmed to be located within the same gene cluster by searching the *P. vivax* genome sequence database. The predicted protein sequences of all eleven family members have the common feature of a central alanine-rich domain containing heptad repeats known to form coiled-coil tertiary structure. These structural features along with the presence of the common N-terminal motif were the basis for inclusion in the Pvmsp-3 gene family. Increased knowledge on the expression of the Pvmsp-3 transcripts throughout the erythrocytic life cycle will be beneficial to better understand the biological function of this gene family. RT-PCR and quantitative RT-PCR methods were used to analyze the total RNA from trophozoite and schizont-infected erythrocytes. The results of RT-PCR amplification with gene-specific primers demonstrated that full-length transcripts of all eleven Pvmsp3 genes were produced in both life cycle stages. Quantitative RT-PCR results showed a broad range of 0.7 to 111.4 fold increases in transcripts for different gene family members compared to a genomic DNA standard. Transcripts for all gene family members except gene #4 were generated 1.2 -18.2 times higher in the trophozoite stage compared to the schizont stage. Current experiments are being

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conducted to verify whether all eleven genes are concomitantly expressed on the merozoite surface. These data are relevant for gaining a molecular biological understanding of this gene family and towards choosing which members may be of interest for follow-up diversity and immunogenicity investigations pertinent to malaria vaccine studies.

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ANTISENSE RNA AND ANTIGENIC VARIATION IN PLASMODIUM KNOWLESI

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Malaria parasites are able to establish chronic infections in their hosts due to the ability to vary antigens expressed on the surface of red blood cells in the face of immunological challenge. The mechanisms that control the switching and silencing of *Plasmodium falciparum* var and *P. knowlesi* SICAvar genes are still being elucidated. Antisense RNA has been previously shown to be ubiquitous in *P. falciparum*. A good deal of debate has occurred as to the source of these transcripts, and whether they are meaningful in a genetic context. With regard to *P. falciparum* var antisense only partial transcription has been observed in the context of microarray data, with the conclusion that antisense transcripts are not involved in silencing. Using the non-human primate malaria model, *P. knowlesi*, by quantitative RT-PCR and other standard methods, we show that (1) SICAvar antisense RNA is produced in a stage-specific manner, (2) the antisense transcripts are lacking SICAvar intron sequence, and (3) SICAvar antisense transcripts can be detected in northern blot experiments. These data suggest that the antisense SICAvar RNA transcripts may be produced with the SICAvar sense strand functioning as a template. Additionally, SICA+ parasites are known to lose expression of their SICA antigens when passaged in splenectomized monkeys. Our data shows that SICA- parasites that originally expressed a 205 KDa protein prior to passage in the splenectomized monkey exhibit higher levels of the 205 KDa gene-specific antisense RNA. Together these data suggest a possible regulatory role for SICAvar antisense RNA in *P. knowlesi* antigenic variation, and also open discussion regarding the potential role of antisense RNA in *P. falciparum* var gene expression.

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ASSOCIATION OF PATIENT CHARACTERISTICS AND *P. FALCIPARUM* STEADY STATE MRNA ABUNDANCE

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Host factors such as age, cytokine profiles and hematocrit are associated with *Plasmodium falciparum* disease severity and blood gametocyte prevalence. To explore host influence on parasite biology, we analyzed 43 *in vivo* derived transcriptomes in association with host factors. We isolated RNA from blood samples of infected patients residing in Senegal. Twenty percent of these patients had severe disease. Steady state mRNA levels were analyzed using a custom made oligonucleotide array with probes based *P. falciparum* 3D7 genome. The *in vivo* transcriptomes along with the 3D7 life cycle stages were normalized. A Non negative Matrix Factorization (NMF) clustering algorithm defined two main clusters. Cluster One contained the 3D7 early ring reference strain transcriptome and one half of the *in vivo* transcriptomes while Cluster Two contained gametocyte transcriptomes and the remaining *in vivo* transcriptomes. Patient data that significantly correlates with this clustering will be presented. A second approach to the analysis involved grouping samples by patient features and determining if there are significant differences in transcript abundance. Parasite transcripts were compared from samples derived from patients who had the highest parasite load (quartile 4) and compared to samples derived from patients who had the lowest parasite load (quartile 1). To determine if a subset of genes or pathways are differential expressed Gene Set Enrichment Analysis (GSEA) was carried out. We found that GO clusters involving transcription and energy metabolism to be significantly enriched in samples derived from patients with the highest parasitemia. Similar analysis carried out on samples organized by other host features will be presented. Finally we examined the effect of anti-malarials on *in vivo* transcript abundance. Transcriptomes were derived from blood draws taken at presentation (pre drug) and again at twenty four hours on drug in ten patients. Seven paired samples correctly clustered into a pre and post groups and two pairs of pre and post samples clustered into a third group. The third group pre drug samples had fallen into the Cluster 2 (gametocyte like) and demostrated minimal transcriptional change in their matched post drug samples. The exploration of host features in association with parasite *in vivo* transcriptome analysis provides insight into pathogenesis and sexual stage development.

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CHANGES IN HUMAN GENE EXPRESSION DURING UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Understanding the pathogenesis of malaria is a potentially essential step in reducing the morbidity and mortality of the disease. Factors that may contribute to the pathogenesis of malaria include host factors (protection against high parasitemias [e.g. sickle cell], host gene expression in response to infection) and parasite factors (cytoadherence and other virulence determinants, copy number [parasitemia], other parasite antigens on the host red cell surface). To examine the role of host factors in the pathogenesis of malaria, the studies reported here examined changes in gene expression in human subjects with uncomplicated *Plasmodium falciparum* malaria. Because the interpretation of microarray data is potentially confounded by genomic differences among individuals and by inter-individual variation in gene expression, we used each subject as his/her own control. Three samples were obtained from each subject: 1] Acute, or Illness sample at the time of presentation with acute illness (when subjects were begun on standard antimalarial treatment), 2] Treatment sample after 3-4 days of treatment, and 3] Recovery sample 7-10 days after treatment (when there were no signs or symptoms of malaria, or asexual parasites on thick smear). Because it was thought to represent a subject's healthy level of gene expression, the Recovery sample was used as the baseline and was therefore the sample to which the Acute Illness and Treatment samples were compared to identify changes in gene expression. Using labeled cRNA prepared from these specimens, we performed oligonucleotide arrays and low-density arrays, followed by gene ontology and pathway analyses to identify genes, networks, and pathways with altered expression during uncomplicated malaria. The results indicate up-regulation of innate immune response and inflammation-related genes such as IL-1, IL-6, TNF, and IFN- (and thus implicate monocytes, macrophages, neutrophils and Natural Killer cells). The data also indicate up-regulation of pathways involved in apoptosis. Finally, increased expression of these genes was associated with greater parasitemia and clinical severity, suggesting that modulated increases in the expression of genes in these pathways may determine the severity of disease.

(ACMCIP Abstract)

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ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA IN UGANDA: A RANDOMIZED CLINICAL TRIAL AT A SITE WITH HIGH TRANSMISSION INTENSITY

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Artemisinin-based combination therapies (ACTs) have been strongly advocated for use in Africa but obstacles to widespread implementation of these regimens exist. In Uganda, artemether-lumefantrine (AL) has been chosen as the new first-line therapy, but data on the efficacy and safety of this regimen are limited, and its substantial cost, limited availability, twice a day dosing, and high reinfection rates after therapy remain significant obstacles. Dihydroartemisinin-piperaquine (DP) is a new ACT that has shown excellent efficacy in Southeast Asia and has the benefit of once a day dosing and potentially better post-treatment prophylactic effect compared to other ACTs. There have been no published studies of the efficacy of DP in Africa. We are conducting a randomized, single-blinded trial of AL versus DP for the treatment of uncomplicated falciparum malaria at a very high transmission site in Uganda. A total of 400 patients aged 6 months to 10 years shall be recruited in the study and followed for 42 days. Genotyping will be used to distinguish recrudescence from new infections. A total of 250 patients have been recruited and 65 have completed the study. Of the patients enrolled, 95% have been under the age of 5 years. Preliminary results show no early treatment failures. After 28 days of follow-up, the risk of recurrent malaria (LCF) is 13% and the risk of recurrent parasitemia (LCF or LPF) is 27%; after 42 days the risk of recurrent malaria is 46% and the risk of recurrent parasitemia is 59%, unadjusted by genotyping. No serious adverse events related to study drugs have been reported to date. It is anticipated that recruitment of patients shall be completed in early June, and the study completed in July. Full unblinded results of the trial will be presented.

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ARTESUNATE (AS) PLUS AMODIAQUINE (AQ) FOR TREATING FALCIPARUM MALARIA - ASSESSING ITS EFFICACY AND TOLERABILITY DURING SIX YEARS OF FIELD DEPLOYMENT IN SOUTHERN SENEGAL

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Data are lacking on the efficacy and tolerability of artemisinin based combinations (ACT) when deployed widely in Africa. Our study was conducted in Oussouye district, southern Senegal, an area of moderate malaria transmission. We assessed the feasibility and effect (efficacy, safety, drug use, ***in vitro*** drug sensitivity) of introducing a new practice of treating only microscopically confirmed falciparum malaria with AS+AQ. Data were extracted from clinic registries for the period 1996-1999 when malaria was diagnosed clinically and treated with either intramuscular quinine or oral chloroquine. From 2000 to 2005, we introduced gradually, in district clinics, AS+AQ for microscopically confirmed ***P.falciparum***. A total of 3037 treatments were given, of whom 2071 were followed clinically for 28 days and 966 underwent intensive parasitological, biochemical, and haematological assessments. Children <5kg and pregnant women were excluded. We used loose and co-blistered AS+AQ. Target doses were 4mg/kg/dx3d (AS) and 10 mg/kg/dx3d (AQ). Microscopic diagnosis (applied to 71% of fevers during 2000-05) reduced the numbers of malaria treatments from a mean of 5424 per year (1996-99) to 2518 in 2005, while the slide positive rate remained constant (ca. 40%). AS+AQ was well tolerated. Provisional analysis in 2521 patients show that 252 patients experienced a total of 317 events: vomiting (57%), vertigo (20%), pruritus (12%). There were 4 serious adverse events: 2 deaths, 1 deterioration, 1 hospitalization.

AS+AQ was very effective: the mean, crude, per protocol, D28 cure rate was 811/855 (97%; range 94-99%). *In vitro* drug sensitivities for CQ, AQ, QN, and AS were similar pre- and post ACT.Microscopy reduced antimalarial drug wastage for treating undifferentiated fevers. AS+AQ was efficacious and well tolerated. Efficacy and parasite sensitivity remain high after this initial phase of deployment. Long term efficacy testing and pharmacovigilance are possible in an under resourced, African setting.

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ARTESUNATE + AMODIAQUINE (AS+AQ) FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA: AN INVENTORY AND SYSTEMATIC REVIEW OF SAFETY AND EFFICACY DATA.

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AS+AQ is currently adopted by 16 countries (15 in Africa) to treat uncomplicated falciparum malaria. To inform better drug policy and research on the use of this combination, we compiled and analysed an inventory of AS+AQ clinical trials by searching PubMed, the Cochrane Registry and requesting unpublished data from investigators. The primary efficacy parameter was parasitological and clinical success on Day 28 (crude and PCR-corrected). 30 studies (27 comparative, 3 non-comparative), conducted between 1999-2006 in 19 countries (Africa=18) were suitable for efficacy and safety analyses. A total of 11,751 patients were recruited: (i) AS+AQ = 5,272 (4,173 in comparative and 1,099 in non-comparative studies), and (ii) 6,479 comparator drugs.18 studies genotyped recurrent parasites: (i) 2543 AS+AQ, and (ii) 3191 others.

AS and AQ were given together as loose tablets (28 studies) or in a co-blister. Most patients received doses of 4 mg/kg/d (AS) and 10 mg/kg/d (AQ) for three days. 88% of the AS+AQ patients in controlled trials were evaluable on Day 28.The Day 28, uncorrected rates for AS+AQ varied considerably. The Day 28, corrected rates were > 90% in 14 of 18 studies. Overall, AS+AQ efficacies (crude and PCR corrected) were: (i) significantly better than single agents or non artemisinin combinations, (ii) similar to AS+SP; artemether + lumefantrine and dihydroartemisinin + piperaquine were more effective when crude rates are considered, but not different after PCR correction. AS+AQ was reportedly well tolerated but detailed safety data were often lacking.

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A DOUBLE BLINDED RANDOMISED CONTROLLED TRIAL COMPARING SULFADOXINE-PYRIMETHAMINE (SP) + PLACEBO TO SP+CHLOROQUINE, SP+ARTESUNATE OR SP+AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN MALAWI

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Malawi changed its first line treatment for uncomplicated malaria from chloroquine (CQ) to sulfadoxine-pyrimethamine (SP) in 1993. Since then, the prevalence of DHFR DHPS mutations has risen, while that of PFCRT 76T, the molecular marker of CQ resistance, has fallen (85% in 1992 to 0% in 2004). Amodiaquine (AQ) is increasingly being used in Africa despite concerns about its toxicity when used prophylactically.

The aims of this study were to test the hypothesis that there has been a return to CQ sensitivity in Malawi and to compare the effect of adding a 4-aminoquinoline or artemisinin to SP on the efficacy and selection pressure for resistance mutations. We compared SP alone with SP + 3 days of CQ, AQ or artesunate (ART) in 455 children aged 1-5 years in a double blinded RCT. The day 28 ACPR rates (per protocol analysis, PCR corrected) with 95% CI were; SP alone 26% (18-36), ART+SP 74% (64-82), CQ+SP 86% (78-93) and AQ+SP 97% (91-99). Parasite clearance was significantly shorter in the 3 combination groups than with SP alone. Haemoglobin rose in all groups after treatment, with significantly larger rises with AQ+SP and CQ+SP There was no difference in the rates of gametocyte carriage after treatment between the groups. DHFR *Triple* mutants rose from 92.8 to 99.2% and *Quintuple* mutants from 89.3 to 94.3% with treatment. Pfcrt, pfmdr1data will be presented. Adverse event self-reporting was similar in all 4 groups. 2 children (AQ+SP group) developed significant rises in ALT. Neutrophil counts fell from day 0 to day 14 without significant differences between the groups. These results show a profound lack of SP efficacy in Malawi. SP+ART was inferior to the other 2 combinations, and CQ+SP was significantly inferior to AQ+SP even in the absence of parasites carrying the pfcrt 76 mutation. The laboratory "safety data" in the AQ group warrant further investigation. The selection of resistance mutations by the different treatments will be discussed.

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A CONTROLLED TRIAL ON EXTENDED INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE FOR MALARIA CONTROL IN INFANTS IN AN AREA OF INTENSE PERENNIAL TRANSMISSION

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Intermittent preventive antimalarial treatment of infants (IPTi) with sulfadoxine-pyrimethamine reduces the frequency of falciparum malaria in areas of moderate transmission. We investigated the protective efficacy of IPTi in a holoendemic area and evaluated whether an additional drug administration in the second year of life prolongs protection. A randomized, double-blinded, placebo-controlled trial on IPTi with sulfadoxine-pyrimethamine at three, nine and fifteen months of age was conducted with 1070 children in the Ashanti Region of Ghana. Participants were actively monitored for 21 months. Primary endpoint was the incidence of malaria. Additional endpoints were anemia incidence, outpatient visits, hospital admissions, mortality, and safety. Stratified analyses for six-month periods after each treatment were applied. Overall protective efficacy against malaria episodes was 20% (95% confidence interval [CI] = 11-29). Clinical malaria episodes were reduced by 23% (95% CI = 6-36) after the first and by 17% (95% CI = 1-31) after the second sulfadoxine-pyrimethamine application. After the third IPTi dose at month 15 no protective efficacy was achieved. Protection against the first or single anemia episode was conferred only by the first IPTi dose (30%, 95% CI = 5-49). The frequency of anemia episodes increased by 24% during the period following the last IPTi application (95% CI = -50- -2). The incidences of severe anemia, hospital admissions, outpatient visits, and all-cause mortality were similar in both study-arms. Vomiting was more frequent in the sulfadoxine-pyrimethamine group (4.7% *versus* 2.1%; risk ratio 2.26; $P < 0.001$). Three individuals developed a Stevens-Johnson syndrome; two were attributable to sulfadoxine-pyrimethamine. In conclusion, IPTi confers considerably lower protection in holoendemic areas than reported from an area of moderate endemicity and was associated with anemia in the time after cessation of the intervention. Protective efficacy is age-dependent and extension of IPTi into the second year of life does not provide any benefit. This study is registered at www.ClinicalTrial.gov, registration number NCT00206739.

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CHANGES IN HOSPITAL CASES OF MALARIA AFTER THE INTRODUCTION OF ARTEMISININ COMBINATION THERAPY IN ZAMBIA

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The World Health Organization has recommended the adoption of artemisinin combination therapy (ACT) in countries that have drug resistant parasites, and Zambia was one of the first countries in sub-Saharan Africa to adopt this policy on a nationwide basis using the drug artemether/lumefantrine.

Macha Mission Hospital, a district level hospital serving a population of approximately 120,000 people where widespread use of Insecticide Treated Nets (ITNs) has not yet been scaled up, and with a 10 year history of accurate diagnosis and documentation of malaria cases, noted a dramatic decrease in pediatric hospital admissions for both uncomplicated and severe malaria, subsequent to the widespread adoption of ACT in 2003/2004. During the most recent malaria transmission season of 2006, as stock-outs of ACT developed at the many referring rural health centers as well as at the hospital, the number of malaria cases, and especially severe cases, increased, as did malaria associated mortality and the number of blood transfusions. The data documenting this experience will be presented. We believe that this represents early evidence that widespread use of ACT can decrease morbidity and mortality of malaria in children, and should be used together with ITNs and Indoor Residual Spraying (IRS) for an effective program to decrease malaria transmission levels.

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COUNTERFEIT ARTESUNATE AND MALARIA IN ASIA AND AFRICA

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Artesunate is a crucial drug in the control of malaria. Since 1998 a worsening epidemic of sophisticated counterfeit 'artesunate' tablets

containing no artesunate has plagued mainland SE Asia. There are at least 12 different types of counterfeit artesunate, based on analysis of the packaging and fake holograms. Using chemical 'fingerprinting' a wide range of different compositions have been described and these are associated with different packaging types. The epidemiology of counterfeit artesunate in Asia, using information from the analysis of the packaging and tablets from different countries will be reviewed. The discovery of types of counterfeit artesunate containing small, sub-therapeutic amounts of artesunate is of great concern as a potential contributor to drug resistance. The recent discovery of counterfeit artemisinin derivatives in Africa and the high cost and shortage of artemisinin derivatives provide a favourable situation for the spread of fake artemisinins that could put the lives of thousands of African children at risk. Strategies to combat this lethal trade will be discussed.

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SEVERE OUTBREAK OF DIARRHEAL DISEASE AND ACUTE MALNUTRITION AMONG YOUNG CHILDREN - BOTSWANA, 2006

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From January - March, 2006, Botswana reported 22,500 cases and 470 deaths from diarrhea in children <5 years, reflecting a doubling of diarrhea morbidity and a 22-fold increase in mortality compared to the same period in 2004 and 2005. We investigated pediatric diarrhea admissions in a large hospital during the outbreak. Caregivers of all children <5 years old admitted for diarrhea from February 20 - March 1, 2006 were administered a standardized questionnaire. Patient records were reviewed and stool specimens tested for enteric pathogens. We defined severe marasmus as weight-for-height z-score $\leq$-3 and kwashiorkor as bipedal edema. Among 156 inpatients, the median age was 10 months (interquartile range 4.8 - 14.5); 47 (30%) had diarrhea for $\geq$3 weeks. Of those with laboratory results, 93/144 (65%) had HIV-positive mothers, 22/131 (18%) were HIV-infected, 19/37 (51%) had enteropathogenic *Escherichia coli* (EPEC), and 49/76 (64%) had *Cryptosporidium*. Medical records indicated that 8/25 (32%) had severe marasmus, 30/147 (20%) developed kwashiorkor, and 33/156 (21%) died. Of those who died, the median age was 8 months and median hospital stay was 5 days. Of children <2 years old, 14/136 (10%) breastfed in the week before illness. No breastfed children <2 years old died compared to 28/122 (23%) non-breastfed children (p=0.04). Death was not associated with *Cryptosporidium*, EPEC, or HIV infection; however, children who developed kwashiorkor were more likely to die than children who did not (37% vs. 17%, p<0.03). In conclusion, despite hospitalization, development of kwashiorkor was associated with very high mortality during a multi-pathogen outbreak of protracted diarrhea in children. Breastfeeding may have protected some children from death. Further laboratory testing and data analysis may identify additional risk and protective factors.

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CHANGES IN THE SPATIAL DYNAMICS OF SEASONAL DIARRHEA IN MEXICO IN 1979-2001

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Mexico represents one of those rare confluences of dramatic improvements in the health of its population in a relatively short period of time with excellent data documentation by surveillance and other socio-economic indicators. During the last decades, diarrhea mortality has decreased and shifted from a spring-summer peak to an autumn-winter period. In the present study we investigated the temporal/spatial dynamics associated with these annual episodes of diarrhea in Mexico, taking into account two epidemiological stages characterized by putatively bacterial and viral infections, respectively. By doing so, we provide the first account of diarrhea mortality moving in annual waves from a large urban center (the megalopolis containing the country's capital, Mexico City) to surrounding regions - a phenomenon present during those earlier years associated with the spring-summer peaks of mortality. The investigation of putative factors underlying this geographical pattern indicates that climate probably drives the summer waves of infection originating from Mexico City, although population factors might play a role as well. No significant geographical pattern of spread was detected for the more recent autumn-winter dominated period of diarrhea in Mexico.

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ETIOLOGY OF ACUTE DIARRHEA IN A POPULATION LIVING IN UZBEKISTAN

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To detect the etiological pattern of acute diarrhea there is project since 2004 focused on identifying the causative agents of diarrheal disease. Totally 2472 stool samples have been obtained from five regions of Uzbekistan (2070 from infectious hospitals and 402 from out-patients institutions). In 433 cases the causative diarrheal agents was not detected. The Rotavirus was detected 17.5% and 45% (2070/569 and 402/181) of hospitalized and out-patient cases, respectively. Amongst the bacterial enteropathogens isolated, antimicrobic resistance is emerging performing resistance for gentamycin, chloramphenicol and polymyxin, moderate resistance for tarivid, netilmicin sulfate and remains sensitivity for quinolones. In north regions (Khorezm region and Republic of Karakalpakstan) Rotavirus was detected in 25% (1039/264) and in south regions - 39.4% (1008/397). Thus, south territories, bacterial pathogens are isolated with highest frequency while in north territories viruses outrank bacteria in detected causes of acute diarrhea. In Uzbekistan in the last decade there has been an etiological shift from bacterial agents causing acute diarrhea to viruses, like rotavirus, maybe due to improved and enhanced laboratory techniques. Primary causes of diarrhea vary geographically. Ecological and social risk factors were correlated with duration and outcome of acute diarrhea.

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DIARRHEAL DISEASE AND HOUSEHOLD DRINKING WATER QUALITY IN BONAO, DOMINICAN REPUBLIC

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Consumption of unsafe water causes gastrointestinal illnesses killing >1.4 million people annually, and reducing quality of life affecting millions more. After performing a cross-sectional survey in summer 2005 in under-served communities near Bonao, Dominican Republic, we enrolled ~150

households to participate in a longitudinal, cohort study examining water source, water quality and diarrheal disease over a four-month period from Sept., 2005-Jan., 2006. Weekly field interviews asked questions on diarrheal disease, water source and hygiene. Household drinking water samples were taken approximately every two weeks. Household drinking water samples were distributed as follows: 44% piped source, 27% wells, 10% rain water, 5% springs, 11% bottled water, and 3% river water. Of the >1500 samples processed, 31% were exposed to some type of treatment of which 75% were boiled and 23% chlorinated. Geometric mean *E. coli* MPN concentrations/100 ml were: 20 for untreated waters, 7 for treated waters and 5 for bottled waters. The distribution of *E. coli* contamination/100 ml was: 21% zero (0), 25 % 1-10, 32% 10-100, and 22% >100. Overall, household diarrheal disease incidence was estimated to be 1.6 cases per person year. However, diarrhea incidence rates in children under 5 years of age were estimated at 4.6 cases per person year. Households reporting diarrhea had a geometric mean of 32 MPN *E. coli*/100 mL as compared to 20 MPN *E. coli*/100 mL in households not reporting diarrhea, a significant difference at the p 0.05 level. In addition, when comparing the distribution of drinking water contamination in households with/without diarrhea: *E. coli* at >100/100 mL (a high contamination level) was 36% of the drinking water samples in households reporting diarrhea as compared to only 26% of the drinking water samples in households that did not report diarrhea. While many households report taking some action to improve drinking water (treatment or purchasing of bottled water), *E. coli* contamination and diarrheal disease remain high. We suspect that fecally contaminated drinking water is contributing to the observed high burden of diarrheal disease. Therefore, we have implemented an intervention study to determine if *E. coli* levels in water and burdens of diarrheal disease are significantly reduced by implementing household, point-of-use water treatment with a biological sand filter.

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INTEGRATING PUBLIC HEALTH CONTROL STRATEGIES: BALANCING WATER SANITATION, AND HYGIENE INTERVENTIONS TO REDUCE DIARRHEAL DISEASE BURDEN

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The overall burden of diarrheal disease due to inadequate water, sanitation and hygiene remains high in spite of the reduced mortality due to Oral Rehydration Therapy. Despite this need to focus on environmental interventions, understanding of integrated control strategies remains poor. We seek guidance on integrating water, sanitation and hygiene control strategies by pursuing a modeling framework that captures the interdependent transmission pathways of enteric pathogens. Here we show that the benefits of a water quality intervention depend on sanitation and hygiene conditions. When sanitation conditions are poor, water quality improvements may have minimal impact, regardless of the amount of water contamination. If each transmission pathway alone is sufficient to maintain the disease, single pathway interventions will have minimal benefit and ultimate success is obtainable only by eliminating all sufficient pathways. However, when one pathway is critical to maintain the disease, public health efforts should focus on this critical pathway. Our findings provide guidance in understanding how to best reduce and eliminate diarrheal disease through integrated control strategies.

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DETERMINANTS OF HOUSEHOLD WATER QUALITY IN COASTAL GHANA

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Unsafe water, sanitation and hygiene produce almost 6% of the total disability adjusted life years in high mortality countries. Provision of clean water through municipal or private systems has not yielded anticipated health improvements, suggesting the need to better understand socio-demographic determinants of water use and quality. This paper examines associations between socio-demographic characteristics and household drinking water quality in a representative sample of households residing in coastal districts of Ghana's Central Region. Data were collected in 2004 in 36 enumeration areas (EA) stratified by three levels of urbanization. In each EA, 24 households were chosen for water quality study and socio-demographic interview, with a final sample of 703 households. Drinking water was collected within households and came directly from the vessel used for water consumption. Drinking water was put in sterile plastic containers, stored on ice and transported to the laboratory in ≤ 6 hours. Total coliforms and *Escherichia coli* were quantified using enzyme-based defined substrate technology (IDEXX Colilert®). We report here on the association between *E. coli*/100ml H_2O after linear regression adjustment for rural/urban residence, household socioeconomic factors, and all other sanitation factors. About 26% of households had <2 *E. coli*/100ml, and 23% had >250 *E. coli*/100ml. Drinking water from a tap has significantly lower *E. coli* levels than well water, and surface or rainwater had the highest *E. coli* levels. Households with a water closet toilet have significantly lower *E. coli* compared to those using pit latrines or no toilets. Household size is positively associated, and the household asset (socioeconomic status) index is negatively associated, with *E coli*. Neither rural/urban residence nor presence of home electricity is associated with *E. coli.* Variations in community and household socio-demographic and behavioral factors are key determinants of drinking water quality, net of water source. These behavioral factors should be included in planning health education associated with investments in water systems.

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CERAMIC FILTERS FOR HOUSEHOLD-SCALE DRINKING WATER TREATMENT IN RURAL CAMBODIA: INDEPENDENT APPRAISAL OF INTERVENTIONS FROM 2002-2005

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This study is an independent follow-up assessment of several implementations of the household-scale ceramic drinking water purifier (CWP) conducted by two NGOs over a period of four years (2002-2005) in rural Cambodia. Approximately 1000 household filters were introduced by Resources Development International (RDI) in Kandal Province from December 2003 and 1000+ filters by International Development Enterprises (IDE) in Kampong Chhnang and Pursat provinces from July 2002. This study assesses the water quality and health impacts of the CWP interventions to date. The study design was a longitudinal prospective cohort study of 80 households using filters (selected from all households receiving the intervention over the 4 year period) and 80 control households. We measured (i), the microbiological effectiveness *in situ* of the filters, as determined by the reduction of the indicator bacterium *E. coli*; and (ii), the health impacts of the filters as determined by data on diarrheal disease prevalence proportions among filter users versus non-users. Stratified analyses and log-risk regression with Poisson extension of generalized estimating equations (GEE) were employed in analysis of longitudinal data. Major findings are that (i), the filters reduced *E. coli*/100ml counts by a mean 95.1% in treated versus untreated household

water, although demonstrated filter field performance in some cases exceeded 99.99%; (ii), microbiological effectiveness of the filters was not observed to be closely related to time in use; (iii), the filters may be subject to recontamination, probably during regular cleaning; and (iv), the filters were associated with an estimated 46% reduction in diarrhea in filter users versus non-users (RR: 0.54, 95% CI 0.41-0.71).

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DIAGNOSIS AND QUANTIFICATION OF PEDIATRIC HIV-1 INFECTION BY AN ULTRASENSITIVE HIV-1 P24 ASSAY ADAPTED TO DRIED BLOOD SPOT SPECIMENS

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The assessment of the HIV-1 status of children in resource-limited countries is difficult and occurs often only in advanced disease. We report the adaptation of the ultrasensitive HIV-1 p24 antigen assay to dried blood spots (DBS-p24) for diagnosing pediatric HIV-1 infection. Seventy two untreated Tanzanian children, as well as 95 HIV-infected and 159 HIV-negative subjects from Switzerland were enrolled for this study. DBS-p24 results were compared to plasma-p24 results and to results obtained by real-time PCR to detect DBS HIV-1 DNA or plasma HIV-1 RNA. Thirty eight Tanzanian children were HIV-1 infected, and displayed following subtypes: 18 C, 9 A1, 8 D, 1 AC, 1 J-like and 1 unidentified. Among subtype C (n = 18), all samples except one (389 RNA copies/mL) were DBS-p24 positive, disclosing a sensitivity of 94.4% (CI95% = 76 - 99%). Observed specificity was 100%. Correlation between DBS-p24 and plasma-p24 concentrations was excellent (R2 = 0.83; P < 0.0001). DBS-p24 antigen sensitivity for non-D subtypes was 93% (CI95% = 81 - 99%). False-negative results obtained with the DBS-p24 assay were clearly sequence-related and significantly associated with subtype D (P<0.01). Thus, in subtype C predominant areas, DBS-p24 assay is an inexpensive and valid alternative to PCR-based tests for early diagnosis of pediatric HIV-1 infection.

(ACMCIP Abstract)

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THE EFFECT OF CO-TRIMOXAZOLE PROPHYLAXIS AND INSECTICIDE-TREATED BEDNETS ON THE RISK OF MALARIA AMONG HIV INFECTED UGANDAN CHILDREN

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Studies from Africa have shown that HIV infection is associated with an increased risk of clinical malaria and parasitemia among pregnant and non-pregnant adults. Use of co-trimoxazole (CTX) prophylaxis in HIV infected persons is associated with decreased morbidity and mortality and is now widely recommended for all HIV infected patients in Africa. Among HIV infected adults, one of the benefits of CTX prophylaxis is a documented decrease in the risk of malaria. However, limited data exist on the effect of CTX on malaria risk in children, the group that suffers the greatest burden of this disease. From October 2005 through April 2006 we have enrolled 172 of a planned 300 HIV infected children cohort ages 1-10 years from a dedicated HIV clinic in Kampala, Uganda where all children are given CTX prophylaxis and 89% are using insecticide treated bednets (ITNs). A concurrent cohort of 563 children aged 1-10 years recruited from the surrounding community has been followed during this same time period. Children from this community based cohort are not taking CTX prophylaxis and 6% report ITN use. Similar protocols are being used to follow both cohorts. Parasite prevalence is measured at enrollment and children are followed for all of their health care needs. During follow-up thick blood smears are done in all children who present with a new episode of fever and an episode of malaria is diagnosed if the thick smear is positive for malaria parasites. None of the 172 HIV infected children prescribed CTX prophylaxis had a positive blood smear at enrollment compared to a parasite prevalence of 20% among children recruited from the community (p<0.0001). Between October 2005 and April 2006, only 2 episodes of malaria (incidence = 0.05/person year) were diagnosed among HIV infected children compared to 236 episodes (incidence = 0.79/person year, p<0.0001) among children recruited from the community for an incidence rate ratio of 0.06. The use of CTX prophylaxis and ITNs among HIV infected children was associated with a dramatic reduction in the risk of malaria.

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ASSESSMENT OF PAEDIATRIC ANTIRETROVIRAL TREATMENT PROGRAM CHARACTERISTICS IN SUB-SAHARAN AFRICA: THE KIDS-ART-LINC COLLABORATION

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Paediatric HIV burden in sub-Saharan Africa is currently estimated at 2.1 million children <15 years and significantly contributes to under-five mortality. Highly active antiretroviral therapy (HAART) improves survival and quality of life, yet programmatic confines may hinder ART universal access. The Paediatric Antiretroviral Treatment Programs in Lower-Income Countries (KIDS-ART-LINC) collaboration is an international network aimed to document and improve pædiatric ART treatment programs in Africa. We assessed programs characteristics of the collaborating clinics in 2006. We evaluated 21 of the 26 participating treatment programs using a standardized assessment tool during site visits in 14 countries On the average, 195 children receive HAART in each of the 23 clinics, 17 of which are based in public facilities. Five programs exclusively treat children. The minimum care package offered by all programs includes medical consultation, cotrimoxazole prophylaxis, HAART, CD4 monitoring and a complete blood count. All programs but two provide free ARVs. Seven programs perform viral load estimates, 12 offer early infant HIV diagnosis at clinic sites and only 8% of children on ART were <2 years old. Standardized record forms are used in 20 sites, but only 12 have a computerized and functional data management system. Clinical criteria for starting HAART essentially correspond to WHO guidelines. Lack of paediatric formulation delayed HAART initiation in 9 programs. There is follow-up of children in 13 programs. 21 programs' first-line HAART regimens are non-nucleoside (NNRTI)-based (13 nevirapine, 8 efavirenz) while 2 provide ritonavir-boosted lopinavir and nelfinavir-based regimens. The nucleosidic (NRTI) drugs used are lamivudine and either zidovudine or stavudine. In conclusion, there is good standardization of clinical practices in the pædiatric treatment programs surveyed across sub-Saharan Africa but monitoring and patient information systems are commonly lacking. In most sites, access to DNA-PCR technology for early infant HIV diagnosis has not yet been operationalized and the number of infants receiving ART is still limited. Information systems are needed to accurately estimate and document impact of HAART in children. KIDS-ART-LINC will enhance

information systems, describe models of care and evaluate the impact of program characteristics on treatment outcomes

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CD4 T COUNT AND HIV-1 INFECTION IN PATIENTS WITH UNCOMPLICATED MALARIA

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HIV-1 negative children with malaria have reversible lymphocyte and CD4 count decrease. We assessed the impact of malaria parasitaemia on the absolute CD4 count in both HIV-1 infected and non-HIV infected adults. In Ndola, Zambia, at health center level, we treated 327 non-pregnant adults for confirmed uncomplicated clinical malaria. We assessed HIV-1 status, CD4 count and HIV-1 viral load (if HIV-1 infected) at enrolment, 28 and 45 days after treatment. After successful antimalarial treatment, median CD4 count increased from 468 to 811 cells/µl in HIV-1 negative and from 297 to 447 cells/µl in HIV-1 positive patients (paired t-test P <.001 for both). CD4 count increment was inversely correlated with CD4 count at day 0 in both HIV-1 negative (P<0.001) and HIV-1 positive patients (P=.03). the proportion of patients with CD4 count <200/µl decreased from 9.6% to 0% in HIV-1 negative and from 28.7% to 13.2% (P<.001 for both) in HIV-1 positive malaria patients. In patients with detectable parasitaemia, CD4 count and viral load at day 45 were similar than those at day 0. In conclusion, the interpretation of the absolute CD4 count might be biased during or just after a clinical malaria episode or even in case of asymptomatic parasitaemia. Therefore, in malaria endemic areas before taking any decision on the management of HIV-1 positive individuals, their malaria status should be assessed. Furthermore, as malaria in itself causes a reversible decrease in CD4 count, HIV-1 infected individuals might be at risk for clinical malaria and malaria treatment failure at higher CD4 levels than reported so far. The threshold for an increased risk of malaria treatment failure and clinical malaria is estimated around 400 CD4 cells/µl.

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MATERNAL HIV LOAD AND LOW CCR5 EXPRESSION HAPLOTYPES ARE ASSOCIATED WITH REDUCED MOTHER-TO-CHILD HIV TRANSMISSION IN MALAWI

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CCR5 promoter and CCR2b gene single nucleotide polymorphisms (SNP) have been associated with protection against HIV transmission in adults and with delayed HIV progression to AIDS. Previous studies have reported that SNP CCR5 -2132T was associated with protection against mother to child transmission of HIV-1 (MTCT). We investigated the association between infant CCR2/CCR5 genotype and MTCT in Malawi. 552 blood samples from infants of HIV positive women were genotyped using a multiplex ligase detection reaction and PCR. Following verification of Hardy-Weinberg equilibrium, log linear regression was performed to determine the association between mutations and MTCT. Maternal Viral Load (MVL) was dichotomized at the median (above/below 70,000) and evaluated for interaction with SNPs following a dominant model. Risk difference modification by MVL was assessed by comparing the risk ratio at each joint level of mutation carrier status (yes/no). The Likelihood Ratio Test (LRT) was used to determine homogeneity of the association across MVL categories. Overall, protection against MTCT was weakly associated with CCR5 -2459G (Risk ratio [RR], 0.77; confidence interval [CI], 0.54-1.12), and CCR5 -2135T (RR, 0.78; CI, 0.54-1.13). These SNPs are linked, and present in CCR5 haplotypes A - D (in contrast to E - G2), and have been associated with lower CCR5 expression and HIV-1 propagation. Among mothers with low MVL, statistically significant protection against MTCT was observed for -2459G (RR, 0.50; CI, 0.27-0.91), and -2135T (RR, 0.51; CI, 0.28-0.92). Statistically significant protection was not found at high MVL. Borderline evidence of non-homogeneity of the association across MVL categories was observed for -2459G (LRT Chi square=3.83, p=0.050) and -2135T (LRT Chi square=3.60, p=0.058). This analysis suggests that CCR5 haplotypes A - D may protect against MTCT of HIV at low MVLs, whereas high MVLs may over-ride differences in coreceptor availability.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA ON MOTHER-TO-CHILD TRANSMISSION OF HIV IN MOZAMBIQUE

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Malaria and AIDS are the most prevalent infections in sub-Saharan Africa and there is evidence that suggests that malaria infection may increase mother-to-child transmission (MTCT) of HIV. Several studies have suggested that placental malaria (PM) may impact MTCT of HIV but the few studies performed thus far have given contradictory results. The objective of this study was to determine whether, intermittent preventive treatment (IPT) with sulphadoxine-pyrimethamine (SP) given to HIV-infected pregnant women has an impact on MTCT of HIV in a malaria endemic region. This study was integrated into a randomized double blind, placebo-controlled trial of two-dose IPT given to pregnant Mozambican women in conjunction with insecticide treated bed nets. After obtaining informed consent, two doses of SP were administered at the beginning of the second trimester of pregnancy one month apart. At the time of the study, anti-retroviral treatment was not available and nevirapine was self-administered at the onset of labor and given to the infant. HIV status of infants was evaluated by HIV-DNA PCR at 1 month of age.

Of the 207 HIV-infected women enrolled between 2003 and 2005, 90 received placebo and 117 received SP. The median viral load at delivery was not significantly different between groups (***Placebo***: 18086 copies/mL, ***SP***: 16312 copies/mL p=0.62) nor was the median CD4 count one-month after delivery (***Placebo***: 591, ***SP***: 661 p=0.327). MTCT of HIV did not differ significantly between treatment groups (***SP***: 15%, ***Placebo***: 19% p=0.59), although IPT appeared to decrease active PM. Histological analysis of placentas suggested that there was no significant difference in MTCT in women with active PM as compared to women negative for PM. Interestingly, analysis of different categories of PM suggested that active PM (presence of parasites) and past PM (presence of pigment) may have different effects on MTCT. These factors and associations with cytokine and chemokine levels in the placenta will be discussed.

These results indicate that two-dose IPT may not have a significant impact on MTCT of HIV, but reveal a potentially complex interaction between PM and MTCT. Possible mechanisms will be discussed.

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THE EFFECT OF HELMINTH INFECTIONS AND THEIR TREATMENT DURING PREGNANCY ON VERTICAL TRANSMISSION OF HIV INFECTION IN UGANDA: RESULTS OF A RANDOMISED, DOUBLE-BLIND, PLACEBO CONTROLLED TRIAL

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It is suggested that helminths may promote vertical HIV transmission through effects on lymphocyte activation *in utero* or in infancy. The objective of this study was to examine effects of helminths and their treatment during pregnancy on vertical HIV transmission. A cohort of 302 HIV-1-infected women was recruited within a larger trial of anthelmintic treatment in pregnancy. After providing stool and blood samples for analysis for helminth infection and malaria, women in the second or third trimester of pregnancy were randomised to receive albendazole or placebo and praziquantel or placebo in a 2x2 factorial design. In keeping with the government programme for prevention of mother-to-child HIV transmission, women were counselled and given nevirapine as a single dose, to be taken during labour; neonates were treated with nevirapine syrup. Maternal viral load was measured at enrolment and delivery, infant viral load in cord blood and at age six weeks, by viral RNA assays. The prevalence of helminths among HIV-infected women was 68% (hookworm (39%), *Mansonella perstans* (22%), *Schistosoma mansoni* (19%) and *Strongyloides* (12%)). Preliminary data show no significant associations between helminth infections or malaria and maternal viral load at enrolment. The incidence of vertical transmission at six weeks of age is 17% (14% among those who report using nevirapine as recommended). Vertical transmission shows an expected, highly statistically significant positive association with maternal plasma viral load ($p<0.001$) and a possible association with malaria at enrolment ($p=0.16$). As yet there is no evidence of an association between vertical transmission and socio-demographic factors that might confound an association with helminths. All infants will reach age six weeks in June 2006. An unblinded analysis of effects of helminths and their treatment will be performed thereafter. The study has power to detect just over a doubling or halving of transmission by helminths, or by either intervention. Results will be presented at the meeting.

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GENOME-WIDE DIVERSITY MAP OF *PLASMODIUM FALCIPARUM*

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Understanding the extent and degree of genetic variation in *Plasmodium falciparum* has important implications for the development of intervention strategies. To assess genome-wide genetic diversity in this important human pathogen, we took a three-pronged sequencing approach that included full genome sequence (8X coverage) of the HB3 and Dd2 strains; whole genome shotgun sequencing of 12 additional strains isolated from globally diverse populations at low coverage (0.25X); and sequencing PCR products derived at defined intervals across twenty genomic regions from 16 geographically diverse strains. Collectively we identified 46,937 single nucleotide polymorphisms, and successfully genotyped and analyzed a preliminary set of 372 SNPs across the 20 loci in 54 unique strains from around the world. The data reveal a very rich diversity among *P. falciparum* parasites ($= 1.04 \times 10^{-3}$) and very little correlation between linkage disequilibrium and distance across a global parasite population. Markers separated geographically distinct parasite populations, and several potential signatures of selection, including a previously identified selective sweep on chromosome 7, were identified across the genome. As the first genome-wide sequence survey of genetic diversity in *P. falciparum*, these data provide the foundation for a map of genetic diversity in the organism. Such a map will create a powerful tool to determine population structure, estimate linkage disequilibrium, and identify polymorphisms for subsequent association studies to identify genes that mediate drug resistance or virulence. Finally, such a map enables the identification of specific loci that are subject to natural selection and provides the basis for tracing the historical and future spread of malaria worldwide.

(ACMCIP Abstract)

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BXB1 MYCOBACTERIOPHAGE INTEGRASE-MEDIATED SITE-SPECIFIC INTEGRATION INTO *PLASMODIUM FALCIPARUM* AND ITS APPLICATION TO THE STUDY OF THE VAR GENE FAMILY

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We recently reported the development of an efficient, site-specific system for genetic integration into *Plasmodium falciparum* chromosomes, mediated by Bxb1 mycobacteriophage integrase. Parasite lines were developed with an *attB* site integrated into the non-essential glutaredoxin-like *cg6* gene or the *hrp3* gene, and transfection of these lines with a dual plasmid system produced recombinant parasites that were detectable within 14-25 days, and were genetically uniform for single copy plasmid integration. In contrast to episomally transfected lines, recombinant *attB* × *attP* parasites were phenotypically homogeneous and genetically stable in the absence of drug. We are now applying this system to study gene regulation and cytoadherent domain properties of the *var* variant antigen family. Parasites were generated with single-copy reporter cassettes integrated in central and subtelomeric loci to quantitate levels of expression from a *var* promoter, with or without the *var* intron. Additionally, we targeted the *attB* site to a subtelomeric *var* locus in two different parasite strains, such that an open-reading frame could be driven from a constitutive promoter upon integrase-mediated recombination. These parasites should be amenable to rapid *var* domain shuttling and analysis of cytoadherence properties. Bxb1-based integrative recombination thus provides a powerful new tool for genetic studies of intracellular eukaryotic organisms.

(ACMCIP Abstract)

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MICROSATELLITE DIVERSITY OF *PLASMODIUM VIVAX* ISOLATES FROM SRI LANKA

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Plasmodium vivax is the most globally widespread of the four malaria parasite species that infect humans. Over one billion people live in ***P. vivax*-**endemic areas and 70-80 million clinical cases are reported each year. Although extensive polymorphism has been observed in several antigen-coding loci previous studies with the use of microsatellite arrays in natural ***P. vivax*** populations have yielded contradictory results.

We have optimized a novel set of 14 *P. vivax* microsatellite markers with repeat units of either three or four nucleotides and identical amplification protocols for all loci, enabling multiplex PCR easy to standardize. These markers were used to characterize 25 field isolates of *P.vivax* from Sri Lanka and 3 reference strains of *P.vivax* with measurement of length variation of labeled PCR products using ABI PRISM 3730XL DNA Analyzer. All markers were found to be highly polymorphic. The number of alleles per locus ranged from 6 to 13 (average, 7.8), with expected heterozygosity *(H_E)* estimates in the Sri Lankan population ranging between 0.627 and 0.913 (average, 0.790). More than one allele was amplified for at least one marker in 52% of isolates. We also compared sizes of PCR products, for all 14 markers, amplified from: (a) genomic DNA from two field isolates and (b) whole genome amplification (WGA) products derived from the same isolates by performing multiple displacement amplification using a REPLI-g Mini-kit (Qiagen). Exactly the same alleles were scored when either template was used for PCR, suggesting that WGA would enable microsatellite analysis of limited amounts of DNA. This is particularly useful for organisms that cannot be efficiently propagated *in vitro*, such as *P. vivax*.

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TRANSFERRIN POLYMORPHISM INFLUENCES THE RISK OF SEVERE MALARIAL ANAEMIA IN GABONESE CHILDREN

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Severe malarial anaemia (SMA) is one of most complications of severe malaria in African children. Although the factors that determine the patient outcome of malaria have not been completely defined, host genetic factors are implicated. SMA is often associated with iron deficiency, but investigations of soluble transferrin revealed controversial data. Several studies have involved transferrin polymorphisms in pathogenesis of diseases associated with iron deficiency. We investigated polymorphisms of transferrin and TfR1 (transferrin receptor 1) from 208 Gabonese children with uncomplicated malaria, mild malarial anaemia or SMA. The mean age of children with SMA was 27.7 months confirming that SMA is associated with young age ($p<0.05$). Transferrin exons (7, 8, 12, and 15) and TfR1 exon 4 were analysed by PCR-RFLPs. For transferrin exon 7, the C3 allele characterized by the G258S mutation (A879G transition), is associated with the development of SMA (10.3 % vs 0 %, $p<0.05$). This is consistent with observations showing that the C3 allele increased the risk of haemolytic anaemia. The C2 allele, characterized by amino acid mutation P570S (T1815C transition) is associated with the decreased risk of development of SMA (25.6 % vs 2.6 % and 10.8 %, $p<0.05$). Frequencies of C2 and C3 alleles are respectively 2 % and 12% in the population of the study. Polymorphism of TfR1 (S142G) is not associated with the development of SMA and polymorphisms of transferrin exons 8 and 12 were not detected. This is the first evidence of the involvement of transferrin polymorphism in the pathogenesis of SMA. Further studies to investigate the implication of transferrin genotypes on the frequency of SMA are needed.

(ACMCIP Abstract)

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MOLECULAR CLONING OF A NOVEL *TRYPANOSOMA CRUZI* CELL SURFACE CASEIN KINASE II SUBSTRATE, TC-1, THAT MEDIATES EARLY CELLULAR INFECTION

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We report the cloning and characterization of the first cell surface casein kinase (CK) II substrate (Tc-1) of *Trypanosoma cruzi*. The gene has a 1653 base pair ORF coding for 550 amino acid residues. Northern blot analysis shows a 4.5 kb transcript that is expressed in invasive trypomastigotes but not in non-invasive epimastigote forms of *T. cruzi*. Southern blot analysis indicates that Tc-1 is a single-copy gene. The coded protein has a putative transmembrane domain with multiple cytoplasmic and extracellular CKII phosphosites. Human exogenous CKII phosphorylates serine residues on both recombinant Tc-1 and Tc-1 of intact trypomastigotes. This phosphorylation was inhibited by the CKII inhibitors. Immunoblot analysis indicates that Tc-1 is only expressed in infective trypomastigotes. Immunoprecipitation of labeled surface proteins of trypomastigotes indicates that the 62-kDa protein is a surface protein. Antibodies to Tc-1 effectively blocked trypomastigote invasion of host cells and consequently reduced parasite load. Pre-incubation of either trypomastigotes or myoblasts with CKII Inhibitors blocked *T. cruzi* infection. Thus, we describe for the first time a cell surface CKII substrate of a protozoan parasite that is phosphorylated by human CKII and is involved in early cellular infection.

(ACMCIP Abstract)

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NORMAL RANGES FOR CHEMISTRY AND HEMATOLOGY PANELS IN MALI

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In order to perform Phase 2 Studies of a candidate antimalarial, it is necessary to establish a clinical laboratory on-site to obtain accurate results with reasonable turn-around times. For example, the protocol approved for these studies by IRBs in Mali and the US requires that persons with laboratory values sufficiently abnormal to require treatment must be identified and excluded, even if they are otherwise eligible to participate. To accomplish this goal, we have purchased two Piccolo Abaxis analyzers and two AcT10 Coulter Counters, and have established that the values they yield are indistinguishable from those obtained at the Tulane University Hospital. Now that these instruments have been shipped to Mali, the purpose of the study reported here was to determine whether the frequency of abnormal results (by American standards) was similar in normal healthy Malian adults (persons who have volunteered to donate blood at the National Transfusion Center in Bamako). The results obtained with 45 normal subjects indicate that abnormal results were more frequent with determinations of blood glucose (8/45=18%), creatinine 11/44=25%), BUN (10/46=22%), creatine kinase (6/46=13%), albumin (7/36=19%), total protein (16/34=47%), alkaline phosphatase (5/23=22%), ALT (7/35=20%), AST (5/36=14%), GGT (3/36=8%), amylase (7/36=19%), Na^+ (14/55=25%), K^+ (7/45=16%), and Cl^- (11/45=24%),

but not CO_2 (1/42=2%). These results suggest that the range of chemistry values in healthy clinically normal subjects may be greater in mali than in the United States.

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IDENTIFICATION AND CHARACTERIZATION OF A NOVEL *PLASMODIUM* PROTEIN RESPONSIBLE FOR HEMOZOIN FORMATION - IMPLICATIONS FOR ANTIMALARIAL DRUG DEVELOPMENT

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During the intraerythrocytic stages of infection, malaria parasite rapidly cannibalizes host hemoglobin. The globin chain is proteolytically processed by parasite proteases and the amino acids recycled to support its rapid development. However, heme released as part of this process is extremely toxic for the parasite and to protect itself, parasite undertakes its detoxification primarily by converting it into an inactive product called hemozoin. The process of hemozoin formation is targeted by some of the most effective antimalarial drugs which primarily act by binding to the free heme and thereby prevent its detoxification into hemozoin. However, parasite factors responsible for hemozoin formation are poorly identified and remain controversial. We have identified and characterized a novel *Plasmodium falciparum* protein that efficiently converts free heme into hemozoin. A single molecule of this protein converts greater than 500 molecules of heme into Hz, a conversion rate at least an order of magnitude higher than any of the known parasite factors capable of Hz synthesis. Due to its activity, we have named this molecule as the heme detoxification protein or HDP. Orthologs of this protein have been identified in rodent, simian and avian *Plasmodium* species and the protein was found to be functionally conserved. We also found that after merozoite invasion, ring form parasites express and secrete this protein into the erythrocyte cytosol before any detectable amount of Hz is visible inside the parasite. Subsequently, HDP, accompanied by host hemoglobin, is delivered to the parasite food vacuole, the site of Hz formation. These results establish that HDP is a key parasite factor responsible for hemozoin formation and targeting this molecule could lead to the discovery of new anti-malarial drugs effective against all *Plasmodium* species.

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STRUCTURE-BASED DRUG DESIGN TARGETING *PLASMODIUM FALCIPARUM* HSP90

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Heat shock protein 90 (hsp90) is an essential chaperone involved in the trafficking of proteins in *Plasmodium falciparum*. Drugs such as radicicol and geldanamycin which target hsp90 appear to have anti-malarial activity that synergizes with traditional quinoline-based antimalarials. Here we present the crystal structure of the N-terminal ATP-binding domain of *P. falciparum* hsp90 resolved to 2.7Å. Using this structural data, we also demonstrate a chemical synthetic approach to generate a library of compounds based on a radicicol scaffold, for which preliminary data on anti-malarial activity is presented.

(ACMCIP Abstract)

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PHARMACOKINETIC STUDY OF INTRAMUSCULAR AND INTRARECTAL ARTEMETHER APPLICATION FOR THE ACUTE ATTACK TREATMENT OF MALARIA

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Vomiting and gastrointestinal upset are common features in patients with malaria. Alternative ways for administration the drugs are needed to create a fast therapeutic effect. With this knowledge two different formulations for parenteral applications were investigated. The first is an intramuscular injection and the second a soft gel capsule (suppogel) for intrarectal application, both containing artemether dissolved in oil. The study medicines were administrated to healthy volunteers of Ivory Coast in the Urban Sanitary Formation in the Suburban Community of Abidjan. From 11 patients blood samples were taken after i.m. administration of artemether (4 mg/kg). The same 11 volunteers together with 4 new volunteers were included in the study were artemether (4 mg/kg) was administered rectally using suppogels. Plasma samples were taken at regular time intervals after administration and the concentration of artemether and DHA was determined by Liquid Chromatography-Mass Spectrometry. After intramuscular injection, within 1 hour therapeutic levels were obtained and the Tmax for both compounds, Am and dha, was found at 12 ± 2 hours. Therapeutic levels were maintained throughout the observed 24-hour period. After rectal application, therapeutic levels were obtained within 1 h and the Tmax for DHA was 3 ± 2 hours and for artemether 5 ± 2 hours. Therapeutic levels were maintained throughout a 12-hour period. Maximum plasma levels of the sum of dha and of Am were about the same in both groups. When comparing the outcome of these two studies it is obvious that rectal administration leads to an early Tmax. The i.m. injection gives a much more protracted plasma curve. In conclusion, it can be concluded that both product are present in a satisfactory concentration in blood plasma within one hour after application, but the artemether release from its formulation and the uptake in the blood is much faster in case of intrarectally used suppogels. This observation can also be useful when treating patients with complicated malaria.

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EVALUATION OF ALKYLAMINOQUINOLINYL-METHANOLS AS NEW ANTIMALARIALS

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The quinolinyl methanol drug, mefloquine, is an important antimalarial agent with a long half-life in humans, which allows for its use as an effective single dose treatment for malaria and as a once weekly dosing for prophylaxis. However, clinical application of mefloquine is limited by its relative costliness and its debilitating neurological side effects. We, therefore, evaluated a series of 2-phenyl substituted alkylaminoquinolinyl-methanols (AAQMs) for their potential as novel anti-malaria agents that retain the efficacy of mefloquine, yet lack neurotoxicity. These AAQMs were obtained from the U.S. Army's Chemical Information System and tested in a series of *in vitro* and *in vivo* efficacy and toxicology screens and in a theoretical cost of goods analysis. In general, we found that the AAQMs were less neurotoxic, exhibited greater anti-malaria potency and are potentially cheaper than mefloquine, but showed poorer metabolic stability and pharmacokinetics and the potential for phototoxicity. From

this series we identified a lead compound, referred to as WR069878, and determined structural modifications expected to result in a novel anti-malaria agent lacking neurotoxicity and phototoxicity. In particular, we anticipate that modification of WR069878 by substitution of an appropriate N functionality at the 4-position, optimization of quinoline ring substituents at the 6 and 7-positions, and deconjugation of quinoline and phenyl ring systems should yield a valuable new antimalarial drug.

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EVALUATION AND LEAD OPTIMIZATION OF ANTIMALARIAL AROMATIC KETONES

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Our research group recognized the antimalarial potential of xanthones and demonstrated through structural alteration that tricyclic molecules could be modified to improve their affinity for heme, to block the process of hemozoin formation, and to effectively kill *Plasmodium falciparum* parasites with 50% inhibitory (IC_{50}) values in the nanomolar range. More recently, we became interested in merging two drug design strategies to develop a tricyclic heme complexing molecule with powerful antimalarial properties that also exhibits resistance reversal activity. In order to effect this strategy we needed to switch to the structurally related acridone system to gain the ring nitrogen atom, essential for introducing resistance reversal functionality into the construct. While this objective is still being pursued, we were surprised to discover that a chemical intermediate formed in the course of this work but before it had been functionalized to bind heme, exhibited remarkably potent antimalarial activity. Over 30 acridone derivatives were synthesized to explore the structure-activity profiles. The most potent compounds contained extended alkyl chains terminated by trifluoromethyl groups and located at the 3-position of the tricyclic system. Acridones optimized in the length of the side chain and the nature of the terminal fluorinated moiety exhibited *in vitro* antimalarial IC_{50} values in the nanomolar and picomolar range and were without cytotoxic effects on the proliferation and differentiation of mitogen-activated murine lymphocytes at concentrations up to 100,000-fold higher. Based on a structural similarity to known antimalarial agents it is proposed that the haloalkoxyacridones exert their antimalarial effects through inhibition of the *Plasmodium* cytochrome bc1 complex. We will also present data on attempts to reduce the tricyclic pharmacophore to its minimal structural elements.

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NOVEL ANTIMALARIAL ACRIDONE DERIVATIVES WITH BOTH INTRINSIC POTENCY AND SYNERGY WITH SELECTED QUINOLINES: IN VITRO AND *IN VIVO* STUDIES

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Preventing or delaying emergence of drug-resistance is an essential goal of antimalarial drug development. Drug monotherapy and highly mutable drug targets have each facilitated resistance, and both are undesirable in effective long-term strategies against multidrug-resistant (MDR) malaria. Resistance to heme-binding drugs (e.g., chloroquine) is now extensive, but unlike examples of resistance due to target mutation (e.g., antifols, atovaquone), resistance to heme-binding drugs appears to result from diminished target access and, even in resistant parasites, heme remains an immutable and vulnerable drug target. As one arm of the development of novel antimalarial acridones that exploit heme as a target, we have sought drugs that are active alone against MDR parasites, but that also enhance the activity of potential partner antimalarials. Here we report the discovery of novel acridone derivatives that are intrinsically potent *in vitro* against both sensitive (D6) and MDR (Dd2) *Plasmodium falciparum*, synergistic with other antimalarials presumed to interfere with hemozoin formation, and orally active *in vivo*. To date, *in vitro* testing of the first of this chemotype (T3.5) has included drug susceptibility assays alone and in a series of fixed-ratio combinations with chloroquine, quinine, mefloquine and amodiaquine against *P. falciparum* D6 and Dd2. Intrinsic T3.5 IC_{50} values are submicromolar and, in combination, synergy is evident against both Dd2 (with quinine>>amodiaquine, chloroquine) and D6 (with quinine). In a model of patent *P. yoelii* infection, mice given a single gavage dose of T3.5 (100mg/kg) showed a rapid fall in parasite burden with residual 80% inhibition of parasitemia 72 hours after treatment. The unique profile of T3.5 (*in vitro* intrinsic potency, quinine-predominant synergy, and oral efficacy *in vivo*) as the first candidate drug of its chemotype, indicates that expanded therapeutic options and mechanistic understanding are both likely to result from study of this and related compounds. Synthesis and comparative testing of other acridone derivatives and mechanistic studies are underway and will be presented.

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UPDATE ON MAKING CGMP INTRAVENOUS ARTESUNATE AVAILABLE IN THE UNITED STATES

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Artemisinins are antimalarials derived from the Chinese herb, ***Artemisia annua***. These compounds clear the parasites from the blood more rapidly than other antimalarial agents. Intravenous formulations of artemisinins have been used in much of the world and represent an improvement in both efficacy and safety for severe malaria. There are however, currently no United States Food and Drug Administration (USFDA) approved artemisinin products available in the United States for the treatment of malaria.

Quinidine is the only currently USFDA approved intravenous drug for the treatment of severe malaria. While it is commercially available and effective against malaria, it is not an ideal drug. Most significantly, quinidine is no longer the drug of choice in treating certain electrocardiac disturbances and may soon cease to be available in the U.S. The Walter Reed Army Institute of Research has a long history with the artemisinins, which has culminated in an intravenous artemisinin product. Between 2000 and 2002, two derivatives were in consideration (artesunate and artelinate) for development to licensure. After intense scrutiny and eventual consideration in committee at the Division of Experimental Therapeutics, artesunate was selected as the compound to put forward into advanced development. Artesunate is currently in use in much of the malarial world as a non-ICH cGMP intravenous formulation produced in China, providing a potential wealth of clinical data. We envision our formulation of a cGMP-produced, FDA-licensed, intravenous artesunate being available for use in the very near future for US military and civilians, as well as for eventual use worldwide. We have successfully filed an IND for this product; clinical trials are underway. Our presentation will update 1) the progress that has been made in preclinical studies, 2 data from early cGCP Phase 1 trials (the first regulated Phase 1 work ever done with artesunate), 3) data from early cGCP Phase 2 trials in Kenya, 4) future planned clinical work, and 5) our overall strategy for gaining full licensure of this product in the United States.

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L-ARGININE INFUSION INCREASES NO PRODUCTION AND REVERSES ENDOTHELIAL DYSFUNCTION IN ADULTS WITH MODERATELY SEVERE FALCIPARUM MALARIA IN PAPUA, INDONESIA

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Severe falciparum malaria is associated with impaired nitric oxide (NO) production by the vascular endothelium and low concentrations of the precursor of NO, L-arginine. Severe malaria is also associated with endothelial inflammation, which is thought to exacerbate sequestration of parasitized red cells and thus microvascular obstruction. In cardiovascular disease, supplementation of L-arginine can improve endothelial function. We hypothesized that low concentrations of plasma arginine in falciparum malaria would result in impaired endothelial production of NO and thus cause endothelial dysfunction. We also hypothesized that replacement of L-arginine in falciparum malaria would increase NO production and reverse endothelial dysfunction. Thirty adults (18-60 yrs) hospitalized with moderately severe falciparum malaria (but without WHO criteria of severe malaria) at Mitra Masyarakat Hospital in Timika, Papua, Indonesia were enrolled in a single ascending dose response study to assess safety and preliminary efficacy of L-arginine. Endothelial function was measured by peripheral arterial tonometry (PAT) of the digital microvasculature before and after an ischemic stress, generating a reactive hyperemia PAT (RH-PAT) ratio. A ratio less than 1.67 has previously defined endothelial dysfunction. L-Arginine hydrochloride was given intravenously over 30 mins at doses of 3g (n=10), 6g (n=10) and 12g (n=10). Endothelial function and exhaled NO and plasma L-arginine were compared before and after arginine infusion. Results were also compared with a control group (n=42). Arginine infusion resulted in a 55% increase in exhaled NO (p=0.0001) and a 19% improvement in endothelial function, with mean RH-PAT index increasing from 1.76 (95% CI 1.62-1.89) to 2.06 (95% CI 1.84-2.25) (p=0.01). In the prospectively defined patient subgroup with impaired endothelial function (RH-PAT index <1.67; n=14), endothelial function improved 38% (p=0.004), with the increase in RH-PAT index being dose-related. There were no clinically significant changes in vital signs, pH, glucose or potassium after infusion. Arginine doses up to 12g appear safe and can improve NO production and endothelial function in adult patients with moderately severe malaria. Arginine has potential for adjunctive treatment of severe malaria where NO production and endothelial function are impaired to a greater extent.

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CLINICAL UTILITY OF MALARIA-SPECIFIC RETINAL FINDINGS IN PEDIATRIC CEREBRAL MALARIA

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Malaria-specific retinal findings are sensitive and specific markers of cerebral sequestration of malaria parasites in children with clinically defined cerebral malaria (CM). We postulated that children with clinically defined cerebral malaria (coma and peripheral parasitemia) without malarial retinopathy, in whom cerebral sequestration of parasitized red cells is unlikely, might therefore be similar to aparasitemic children with comas of other (usually unknown) causes (COC) in terms of clinical course and outcome. We examined data from 958 comatose children admitted to our ward from 1996-2005 who had retinal exams by direct and indirect ophthalmoscopy: 885 children with clinically defined cerebral malaria (554 with malarial retinopathy (MR+), 331 without (MR-)) and 73 aparasitemic children with non-malarial comas. We compared these groups in terms of clinical findings on admission, coma resolution time, outcome, and time to death. We excluded children with bacterial meningitis from the analysis. No clinical finding distinguished between the patient groups. Mean coma resolution time for the MR- group (21.9 hours) was similar to that for the COC group (21.8 hours) and was significantly shorter than for the MR+ group (31.3 hours, p<0.0001). However, the MR- group had significantly lower mortality (7.0%) than the COC (26%) and CM (20.4%) groups (p<0.0001). In conclusion, children who meet the clinical case definition of cerebral malaria, but who do not have any evidence of malarial retinopathy, recover more quickly and more frequently than do children who meet the clinical case definition and who have one or more features of malarial retinopathy. These findings highlight the utility of retinal examination in comatose patients with clinically suspected cerebral malaria; while the patients without retinal findings certainly require treatment for malaria, their prognosis is better than for patients with retinal findings, and they might benefit from additional investigations into other potential causes of coma.

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COMPARISON OF RECTAL DIAZEPAM TO BUCCAL MIDAZOLAM IN TREATMENT OF PROLONGED CONVULSIONS IN UGANDAN CHILDREN

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Prolonged convulsions are convulsions lasting over five minutes and are pediatric medical emergencies that warrant urgent and safe treatment. Rectal diazepam is the first-line treatment for convulsions in Uganda. Its effectiveness is limited by erratic absorption and the risk of respiratory depression. Efficacy and safety data on rectal diazepam and alternative anticonvulsants is limited in Uganda. Buccal midazolam has been shown to be efficacious for treatment of prolonged convulsions in other countries. We are currently conducting a randomized blinded trial comparing the efficacy and safety of rectal diazepam to buccal midazolam for treatment of children with prolonged convulsions in the pediatric emergency unit in Uganda's national referral hospital. A blinded interim analysis including 175 patients (target size = 350) is presented here. Consecutive patients aged 3 months to 12 years who presented with a prolonged convulsion or experienced a prolonged convulsion in the unit were randomized to receive buccal midazolam (0.5 mg/kg) plus rectal placebo or rectal diazepam (0.5 mg/kg) plus buccal placebo. The most common cause of convulsions was malaria (66%); meningitis, respiratory tract infections and epilepsy accounting for the others. Treatment success was defined as termination of convulsion within 10 minutes without recurrence within the subsequent hour. Patients were followed up for 24 hours to assess the risk of adverse events. Of 175 patients enrolled, 38% failed treatment. Of the 124 participants in whom convulsions were terminated within the first 10 minutes, 16 (12.9%) experienced a recurrence during the subsequent hour. Two patients (1%) experienced respiratory depression. Four deaths (2.2%) have occurred, all judged to be likely associated with the underlying illness, although a possible association with the study medication could not be ruled out. Unblinded comparative data, including therapeutic success, time to cessation of convulsion, risk of relapse, and risk of respiratory depression and other adverse events will be presented.

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VERY LOW MORTALITY ASSOCIATED WITH ALBUMIN INFUSION IN KENYAN CHILDREN WITH SEVERE MALARIA

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Previous studies have shown that resuscitation with albumin infusion resulted in a lower mortality than saline in severe malaria. Whether the apparent benefit of albumin is due solely to its colloidal properties, and thus might also be achieved with other synthetic colloids, or due to the many other unique physiological properties of albumin is unknown. As albumin is costly and not readily available in Africa examination of more affordable colloids are warranted. In order to inform the design of definitive Phase III trials we compared volume expansion with the synthetic colloid Gelofusine with albumin. Kenyan children admitted with severe falciparum malaria (impaired consciousness or deep breathing), metabolic acidosis (base deficit >8) and clinical features of shock were allocated volume resuscitation with either 4.5% human albumin solution or Gelofusine. Primary endpoint was in-hospital mortality; secondary endpoints included resolution of shock and adverse events including neurological sequelae. 88 children were enrolled: 44 received Gelofusine and 44 received albumin. Mortality was lower in patients receiving albumin (1/ 44; 2.3%) than in those treated with Gelofusine (7/44; 16%) by intention to treat (ITT) (Fisher's exact P=0.06); or 1/40 (2.5%) and 4/40 (10%) respectively for those treated per protocol (P=0.36). Meta-analysis of published trials (all of which utilized identical criteria for enrolment) to provide a summary estimate of the effect of albumin on mortality showed a pooled relative risk of death with albumin administration was 0.19 (0.06-0.59); P=0.004 compared to other fluid boluses. In conclusion, in this trial and our two previous studies, albumin infusion had a consistent benefit in reducing mortality in children with severe malaria. The lack of similar benefit from the synthetic colloid, Gelofusine suggests that the mechanism may involve a specific neuroprotective effect of albumin, rather than solely the effect of administered colloid. Further exploration of the benefits of albumin is warranted in larger clinical trials.

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PRESUMPTIVE ANTIMALARIAL TREATMENT OF FEBRILE EPISODES AMONG CHILDREN LIVING IN URBAN UGANDA. IS IT NECESSARY?

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Presumptive treatment of malaria in febrile children is widely advocated in Africa. This may occur in the absence of diagnostic testing or even when diagnostic testing is performed but fails to detect malaria parasites. Such over-treatment of malaria has been tolerated in the era of inexpensive and safe monotherapy. However, in the new era of artemisinin-based combination therapy (ACT), presumptive treatment becomes economically and clinically less acceptable. We investigated the risks and benefits of only treating children with microscopy confirmed malaria using a prospective cohort design. A representative sample of 601 children aged 1 to 10 years were recruited from a census population in Kampala, Uganda and followed for all of their health care needs in a study clinic. Standard microscopy was performed each time a child presented with a new episode of fever (subjective fever in previous 48 hours or tympanic temperature >= 38.0) and antimalarial therapy given only if the blood smear was positive. After 18 months of follow-up, 5480 visits occurred for new medical problems of which 40% were episodes of fever. Among 2167 new episodes of fever, 1496 (69%) had blood smears initially reported as negative and no antimalarial therapy was given. Six of these initially negative smears were reported to be positive following quality control reading of all blood smears: 4 of these patients were subsequently diagnosed with uncomplicated malaria and 2 cleared their parasites without antimalarial treatment. Of the 1490 new episodes of fever with final blood smear readings classified as negative, only 12 (0.8%) went on to be diagnosed with uncomplicated malaria within the subsequent 7 days. In this setting, malaria was responsible for only 31% of febrile episodes. Withholding antimalarial therapy in children with negative blood smears was safe and saved almost 1,500 malaria treatments in 601 children over an 18-month period. In the era of ACT, resources to expand the use of microscopy or other validated diagnostic methods may provide a cost effective means for promoting rational use of antimalarial therapy.

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IMPACT OF BACTEREMIA ON HEMATOLOGICAL AND PARASITEMIC OUTCOMES IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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Bacteremia is responsible for significant morbidity and mortality, particularly in pediatric populations in sub-Saharan Africa where malaria and HIV are also co-endemic. An improved understanding of interactions between these pathogens is, therefore, of particular public health interest. Here we report findings from our ongoing Severe Malarial Anemia Study examining bacteremia, malaria, and HIV in children (n=826, age <4 yrs) over a three yr period. Upon enrollment, children were screened for sepsis, malaria, and HIV, in addition to comprehensive testing for hematological abnormalities that may contribute to anemia. These investigations demonstrated that children also infected with gram negative (GN) organisms (9,700/μL, n=62) had significantly lower malaria parasitemia than parasitemic, aseptic children (14,600/μL, n=708, p=0.015). A potential mechanism for reduced parasitemia may be due to significantly higher temperatures in the GN/malaria co-infected children (38.3°C) relative to those with malaria alone (37.4°C, p<0.001) and malaria-infected children with gram positive (GP) organisms (37.6°C, p=0.022). The number of parasitemic visits was also substantially lower in GN/malaria co-infected group (5.5) vs. the malaria alone (6.9, p=0.020) and GP/malaria groups (8.0, p=0.008). Although hemoglobin (Hb) concentrations in the malaria-alone and GP/malaria groups were nearly identical (9.1 and 9.0g/dL, respectively), GN/malaria co-infected children had substantially lower Hb levels (8.2g/dL, p=0.002). Additional analyses revealed that HIV status, G6PD deficiency, sickle trait/disease, age, and gender were not significantly different between children with malaria alone, GN/malaria, and GP/malaria. Taken together, results here suggest a significant interaction between GN organisms and malaria that is not present during GP/malaria co-infection. Additional investigations are currently ongoing to determine if variation in host immune response genes condition differing susceptibility and outcomes in children with bacteremia and malaria co-infection.