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The objective of this study was to characterize socio-economic, demographic, environmental, genetic and immunological risk factors for acquiring malaria in a rural setting in the Brazilian Amazon (Granada, Acrelândia, AC). A prospective cohort of 466 subjects (113 families) aged between <1 and 90 y.o. was started in March 2004 in the Granada area with a baseline visit to collect information about personal and family history was selected. Blood samples were taken from subjects > 5 y.o. for parasitological, immunological and genetic studies. The cohort was followed daily for malaria episodes between March 2004 - May 2005. All families were visited again in Set/2004 and Feb/2005 to update personal information and search for asymptomatic infected subjects. 65 (13.9%) lost subjects were censored in the analysis. There were 195 episodes of malaria (48 by *Plasmodium falciparum*, 114 by *P. vivax*, and 33 mixed infections) in 122 subjects (42.27 episodes/100 person-years). Malaria incidence was similar in males and females (44.25 and 40.12 episodes/100 person-years respectively, P=0.4822). Subjects <1 year old had the highest incidence of *vivax* malaria (56.14 episodes/100 person-years, P=0.0035), while *falciparum* malaria incidence was higher in subjects 5 -14 years old and in those >30 y.o (20.1 and 18.4 episodes/100 person-years respectively), although not achieving statistical significance. Preliminary univariate analysis of putative risk factors (demographic, socio-economic, environmental, behavioral, FcγRIIa allotype, Duffy promoter type and previous exposure to malaria) showed that age, place of residence, wealth index, previous exposure to malaria and Fcγ receptor IIA genotype were significantly associated to the relative risk of presenting an episode of clinical malaria of any type during follow-up (P 0.0197 to < 0.0001), while land-clearing activities, fishing and bednet use were associated with a higher RR of presenting *falciparum* malaria episodes(P 0.0304 to < 0.0001). Detection of antibodies a-PvMSP-1 and multivariate analysis are in progress. In conclusion, rural settlements are currently an important keypiece of malaria transmission in the Amazon, and this study shows that such longitudinal studies are feasible. Our results indicate association of individual characteristics and environmental variables to symptomatic malaria infection, with some differences between *P. vivax* and *P. falciparum* transmission.

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INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH MALARIA INCIDENCE IN NAZARETH, ETHIOPIA

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Malaria risk has been shown to vary greatly between households within communities (1), as well as between individuals within the same household (2). To develop effective malaria interventions, it is useful to identify individual and household level risk factors that underlie this variation in risk. Multilevel analysis of cohort data was used to identify individual and household level factors associated with malaria risk in 1,367 individuals in a peri-urban area of highland Ethiopia. Data were also used to estimate the proportion of between-household variance in malaria incidence attributable to explanatory factors. In both adults and children, living within 350 meters of the major vector-breeding site greatly increased malaria risk and was the most influential factor determining between household variance in malaria incidence, accounting for 38.78% and 78.49% of this variance in adults and children respectively. In adults, individual level factors associated with malaria risk were regular or recent travel to rural areas [IRR=12.96, 95% CI=(4.05, 41.48)], and having an indoor job [IRR=0.37, 95% CI= (0.16,0.87)]. Household level factors associated with malaria risk in adults were low vegetation level in compound [IRR=0.27, 95% CI= (0.10, 0.78)], tidy compound [IRR=0.29, 95% CI=(0.12, 0.71)], household use of preventive measures [IRR=0.31, 95% CI=(0.13, 0.74)] and the number of 5 to 9 year olds in household [IRR=1.66, 95% CI=(1.08, 2.53)]. In children, age was the only individual level factor associated with malaria risk; linear age splines revealed that malaria risk was highest at age 9. Only one household level factor, number of adults in household with indoor job [IRR=0.54, 95% CI=(0.34, 0.88)], was associated with malaria risk in children. In conclusion, several individual and household level factors that account for malaria clustering were identified. These factors should be considered when developing malaria interventions for highland urban communities in Africa.

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MULTILEVEL ANALYSIS OF SOCIAL, ECOLOGICAL, AND BIOLOGICAL PREDICTORS OF POLYPARASITISM IN COASTAL KENYA

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Schistosomiasis and malaria are diseases with complex etiologies in which social, ecologic and biologic factors interact to cause pathology. Although both diseases share some common risk factors, few studies have examined the relative importance of these factors in predicting co-infection. We applied multilevel analyses to investigate the relative importance of individual-level behavioral and biological factors and household-level social and environmental factors in prediction of co-infection. Specifically, we studied the contextual determinants of *Schistosoma* and *Plasmodium* infection in southeastern coastal Kenya. Following informed consent, 1,300 residents of Kingwede village (Kwale District) aged 8 years and older participated during April and May, 2006. Presence and intensity of *S. haematobium* infection was determined using standard urine filtration examination. Presence and intensity of *Plasmodium* infection was determined using standard thick and thin blood slide examination as well as by PCR. Participants were administered a detailed questionnaire in Kiswahili addressing schistosomiasis and malaria knowledge, self-reported water use and other health practices, and socioeconomic status (SES). SES scales were developed for individuals and for households based on previous research. Results showed a significantly higher prevalence of co-infection (15.5%, OR = 2.07 (1.5-2.86) than would be expected based on single infection prevalences (23.5% for *S. haematobium* and 52.2% for *Plasmodium spp*.), indicating potential shared risk factors for the two diseases. We used general estimating equations (GEE) models to evaluate individual age, sex, education and health behaviors, as well as household SES, household practices, and household environmental variables (distance to water bodies, housing quality) as predictors of co-infection. Household factors contributed significantly to prediction of *S. haematobium* and *Plasmodium spp*. co-infection, suggesting that household-focused interventions such as latrine construction or improving housing quality may be more effective in disease prevention than current individual-based methods.

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INTEGRATING ONE OF THE NTDS WITH ONE OF THE BIG THREE. AN INTEGRATED MALARIA INDICATOR, PARASITE PREVALENCE, TRACHOMA INDICATOR, AND TRACHOMA PREVALENCE SURVEY IN AMHARA REGIONAL STATE, ETHIOPIA

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Trachoma prevalence in Amhara regional state of Ethiopia is believed to be one of the highest in the world; the region is also subject to unstable and epidemic malaria. The Carter Center is conducting an integrated malaria

and trachoma control program implementing the full SAFE strategy for control and supporting an integrated malaria control program through the distribution of long lasting insecticidal nets (LLIN). To provide baseline data, a large (160 cluster) household survey was conducted during December 2006 (towards the end of the peak malaria season) to estimate malaria indicators, particularly net coverage, malaria parasite prevalence, trachoma risk factors, and prevalence of clinical trachoma signs in all ages in each of the ten zones of Amhara national regional state. A total of 160 clusters of at least 25 households (total households=4122) were surveyed for net coverage and all persons living in these households (N=19,234) were examined for trachoma signs. All individuals in even numbered households were tested for malaria parasites by microscopy (N=7,751). Preliminary analysis showed that the overall prevalence of trachomatous inflammation - follicular (TF) in children aged 1-9 years was 35.7% (95% confidence interval (CI) 34.3 to 37.0%) and prevalence of trachomatous trichiasis (TT) in persons aged 15 years and above was 6.0% (95% CI: 5.5% to 6.5%). For net coverage, 41.0% (95% CI: 39.5 to 42.5%) of households had at least one mosquito net and 20.5% (95% CI:19.3 to 21.8%) had one or more LLIN. The mean number of LLIN per household was 0.32 (SD 0.69). The overall malaria prevalence was 4.3% (95% CI: 3.9 to 4.8%). Integrating relatively poorly funded programs for neglected tropical disease control with relatively well funded programs for the big three (Malaria, HIV/AIDS and TB) can result in reduced costs and enhance the value of both programs. The results generated in this survey will provide extensive data to guide and evaluate the integrated malaria and trachoma control programs in the region as well as to understand the relationship of trachoma and malaria prevalence with altitude, and other household characteristics such use of latrines and proximity of water.

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SEVERE DISEASE ASSOCIATED WITH BOTH *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTION IN PAPUA, INDONESIA

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The aim of this study was to define the burden of hospitalized malaria in in Timika, Papua, Indonesia, an area where drug resistant *Plasmodium falciparum* (Pf) and *P.vivax* (Pv) are highly prevalent. Data were collected from all patients attending the only hospital in the region using a systematic questionnaire and hospital records. Between January 2004 and December 2006, malaria was present in 15% (41055/274009) of hospital outpatients and 30% (8937/29,979) of inpatients, accounting for 23% (28408/123719) of bed occupancy. Of those patients admitted with slide confirmed malaria, 62% of patients had Pf, 27% Pv, 1% *P. malariae* (Pm) and 9.9% mixed infections. The proportion of malaria attributable to Pv was greatest in infants (50%, 284/566), then fell to 23% (199/875) in children 5-10 years old and did not change thereafter. Pv was significantly more common in non-Papuan compared to Papuan patients (OR=1.22 [95%CI1.07-1.40]; p=0.0036). In total 2368 (27%) patients had WHO criteria of severe disease: 1462 (62%) with pure Pf infection, 547 (23%) pure Pv, 22 (0.9%) Pm and 337 (14%) mixed infections. Severe anaemia, respiratory distress and cerebral malaria accounted for 73% (1720/2368) of severe malaria cases with a further 18% of cases having multiple criteria. Overall 23% (547/2385) of patients with pure *P. vivax* had markers of severe disease with severe anaemia (Hb<5g/dl) accounting for 84% (458/547) of cases compared to only 55% (806/1462) of patients with pure Pf; p<0.001. Pure *P. vivax* infection was present in 63 patients with respiratory distress and 34 patients with coma. A total of 182 (2.1%) patients with malaria died during admission: 2.2% (123/5482) for Pf and 1.6% (37/2365) for Pv (p=0.06). In conclusion, in this region where high grade chloroquine resistance is established for both Pf and Pv, both species are associated with a wide spectrum of disease severity and considerable morbidity, mortality and healthcare expenditure particular in young children. The spectrum of severe disease associated with Pf and Pv infections will be presented and further discussed.

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BLOOD GROUP O PROTECTS AGAINST SEVERE *PLASMODIUM FALCIPARUM* MALARIA

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Malaria has been a major selective force on the human population, and several erythrocyte polymorphisms have evolved that confer resistance to severe malaria. *Plasmodium falciparum* rosetting, a parasite virulence phenotype associated with severe malaria, is reduced in blood group O erythrocytes compared to groups A, B and AB, but the contribution of the ABO blood group system to protection against severe malaria has received little attention. We hypothesised that blood group O may confer resistance to severe malaria via the mechanism of reduced rosetting. In a case-control study of 670 Malian children, we found that blood group O was present in only 22% of severe malaria cases compared to 40-45% of controls and non-severe malaria cases. Blood group O was associated with a 66% reduction in the risk of severe malaria compared to the non-O blood groups (odds ratio (OR) 0.34, 95% confidence interval (CI) 0.21-0.54, P<0.0001). In the same sample set, *P. falciparum* rosetting was reduced in parasite isolates from blood group O children compared to the non-O blood groups (P= 0.003, Kruskal Wallis test). A second study of 144 Kenyan children also showed that group O was associated with reduced rosetting (P= 0.0001) and protection against severe malaria (OR 0.37; CI 0.17-0.80, P<0.05). This work highlights the importance of *P. falciparum* rosetting as a pathogenic factor in severe malaria, and suggests that the selective pressure imposed by malaria may contribute to the variable global distribution of ABO blood group types.

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IMPACT OF MATERNAL MALARIA AND UNDER-NUTRITION ON INTRAUTERINE GROWTH RESTRICTION: A PROSPECTIVE COHORT STUDY IN DEMOCRATIC REPUBLIC OF CONGO

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Maternal malaria and under-nutrition are established risk factors for small for gestational age (SGA) at delivery, however, a study to investigate their effects on intrauterine growth restriction (IUGR) has never been performed. We conducted a prospective, longitudinal ultrasound study of 182 pregnant women in Kinshasa, Democratic Republic of Congo from May 2005 through May 2006. At monthly intervals, malaria infection, maternal anthropometrics, and ultrasound estimated fetal weight were measured. All positive malaria cases were treated and intermittent presumptive therapy with SP was provided. IUGR was defined as estimated fetal weight below the 10th percentile of a standardized fetal weight curve. Log-binomial models were fitted separately for malaria and maternal anthropometric exposures, accounting for statistical clustering due to repeat IUGR measurements. Variation in the relationship between malaria and IUGR by under-nutrition was also examined. Sixty percent of women had at least one positive smear during pregnancy. At baseline, 11% of women were underweight (BMI <19.8 kg/m^2), 3% had short stature. Mean monthly weight gain was 1.6 kg (SD 1.5) and mean monthly mid

upper arm circumference was 0.2 cm (SD 0.8). Overall, incident malaria infection was not associated with an increased risk of IUGR (RR=1.2, 95%CI: 0.7, 2.2). Maternal nutritional status significantly modified the association between malaria and IUGR. For example, among women with low baseline BMI, incident malaria infection increased the risk of IUGR over four-fold (RR=4.5 95% CI: 1.0, 19.9) compared to those unexposed to malaria. However, at normal baseline BMI levels, there was no observed association between malaria and IUGR (RR=1.1 95%CI: 0.6, 2.1). A similar pattern was seen for all anthropometric indicators of maternal under-nutrition. Frequent monitoring and case management of antenatal malaria infections may prevent IUGR, suggesting that antenatal malaria screening policies and nutrient supplementation in malaria endemic areas should be bolstered.

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THE COSTS OF HIV TREATMENT IN DEVELOPING COUNTRIES: EFFECTS OF PROGRAM MATURITY, CONTEXT AND DESIGN ON TOTAL AND COMPONENT COSTS

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Efficient scale-up of antiretroviral (ARV) treatment requires accurate estimation of resource needs and understanding of how these are affected by program maturity, context and design. A public health evaluation funded under the U.S. President's Emergency Plan for AIDS Relief (PEPFAR) assessed the cost of treatment across five countries_Nigeria, Uganda, Ethiopia, Botswana and Vietnam_to inform planning, promote efficient use of resources and facilitate future cost projections. This presentation reports findings based on preliminary analyses of data collected from 27 facilities in Nigeria, Uganda and Ethiopia. We collected data on the costs of providing ARV treatment and on the volume and types of patients. Retrospective data were collected since the start of PEPFAR support to the facility. Costs were categorized by source of program support (*e.g.*, PEPFAR, national government), by cost category (*e.g.*, personnel, ARVs) and by program activity (*e.g.*, clinical care, training). Analyses focus on average annual cost per patient, annual building and equipment investment per facility, and how costs are spread across cost categories and change with time. Current annual comprehensive HIV treatment costs range from USD1680 to USD3310 per patient in programs using branded medications; clinical care costs excluding purchases of ARVs for dispensing and establishing buffer stocks are USD385-1520 per patient. Higher cost programs are characterized by investment in large ARV buffer stocks and more intensive laboratory monitoring. Per-patient costs are highest in the first 6 months, when resources for program start-up account for 30-50% of total costs. ARV expenditures are major cost drivers after the first 6 months (55-75% of total costs), while personnel and laboratory supplies represent smaller components (10-20% and 5-15% respectively). Non-drug clinical care costs decline as programs expand and mature. In conclusion, treatment costs vary widely across facilities, especially at program outset. Personnel, laboratory supplies and infrastructure development costs are significant, but ARV costs dominate per-patient costs. Because buffer stock investment is a primary cost-driver during program scale-up, optimal buffer-stock size must balance the need to avoid stock-outs and program costs.

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DRAMATIC REDUCTIONS IN HIV RNA AMONG HIV-INFECTED CHILDREN WITH ACUTE MEASLES IN UGANDA

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Measles remains an important cause of morbidity and mortality in Africa; where HIV is prevalent.The immune dysregulation that accompanies measles might be expected to enhance HIV replication in co-infected children. To determine the effect of measles virus co- infection on plasma HIV RNA levels, we studied a group of HIV infected children with measles between July 2006 and January 2007. Participants were recruited from an ongoing longitudinal study examining the interactions between HIV and malaria in 300 HIV infected Ugandan children aged 1 to 10 years. The WHO case definition of measles was used: fever of ≥ 38.0 C with maculo-papular rash and at least one of: cough, coryza or conjunctivitis. Serological confirmation was also done. Plasma HIV viral load (PVL), absolute CD4 count, CD4% and total lymphocyte count were obtained before, during and after an episode of measles. A total of 17 children were diagnosed with clinical measles; 9 were ART-naïve and 8 were on ART. Plasma viral load declined in all ART-naïve patients, by a mean 1.4 log ($p<0.0001$). Absolute CD4 ($p=0.04$) and TLC ($p=0.01$) declined in most (7 of 8), but CD4% remained stable ($p=0.23$). Notably, the PVL of one patient dropped 1.4 log despite a rise in TLC and absolute CD4. A similar effect was observed among HIV-infected children on ART; children with undetectable viral loads, remained so (n=4) and those with detectable plasma viral load experienced declines (n=4). Plasma HIV viral load returned to baseline levels by 5 weeks in 4/17 patients and to levels above baseline by 11 weeks in 2/17 patients. Our findings demonstrate that during acute measles HIV infected children have a transient but dramatic decrease in plasma HIV viral load levels. Further research is needed to identify the exact mechanism of viral suppression by measles. This knowledge will be useful in the design of new treatment strategies for HIV.

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HIV-1 INFECTION IN PATIENTS REFERRED FOR MALARIA BLOOD SMEARS AT UGANDAN GOVERNMENT HEALTH CLINICS

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Malaria is the most common diagnosis at outpatient clinics in Uganda. HIV is associated with an increased incidence of malaria in adult African populations, presumably due to loss of previously acquired immunity. In children, who have less acquired malaria immunity, the relationship between HIV and malaria is less clear. Understanding interactions between HIV and malaria may have important public health consequences. We investigated the relationship between malaria and HIV infection among adults and children suspected of malaria at government health clinics in Uganda. In this cross-sectional study, we collected dried blood spots and malaria blood smears from 1,000 consecutive patients who presented to each of 7 health clinics and were referred by clinic providers for malaria blood smears, generally due to reported or documented fever. Giemsa-stained blood smears were examined for malaria parasites by clinic personnel and results were confirmed by experienced microscopists. Blood was screened for antibodies to HIV using the Murex HIV 1.2.0 HIV Enzyme Immunoassay. Positive results were confirmed using the Roche Amplicor HIV-1 DNA test. Risk factors for HIV infection were identified using multivariate logistic regression. Of 7,000 samples, 2,703 (38.6%) blood smears were positive for malaria parasites. Among 4467 children aged 16 years or younger, 77 (1.7%) were HIV infected. Of 2533 adults, 270 (10.7%) were HIV infected. In children, having a negative malaria blood smear was associated with a higher odds of HIV infection (OR=1.90, 95% CI 1.18-3.06) after controlling for age and gender. In contrast, in adults, having a negative malaria blood smear was associated with a lower odds of HIV infection (OR=0.71, 95% CI 0.51-0.99) after controlling for age and gender. Thus, the association between HIV and malaria is complex and operates differently in adults and children. Malaria may be a warning

sign for HIV infection in adults, as HIV infection may diminish acquired antimalarial immunity. In children, malaria is very common, and a negative malaria smear suggests other causes of fever, including HIV-related infections. Our results suggest that counseling and testing for HIV is of particular importance in adults with malaria and in children suspected of malaria but with negative blood smears.

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HEMATOLOGICAL AND INFLAMMATORY MEDIATOR ANALYSES IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* AND HIV-1 CO-INFECTION

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Malaria and human immunodeficiency virus (HIV) are co-endemic in many tropical and sub-tropical countries and are leading causes of pediatric morbidity and mortality. Anemia is the most common hematological manifestation of pediatric HIV-1 and *Plasmodium falciparum* malaria in sub-Saharan Africa. However, since the clinical and hematological outcomes, and the immunological profiles associated with these outcomes are largely undefined in co-infected children, interactions between malaria and HIV-1 were investigated in Kenyan children residing in a holoendemic *P. falciparum* transmission area. Children presenting at hospital with *P. falciparum* parasitemia (n=192, aged 3-36 mos) were stratified into three groups: HIV-1 negative [HIV-1(-), n=141], HIV-1 exposed [HIV-1(exp), n=33], and HIV-1 infected [HIV-1(+), n=18]. Complete hematological profiles and monocytic and neutrophilic hemozoin (Hz)-burden were determined. In addition, circulating inflammatory mediator production was measured by a microbead-based 25-Plex assay. Statistical significance of parametric (ANOVA, Student's t-test) and non-parametric (Kruskal-Wallis, Mann-Whitney U) tests was set at $P<0.05$. Relative to the HIV-1(-) group, the HIV-1(+) group had significantly higher pigment containing neutrophils and significantly lower hemoglobin concentrations and hematocrit. Additionally, HIV-1(+) children presented with significantly higher numbers of monocytes, red cell distribution width, and mean platelet volume than the HIV-1(-) group. Levels of IL-12, GM-CSF, MIG, and EOTAXIN were significantly elevated in the HIV-1(+) group vs. the HIV-1(-) group, while the IL-10/IL-12 ratio was significantly lower in HIV-1(+) children. Relative to HIV-1(-) children, the HIV-1(exp) group had significantly elevated IL-1ß, IL-2, IL-7, IFN-γ, GM-CSF, and MIG levels. Results presented here demonstrate important clinical, parasitological, and hematological interactions between *P. falciparum* and HIV-1 defined by an increased level of anemia in co-infected children that is associated with an enhanced pro-inflammatory response, providing further immunological support for a pro-inflammatory basis of childhood anemia.

(ACMCIP Abstract)

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CLINICAL MENTORING: EFFECTIVE AND RAPID TOOL IMPROVES CLINICAL CARE SKILLS FOR TB/HIV IN DEVELOPING COUNTRIES

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The high rate of HIV seroprevalence among TB patients in developing countries is a public health priority. While treatment is increasingly available, there remains a lack of clinical expertise among local healthcare workforce to meet the expanding patient demand. In order to rapidly create practical expertise amongst local health professionals, Ministries of Health in developing countries are including "clinical mentoring" in their healthcare capacity building programs. ICEHA (International Center for Equal Healthcare Access; www.iceha.org) provides clinical mentoring by healthcare providers with HIV and TB expertise to complement national didactic training in resource-poor settings. Its award winning model rapidly and effectively transfers clinical skills from one practitioner to another. ICEHA clinical mentors coach colleagues for defined periods of time, 6-12 weeks, on a pro bono basis in local settings. ICEHA has implemented mentoring program in developing countries in Africa, Asia and Oceana. Mentoring ensures provision of optimal HIV/TB treatment and prevention by local health providers within available resources. ICEHA clinical mentors have been successful in all country programs across Asia and Africa. Mentoring has flexibility and specificity to address the unique issues particular to individual settings. Evaluation consistently shows a significant increase in knowledge and awareness of TB/HIV coinfection among mentored health workforce. Local providers gained expertise in screening procedures, case detection, case treatment and monitoring. Clinical mentoring was critical to rapid improvement in care for people with HIV/TB coinfection. In conclusion, clinical mentoring in patients with TB/HIV coinfection rapidly expands local treatment expertise and is an essential pillar of national treatment and prevention schemes.

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IMPACT OF *SPIRULINA PLATENSIS* SUPPLEMENTATION ON GENERAL HEALTH STATUS OF HIV INFECTED PEOPLE IN BURKINA FASO

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Malnutrition constitutes a public health problem among HIV infected people and particularly in developing countries.The objective of the study is to assess the impact of a daily supplementation of spiruline (*Spirulina platensis)* on the clinical, nutritional and immunologic status of HIV infected people (HIVIP). We enrolled 180 HIV infected patients in a randomized double blind placebo controlled trial. The patients were divided into three groups according to the number of CD4: Group1, 60 HIVIP with CD4≤200/µl; Group2, 60 HIVIP with 200<CD4<400/µl; Group3, 60 HIVIP with CD4>400/µl. Each group was divided into two sub-groups: 30 patients for spiruline supplementation and 30 patients for placebo. Every included patient had a monthly clinical follow-up (treatment, anthropometric parameters) and biological follow-up (haematological, biochemical parameters and CD4 cells count) by first and sixth months. Anthropometrics, hematological and immunological parameters allowed us to appreciate the nutritional and biological evolution of patients: a nutritional recovery of 10,71% among spiruline supplemented patients versus 2,35% for placebo group; the mean of lymphocyte CD4 count after six months supplementation increased from 143.88 ± 45.59 to 174.9 ± 94 for patients of the sub-group spiruline, group I, while this mean has not varied for placebo sub-group of the same group; We have observed also a significantive decrease of the mean of lymphocyte CD4 count passing from 546,60 to 379,92 CD4/µL among placebo groupIII (p=0,0005). *Spirulina* supplementation has therefore positive impact on nutritional recovery (weight gain), gain or stabilization of CD4 cell count and contritbue to HIV infected person well-being.

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CO-INFECTION OF CUTANEOUS LEISHMANIASIS AND HIV IN MALI

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Cutaneous leishmaniasis is endemic in many areas of Mali and most often caused by *Leishmania major*. The most common clinical presentation is a localized ulcero-crusted lesion of the uncovered part of the body. In patients with HIV, the disease outcome and response to treatment might change because of immunodepression. We collated all cases of patients with leishmaniasis seen in our Institution from 2002 to 2006. All cases of cutaneous leishmaniasis were confirmed by microscopy on scratch skin smear stained with giemsa. Of 320 cases of leishmaniasis 11 were HIV positive. 10 of the 11 HIV positives patients were males and their age varied from 23 to 53 years old. The clinical feature of the lesion included nodules lepromatous, large ulcer or crusted, disseminated macules and superinfected plaques. The number of lesion varied from 2 to 6 but reached more than 20 for disseminated macules and lepromatous cases. Most of these lesions were not observed in non-HIV patients. Patients' response to meglumine antimoniate was delayed (1 to 2 months). Two patients died during follow up (superinfected plaques and lepromatous cases) and 1 relapsed after antimony treatment. In conclusion, co-cultures from other fluids such as lymph nodes and organs should be performed for the detection of parasites in HIV patients suspected to have leishmaniasis. The extend of skin involvement and the rapid fatal outcome in some patients seems to be related to a possible trends towards a systemic disease like Kala Azar.

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REPRODUCTIVE TRACT INFECTIONS (RTI) IN FEMALE SLUM POPULATION MUKURU, NAIROBI, KENYA

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STI at an early stage may result in serious complications, including infertility, fetal wastage, ectopic pregnancy, cancer and premature death as well as neonatal and infant infections. Inhabitants of suburb slums in developing countries are highly vulnerable group due to migration, unsafe commercial sex workers, being orphaned, pedophilia, early sexual debut, early marriages, single parenthood, addiction to alcohol, low educational level and unemployment. We studied female patients living in Mukuru slum, Nairobi who presented STI symptoms from October to May 2006 in Mary Immaculate Clinic. Diagnose was based on history, clinical examination,urinalysis, microscopy of native and Gram-stained vaginal discharge, RPR and HIV serology. Significant correlation was looked for in 4 selected risk groups (HIV positive, pregnancy, failure of treatment and history of STI) by univariate analysis. At least one STI was diagnosed in 3.2% of patients (384 from 12 328), 75% were women. VCT centre in our clinic documented 12.4% HIV infected patients in the area. Unusual vaginal discharge was associated with HIV positivity ($p<0.05$), urethral syndrome was associated with STI history and treatment failure ($p<0.05$). The commonest pathogens were *Candida* spp. (44%), *Trichomonas vaginalis* (11%), *Neisseria gonorhoe* (79%) and clue cells (49%). *N. gonorrhoe* was significantly associated with pregnancy and HIV positivity ($p<0.05$). HIV positivity was significantly associated with palpable tumor of internal genital, Candida spp. infection in vaginal secret and also in urine, positive STI history and tratment failure. Pregnant women were treated with erytromycin for gonorrhea. Infection in pregnancy was significantly associated with bacterial vaginosis and gonorrhoea. Gram-staining of vaginal secret and urinalysis was done in patients with recurrent infection and with failure of treatment ($p<0.05$). Failure of treatment within 1 week was caused by bacterial vaginosis due to poor compliance to treatment of partner. Free access to drugs in local pharmacies without consultation leads to high recurrence rate. We propose package system for STI similar to TB treatment. In conclusion, momen in slums of Nairobi are vulnerable group especially for reproductive tract infections.

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CONGENITAL AND NEONATAL MALARIA IN A TERTIARY REFERENCE HOSPITAL IN MALI

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Hopital Gabriel Toure (HGT), a Teaching Hospital in Bamako, Mali experiences an unacceptably high rate of pre-term infant mortality. The exact cause of most of these deaths is not known although infection is very frequently suspected. Malaria is highly prevalent in this setting but the extent to which malaria contributes to mortality or morbidity in preterm and neonates in Mali is not known. The objective of this study was to determine the rate of congenital and acquired malaria in inpatient neonates at HGT. We are performing a cross sectional study in infants aged 0-28 days that were admitted for inpatient care to the Unit of Reanimation and Neonatology of HGT. The study will recruit 300 mother-infant pairs. After informed parental consent was obtained venous blood was collected for malaria diagnosis by OptiMAL® IT, microscopy and PCR. If an infant was enrolled and the mother was available, she was approached for enrolment into the study and asked to provide a blood sample. To date 81 infants and 49 mothers were included between October 2006 and February 2007. The mean age of infants was 3.4 days but, 44.4% of infants were included on their first day of life. The mean weight was 2922g but 23.5% of the infant were low birth weight infants. In all infants both PCR and microscopy for malaria were negative. However, two infants were positive for *Plasmodium falciparum* malaria by the OptiMAL® IT test. The mean age of mothers was 25.5 years. No malaria prophylaxis was used by 18.5% of them during the pregnancy. Of the remaining women that used chemoprophylaxis, 88% used chloroquine while only 12% used IPTp with sulfadoxine-pyrimethamine, the national policy for preventing malaria during pregnancy. All mothers were parasite negative by microscopy, the OptiMAL® IT was positive for *P. falciparum* in one case while PCR was positive in five women (3 cases of *P. falciparum* and 2 cases of *P. ovale*). These preliminary data suggest that malaria is not a significant contributor to neonatal morbidity and mortality in this setting. Data from the completed study will be presented.

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THE ECOLOGY AND BIOGEOGRAPHY OF MELIOIDOSIS IN PAPUA NEW GUINEA

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Melioidosis is an emerging infectious disease caused by the saprophytic bacterium *Burkholderia pseudomallei*. It is a significant yet under recognised cause of sepsis and pneumonia in the tropics and has recently been described in a rural community of the Western province, Papua New Guinea. The most important risk factor for acquiring the disease is environmental exposure where inoculum size and frequency of exposure seem to determine the severity of disease, as is likely the case in times of heavy rainfall. Through study of the factors that determine survival and persistence of the organism in the environment, novel methods of prevention may be established. Using multi locus sequence typing this study describes significant genetic diversity between strains of *B. pseudomallei* recovered from geographically separate but related regions

of Papua New Guinea, Torres Strait and mainland Queensland. Specific plant microbe relationships such as association with rhizosphere have been demonstrated which may explain this localised prokaryote biogeography. These relationships can be analysed with geographic information systems (GIS) to help identify regions that are at risk but not yet described. Furthermore, evidence suggests that antagonism produced by a related but avirulent species of *Burkholderia* may be response for local remediation and could be exploited for novel biocontrol. As therapeutic options are limited in developing countries, measures which better describe risk and prevent exposure are required.

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EFFICACY OF SINGLE DOSE LEVOFLOXACIN FOR TREATMENT OF ACUTE LEPTOSPIROSIS IN A HAMSTER MODEL

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A single dose of doxycycline has the ability to prevent clinical leptospirosis, both in humans and experimental animals. Single dose azithromycin also has proven efficacy in animals. Doxycycline use is limited by toxicity and azithromycin has not been evaluated in humans. The availability of alternative antimicrobial options to prevent clinical leptospirosis is desirable. We evaluated the ability of single dose levofloxacin to prevent mortality in hamsters with acute leptospirosis. On the first day of the experiment, hamsters were infected via intraperitoneal injection with a lethal inoculum (10^5 organisms) of *Leptospira interrogans* serovar Portlandvere. Groups of hamsters were then given single intraperitoneal doses of levofloxacin (50 mg/kg) on either the first, third, or fifth day after infection. A group of untreated hamsters were used as controls. All untreated animals died by the eleventh day after infection. Twenty percent of animals treated on day 1 survived to the end of the 21 day observation period, whereas 40% of those treated on day 3 and 70% of those treated on day 5 survived. Single dose therapy given at any time after infection produced a statistically significant survival advantage when compared to no treatment ($P < 0.05$). No toxicity was seen in hamsters receiving levofloxacin therapy. Single dose levofloxacin is effective in preventing fatal leptospirosis in a hamster model.

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ANTIMICROBIAL SUSCEPTIBILITY OF CLINICAL *LEPTOSPIRA* ISOLATES

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The ideal antimicrobial therapy for leptospirosis is not defined. *In vitro* data suggest that *Leptospira* are susceptible to a broad range of antimicrobials but the majority of this data was developed using laboratory derived strains. It is not fully known if clinical isolates from cases of human leptospirosis exhibit similar susceptibility to antimicrobials. Whether antimicrobial susceptibility varies based on geography is also not clear. A broth microdilution technique using a colorimetric growth indicator (AlamarBlue) was used to determine the antimicrobial susceptibility profile of ten different clinical isolates of *Leptospira*, representing 8 different serovars. The isolates, which were from cases of human leptospirosis, were obtained from Egypt (5 isolates), Thailand (3 isolates), and Hawaii (2 isolate). Leptospires were incubated with serial two-fold dilutions of antimicrobials (concentrations of 32 to 0.016 µg/ml) for 5 days at 30°C prior to determination of MICs. Each isolate was tested in triplicate against 13 different antimicrobials. A laboratory derived strain, *L. interrogans* serovar Icterohaemorrhagiae, was used as quality control. Ampicillin, azithromycin, clarithromycin, and cefepime generated MIC_{90} below the lower limit of detection. Doxycycline and tetracycline had the least activity, with MIC_{90} of 2.0 and 4.0 µg/ml, respectively. The MIC_{90} for the remaining drugs were penicillin, 0.06 µg/ml; cefotaxime, 0.03 µg/ml; ceftriaxone, 0.125 µg/ml; imipenem-cilastatin, 0.250 µg/ml; ciprofloxacin, 0.250 µg/ml; levofloxacin, 0.125 µg/ml; and moxifloxacin, 0.125 µg/ml. No appreciable difference was noted across tested serovars. The results for the quality control serovar were within established limits. *In vitro*, laboratory derived strains of *Leptospira* are susceptible to a broad range of antimicrobials. This appears to be the case for clinical isolates of *Leptospira* as well. Antimicrobial susceptibility does not appear to vary based on geography.

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EFFECTS OF A HIGH FAT MEAL ON THE BIOAVAILABILITY OF THE FIXED-DOSE COMBINATION OF AMODIAQUINE AND ARTESUNATE (ASAQ) IN HEALTHY SUBJECTS

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The objectives of this randomized, open-label, cross-over study were to evaluate the interaction with food of a fixed combination of 135 mg amodiaquine and 50 mg artesunate (ASAQ) in healthy male subjects and to assess the safety and tolerability in fed and fasting states. 22 subjects were randomly allocated to receive four tablets of ASAQ (adult daily dosage) 30 minutes after the start of a high-fat breakfast and in fasted conditions. In each study period, serial blood samples were collected over 10 days. A 6 month interval separated the 2 treatment sessions Plasma concentrations of artesunate (AS), amodiaquine (AM) and their pharmacologically active metabolites: dihydroartemisinin (DHA) and desethylamodiaquine (DAM) were used to determine the pharmacokinetic parameters using non compartmental methods. The assays were performed using a LC-MS/MS method with a 1 ng/mL lower limit of quantification. Safety was evaluated by adverse events monitoring, laboratory assessments, vital signs, electrocardiogram parameters, and physical examinations. Clinical and biological tolerance was good for both administration conditions. In fed state, T_{max} was significantly delayed for DMA (2 h), AS (0.4h) and DHA (1.1 h). Fed/fasted point estimates (90% confidence intervals) for C_{max} and AUC0-t were: - AM: C_{max} 1.22 (1.07-1.39), AUC0-t 1.59 (1.39-1.83); - DAM : C_{max} 1.21 (1.05-1.39), AUC0-t 1.13(1.03-1.24); - AS : C_{max} 0.36 (0.28-0.47), AUC0-t 0.88 (0.73-1.05); - DHA : C_{max} 0.51 (0.43-0.60), AUC0-t 0.93 (0.84-1.02). It is assumed that intake of ASAQ with a high fat meal has two consequences:(1) increased amodiaquine and its metabolite blood levels may affect safety and tolerability whereas (2) decreases in artesunate and dihydroartemisinin blood levels may affect efficacy. These results suggest that the fixed combination ASAQ should not be administered with a high-fat content meal.

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A MULTINATIONAL, RANDOMIZED COMPARATIVE STUDY TO ASSESS THE EFFICACY AND TOLERABILITY OF A FIXED DOSE COMBINATION OF ARTESUNATE PLUS AMODIAQUINE ONCE OR TWICE DAILY VERSUS A FIXED DOSE COMBINATION OF ARTEMETHER PLUS LUMEFANTRINE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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A multinational, randomized, observer blind comparative Phase III trial was carried out in Cameroon, Madagascar, Mali, and Senegal, in order to

assess the non inferiority of the new fixed-dose formulation of artesunate and amodiaquine (ASAQ) versus a fixed dose combination of artemether and lumefantrine (AL) according to WHO 2003 D28 protocol and to assess the optimal dosing regimen (one or two daily doses). Any subject with confirmed malaria attack (parasitemia ≥1000/µl) was randomly allocated in one of the three arms, after informed consent. Treatment doses were according to bodyweight range. All treatments were administered by an authorized person, without the knowledge of investigators. In each bodyweight range the total number of tablets was the same, based on the AL reference group, with placebo tablets given as necessary. A total of 941 patients, including 437 children < 5 years old, weighing more than 10 kg, were included between March and December 2006. Day 28 ACPR after PCR correction were in ITT population 95.2%, in ASAQ one daily dose group (n = 310) , 94.9% in ASAQ two daily doses group(n= 315) and 95.5% in AL group (n= 311) . Results calculated on PP population were 98.9%(n=283),100%(n= 285) and 98.6%(n= 289) respectively. In children< 5 years old day 28 ACPR after PCR correction in ITT population were 94.4 %(n=146) in ASAQ one daily dose group, 95.9%(n=148) in ASAQ two daily doses group and 93.7%(n=142)in AL group; while in the PP population results corrected D28 ACPR were 98.5 (n=134), 100% (n=137) and 97% (n=133),respectively. This study shows the non inferiority of ASAQ versus AL, in each population studied. No unexpected adverse event occurred, clinical and biological safety was good in the three treatment groups.

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DEPLOYMENT OF ARTEMETHER LUMEFANTRINE (AL) AT COMMUNITY LEVEL AND ITS IMPACT ON MALARIA SPECIFIC DEATH RATE DURING AN EPIDEMIC YEAR

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This study was undertaken to assess the impact of deploying artemether-lumefantrine at community level on mortality during the malaria epidemic which occurred in 2005 in Tigray, Northern Ethiopia. The study was conducted in the Alamata and Raya Azebo districts (total population of 250,000) in Raya valley of Tigray, Northern Ethiopia, where malaria is meso-endemic. In both districts artemether-lumefantrine (AL) was deployed as 1st-line drug in all public health facilities according to the national treatment policy. In the intervention district (Alamata) early diagnosis and treatment with AL was provided at village level through community health workers. From May to October 2005 a major malaria epidemic occurred in Raya valley, striking both districts. In addition to partial protection with insecticide-treated nets (ITNs), in both districts blanket coverage of indoor residual spraying with DDT 75% was applied during the epidemic. In May/June 2006, a mortality survey was conducted using verbal autopsy (InterVA model) to measure crude mortality rate and malaria-specific mortality rate for the period from May 2005 to April 2006. Poisson regression models of incidence rate ratios for malaria-specific mortality, allowing for clustering effects at the village level revealed a major difference between the two districts, in terms of adjusted incidence rate ratio (adjusted IRR) for malaria specific mortality of 0.52x (P=0.014 and 95% CI 1.1 to 5.4). The malaria-specific mortality rate in women aged 15-49 was 3.5 times higher than for men in the same age group, with a strong association between malaria- and pregnancy-related deaths among women in this age group (relative risk = 2.4 and 95% CI 1.1 to 5.4). In conclusion, onsidering the homogeneity of the two districts, the deployment of artemether-lumefantrine at community level by health volunteers had a major impact on the malaria specific mortality, documented during a malaria epidemic in Tigray, Northern Ethiopia.

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CLINICAL IMPACT OF ENHANCED AMPLIFIED MYCOBACTERIUM TUBERCULOSIS DIRECT TEST (E-MTD) FOR RAPID IDENTIFICATION OF *MYCOBACTERIUM TUBERCULOSIS ON* RESPIRATORY SAMPLES

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The resurgence of tuberculous and non-tuberculous mycobacterial infections as a consequence of the AIDS epidemic has led to the need for more rapid and accurate diagnostic tests. Rapid non-culture molecular diagnostic assays are anticipated to facilitate earlier diagnosis, appropriate therapy and quicker discontinuation of respiratory isolation in NTM infections. This could lead to a significant cost saving and improved outcome. Patients admitted to Detroit Medical Center from April 2003 to March 2007 with at least one respiratory sample (sputum or bronchoalveolar lavage) positive for acid-fast bacilli (AFB) were included in the study. The cases with the E-MTD (Gen-Probe, San Diego, CA) testing done directly on sputum were compared with controls that have no E-MTD available. This test uses transcription-mediated amplification to detect MTBC ribosomal RNA. All the samples were confirmed by mycobacterial culture. The E-MTD results were compared with culture. The clinical outcome looked at were duration of respiratory isolation, appropriateness of therapy and estimated cost. The mean age (42 males and 17 females) was 47.5 years. Of the fifty-nine AFB smear positive samples, 58 were culture positive for mycobacteria (MTBC-51, *Mycobacterium avium complex*-12, *M. kansasii*-5). Among the thirty-seven who had E-MTD available, 26 were positive and 11 negative. The sensitivity, specificity and positive and negative predictive values were 100%. The mean duration of isolation were 5.4 and 19 days in E-MTD and conventional group respectively among the NTM patients ($p<0.05$). Thus an estimated cost saving per patient is $32640 ($2400/day for respiratory isolation) as compared to the test cost of $26. All three patients with *M. avium complex* in the conventional group were inappropriately treated with anti-tuberculous therapy initially. In conclusion, E-MTD is an extremely reliable and rapid method for the direct detection of MTBC to differentiate it from NTM in smear positive respiratory samples. This can lead to earlier discontinuation of respiratory isolation in patients with NTM infection and resultant cost-savings.

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INTEGRATION OF NEGLECTED DISEASE PROGRAMS IN TOGO: EVALUATION OF A PILOT PROJECT

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The integration of neglected disease programs is considered essential for achieving an effective and sustainable public health impact. Though frequently discussed at the international level, there is limited field experience with integrating more than two vertical programs. In Togo, a West-African country with 5.7 million inhabitants, the national program coordinators for malaria, lymphatic filariasis, onchocerciasis, guinea worm, schistosomiasis, geohelminths, and trachoma have collaborated since January 2006, to define integration, decide which activities can be integrated and develop practical guidelines and tools. Integration was defined as the integration of community-based services executable by village volunteers (VV). Activities deemed appropriate for integration included health education, distribution of services and items, training, supervision, monitoring, evaluation and budget. Tools were developed such as a training manual for VV, combined mass drug administration (MDA) guidelines, health education materials, and integrated record-keeping and supervisory forms. In May 2007, a pilot phase of the project

was implemented in Binah district, where the prevalence of several of the targeted diseases warranted MDA with albendazole, ivermectin, and praziquantel. An evaluation of the pilot project will be conducted in June 2007, including a cluster sample survey of the target population to assess MDA and bed net coverage, knowledge, attitudes, and practices. A survey of VV will assess knowledge on the integrated topics, measure process indicators, and gather information about logistics, supplies, and work load. Semi-structured interviews will be conducted with national program coordinators, the medical director and dispensary nurses in Binah. The project represents the first example globally of a truly integrated neglected disease program. The results of the evaluation will be used to strengthen the integration project prior to implementation on a national level in Togo, and are relevant to other countries planning to integrate multiple disease programs

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THE U.S. EPA'S MULTIDISCIPLINARY APPROACH TO EXAMINING THE LINKS BETWEEN BIODIVERSITY AND HUMAN HEALTH

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Changes in biodiversity can profoundly impact the ability of ecosystems to provide clean water, energy, food, recreation and other services that contribute to human well-being. In addition, changes in biodiversity can affect the transmission of infectious disease to humans, particularly vector-borne diseases such as malaria, West Nile virus and Lyme disease. Characterizing the relationship between biodiversity and health can reveal the root causes of disease emergence and spread, modifiable environmental factors that contribute to disease, and opportunities for managers to intervene along the causal chain. EPA has developed a new interdisciplinary initiative to better understand the dynamics and mechanisms underlying the relationship between anthropogenic stressors, changes in biodiversity, and disease transmission to humans. EPA's initiative is unique in its interdisciplinary approach; its focus on systems where changes in biodiversity are hypothesized to be important drivers of risks to human health; and in encouraging the coordination of earth observations with field data. Through the sponsorship of long-term research studies and pilot projects in and outside of the U.S., EPA is particularly interested in testing the relationships between biodiversity decline and increased incidence of vector-borne diseases; deforestation and increased risk of vector-borne diseases such as malaria; and potential effects of climate change on biodiversity and human health. Expected results from our initiative include the development of new approaches to analyze the vulnerability of biodiversity and human health to anthropogenic drivers such as climate change, land-use change, and biological invasions; use of earth observation and field data to track and analyze the global relationship between habitat alteration, biodiversity loss, vector ecology, and the emergence and spread of infectious disease; and the development of tools that can help forecast risks to ecosystem services that directly impact human health.

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PREVALENCE OF BURULI ULCER CASES IN THE HEALTH DISTRICT OF AKONOLINGA, CAMEROON: A CROSS SECTIONAL SURVEY USING CENTRIC SYSTEMATIC AREA SAMPLING

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Buruli ulcer (BU) is a chronic, indolent necrotizing disease of the skin and underlying tissues caused by *Mycobacterium ulcerans* (1), which may result in functional incapacity. In 2002, MSF opened a Buruli programme in Akonolinga hospital, Cameroon, offering antibiotic treatment, surgery and general medical care. Five hundred patients have been treated in the project to date. The objective of this survey was to estimate the prevalence of BU in the health district of Akonolinga describe the geographic extension of the highly endemic area within the health district and determine the programme coverage its geographical distribution. A cross-sectional population survey was conducted, using centric systematic area sampling (CSAS). A 15 x 15 km grid (quadrats of 225 km²) was overlaid on to a map of Akonolinga district with its position chosen to maximize the area covered by the survey. Quadrats were selected if more than 50% of the quadrat was inside of the health district. The chiefdom located closest to the centre of each quadrat were selected and Buruli cases were identified using an active case finding strategy (the sensitivity of the strategy was estimated by capture-recapture).WHO-case definitions were used for nodules, plaque, ulcer, oedema and sequellae. Out a total population of 103,000 inhabitants, 26,679 were surveyed within the twenty selected quadrats. Sensitivity of case finding strategy was estimated to be 84% (95%CI 54% - 97%) by capture recapture. The overall prevalence was 0.47% (n=105) for all cases including sequellae and 0.25% (n=56) for active stages of the disease. Five quadrats presented with high prevalence, >0.6% to 0.9%, 5 with prevalence >0.3% to 0.6%. and 10 <0.3%. The quadrats with the higher prevalence were situated along the river Nyong and Mfoumou. Overall coverage of the project was 18% (12-27%) for all cases and 16% (9-18%) for active cases, but was limited to the quadrates neighbouring Akonolinga hospital. In onclusion, prevalence was highest in the area neighbouring the river Nyong. Coverage was limited to the area close to the hospital and efforts have to be made to increase access to care in the high prevalence areas. This method was particularly interesting for project planning and to identify priority areas of intervention.

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THE EFFECTIVENESS OF AN OUTBREAK RESPONSE COURSE IN THE AMERICAS

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In 2002 NMRCD initiated an outbreak investigation training program that reached over 1300 professionals throughout the Americas, but the contributions of the course to public health capacity building has not been quantitatively documented to date. We assessed the effect of the course using a post-training e-survey. A random sample of 240 graduates from 4 or 5-day courses organized by NMRCD more than 2 years before the survey were invited to participate. Participants were sent an email with a link to the questionnaire posted on a commercial survey website, surveymonkey.com. The effect of the course was assessed by comparing pre/post course reports of outbreak investigations, the quality of investigations, professional advancement and replication of training. Preliminary results obtained to date are reported here. Ninety graduates (37%) responded to the survey within two weeks. The average age was 41.6 years, 57.5% were females, and they have worked in the field for 13.7 years. There was an increase in the number of outbreak investigated per year before and after the course (0.3±0.8 vs. 1.0±1.7, $p<0.001$) and in the number of outbreaks where the source or mechanism of transmission was identified (0.2±0.4 vs. 0.6±1.3, $p<0.001$). More participants engaged on graduate epidemiology training after the course (33% vs 42%, $p=0.30$). The overall retrospective evaluation of the course was 4.3±0.6 on a six-item, 5-point scale. The overall perceived impact of the course was 3.9±0.8 on a 12-item, 5-point scale. After training, 37% of participants taught replication courses, 80%% used course materials for teaching and 20% used the course methodology in teaching at a university. Consistent suggestions of improvements in the quality of investigations and career advancement after the course were observed with less than half of the target sample size. Years after training, these experienced health

professionals were very satisfied with the course, and the high rates of replication of the course demonstrated that it is practical, sustainable, and reproducible. Outbreak investigation training courses could be a valuable strategy to developing response capacities in the developing world.

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FETAL TOXOPLASMOSIS: OUTCOME AND MANAGEMENT OF PREGNANCY IN 193 ROMANIAN FEMALE SURVEYS

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Toxoplasma gondii (*Tg*) infection is a well-known cause for fetal disorders. In Romania, there is a high *Tg* human exposure, certified by over 58% IgG anti*Tg* seroprevalence in general population. This study was undertaken too assess the failure in fetal developpment in maternal *toxoplasma* infection acquired during pregnancy and to establish a practical attitude for toxoplasmosis management. A multidisciplinary study was performed during 2001-2005 period, data being prospectively analyzed on 193 pregnancies followed-up in our departaments due to *Tg* Infection: 65 cases<12 weeks of gestation, 84 cases in 12-20 weeks of gestation and 44 ones> 21 weeks of pregnancy. Each case was evaluated by seroimmunologic tests (ELISA-ELFA IgM, IgG avidity test and PCR in amniotic fluid sporadically), ultrasonographic findings, clinical, neurological and ocular assessments. From 193 pregnancies, 74(38.3%) underwent an abortion procedure, 12 (16.2%) with severe abnormalities (hydrocephalus, cerebellum and renal agenezia). A number of 119 live infants were born: 4 cases (3.36%) presented an early overt disease, 104 (87.3%) infants were free of infection and 11 (9.2%) ones were lost from evidence. All 119 cases have received "*in utero*" prolonged *azithromycine* (*AZM*) chemotherapy. After a mean follow-up period of 18 months, 7(5.8%) additional children had evidence for *Tg* infection, including hypotonia, intracranial calcifications and eye abnormalities. In conclusion, a national screening programme for *Tg* infection during pregnancy is an imperative need for Romanian health services using a qualified methodology with increased sensitivity.*AZM* chemotherapy is an efficient and safe drug regimen, but long-term evaluation is essential for a definite final outcome of fetal toxoplasmosis.

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RIFT VALLEY FEVER IN A MALARIA EPIDEMIC-PRONE AREA, IJARA DISTRICT, KENYA, JANUARY 2007

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In December 2006, a Rift Valley Fever (RVF) outbreak was detected in north-eastern Kenya after massive flooding. In 1998, a RVF epidemic with similar environmental conditions was followed by a malaria outbreak. Our objectives were to support an MSF team in investigating the RVF epidemic in Ijara district and by implementing a malaria surveillance/alert system for early outbreak detection. We conducted active RVF case finding by visiting settlements and collected blood specimens from suspected and probable cases (definitions according to MoH/WHO recommendations). RVF virus and antibody testing was performed at the Kenyan Medical Research Institute. For malaria, we analysed previous years' data (source: Kenyan Health Management Information System [HMIS]), performed an initial survey to estimate the prevalence of malaria among febrile patients (using rapid diagnostic test [RDT]), and implemented an integrated sentinel (including 3 health facilities and 3 population settlements) and exhaustive surveillance system (including all 9 health facilities in Ijara district). In sentinel sites, febrile patients were tested with RDTs. Health facilities reported data on number of consultations, febrile patients, patients treated with antimalarials, and deaths due to any reason. With a total of 139 RVF cases (28 deaths, 20%), Ijara was the second most affected district of North-eastern Province (total of 333 cases) after Garissa (175 cases). Symptom onset among the first and last cases in Ijara was noted on 7 December 2006 and 28 January 2007, respectively. The peak occurred from 26-28 December 2006 (27 cases). The majority of cases were male (61%) with a median age of 30 years (IQR 21-40 years). The malaria surveillance/alert system lasted from 22 January to 11 February 2007. During this exhaustive surveillance the proportion of febrile patients decreased from 22% to 4%. The initial survey and sentinel surveillance confirmed respectively 1.0% and 1.5% malaria prevalence in febrile patients. In conclusion during the 2006-07 RVF outbreak in Ijara district, the majority of cases were males aged 20-40 years. Interventions to control human RVF outbreaks should target this high-risk group to avoid contact or consumption of sick animals' meat or blood. Malaria cases were far fewer than expected. No malaria epidemic occurred during the observed period.

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COMPLIANCE TO ARTESUNATE - AMODIAQUINE COMBINATION FOR THE TREATMENT OF UNCOMPLICATED MALARIA IN THE MIDDLE BELT OF DISTRICT OF GHANA

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It is estimated that, about 2.7 million cases of malaria deaths are reported each year, 90% of which are in Africa. The most important drawback to successful control of malaria is the development of resistance by *Plasmodium* species to commonly used antimalarial drugs. To enhance effective treatment, Ghana changed its malaria treatment policy from monotherapy to combination treatment with artesunate-amodiaquine. However one of the main challenges to its implementation is compliance to the therapy. It was therefore important to investigate compliance to artesunate-amodiaquine combination therapy and reasons for non-compliance to help with education in the policy change process. The objectives of this study were to determine compliance, side-effects, and parasite clearance among patients treated with artesunate - amodiaquine combination therapy for acute uncomplicated malaria. The study was conducted in the Kintampo North and South Districts in the middle belt of Ghana using both qualitative and quantitative methods. Patients diagnosed with acute uncomplicated malaria as per the Ghana Ministry of Health malaria case definitions were randomised into two groups. Treatment with artesunate-amodiaquine was supervised in one group while the other group was not supervised. Exit interviews were conducted to assess patient's perception about treatment with artesunate-amodiaquine. Compliance in both supervised (95.9%) and unsupervised (92.0%) groups were statistically similar (p=0.1). However, women in the supervised group (97.9%) complied with treatment more than in the unsupervised group (91.3%), (p = 0.02). The main reason for non-compliance was as a result of side-effects and forgetfulness. The commonest side-effect reported among both groups were headaches (supervised = 28.8%, unsupervised = 35.2%), body weakness (supervised = 26.8%, unsupervised = 27.2%) and body pain (supervised = 19.7%, unsupervised = 21.0%). Parasite clearance by day 28 was >95% in both groups. Perceptions of artesunate-amodiaquine treatment were diverse regarding the number of tablets and frequency of taking tablets. In conclusion, compliance to artesunate-amodiaquine is good in the middle belt of Ghana. However, there is the need for intense education on the commonest side-effects of the drug to avoid non-compliance.

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LONG LASTING HUMORAL AND CELLULAR RESPONSES TO *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 IN THE LOW-TRANSMISSION AMAZON REGION OF PERU CORRELATE WITH LONG-TERM CLINICAL PROTECTION

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Antibody responses to vaccine candidate antigen Merozoite Surface Protein-1 (MSP-119kD) are short-lived (<2 months) in high-transmission areas such as sub-Saharan Africa, and clinical protection does not develop despite constant, overlapping infections for the first 5 years of life. Thus, we investigated IgG and IgG subclass responses to MSP-1 19kD in the *low*-transmission Amazon region of Peru, where we examined antibody responses before, during, and after spaced *Plasmodium falciparum* infections. In addition, we performed plasmablast flow cytometry on a select group of *P. falciparum*-infected individuals to examine the potential for anti-MSP-119kD memory responses. Longitudinal blood samples and comprehensive epidemiologic data were obtained from a cohort of ~2000 individuals living near Iquitos, Peru. Between 2003-2006, 111 individuals who have had successive *P. falciparum* infections spaced ~8 months apart were evaluated. ELISAs were performed on sera collected 1 month before, during, and 1 month after a detected infection to evaluate the IgG, IgG subtype, and IgM levels to MSP-1 19kD. Also, available samples from Day 5 and Day 8 after infection were evaluated for plasmablast content. We then compared antibody responses and plasmablast production with clinical symptoms and parasite density. We found that the anti-MSP-119kD IgG responses were long-lasting (> 8 months) in individuals with a history of relatively frequent or early *P. falciparum* exposure. Children had a lower and slower IgG response than adults, which is likely due to lack of prior exposure. IgG1 and IgG3 responses were high in individuals with fewer symptoms, lower parasite loads, and longer aparasitemic periods. Also, our data indicate that plasmablasts, which were assumed to indicate a memory B-cell population being formed to *P. falciparum* antigens, are more likely to be present 6-8 days after infection in asymptomatic, older individuals than in younger individuals or those responding to a first infection. Thus, natural low-transmission *P. falciparum* exposure seems to elicit long-term clinical protection.

(ACMCIP Abstract)

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SELF-EVALUATION OF VSS, A SYNDROMIC SURVEILLANCE SYSTEM FOR OUTBREAK DETECTION IN PERU, A DEVELOPING COUNTRY

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Conventional disease surveillance may be too slow and insensitive to rapidly detect disease outbreaks. To meet this need, in Jun 2005, the Direccion General de Epidemiologia (DGE) supported by the US Naval Medical Research Center Detachment (NMRCD) implemented a syndromic surveillance system named VSS (Vigilancia de Signos y Sintomas), based on the Early Warning Outbreak Recognition System (EWORS) developed by the US Naval Medical Research Unit 2 (Indonesia). Two health centers were selected in Tumbes. In Jan 2006, 7 sites were added in Lima. We performed an evaluation of the system after its implemetation. VSS is based on the recording of 33 symptoms compatible with epidemic infectious diseases. Data is collected using a paper form filled out by technical personnel at Triage. Data is entered into the software and sent by email to DGE, a regional hub and NMRCD. Data is analyzed daily at the local level. The system identifies any unusual increase in symptoms through the C2 EARS algorithm. Using CDC criteria, we retrospectively evaluated VSS covering the period from Jan 2006 to Mar 2007 (15 months), using the data collected, on site evaluations and focus groups. During this period, 4756 forms were collected in Tumbes, 8.1 forms per site per day. The most frequent symptoms were cough (70.8%), fever (68.8%), and rhinorrhea (44.2%). In Lima, 5330 forms were collected, 2.9 forms per site per day. The most frequent symptoms were fever (97.6%), cough (54.4%), and rhinorrhea (27.6%). The system detected 29 peaks of febrile syndrome in Tumbes and 83 in Lima, but only 8 of those were considered relevant for further investigation. Reporting rate was 83.4% in Tumbes and 80.6% in Lima. Information was sent on average each 7.3 days in Tumbes and 3.6 in Lima. An evaluation held in Feb 2006 in Tumbes showed 92.5% complete forms and 0.49 errors per form. Fever reports had good correlation with the MOH febrile surveillance. Time was estimated for every part of the process, which users felt comfortable with. VSS was considered useful by 100% and easy to operate by 81%. VSS sustainability is based on the free software and the use of technology already in place. In conclusion, VSS was rapidly implemented with good performance reached during the first year. VSS proved to be a simple, stable and an acceptable system, but timeliness needs to be improved. VSS has been successful in enhacing the local level surveillance and response.

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URBAN FARMING AND RISK FACTORS FOR *SCHISTOSOMA MANSONI*, HOOKWORM AND MALARIA IN WESTERN CÔTE D'IVOIRE

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Urban agriculture is common across sub-Saharan Africa, yet little is known about the risk of malaria, schistosomiasis and hookworm infections in urban settings. Our objective was to identify risk factors for an infection with *Plasmodium*, *Schistosoma mansoni* and hookworm in farming communities in the town of Man, Côte d'Ivoire, and to assess small-scale patterns of infection. We carried out two cross-sectional surveys in six agricultural zones with 112 farming households and 586 individuals involved. Heads of households were interviewed on agricultural activities, water use, sanitation facilities, and socioeconomic status. Geographic coordinates were recorded of house location, farming plots and potential mosquito breeding sites. A finger prick blood sample was taken from each household member and examined for *Plasmodium*. Stool samples were examined for *S. mansoni* and hookworm using two diagnostic approaches. We found a prevalence of *S. mansoni*, *P. falciparum* and hookworm of 51.4%, 32.1% and 24.7%, respectively. Risk factors for a *S. mansoni* infection included living in close proximity to the main river, water contact with irrigation wells and low education attainment. Risk factors for a *P. falciparum* infection among children aged < 15 years comprised of living in close proximity to permanent ponds, periodic stays overnight in temporary farm huts and low socioeconomic status. Risk factors for a hookworm infection were using water from domestic wells, and low socioeconomic status. We observed considerable heterogeneity over small spatial scales; the highest prevalence of *S. mansoni* and hookworm was observed in a zone of a large rice parameter, and the highest prevalence of *P. falciparum* occurred in a zone of mixed crops. Our data call for an enhanced understanding of the epidemiology of malaria, schistosomiasis and hookworm in urban settings for the design and implementation of sound control strategies.

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INACTIVATION OF CHIKUNGUNYA VIRUS IN PLASMA AND PLATELETS USING THE INTERCEPT BLOOD SYSTEM

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Chikungunya virus (CHIKV), an enveloped virus in the alphavirus genus of the family Togaviridae, has recently emerged as a significant epidemic pathogen in the Indian Ocean region. As a result of travel, numerous infections with the virus have been identified in at least six European countries. Although transmission is most commonly by mosquito bite, the infection can also be spread by blood transfusion from an infected donor. CHIKV poses a significant threat to the blood supply in the epidemic region, particularly on the island of La Réunion where approximately one third of the population had been infected by April 2006. The INTERCEPT Blood System™ for platelets and plasma has been extensively tested and shown to inactivate a wide variety of enveloped viruses. To reduce the risk of transfusion-transmitted CHIKV infection from locally collected platelets, the INTERCEPT Blood System for platelets was implemented in La Réunion, as previously reported (Rasonglès). Because the INTERCEPT Blood System for platelets was implemented in La Réunion specifically to address the CHIKV epidemic, these studies were undertaken to evaluate the efficacy of this system for inactivation of CHIKV in platelets and in plasma. The platelet study used CHIKV strain LR2006 OPY1, isolated in La Réunion in 2006. The plasma study used strain 27, isolated in Tanganyika in 1953. Platelet units consisted of 2.5-6.0 $x10^{11}$ platelets suspended in ~300 mL of 35% plasma/65% PASIII, and plasma units were ~600 mL. The inoculated platelets and plasma were treated with 150 µM S-59 and 3.0 J/cm^2 UVA. Samples taken pre- and post-treatment were diluted in assay medium prior to inoculation of vero cells for determination of infectivity endpoint titers. >6.3 ±0.7 logs of CHIKV was inactivated in platelets and greater than or equal to 7.2 ±0.9 logs was inactivated in plasma. In conclusion, high titers of CHIKV in platelets and in plasma were effectively inactivated by treatment with the INTERCEPT Blood System.

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SAFETY AND EFFICACY OF SILYMARIN ON PATIENTS WITH ACUTE HEPATITIS: A RANDOMIZED, CONTROLLED TRIAL

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Milk thistle or its purified extract, silymarin, is widely used for patients with acute or chronic viral hepatitis, but efficacy studies have been inconclusive. We evaluated the effect of silymarin (*Silybum marianum*) and a multivitamin placebo on symptoms, signs and laboratory test results in patients with acute hepatitis, regardless of etiology, with a particular focus on markers of biliary excretion. In this randomized, double-blinded, placebo-controlled trial, 105 individuals with symptoms compatible with acute hepatitis and serum alanine aminotransferase levels > 2.5 times the upper limit of normal were enrolled from two fever hospitals in Egypt from July 2003 through October 2005. They were randomized to three times daily oral ingestion of either a standard recommended dose of 140 mg of silymarin or a placebo for four weeks with an additional four-week follow-up. Primary outcomes were symptoms and signs of acute hepatitis and results of liver function tests on days 3, 5 and 7 and weeks 2, 4, and 8. Side-effects and adverse events were ascertained by self-report. Patients randomized to the silymarin group had a quicker reduction in direct *(p=0.042)* and total *(p=0.027*) bilirubin, scleral icterus (*p=0.008*), jaundice *(p=0.016)*, dark urine *(p=0.033)*, malaise (*p=0.077*), fatigue (*p=0.11*) and anorexia *(p=0.19)*. The major improvement of silymarin over placebo was on those findings characteristic of biliary excretion *(p=0.021).* No serious adverse events were noted. The major limitations are the heterogeneous etiologies of acute clinical hepatitis and in clinical settings patients may not be as compliant in taking the silymarin. In conclusion, regardless of etiology, the participants receiving silymarin had earlier improvement in markers of biliary excretion, whereas improvement in variables associated with hepatic inflammation and systemic manifestations were less influenced. The fact that the recommended dose of silymarin was effective and safe, although still a fraction of that used in successful animal studies, supports evaluating higher doses in patients with acute or chronic hepatitis.

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DROUGHT WAS A CONSTANT FACTOR IN THE INITIATION OF LARGE EPIDEMICS OF LOUSE-BORNE TYPHUS

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Louse-borne typhus is an acute infectious disease caused by bacteria *Rickettsia prowazekii*. The infection is transmitted among humans by the body lice (*Pediculus humanus corporis*), which is highly contagious in populations subject to crowding and poverty. For centuries, louse-borne typhus caused devastating epidemics in many parts of the world, particularly in periods of war and famine. The great Irish famine of 1846-1849, Napoleon's catastrophic Russian expedition of 1812 and the Serbian epidemic of 1915 are some of the best-documented examples. The frequent association of typhus with winter, famine and war can be explained by the existence of large military and civilian populations subject to deplorable sanitary conditions such as crowding, lack of regular bath, and using the same cloths for extended periods of time, conditions that facilitated the transmission of body lice. Drought has been associated to some historically important typhus epidemics. However, the coexistence of both, have been considered as casual concurrent events. In this report we demonstrate that all 22 major louse-borne typhus epidemics in the highlands of Mexico between 1650 and 1950 were associated with drought in the first year of the outbreaks.

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ASSESSMENT OF CLINICAL TROPICAL MEDICINE COMPETENCY AMONG U.S.-TRAINED MEDICAL STUDENTS AND RESIDENTS

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There is general agreement that U.S.-based medical training does not adequately prepare students in the field of clinical tropical medicine. Our aim was to assess the clinical tropical medicine competency of U.S.-trained medical students and residents. We did so by creating a quiz based on clinical cases seen by the first author as a third-year medical student on clinical elective in India in January 2007. The quiz--distributed to individuals at various levels of medical training--consisted of photographs of 12 different clinical findings seen in a tropical setting, including classic diagnostic images of scabies, lymphatic filariasis and meningococcemia. Students/residents at Tulane University School of Medicine were shown a clinical finding and asked to write down their response. Then they were shown 5 possible answer choices and were asked to select from these options, without changing their previous answers. Results were analyzed and stratified by level of medical training, specialty and infectious vs. non-infectious etiology. Preliminary results showed the following: 1st-year medical students answered 33.55% correctly; 2nd-year medical students, 35.56%; 3rd-year medical students, 51.47%; 4th-year medical students,

63.89%; and surgery residents and interns, 56.25%. Upon stratification by Surgical vs. Medical/Pediatric, surgery residents and interns correctly identified both types of findings more frequently than all medical students combined (77.8% and 51.0%, respectively). Among medical students, performance was highest among 4th-year students and was proportional to level of undergraduate medical training. In conclusion, medical students with higher levels of training, not surprisingly, were more successful at recognizing clinical signs. However, the levels of clinical awareness at all stages were still inadequate. Given that, at best, students/residents answered approximately 60% of the questions correctly, it is recommended that microbiology and infectious disease components be emphasized in both undergraduate and post-graduate curricula.

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REDUCED PEDIATRIC HOSPITALIZATIONS FOR SEVERE MALARIA FOLLOWING IMPLEMENTATION OF COMMUNITY-BASED PREVENTION AND EARLY TREATMENT PROGRAMS IN RURAL RWANDA

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Poor access to medical care and preventive measures contributes significantly to the burden of severe pediatric malaria in Sub-Saharan Africa. To assess whether reduction of these barriers led to a decline in severe malaria, we evaluated admissions to the pediatric ward of Rwinkwavu Hospital, in a malaria-endemic zone of rural Rwanda, before and after rollout of community-based prevention and early treatment programs. Pre- and post-intervention hospitalization and laboratory data were collected during the high transmission seasons of December 2005 through February 2006 and December 2006 through February 2007. Implementation of community programs occurred from July through September 2006. The prevention arm involved distribution of over 25,000 insecticide-treated bed nets to children 5 and under. The early treatment arm was conducted by 300 community health workers trained to administer antimalarial medications (sulfadoxine-pyrimethamine and amodiaquine) to children ages 1-5 with clinical malaria in the community. Children with severe disease were referred to our hospital. Evaluation was based on discharge registry, laboratory records, and chart review. The main outcomes were the number of hospitalized cases of 1) clinical malaria, and 2) microscopically-confirmed malaria. There was a significant decline in hospital admissions for clinically diagnosed malaria (287 vs 151, Chi-squared test p=0.000). The analysis to slide confirmed cases demonstrated an even greater reduction (205 vs 63, Chi-squared test p=0.000). Additionally, there was a significant trend towards lower parasitemia post-intervention. Continued monitoring over time and measurements of vector capacity and other variables that may impact disease prevalence will be necessary. However this data suggests that intensive community-based prevention and early treatment programs can rapidly result in a reduction of severe pediatric malaria in rural Africa.

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PROSPECTIVE ASSESSMENT OF SEVERE MALARIA FOR CLINICAL TRIALS AT INSTITUTIONS IN WESTERN KENYA

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Malaria continues to be a significant cause of morbidity and mortality in Africa. Effective treatments and outcomes for complicated malaria are difficult due to many factors including physiologic, cultural and financial. This study sought to evaluate the demographics, clinical and laboratory presentation, hospital course, and outcomes among Kenyans admitted to the hospital in Sub-Sahara Africa. Four hospitals in Western Kenya were selected to participate in recruitment of individuals who were admitted with confirmed malaria. Individuals were followed throughout their hospital course until discharge or death. 368 individuals were recruited (44 Adults/ 324 Children). On averaged, the inpatient stay was 7 days while fever duration was 3.4 days. The proportion of individuals with one episode of the following: Seizure 164(44.5%), respiratory distress 76(20.6%), congestive heart failure 4(1%), pulmonary edema 3(0.8%), jaundice 102(27.7%), oliguria 27(7.3%), hemorrhage 15(4%), meningitis 8(2.1%), and pneumonia 31(8.4%). Laboratory evaluation included: CBC, AST, ALT, bilirubin, albumin, BUN, creatinine, glucose and lactate on admission. Of 368 admissions there were 21 deaths (20/21 were children, 3 months to 14 years) for a total death rate of 5.7% and occurred on average 4.9 days after admission. Of those that died, the following parameters were noted at admission: 17/23 (74%) were initially started on IV Quinine, 9/23 (39%) had jaundice, 1/23(4.3%) meningitis, 4/23(17.4%) evidence of shock, 11/23 (47.8%) respiratory distress and 7/23(30.4%) seizures and an average Child Blantyre score of 2.7. Death was highly correlated to age, leukocytosis, increased prothrombin time, AST, total Bilirubin, BUN and lactate. Malaria is a complex infection involving multiple organ systems. Our study illustrates the wide range of clinical and laboratory findings associated with individuals effected by malaria and the challenges faced by the treating health care provider. Given the enormous relative expense of hospitalization, parameters for the admission and administration of health resources would be extremely helpful for resource management and improved health care. Based on the prospective data obtained in this study, clinicians can utilize these data to assist in triaging individuals and in the allocation of resources for those with the greatest need.

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MALARIA INFECTION AND ANEMIA AMONG PREGNANT WOMEN AND CHILDREN UNDER FIVE YEARS OF AGE: A PREVALENCE SURVEY FROM FIVE DISTRICTS IN EASTERN INDONESIA

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The eastern islands of Indonesia have long been known for their high levels of malaria transmission. Nevertheless, rigorous surveys to measure the burden of malaria in this part of the world have rarely been done. To obtain baseline data for an integrated program of malaria prevention for pregnant women and children under five years old, Unicef/Indonesia supported health and nutrition surveys in five districts in Eastern Indonesia. Using hand-held computers (PDAs) and GPS, surveys were done in 2006 in Alor, East Sumba, and West Sumba Districts of East Nusa Tenggara Province, and in Jayapura District, Papua Province. Sorong, in West Irian Jaya Province, was surveyed in 2007. In each district 30 villages were chosen randomly with probability proportional to size. Twenty-eight households were simple randomly selected from each selected village. Health and nutrition data were collected from each household through questionnaires, with the data entered directly into PDAs. Children under 5 years of age, pregnant women, and women of reproductive age were examined for malaria and geohelminth infection as well as anemia. Anthropometric measures to assess nutrition status were also taken. The result showed that malaria prevalence varied widely among districts. For pregnant women, infection rates varied from 1.4% to 30.1% while among children under five prevalence varied from 1.8% to 23.8%. Both

Plasmodium falciparum and *P. vivax* were common. Anemia, as defined as Hb < 11 g/dl, was strikingly high, with prevalence varying from 40.0% to 70.2% and 51.9% to 63.6% among pregnant women and children under five, respectively. Overall, results showed high levels of malnutrition and malaria, demonstrating a clear need for intensified malaria control efforts in this region of Indonesia.

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ACHIEVING EFFECTIVE COVERAGE: THE IMPORTANCE OF QUALITY AND USE CONSIDERATIONS IN SCALING UP BED NET DISTRIBUTION PROGRAMS FOR MALARIA CONTROL AND PREVENTION

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Global malaria control and prevention efforts have received a substantial infusion of new resources over the past decade, and much of this has been directed toward the purchase and distribution of insecticide-treated bed nets which have been proven one of the most cost-effective interventions currently available. Thus, in many respects, the vigor and enthusiasm with which funding and implementing agencies have focused on increasing bed net coverage represents an impressive example of "research to practice" in action. However, in an ironic turn of events, public health practice has gotten ahead of the science. International targets and standard coverage indicators that once provided a reasonable guide for malaria control program managers to plan and assess their progress may quickly become less useful in the context of a rapidly changing market for nets and increased availability. A measure of "effective coverage," - that takes quality into account as well as intra-familial and community use patterns - is needed to be able to adequately assess progress as countries begin to achieve early milestones and targets. In this paper, we will: 1) provide a review of the coverage indicators commonly used by the major implementing agencies to assist planning efforts and to assess progress; 2) describe the inadequacies of commonly used coverage indicators, referring both to the published literature and original field data from Vanuatu; and 3) propose a new measure of effective coverage that can be used to supplement existing international targets and standard coverage indicators to guide malaria control planning efforts and to assess progress and achievements in scaling up bed net distribution programs. Evidence in support of this new measure will be drawn from the environmental health literature on "dose response" (e.g. what dosage of insecticide remaining on an aging bed net causes what effect?), epidemiological studies estimating community-wide effects of widespread bed net coverage, and original data from a comprehensive household survey conducted in Vanuatu.

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ASSOCIATED INFECTIONS TO HUMAN BARTONELLOSIS (ACUTE CARRION'S DISEASE) INPATIENTS IN AN ENDEMIC AREA OF THE NORTHERN FOREST OF PERU

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Carrion's disease is a reemerging disease in Peru; one of the main causes of complication and death among cases of Acute Carrion's Disease (ACD) is the coinfection or superinfection. Nevertheless, these conditions has not been previously researched in endemic areas. The aim of our study was to describe the associated infections to ACD inpatients in an endemic area of the Northem forest of Peru. A prospective study was performed in the Jaen General Hospital, Cajamarca department located in the Northern forest of Peru, between July 2005 and February 2007. All inpatients with clinical picture of ACD were enrolled. A clinical-epidemiological questionnaire was administered and blood samples were collected for thin smear, culture and PCR for *Bartonella bacilliformis* (Bb). Sera were tested for evidence of hepatitis B, *Leptospira*, Dengue virus, toxoplasmosis, Chagas disease, brucellosis and *Salmonella*. Urine culture, stool examination for parasites, and thick smear for malaria were also performed. Those who had thin smear, culture or PCR positive for Bb were considered as confirmed cases of ACD. A total of 115 subjects were enrolled; from them 75 were confirmed cases of ACD. The median age was 9 years old (3months-62 years), 61% were male. The main associated infections were: leptospirosis 54% (29/53), toxoplasmosis 8,3% (4/48), Chagas disease 1,7% (1/60), *P. vivax* 1.3% (1/75) and *P. falciparum* malaria 1.3%(1/75). The main intestinal parasites were: *A. lumbricoides* 23.7% (14/59), *T. trichiura* 20.3% (12/59), *A. duodenale* 6.9% (4/59), and *G. lamblia* 13.6 % (8/59). One blood culture was positive for Staphylococcus sp. and all urine cultures was negative. The lethality rates was 5.3%(4/75). In conclusion, the main associated infections to ACD inpatients were leptospirosis and toxoplasmosis. Local endemic infectious diseases such as malaria, Chagas disease and intestinal parasites should be also considered into the therapeutic approach of these cases.

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ADIPONECTIN AND LEPTIN - YIN AND YANG MEDIATORS OF THE MACROPHAGE INFLAMMATORY RESPONSE, DEPENDENT ON HOST NUTRITIONAL STATUS

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Malnutrition contributes to 55% of all childhood deaths from infectious diseases in developing countries. We have previously described a murine model of malnutrition that mimics moderate human malnutrition. Compared to macrophages from control mice, resident peritoneal macrophages from the malnourished mice produce, after stimulation with interferon-γ (IFN- γ), and lipopolysaccharide (LPS), lower levels of TNF- α, NO, and cysteinyl leukotrienes (cysLT), and higher levels of prostaglandin E2 (PGE2) and prostacyclin (PGI2). Serum levels of adiponectin were 95% higher in the malnourished mice than in the well-nourished mice (31.0 mcg/mL vs 16.0 mcg/mL), whereas leptin levels were 48% lower (2.6 ng/mL vs 5.0 ng/mL). In prior experiments, we found that leptin increased IFN-γ/LPS-stimulated macrophage release of NO, TNF-α, and cysLTs, and reduced IL-6, PGE2 and PGI2 secretion, whereas adiponectin decreased cell culture supernatant levels of both TNF–α and NO and increased levels of PGE2 and PGI2. We hypothesized that adiponectin may have a direct antagonistic effect on macrophage stimulation by leptin. When macrophages were pre-incubated with adiponectin prior to IFN-γ/leptin/LPS stimulation, macrophage production of TNF- α and NO decreased 54 and 70%, respectively ($P<0.01$), whereas the levels of PGE2 and PGI2 significantly increased (267% and 247%, respectively; $P < 0.01$). Thus, adiponectin has a potent modulatory effect on the leptin stimulation of the macrophage pro-inflammatory response. The excessive levels of adiponectin observed in malnutrition may contribute to defective macrophage activation and may be a factor in the increased susceptibility to infection seen in the malnourished host.

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ETIOLOGY OF DIARRHEA AMONG 0- TO 59-MONTH OLD CHILDREN IN BAMAKO, MALI - A PILOT STUDY

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Diarrhea is one of the most common causes of childhood mortality in developing countries. However, few systematic studies have been conducted to identify the etiology. This pilot study has been undertaken to identify the most common causes and to prepare for a large-scale multicenter study in developing countries. All 0- to 59-month old children presenting to Hôpital Gabriel Touré, the primary pediatric hospital in Bamako, with diarrhea (3 or more loose stools passed within the last 24 hours) are recorded in a registry. From Monday through Thursday, after obtaining consent, stool samples were collected from the first 2 children who presented with diarrhea which began within the last 5 days and provided a stool sample by 1:30 pm. Stool samples were stored in cold packs and then processed using standard microbiologic techniques to isolate *Salmonella, Shigella* and pathogenic *Escherichia coli* and tested by immunoassay for rotavirus. Results were provided to the treating physician. From June 2006 to November 2006, 1713 cases of diarrhea were recorded, 852 (49.7%) 0- to 11-month olds, 611 (35.7%)12- to 23-month olds and 250 (14.6%) 24- to 59-month olds and stool samples were collected from 208 children, 107 (51.4%), 67 (32.2%) and 34 (16.3%) per age group, respectively. Pathogens were isolated from 49 (23.6%) samples, *Shigella spp.* were isolated from 9 (4.3%), *Salmonella* from 6 (2.9%) and rotavirus was positive in 34 samples (16.3%). *E. coli* typing results are pending. Among the 15 children who provided a stool sample and reported bloody stools, *Shigella* was isolated from 3 (20%) and *Salmonella* from 1 (6.7%). Among the 135 children who provided a stool sample and reported stools with mucus, *Salmonella* was isolated from 2 (1.5%) and *Shigella* from 6 (4.4%) and 20 (14.8%) were positive for rotavirus. Among the 25 (23.4%) infants who had a pathogen, 4 (16%) had *Salmonella*, 1 (4%) *Shigella* and 20 (80%) had rotavirus. Among the 18 (26.9%) toddlers who had a pathogen, 4 (22.2%) had *Shigella*, 2 (11.1%) *Salmonella*, and 12 (66.7%) rotavirus. Among the 6 (17.6%) 24- to 59-month olds who had a pathogen, 4 (66.7%) had *Shigella* and 2 (33.3%) had rotavirus.In conclusion, rotavirus is an important pathogen in the first 2 years of life and a vaccine is likely to offer great benefit. *Shigella* becomes the leading pathogen among children 24-59 months of age. Additional analysis will reveal the role of diarrheagenic *E. coli* and viral enteropathogens.

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EMERGENCE AND CLONAL EXPANSION OF INTESTINAL QUINOLONE-RESISTANT *ESCHERICHIA COLI* IN SOUTHWESTERN NIGERIA

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Resistance to several antimicrobials became increasingly prevalent among fecal *Escherichia coli* from Ile-Ife, southwestern Nigeria between 1986 and 2000. An exception to this trend was the quinolone antibacterials to which resistance was rarely detected. In 2005, we sampled fecal *E. coli* from the same location and found that carriage of quinolone resistant *E. coli* (QREC) by healthy adults had increased from 0-1%, before 2000, to 20% in 2005. Multi-locus sequence typing of 21 QREC isolates, with other complementary tests for clonality, distinguished at least two highly related groups, which accounted for two-thirds of the QREC strains identified. Ten isolates belonged to the Sequence Type (ST) complex 10 and four strains to a previously undescribed, and as yet uncatalogued, ST complex. The identification of strains belonging to ST 10, an enteroaggregative *E. coli*- and enterotoxigenic *E. coli*-associated group, and the exhibition of localized, aggregative-diffuse or diffuse adherence by ten isolates, led us to screen the QREC strains for virulence genes associated with diarrheagenicity. Genetic loci and/or phenotypes associated with enteroaggregative, diffusely-adherent, enterotoxigenic or enteropathogenic *E. coli* were detected in all but three QREC isolates, suggesting that, although recovered from apparently healthy adults, the isolates were predominantly sub-clinical diarrheal pathogens. Recently-emerged QREC appear to be exceptional colonizers with diarrheagenic potential, which could have important implications for enteropathogen spread as well as for the management of persistent and invasive diarrhea in the region.

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CHARACTERISTICS OF CHOLERA OUTBREAK IN DELHI (2000-2006)

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Cholera is highly seasonal disease which constitutes major burden of disease in the hospitals of developing nations. We studied the characteristics of cholera outbreak in Delhi and the preventive measures taken by health authorities to reduce the impact of the disease. Retrospective cross sectional study was conducted with the cooperation of the Registry of Health Department, Municipal Corporation of Delhi. The duration of the study was 7 years, from 2000 to 2006). The suspected cases were defined by the sudden appearance of watery diarrhea, with or without dehydration. Rectal swabs from all suspected cases were bacteriologically examined to confirm the disease. SPSS version 10 was used for statistical analysis. 663 confirmed cases with mean age of 16.1 ± 14 years, (range 3 months to 85 years), 63% males, and 44% children(<12 years) were included. Mean declaration time was 3 ± 1 days. A decrease in the cases was reported in 2003(66 vs. annual average of 100). 82% cases were detected between months of April and August which represent summer and monsoon (rainy) season. The risk factors included living in villag e(51%) or resettlement colonies(22%), poor personal hygiene(60%),contaminated drinking water (22%), poor sanitation (95%), and government piped water supply- (75% of contaminated water cases).The first case isolation was done in March and the last was in December each year. 21% (116/553) cases were untraced after initial detection. Preventive measures included distribution of- ORS (35 packets/case), Chlorine tablets (mean 4130 /case), posters, pamphlets, hand bills in the affected area. In conclusion. considering the frequency of cholera in Delhi, interventions should be designed to prevent and control the reappearance of the disease and its spread to neglected areas. Seasonal variations should also be considered.The hygienic practices were more important than contaminated water sources for transmission of cholera. Significant, untraced cases warrant strengthening the surveillance.

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ELECTROLYTE AND MIXED ACID-BASE DISTURBANCES IN CHOLERA

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The presence of metabolic acidosis with an increased anion gap during the diarrheal phase of cholera has been documented but there is no notification on mixed acid-base disturbances in cholera. Acid-base disturbances in 56 patients with *Vibrio cholerae* 9serotype Ogawa) who were admitted in emergency ward of Imam-Khomeini Hospital/Tehran in the summer of 1998 were studied. At time of admission and 24 hours after rehydration, electrolytes, renal function tests, PO_2, PCO_2, pH and serum bicarbonate were measured. At time of admission, the mean serum concentrations of sodium was 136.6 meq per liter, that of potassium was 3.47 meq per liter, and that of BUN was 58.1 meq per deciliter. All of the patients had metabolic acidosis with a mean serum bicarbonate value of 11.33±3.13 mmol per liter and PH of 7.19± 0.08 which were changed to 14±2.7 mmol per liter and 7.28±0.06; respectively ($p<0.05$).Thirty-three (58.9%) of patients had mixed respiratory acidosis and metabolic acidosis.

In comparison, the severity of acidosis and the mean of logPCO2 of those with mixed acidosis were more than the patients with pure metabolic acidosis ($p<0.05$). In conclusion, acid-base disturbance in cholera is mixed respiratory and metabolic acidosis and the paradox of the presence of sever acidosis and low bicarbonate loss in diarrhea pf patients with cholera is due to respiratory acidosis.

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PREVALENCE OF DIARRHEAGENIC *E. COLI* AMONG BACTERIAL ISOLATES IN PATIENTS WITH ACUTE DIARRHEA IN UZBEKISTAN: THREE YEARS SURVEILLANCE PROJECT RESULTS

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Recent comprehensive data on diarrhea pathogen distribution and antibiotic susceptibility patterns is important for the public health of Uzbekistan. To determine the nature of diarrheal diseases. A surveillance program in Uzbekistan was undertaken to identify bacterial pathogens associated with diarrhea. Stool samples were collected from adults (741 samples) and children (1709 samples) presenting with diarrhea in five regions of Uzbekistan in 2003-2006. Stools were refrigerated and transported to a central laboratory where culture for *E. coli*, *Salmonella*, *Shigella*, and *Citrobacter* using standard methods with utilization of Endo, Kligler and SS media with following differentiation by biochemical tests (Merck, Germany; Microgen, Russia). Diarrheogenic *E. coli* was identified with polyvalent serums (BioMed, Moscow, Russia), *Shigella* species and *Salmonella* serotypes were identified by serotyping with commercial available serums (Pasteur Research Institute, Saint-Petersburg, Russia). A total of 2450 stool samples (741 from adults and 1709 from children) from the five regions were received at the central laboratory. At least one bacterial species was isolated from 579 samples (24%). Major pathogens identified included diarrheogenic *E. coli* in 129 (22%); *Salmonella enterica* serovar *tymphimurium* 49 (8%); *Shigella spp.* 47 (8%) (*S. flexneri* 34 (6%), *S. sonnei* 11 (2%), *S. boydii* and *S. dysenteriae* (1 each), other *Salmonella enterica* serovars 15 (3%). Two bacterial pathogens (duel infection) were identified in 12 (2%) of stool samples. *Citrobacter spp.* were identified in 119 (21%). In conclusion, diarrheogenic *E. coli* was the most common bacterial pathogen identified although *Citrobacter* was also frequently isolated. To determine the pathogen group of *E. coli* the further development of DNA specific probing is proposed.

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CRYPTOSPORIDIUM AND MALNUTRITION ADDITIVELY INCREASE ILEAL DAMAGE AND PRO-INFLAMMATORY CYTOKINE RESPONSES

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Cryptosporidium is a leading pathogen in children in developing areas and in immune-compromised patients with AIDS. In order to investigate whether early post-natal malnutrition leads to heavier cryptosporidium infections, we conducted studies in a malnourished mouse model to assess intestinal adaptation and infection loads during the first two weeks of life, similar to the first two formative post-natal years in humans. C57BL6J litters were adjusted to 7 to 8 pups. Malnutrition was induced by daily separation of the pups from their lactating dams. Half of the litter was separated daily, starting with 4 h at D4, 8 h at D5 and 12h from D6 until D14. Nourished controls stayed with the mothers. Body weight was recorded daily until sacrifice. At D6, each pup received an oral inoculum of either 10^5 or 10^6 in 10-25µl of PBS. Littermate controls received PBS orally at the same age. Stools were collected from day 8, 11 and 14 for shedding and oocyst counts. Euthanasia was performed at D14, eight days post-inoculation, at the peak of the infection. Ileal segments were immersed in 10% zinc formalin for histology or frozen in liquid nitrogen for real time and reverse transcriptase-PCR and TNF-α and IFN-γ immunoassays. Villus and crypt length and area were measured. Both malnourished and nourished mice infected with 10^6 oocysts exhibited the poorest developmental outcomes compared with their non-infected controls. Nourished 10^6 infected mice have comparable weight decrements to non-infected malnourished mice. Body weight and villus surface area were additively affected by malnutrition and cryptosporidiosis. Hyperplastic crypts and heavier inflammatory responses were found in the malnourished 10^6 -infected mice with higher rates of oocyst shedding, measured by immunofluorescence and real time-PCR compared to malnourished and nourished mice innoculated with 10^5 oocysts and nourished mice infected with 10^6 oocysts. Malnourished 10^6 -infected mice also had higher TNF-α and IFN-γ cytokine levels and mRNA expression in the ileum, compared to non-infected and nourished 10^6-infected mice. Taken together these findings suggest additive malnutrition and Cryptosporidium effects on intestinal morphology and growth that are also associated with accentuated inflammatory responses. This model can help elucidate the mechanisms of infection and malnutrition and potential interventions to ameliorate these effects.

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ROTAVIRUS DIARRHEA IN GHANA: EMERGING IMPORTANCE OF ZOONOTIC STRAINS

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Presently, there is a concerted global effort with the objective of introducing rotavirus vaccines. The vaccine is to be used as a means to change radically childhood morbidity and mortality due to diarrhea. With the benefits of substantial reduction of morbidity and mortality associated with rotavirus infection in developing countries and the huge cost of hospitalisation in developed countries. From 1998 to 2007 we have characterised circulating rotavirus strains amongst children less than 5 years of age in Ghana using polyacrylamide gel electrophoresis and RT-PCR. The results indicates the presence in the population of rotavirus strains bearing the unusual genotypes P[6]G2, P[4]G3, P[8]G8, P[10]G9 and P[10]G10. These strains bear characteristics typical of animal rotaviruses such as long electrophoretic patterns and nucleotides with high homology to animal rotavirus strain. These strains could be due to an active reassortment between human and animal strains process in the study area. The presence of these genotypes which differ in their antigenic properties may have serious implication for the future introduction of a vaccine in Ghana. The emergence of zoonotic rotavirus strains in Ghana and their possible impact on vaccine introduction are discussed.

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THE EFFECT OF PREPARATION OF CEBICHE ON THE SURVIVAL OF *ESCHERICHIA COLI, AEROMONAS HYDROPHILA* AND *VIBRIO PARAHEMOLYTICUS*

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Cebiche is a common seafood dish in Latin America, prepared using raw fish mixed with vegetables and "cooked" with lime juice. It is commonly

believed by the general population that the lime juice effectively sterilizes any bacterial contamination and a previous study in Costa Rica demonstrated significant reductions in *Vibrio cholera* contamination using a Costa Rican cebiche recipe. Although little data exists concerning rates of cebiche-associated outbreaks, these may be quite high, as cebiche is commonly consumed by both Peruvians and tourists. To determine the effect of the cebiche preparation process we inoculated raw fish with bacterial agents that commonly contaminate fish, *Aeromonas hydrophila* and *Vibrio parahemolyticus,* or commonly cause diarrhea, *Enterotoxigenic E. coli (ETEC)*. Raw fish were exposed to an initial concentration of 10^8 colony forming units (CFUs) of each organism prior to addition of cebiche ingredients. A typical Peruvian cebiche recipe was used combining limes, onions, sweet potatoes, cilantro and hot peppers marinated together for 10 or 30 minutes periods (the usual marination time for Peruvian cebiche). The initial average pH of the fish was 6.4 prior to adding cebiche ingredients and 5.0 immediately afterwards. pH at 10 and 30 minutes had risen slightly to 5.4 and 5.2 respectively. All ingredients were homogenized in a blender and 100µl inocula were streaked onto TSA agar plates and incubated for 24 hours. No significant reduction in bacterial counts was observed at either the 10 or 30 minute time periods. This study demonstrates that the "bactericidal" role played by lime juice in the Peruvian cebiche preparation process is not sufficient to reduce the microbial population present in cebiche and that pathogenic strains may remain viable even after exposed to these acidic conditions. The increasing popularity of Peruvian cuisine in North America and Europe may also lead to cebiche-associated outbreaks outside of Latin America.

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THE RETINOL EFFECT IN PROTECTING THE INTESTINAL EPITHELIAL DAMAGE INDUCED BY *CLOSTRIDIUM DIFFICILE* TOXIN A

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Vitamin A (retinol) is an essential nutrient needed in small amounts for normal functioning of the visual system, immune function and reproduction. In this study, we have explored the role of retinol supplementation in intestinal cell lines, following *Clostridium difficile* toxin A (TxA) cytotoxic challenge. *C. difficile* is the most common anaerobic pathogen that causes antibiotic-associated diarrhea and pseudomembranous colitis. We have focused on changes in transepithelial electrical resistance (TEER) in Caco-2 and on models of proliferation, migration and cell death in IEC-6 cells. To study the retinol effect on the TxA-induced loss of TEER, cells were exposed during 24h to 0.1µg/mL TxA. Retinol improved TEER (% of initial value) at concentrations of 0.1nM and 0.3nM at 3h (59.3±1.3, 69.8±0.6 vs 59.3±1.3Ωcm^2, respectively), and at 4h (36.1± 0.02; 33.5± 1.8 vs 27.3±0.2Ωcm^2, respectively) in relation to the untreated control challenged with TxA. Retinol increased cell proliferation after TxA-induced cell damage (0.1µg/mL) at a rate of 14.2%, 23.8%, 59.8%; 8.4%; 30.2%; 44.1% after 24h (doses of 0.01; 0.03; 0.1; 1.0; 10; 100nM of retinol, respectively), compared to controls only with TxA. After 24h of TxA exposure (0.01µg/ml), following plate scraping, the retinol supplementation improved significantly IEC-6 migration at all concentrations tested. Retinol concentrations of 0.1 and 1nM resulted in an enhancement in post-challenged cell migration of 41.40% and 18.75%, respectively. Retinol supplementation (0.1-100nM) enhanced cell migration at 12 and 24h, as compared to the control with TxA. Retinol reduced TxA-induced apoptosis and necrosis at all doses tested (0.1; 1; 10 and 100nM), $p<0.05$, in comparison to retinol-free medium. These results suggest that retinol has a critical role in reducing apoptosis, improving cell migration and proliferation and preventing the reduction in TEER, following TxA challenge, suggesting that vitamin A is an essential nutrient to protect the intestinal epithelial barrier function.

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HAND WASHING AND WATER USAGE IN A PERUVIAN SHANTYTOWN

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This study was undertaken to describe hygiene behaviors in a community with limited water availability. We conducted a community-based cross-sectional study to document hygiene practices and water usage of mothers and children living in a shantytown without piped water connections in Lima, Peru. The amount of water used for domestic and personal activities by mothers and their youngest child was measured through continuous monitoring by trained fieldworkers during three 12-hour sessions. Three types of events were also recorded: 1) fecal-hand contamination due to defecation, cleaning the child after defecating, or handling stools; 2) possible fecal-oral contamination events, including food handling, eating, and breastfeeding; and 3) interruption of fecal contamination by hand washing or bathing. Thirty-two mothers and children were observed for 1,008 hours. The mean 12-hour amount of water used by the mother was low, only 48L, 11% of which was used for personal hygiene. Only 45% (54/119) of mothers' fecal-hand contamination events and 30% (16/54) of children's contamination events were followed within fifteen minutes by hand washing or bathing. Mothers and children frequently handled food, ate, or breastfed while having fecally-contaminated hands, 36% (43/119) and 44% (24/54) respectively. Mothers' hand washing after fecal-hand contamination was more-likely on days they used less water for their other household tasks compared to days with higher water usage (OR 2.61, 95%CI 1.27, 5.35). In conclusion, in this water-scarce community, both mothers and children exhibited fairly poor hygiene, which led to frequent chances of fecal-oral contamination. Mothers appear to rely upon non-hygiene related water usages, such as laundry or dish-washing, to decontaminate hands, possibly due to the limited water supply. Further improvements in water availability and hygiene knowledge are still needed for better hygiene to be practiced.

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SURVEILLANCE FOR ACUTE DIARRHEAL DISEASES AMONG PERUVIAN MILITARY RECRUITS AT THE VARGAS-GUERRA ARMY (VGE) BASE IN IQUITOS, PERU: FEBRUARY 2004-FEBRUARY 2007

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Between FEB 2004 and FEB 2007, 1313 asymptomatic Peruvian military recruits were enrolled in an active surveillance study of diarrhea at the VGE Base in Iquitos, Peru. Of the 1311 baseline stool samples provided, the most common parasites were *Ascaris lumbricoides* (520/1286; 40%), *Trichuris trichiura* (209/1285; 16.3%), *Giardia lamblia* (188/1285; 14.6%) and *Cryptosporidium* (162/1140; 14.2%), while the most common bacteria were enterotoxigenic *Escherichia coli* (ETEC) (58/1311; 4.4%), *Shigella* spp. (51/1311; 3.8%), and *Campylobacter* spp. (12/1311; 0.9%). Of the 1313 volunteers with at least 30 days observation, 258 (19.7%) reported one or more episodes of diarrhea. At least one enteropathogen was identified in 250 of 312 (80.1%) diarrhea stool specimens. *A. lumbricoides* (115/300; 38.3%) and *Cryptosporidium* (99/293; 33.8%) were the most common pathogens identified by microscopy or by the

ELISA, respectfully. Of the 312 diarrhea samples submitted for culture, *Shigella* spp. were isolated from 65 (20.8%). Thus, the incidence of shigellosis in this population is conservatively estimated to be 0.08 episodes per person-year. Most (57 of 65; 87.6%) of the *Shigella* isolates were *S. flexneri* (Gr. B) of which 22 (38%; 0.03 episodes per person-year) and 17 (29.8%) were serotype 2a and 3a, respectively. These isolates were resistant to multiple antibiotics including tetracycline (92.2%), sulfamethoxazole/trimethoprim (72.5%), ampicillin (64.7%), chloramphenicol (68.6%) and azithromycin (25.5%), while no resistance was observed to ciprofloxacin and ceftriazone. In addition to *Shigella*, ETEC was isolated from 36 (11.5%) and *Campylobacter* from only 6 (1.9%) of the 312 diarrhea samples. To evaluate correlates of protection, IgG seroconversion against *Shigella* spp. virulence determinants (e.g. Ipa proteins) was monitored at 3-month intervals. In initial screening, 8 of the 247 volunteers (3.2%) seroconverted but detection was hampered by the high titers observed in 71% of baseline samples, indicating that the majority of the population has been previously infected with *Shigella* spp.. In summary, asymptomatic carriage of pathogenic parasites and bacteria continues to be high at the VGE Base resulting in high incidence of diarrheal disease among the Peruvian military recruits. This population should be considered for evaluating efficacy of *Shigella* vaccines in advanced development.

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DETECTION OF A HIGHLY SENSITIVE HUMAN FECAL BIOMARKER (10^{-10}) IN ≤ 10 ML CONTAMINATED DRINKING WATER SAMPLES USING IMMUNOMAGNETIC SEPARATION

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Due to the increased prevalence of pathogens and the high risk of transmissible disease in human-contaminated water supplies, there is a pressing need to develop a rapid yet sensitive assay that detects human fecal contamination. Water assays, such as coliform counts, are time-consuming and are not specific for human fecal contamination. Others require filtering large volumes. Lactoferrin and immunoglobulin (Ig), however, are human-specific biomarkers found in normal feces. Lactoferrin however are not consistently excreted in large amounts like immunoglobulin. We therefore focused on developing an Ig-specific assay as an indicator of water contamination. Isolation of human Ig from 8 to 10 ml of water sample was done by immunomagnetic separation (IMS). The beads with the captured Ig were incubated with conjugated detecting antibodies specific for the Ig and a colorimetric substrate to yield a visible color change in the presence of human Ig. Absorbance of the samples was measured at OD_{450}/OD_{620} using a microplate reader. The detection level for the human fecal biomarker, Ig was as low as 0.1 ppb (which approximates standards for fecal coliform levels). Tests done on several highly contaminated (based on coliform counts) drinking water samples taken from households in Fortaleza, Brazil were positive for the biomarker and that one was positive for *Cryptosporidium*. Preliminary experiments did not show cross-reactivity with horse, donkey, cow, pig, goat, sheep, chicken and geese at 10^{-5} dilution. Preliminary findings looking at the stability of the biomarker at different temperatures showed that it is stable at room temperature, refrigeration at 4°C, freezing at -20°C, and even heating at 50°C for 24 hours. Antibody-coated immunomagnetic beads rapidly detect human fecal contamination in water samples less than 10mL. Pathogens can also be detected with this method. Using the assays together to rapidly detect human fecal contamination and transmissible enteric pathogens can provide an early warning of water supply contamination helping to limit disease transmission.

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GLUTAMINE AND ZINC SUPPORT BRAIN DEVELOPMENT IN SUCKLING SWISS MICE CHALLENGED BY UNDERNUTRITION

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Glutamine and zinc have been implicated as critical nutrients for repairing the intestinal mucosa following injury. However, few studies have addressed the possible additive role of zinc and glutamine in protecting brain maturation in murine models of malnutrition. Thus, we conducted a malnutrition protocol, by clustering the litter size within different experimental groups, including 7-8 pups (nourished PBS controls) and 14-16 pups (treated and non-treated malnourished groups)/per dam, N=14 for each. The zinc supplementation was done by adding 500 mg/L of zinc acetate in distilled water given to the lactating dams. Glutamine (100 mM) was administered to the suckling pups since birth by daily s.c. injection. We have conducted behavioral tests, starting at post-natal day 4, assessing, as follows: swim behavior (head position, navigation, and limb movements), surface righting, and dorsal immobility. In addition, by means of 3-D design-based stereological methods, i.e., Cavalieri principle, optical disector as a counting-probe and the combination of both as well as the vertical rotator method, the volume of mouse hippocampus, volume of CA1 hippocampal layer, numerical density of CA1 neurons, total number of CA1 neurons and, the number-weighted mean volume of CA1 neurons were estimated. All study animals were euthanized at day 14 by overdose of ketamine/xilazine solution given i.p. Immediately after euthanasia, we perfused the brains with the Palay fixative for stereological analyses. In this model, we have found significant growth deficits, during the suckling time, as measured by tail length and weight gain, in the malnourished compared to the nourished mice ($p<0.001$), but there was no difference in the treated and non-treated malnourished groups. In addition, we have found significant deficits with all behavioral tests conducted in the malnourished groups as compared to the nourished groups ($p<0.001$). Furthermore, we were able to find a protective role of zinc plus glutamine in the head position during swimming at day 10. The total number of neurons of CA1 hippocampal layer was $0.12x10^6$ (0.22) for undernourished and Zn-treated mice, $0.19x10^6$ (0.36) for undernourished and glutamine-treated animals, $0.10x10^6$ (0.43) and for the control group ($p=0.023$). These findings suggest that zinc and glutamine have critical additive role during brain plasticity following undernutrition.

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APOE KNOCK-OUT MICE HAVE DISRUPTED INTESTINAL TIGHT JUNCTIONS, FOLLOWING EARLY POST-NATAL MALNUTRITION

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Apolipoprotein E (apoE), a plasma protein involved in cholesterol metabolism, has been found to play a critical role during brain and

nerve plasticity following injury. The blood-brain barrier, which is mainly composed of tight junctions, is disrupted in apoE knock-out mice. In order to investigate whether the intestinal epithelial barrier integrity, also maintained by tight junctions, is compromised in apoE knock-out mice, we conducted studies in our neonatal murine model to assess challenges to the intestinal epithelial barrier due to early post-natal malnutrition. C57BL6J wild-type and apoE knock-out had final, adjusted litter sizes of 7 to 8 pups. Wild-type mothers were used to foster both litters in order to avoid variations in breast-milk. Early post-natal malnutrition was induced by separating pups from their lactating dams. Half of the litter was separated daily, starting with 4 h on D4, 8 h on D5 and 12h thereafter. Nourished controls, stayed with the mothers for the entire experiment. Body weight and tail length were recorded daily until sacrifice. Ileal segments were obtained and carefully washed with PBS, immersed in 10% zinc formalin for histology, or frozen in liquid nitrogen for reverse transcriptase-PCR and imunoblots to assess e-cadherin, occludin, and ZO-1 protein and mRNA expression. Villus and crypt length and area were measured to address mucosal surface. We found a significant decrement on the daily gain of body weight (after day 7, $p<0.05$) and tail length (after day 10, $p<0.05$) in malnourished apoE-ko mice compared to malnourished wild-types. Furthermore, we found shorter crypts and villi in the apoE malnourished mice as compared to malnourished and nourished wild-type controls. ZO-1 expression was significantly reduced in the malnourished apoE-ko mice as compared to the wild-type control. These findings suggest a critical role of apoE in the intestinal barrier function and may be helpful in elucidating potential mechanisms by which to improve development and mitigate the long-term effects of malnutrition in young children.

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DENGUE VIRUS SEROTYPE 2 (SE ASIAN STRAIN) IS STRONGLY ASSOCIATED WITH CLINICALLY DEFINED SECONDARY INFECTIONS IN PUERTO RICO

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Dengue virus serotype 2 (DENV-2) Southeast Asia/Jamaican strain (subtype IIIb) has emerged in Puerto Rico three times in the last 15 years causing outbreaks increasingly associated with hospitalization. The establishment of DENV-2 IIIb in the island correlates with remarkably high occurrence of secondary infections linked to this virus during the last 20 years (84% of viremic DENV-2 patients have detectable levels of IgG antibodies during acute illness indicative of previous infections). The association between DENV-2 IIIb and the occurrence of DHF in the Americas has been previously documented, and a possible connection between secondary infections and a higher viremia or severe disease has been proposed. To better understand the association between viremia in confirmed primary and secondary cases, we have run quantitative RT-PCR on 100 acute serum specimens from patients with confirmed DENV-2 infections and positive IgG test at days 0, 1, 2, 3, 4 and 5 after the onset of symptoms. Among all DENV-2 cases, secondary infection specimens showed a significantly higher concentration of DENV-2 RNA in serum. We have also conducted genomic analyses of DENV-2 rescued from primary and secondary infections in order to evaluate genetic variability among specimens. Our results point towards establishment of DENV-2 IIIb correlated with secondary infections and higher viremia, supporting a more invasive and pathogenic role for DENV-2 during secondary infection.

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GENETIC CHARACTERIZATION OF DENGUE 3 VIRUS ISOLATES RECOVERED FROM PATIENTS WITH ENCEPHALOMYELITIS, RONDÔNIA STATE, NORTHERN BRAZIL

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Dengue virus (DENV), *Flaviviridae*: *Flavivirus*), has four distinct serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), the causative agent of dengue fever (DF) and dengue hemorrhagic fever (DHF). In 2005, an outbreak of DF was reported in Rondonia state, Northern Brazil, and DENV-3 was isolated from the patients including pictures of encephalomyelitis. The study was undertaaken to perform the genetic characterization of DENV-3 by sequencing the entire structural genes (C-prM-E) and the non structural protein 1 (NS1) of the isolates obtained from patients with encephalomyelitis. Two DENV-3 isolated from patients showing clinical pictures of encephalomyelitis, 3 DENV-3 isolates from DF patients of the same outbreak and 5 isolates obtained in 2007 from DHF patients living in Northern Brazil, were used in this study. All of them recovered directly from C6/36 cell culture supernatants from patient samples. Viral RNAs were extracted and used to prepare cDNA which were amplified. Amplicons were directly sequenced and compared with DENV-3 strains isolated in South/Central Americas, and in Asia. To further analyze the genetic relationship of the aforementioned viruses, comparative phylogenetic analysis was conducted using the MP and NJ method including selected DENV-3 strains belonging to all DENV-3 genotypes. The sequence analysis carried out along the structural genes of the encephalitogenic DENV-3 strains revealed silent mutations basically concentrated in the E gene. Of interest, two synonymous mutations were found in the domain III of the E gene generating an amino acid substitution I-380-R modifying the common DENV-3 motif IGD to RGD that is speculated to be related to virus-host cell interaction in some members of the JEV group (Murrey Valley and Japanese encephalitis viruses). Furthermore, the phylogenetic analysis identified the strains isolated in Rondonia state as members of the genotype III and genetically related to strains that circulated in the Brazilian Amazon basin in the same year, but constituted a distinct phylogenetic group. In conclusion, our results revealed that the two DENV-3 strains obtained from encephalomyelitis patients are members of the genotype III that have been commonly isolated in Brazil, and that they presents an interesting mutation at the domain III of the E gene that could be associated with the neurological invasiveness presented during the dengue outbreak in Rondonia state.

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EVALUATION OF A COMMERCIAL NS-1 ANTIGEN CAPTURE ELISA FOR THE DIAGNOSIS OF ACUTE DENGUE INFECTION

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Current methods used to diagnose acute dengue (DEN) infection are costly, difficult to perform, and results may be delayed by up to two weeks (in the case of virus isolation). Consequently, diagnosis of an infection can be delayed or missed, resulting in poor patient care. The dengue non-structural protein 1 (NS-1) may be a valuable diagnostic tool, as it appears earlier in the course of infection than IgM, is more readily detectable in the serum than virus, and may be a reliable predictor of severe disease. This study evaluated the efficacy of a commercially available NS-1 antigen capture ELISA test. This study utilized 225 well-characterized Taqman RT-PCR and/or virus isolation positive samples and 6 negative samples obtained from island-wide surveillance at the CDC- Dengue Branch in San Juan, Puerto Rico. Samples were screened for NS-1 using the *pan-E Dengue Early ELISA* kit (Panbio diagnostics, Brisbane, Australia), and

results were compared to the presence of IgM/IgG antibody, virus, and epidemiological data. The test detected NS-1 in 131 samples (58% overall sensitivity). Sensitivity was better for DEN1 and DEN2 (both 78%) than for DEN3 (51%) or DEN4 (28%). Anti-dengue antibodies (IgM and IgG) in the serum did not seem to interfere with NS-1 detection for DEN1 or DEN2, and RNA load (based on quantitative RT-PCR) did not seem to interfere with detection for any of the 4 serotypes. NS-1 was detected in 82 of 124 (66%) samples taken 3 to 5 days following the onset of symptoms (the critical period of illness). Finally, the test detected NS-1 in 38 of 63 (60%) of samples from cases of DF with hemorrhagic manifestations. In conclusion, while NS-1 remains a promising marker for diagnosing acute dengue infections, the *pan-E* test has reduced sensitivity based on serotype, especially in DEN3 and DEN4. The value of the test as a diagnostic tool may be limited as evidenced by relatively low detection of NS-1 in samples between 3 and 5 days post infection, as well as those from cases with more severe manifestations.

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A BIVALENT DNA VACCINE CANDIDATE AGAINST DENGUE-3 AND DENGUE-4 EXPRESSING THE STRUCTURAL PRM/E PROTEINS ELICITS CELLULAR IMMUNE RESPONSE AND PROTECTS MICE AGAINST LETHAL CHALLENGE

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Dengue is the most important flavivirus infection worldwide. The only method currently available to prevent dengue virus infections is controlling the mosquito vector, but this approach is expensive and very often unattainable. Thus, a dengue vaccine is urgently needed because there is hope that a dengue vaccine will bring this infection to control. However, the development of a dengue vaccine is extremely complicated due to the fact that a protection against all 4 serotypes is needed, since an incomplete immunization could induce the serious form of the disease in individuals experiencing a secondary infection. At present, there are no licensed vaccines for dengue viruses, but several vaccine candidates are in the late-stage of development. Genetic immunization using plasmid DNA expressing immunogenic regions of dengue E protein consists of a new strategy on the manufacturing of a vaccine against dengue. The bivalent vaccine candidate (pVAC3WDEN3/pCID4/CL10) was analyzed *in vivo* to determine its ability to induce a specific immune response in mice. Groups of 10 3-week-old mice were immunized three times in a 15-day interval, and 30 days after the last immunization, the mice were sacrificed. Blood from these animals was collected through the retro-orbitary route, processed for separation of the serum and stored -70°C. For the lymphoproliferation assays, spleen of the immunized animals was isolated and the cells were cultivated in a concentration of $1x10^6$ cells/well and stimulated with either dengue-3 or dengue-4 virus. The cellular proliferation rates from animals immunized with pVAC3WDEN3/pCID4/CL10 were as good as those inoculated with dengue-3 and dengue-4 viruses. The cytokines produced indicate that this vaccine induces a Th-1 profile. Data obtained from challenge experiments show that pVAC3WDEN3/pCID4/CL10 protected 55% of the immunized animals against challenge with a lethal dose of dengue-3 (H87) virus, and 65% of the immunized animals against challenge with a lethal dose of dengue-4 (H241) virus. These protection levels were similar to those obtained with the immunization with dengue-3 and dengue-4 viruses. In summary, the data presented here confirm that the pVAC3WDEN3/pCID4/CL10 vaccine candidate may be part of a tetravalent preparation as long as their immunogenicity is improved.

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SERUM LEVELS OF TNF-α, IL-6 AND IFN-γ AMONG TWO ETHNIC GROUPS INFECTED WITH DENGUE IN COLOMBIA

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It has been postulated that IL-6, TNF-α and IFN-γ play an important role in the pathogenesis of the severe dengue syndromes. In addition, genetic background has been implicated in host response to dengue. This study was undertaken to compare the serum levels of IL-6, TNF-α and IFN-γ in patients infected with dengue from two ethnic groups in the State of Antioquia, Colombia. Methods. Serum samples from forty-three Mestizos and thirty-five African descendants were obtained within five days of the acute phase and in the in the convalescent phase. Both groups had dengue illness confirmed for commercial test dengue IgM capture ELISA. In these samples the measurement of the cytokines was made using commercial enzyme-linked immunosorbent assay kits. The average TNF-α and IL-6 levels were significantly higher in African descendants than in Mestizos (7.14±11.21 pg/ml vs 1.56±3.54 pg/ml, p=0.001 for TNF-α and 14.38±42.30 vs 12.51±18.21, p=0.001 for IL-6). There was no significant difference in the levels of IFN-γ among the two ethnic groups (38.44±163.8 vs 40.06±118.8, p=0.525). According to the clinical form TNF-α level was significantly higher in African-descendants with dengue fever than in Mestizos (6.88±10.92 vs 1.28±3.34, p=0.001). In the cases of dengue hemorrhagic fever TNF-α level was significantly higher in African-descendants than in Mestizos (9.02±13.18 vs 2.42±4.21, p=0.015). IL-6 level was significantly higher in Mestizos with dengue fever (13.77±19.7 vs 12.17±38.6, p=0.001). Although more elevated number of cases with dengue hemorrhagic fever were observed in the Mestizos than in African-descendants (10 vs 4) this differences were no significant, possibly by the sample size. In conclusion, these results suggest that in dengue patients serum levels of IL-6 and TNF-α differ among ethnic groups and emphasize the importance of studying the genetic aspects of this cytokine response in dengue.

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DIFFERENTIAL EXPRESSION OF EFFECTOR-MEMORY CD8⁺T CELL SUBSETS IN PERIPHERAL BLOOD CORRELATES WITH DENGUE HEMORRHAGIC FEVER

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The contribution of memory $CD8^+$T cells to disease pathogenesis in dengue is unclear. In this study, frequencies of the memory $CD8^+$T cell subpopulations in PBMC were measured during and after acute dengue disease. A higher frequency of the effector-memory $CD8^+$T cell subpopulation $CCR7^-CD45RA^-$ and the prolonged presence of IFN-γ-expressing T cells correlated with the disease severity. Consistently with the increased T cell activation during dengue hemorrhagic fever (DHF), viral replication was inhibited more rapidly in the DHF patients as compared with the dengue fever (DF) cases. Differential expression of memory $CD8^+$T cell subsets seems to distinguish DHF from DF.

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SEROTYPE-SPECIFIC CLINICAL CHARACTERISTICS OF HOSPITALIZED DENGUE IN THE PHILIPPINES

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Dengue is the causative agent of dengue fever (DF) and the more severe dengue hemorrhagic fever (DHF). It consists of four distinct serotypes (DEN 1-4). In the Philippines, data from laboratory-confirmed dengue infection is not commonly available. Association of specific clinical or laboratory characteristics with the distinct dengue virus (DENV) serotypes in the Philippines has also remained elusive. A cross-sectional study was conducted at a tertiary-care hospital in Manila between 2005 and 2006 in which patients admitted with dengue-like illness were enrolled. A total of 227 patients with dengue-like illness (89% of all enrolled patients) were confirmed to have DENV by polymerase chain reaction (PCR) or enzyme-linked immunosorbent assay (ELISA). Serotype data was available for 168 of these patients. Of these 168, there were 91 (54%) males. Secondary dengue infection was documented in 151 (90%). Among the primary dengue infections, there were 2 DEN-1, 2 DEN-2 and 7 DEN-3. All of the 4 serotypes circulated with DEN-3 (55%) predominating. A clinical diagnosis of DF was made in only 7 (4%) patients with the remainder being DHF. Among the patients diagnosed with DHF, DHF grade II (49%) was the most predominant clinical diagnosis. Subgroup analysis was done on 161 DHF cases with serotype data. Serotype distribution were as follows: 3 (2%) DEN-1, 23 (14%) DEN-2, 121 (75%) DEN-3, 14 (9%) DEN-4. Secondary infection and DHF grade II predominated at 147 (91%) and 82 (51%), respectively. DEN-4 serotype was associated with the highest mean age at 18.1 years (SD 7.6). Length of hospital stay, duration of illness prior to admission, and fever duration were highest in DEN-3. There were also 3 DEN-3 patients with presence of mental status change. These findings provide new data to characterize serotype-specific laboratory-confirmed hospitalized dengue illness in the Philippines.

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HEAVY PRECIPITATION IN THE BEGINNING OF THE SUMMER IS ASSOCIATED TO A SMALLER NUMBER OF DENGUE CASES IN RIBEIRÃO PRETO, SÃO PAULO STATE, BRAZIL

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Dengue is disease caused by all four serotypes of dengue virus, a flavivirus transmitted to man by the *Aedes aegypti* mosquitoes. Dengue is the most important arboviral disease in the world, and is currently endemic in Brazil. Dengue is a seasonal disease in the southeast region of Brazil, where the number of cases peaks in March and April, and only a few cases are detected from June to December, when a new "dengue season" begins, coinciding with the beginning of the rainy season that starts with the beginning of the summer. The city of Ribeirão Preto is located on the northeast of São Paulo state (southeast region of Brazil) and has experienced two large dengue outbreaks in the last 20 years. Since changes in weather may influence the incidence of several infectious diseases transmitted by vectors, such as dengue, and since other weather parameters, such as temperature, did not show an important variation during the summer, this study compared pluviometric levels to confirmed dengue cases per month of year in the city of Ribeirão Preto, Brazil, from 1995 to 2006. Results show that increased precipitation in December to February, especially in January, resulted in a decreased number of dengue cases in the following four months. On the other hand, in the years in which average rainfall was lower than 200 mm^3/month during those three months, the number of confirmed cases of this infectious disease increased in the following four months. The possible explanation for these results is that the increased rainfall washed off the larvae of the vector, resulting in a smaller mosquito population, and with a lesser number of mosquitoes, the spreading of the disease was reduced.

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THE ROLE OF T CELLS IN DENGUE VIRUS INFECTION

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Dengue virus (DENV), a member of the genus *Flavivirus*, causes the most significant arbovirus disease in the world in terms of illness, death, and economic cost. Adaptive immunity has been implicated in the immunopathology of secondary infections; however, the role of T and B cells in protection is unknown. We therefore investigated the T cell response to PL046 (a non-mouse-adapted DENV2 strain) in AB6 and A129 mice (mice lacking type I IFN receptors on the C57BL/6 and 129/Sv backgrounds, respectively), as these mice support a productive DENV2 infection yet do not succumb. We found PL046 infection results in an increase in $CD8^+$, but not $CD4^+$, T cells in the spleen at day 7 p.i. T cells were activated, as evidenced by upregulation of CD25 and CD69 on $CD4^+$ T cells at days 1 and 3 p.i., and CD69 on $CD8^+$ T cells at days 3 and 7 p.i. At day 7 p.i., approximately 40% of $CD4^+$ and 55% of $CD8^+$ T cells were $CD44^{hi}$. IFN-γ was detected in the sera and supernatants of ex vivo cultured splenocytes from A129 mice at days 1, 3, and 7, with the peak at day 3. Intracellular cytokine staining revealed $CD8^+$ T cells were producing IFN-γ at days 3 and 7 p.i. These data suggest that T cells, in particular the $CD8^+$ subset, contribute to the anti-DENV immune response. Therefore, to determine the protective role of T cells in our mouse model of DENV infection, $CD4^+$ and $CD8^+$ T cells were depleted from AB6 and A129 mice prior to infection with 10^6 PFU of the DENV2 strain, S221, which our laboratory derived by alternate passaging of PL046 between mouse sera and mosquito cells. Viral titers in the serum, spleen, and brain were quantified by real-time RT-PCR on days 3 and 7 p.i. On day 3, viral titers were similar in the sera and spleens of control and T cell-depleted mice. Of the mice that had DENV in the brain on day 3, titers were higher in T cell-depleted mice than control mice. On day 7 p.i., only T cell-depleted mice had detectable DENV in the brain. These data suggest a protective role for T cells in preventing DENV dissemination to the brain, likely via the production of IFN-γ.

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AN ANALYSIS OF REPEAT HOSPITAL ADMISSIONS FOR DENGUE TO ESTIMATE THE FREQUENCY OF THIRD OR FOURTH DENGUE INFECTIONS RESULTING IN ADMISSIONS, DENGUE HEMORRHAGIC FEVER, AND SEROTYPE SEQUENCES

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Immunity to a single dengue virus (DENV) infection does not provide heterologous immunity to subsequent infection. In fact, the greatest risk for dengue hemorrhagic fever (DHF) is with a second DENV serotype exposure. The risk for DHF with a third or fourth dengue infection relative to a first or second exposure is not known. An analysis of our database of children admitted to the Queen Sirikit National Institute of Child Health and Kamphaeng Phet Provincial Hospital with suspected dengue illness revealed that the number of dengue admissions due to a third or fourth DENV infection was extremely low (0.08%-0.8%). Once admitted, the risk for DHF relative to dengue fever was not different for those experiencing third or fourth DENV infections over those experiencing a second DENV infection. We document new dengue serotype infection sequences leading to DHF of 1-4, 2-3, 3-1, and 3-4.

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UTILIZATION OF MEDICAL SERVICES AND QUALITY OF LIFE AMONG DENGUE PATIENTS IN EIGHT ENDEMIC COUNTRIES

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Symptomatic dengue is responsible for extensive use of medical services in endemic countries. Using a standardized methodology, this study measured utilization of health services by symptomatic dengue patients in eight dengue endemic countries. During 2005-2006, this study was conducted in 8 countries: 5 in the Americas (Brazil, El Salvador, Guatemala, Panama, Venezuela) and 3 in Asia (Cambodia, Malaysia, and Thailand). Participants were recruited from suspected or confirmed dengue patients treated at representative, mostly public, hospitals and ambulatory facilities. Each patient (or parent) was interviewed 1-2 times and medical records were abstracted to determine duration of fever and illness, quality of life, number of ambulatory visits, and hospital days. Among the study patients 48% were aged 0-14 years, 55% were ambulatory patients and 71% had laboratory confirmed dengue. Ambulatory patients averaged 4.2 visits (SD 2.7) while hospitalized patient averaged 4.6 visits (SD 2.2), which included ambulatory visits before and after hospitalization. Most of ambulatory care was received in the public sector. Utilization patterns varied by country; the mean number of visits ranged from 2.8 (Guatemala) to 6.3 (El Salvador) among ambulatory patients and from 2.0 (El Salvador) to 7.1 (Malaysia) among hospitalized patients. The average length of stay for hospitalized patients was 3.8 days (SD 2.5) with country means ranging from 2.8 days (Malaysia) to 6.4 (Guatemala). Fever duration averaged 4.9 (SD 2.9) days among ambulatory patients and 5.9 (SD 2.8) days among hospitalized patients. The mean total duration of illness was 11.9 (SD 6.9) days among ambulatory patients and 10.9 (SD 4.6) days among hospitalized patients. In conclusion, the mean number of ambulatory visits, duration of fever and illness, and quality of life were comparable between ambulatory and hospitalized patients. Utilization of medical services is substantial and quality of life is low for both ambulatory and hospitalized dengue cases across 8 countries on two continents.

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ANALYSIS OF NS-1 ANTIGEN AND VIREMIA IN HOSPITALIZED DENGUE HEMORRHAGIC FEVER AND DENGUE FEVER PATIENTS IN THAILAND

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Samples collected during the first 5 days of admission to the hospital with suspected dengue hemorrhagic fever (DHF) in Bangkok were studied to determine the utility of NS-1 as a dengue diagnostic. Samples were assayed for NS-1 antigen using a commercial kit and viremia using quantitative RT-PCR. Diagnoses of DF or DHF were made using WHO criteria and dengue infection was verified by serology. This analysis revealed distinct serotype and severity of disease differences in NS-1 detection. At defervescence, fever day 0, the percentage of positive NS-1 samples from patients diagnosed with DF was 88, 60, 60, and 54 for serotypes 1-4, respectively. For DHF samples, the percentage positive was 57, 0, 71, and 50 for serotypes 1-4, respectively. Generally, NS-1 detection was much more sensitive early in disease regardless of disease severity: 100% for DENV 1, 3, 4, and 90% (80% for DF) for DENV 2 at fever day -2. The qPCR also exhibited differing sensitivities according to serotype and disease severity. For DF at fever day 0, 90% of DENV 1 samples were positive by RT-PCR with an average viremia of 5.3 $x10^6$ GE/ml; for DENV 2, 40% were positive, average viremia 1.7 $x10^5$ GE/ml; for DENV 3, 100% positive, average viremia of 2.3 $x10^5$ GE/ml; and for DENV 4, 44% positive, average viremia of 3.2 $x10^3$ GE/ml. For DHF samples DENV 1, 83% were positive with average viremia 4.6 $x10^4$ GE/ml; for DENV 2 80% were positive, average viremia 2.4 $x10^5$ GE/ml; for DENV 3, 100% were positive, average viremia of 9.7 $x10^3$ and DENV 4, 0 were positive.

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CLINICAL DESCRIPTION OF DENGUE FEVER AND DENGUE HEMORRHAGIC FEVER CASES IDENTIFIED DURING A CLUSTER EPIDEMIOLOGY STUDY IN WEST JAVA, INDONESIA

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We are conducting a cluster investigation study of dengue infections in two large cities in West Java (Jakarta and Bandung), with the goal of identifying patients with dengue early in the course of infection to study disease progression and evaluate factors associated with disease severity. We report here on the clinical features of cases identified at interim analysis. By enrolling volunteers who were household and nearest neighbor contacts of index dengue cases presenting to hospital, we have identified 128 (70 from Jakarta and 58 from Bandung) Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) cases and collected clinical and immunological data. Age ranged from 4 to 47 years, and male to female ratio was 1 to 1.1. According to WHO criteria, 64 cases were classified as DF, 34 as DHF grade I, 16 as grade II, and 14 cases as grade III and IV (Dengue Shock Syndrome). All cases were confirmed by RT-PCR, virus isolation, and serology assays. All four serotypes were identified in Jakarta, while dengue-4 (DEN-4) was not detected in any case from Bandung during the study period. The predominant serotype was DEN-3 in both cities. There was no significant correlation between the severity of disease and dengue serotypes, nor between severity and primary versus secondary infections determined by serological assays. Evaluation of other immunological factors and viral determinants of disease outcome is underway. The predominant clinical manifestations were fever (98%), headache (59%), and nausea (63%). Myalgia, arthralgias, abdominal pain, cough, sore throat and mental status changes were observed less frequently. Twenty-one percent of cases exhibited spontaneous bleeding, including petechiae, epistaxis, hematemesis and melena; one fifth of those cases exhibited multiple bleeding manifestations simultaneously. Plasma leakage was found in fifty percent of cases, manifested by ascites or pleural effusion, with or without increased hematocrit. Complete blood counts showed leucopenia (<4000/mm^3) in 51% of cases and thrombocytopenia (<100,000/mm^3) in 70%. Our data describe the clinical features associated with dengue infection in both children and adults in a hyperendemic region, and may inform the discussion of disease severity stratification schemes.

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SEQUENCE OF INFECTION RATES DETERMINED USING SINGLE DILUTION NEUTRALIZATION ASSAY FROM 1998-2001 KAMPHAENG PHET THAILAND PROSPECTIVE STUDY

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Samples collected during surveillance studies can be important in determining the serological status of those enrolled. The sequence of dengue virus infection surrounding both overt and asymptomatic infections can define the severity of disease that may result from the emergence of new serotypes into the region. In 1998-2002, the Armed Forces Research Institute of Medical Sciences and The University of Massachusetts conducted a prospective study of more then 2000 school age children in Kamphaeng Phet, Thailand. As part of this study blood was collected from each student 4 times per year (January, June, August and November). The January and November blood draw represents the pre and post-dengue season serological baseline respectively. Using 300 random selected serum samples in each year of study, using serum in June (S1) and the following year of January (S2). We will determine the primary and secondary infection rates, the immune status against dengue, and attempt to determine the sequence of infections in asymptomatic infections. To date we have completed analysis of the 300 samples from the 1998 cohort using the single dilution neutralization (SND) assay using a 1:30 dilution and a 70% cut-off to determine the immune status of each serum pair Only 43 of these subjects showed having no detectable dengue antibodies. Using this SND data we observed that 4 subjects (1 DENV-1, 1 DENV-2, 2 DENV-3) had primary infections and 19 had secondary infections during this period. Six of the possible 12 dengue serotype sequences of infections were identified and the rates that these occurred based on the S1 antibody status will be discussed. In addition, we will analyze thes data during these periods with the prospective study results in particular on serotype circulating in the calendar year.

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ESTIMATING THE TOTAL WORLD POPULATION AT RISK FOR LOCALLY ACQUIRED DENGUE INFECTION

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Background. Dengue is a growing public health problem in tropical and subtropical countries. Current estimates indicate that two billion people living in dengue endemic countries. However, the methods used to reach this calculation were not published. Two estimates have suggested that between 50 and 100 million cases of dengue fever occur annually, corresponding to an incidence rate of 2.5% to 5% among the two billion people worldwide at risk. Methods. We identified countries with populations at risk for dengue in the last 25 years using three types of data; [1] PubMed and country Ministry of Health and World Health Organization websites for reports of dengue incidence data, outbreaks, or serosurveys for individual counties to identify laboratory confirmed dengue infection; [2] published literature on travelers where the destination of a laboratory confirmed dengue case was identified; [3] maps to identify additional counties at risk that have no published data but are contiguous to countries reporting dengue disease. We then estimated the total population at risk by summing the 2007 estimated census population of those countries identified. For countries where dengue was limited to certain regions (e.g. China or Argentina) we reduced the population at risk to include only the regions at risk. Results. Using published reports we identified 110 countries with populations at risk for dengue. Published reports identified an additional 3 countries. Using the contiguous-country method we added an additional 11 countries, 9 of which were in Africa. Using the 2007 estimated census data and published reports 3.46 billion people currently live in areas at risk for dengue. The addition of travelers' data to identify at risk populations increased this number to 3.51 billion people at risk; the contiguous country method identified 3.61 billion people at risk. This translates to 52.5-54.7% of the worlds population living in countries at risk for locally acquired dengue infection. Conclusion. Previous calculations of the total and percent of the world population living in countries may have been underestimates. We used published literature for each of the countries of the world to identify countries with at risk populations with confidence. Our estimate of the total population at risk for locally acquired dengue is nearly twice the previous estimate.

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CLIMATE-BASED FORCASTING MODELS FOR DENGUE: A CASE STUDY IN PRACHUAP KHIRI KHAN PROVINCE IN THAILAND

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Infectious diseases have become global public health problems by global climate changes, particularly vector-borne infectious diseases. Dengue, a priority in most of Tropical regions, presents a strong association with climate factors; however, few of them reports association in seasonal changes and annual cycles. This study aimed to identify the important climate factors associated with similar patterns in dynamics of dengue cases over years in Prachuap Khiri Khan Province (PCP) in Thailand. PCP locates at latitude 11° 49′ 0N and longitude 99° 47′ 60E. PCP with the mean incidence rate of 34.03 per 100,000 populations during 1996-2005, was indicated to be the risk area of dengue by the criteria of Ministry of Public Health because incidence rates of dengue in this province were much higher than those medians of incidence of dengue overall Thailand country more than 3 consecutive years Incidence rates of dengue, monthly mean of rainfall (mm), monthly numbers of rainy days, maximum and minimum temperatures (°C) during 1996-2005 were all collected from Department of Disease Control and Department of Meteorology. Our multiple linear regressions demonstrated strongly association between minimum temperature and numbers of dengue cases (β=10.75, $p<0.001$), whereas monthly numbers of rainy days presented highest effects on increasing numbers of dengue cases during epidemic seasons annually in our study years (β=4.980, $p<0.001$). Although, these climate factors presented as the key magnifying factors contributing to the scale and variations of dengue epidemics over the ten years in this area (R^2=0.69). Interestingly, regional variations on the predominant climate factors were also found their associations with dengue incidence rates among four regions of Thailand due to different ecological situations there. In conclusion, future studies on the mechanisms of climate affecting ecology of dengue will be very helpful to predict the up-coming dengue epidemics in Thailand for improving prevention and control of dengue/dengue hemorrhagic fever (DHF) before vaccine era.

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AFTER A DECADE OF ANNUAL DOSE OF IVERMECTIN ON ONCHOCERCIASIS PREVALENCE IN CAMEROON AND UGANDA, TRANSMISSION CONTINUES

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Onchocerciasis is the world's second leading infectious cause of blindness, and a cause of severe skin disease. It is caused by *Onchocerca volvulus*, a parasitic worm that forms nodules under the skin and is transmitted by black flies that breed in fast flowing rivers and streams. Ivermectin kills

the microfilaria, and, while reduces the fecundity of adult worms does not kill them. Mass drug administration (MDA) in Africa with single annual doses of ivermectin has a goal of 'eliminating onchocerciasis as a public health problem.' The required duration for such MDA programs where they can be halted without recrudesce is often cited by the World health Organization's African Programme for Onchocerciasis Control (APOC) cited as being fifteen years. Baseline nodule and skin snip microfilaria prevalence data were available for sentinel villages in Cameroon (from 1996) and Uganda (from 1993). We returned to those sentinel villages in 2005 to repeat cross sectional surveys 10 (Cameroon) and 13 (Uganda) years after ivermectin distribution. Treatment coverage over this time period was reported to be over 65% (of total population) coverage in Cameroon and Uganda respectively exists. Over six hundred persons >10 years were examined in each of the surveys. We also examined children less than 10 years old from Cameroon (1996, n=206; and 2005, n=447) and Uganda (1993, n=234; 2005, n=278). 80 nodules were excised during the Ugandan survey. In Cameroon, nodule (75% to 8%) and microfilaria prevalence (70% to 8%) was significantly reduced between 1996 and 2005. Similarly, for Uganda , nodule (47% to 9%) and microfilaria prevalence (73% to 7%) was significantly reduced between 1993 and 2005 In children under 10 years of age, microfiaria prevalence reduced from 34% to 4% and in Uganda from 20% to 1.5%. Ugandan nodule histological results showed a majority of female (64%) and male (81%) worms were living and 24% of live female worms were inseminated. We concluded that a decade of annual single dose ivermectin treatment has reduced onchocerciasis to below the threshold of being a public health problem (defined as a nodule rate of 20% and a community mf prevalence of 40%), but onchocerciasis transmission continues. We recommend that MDA continue past 15 years if control programs do not wish to risk recrudescence. We also recommend a combination of acceptable methods where feasible for elimination of transmission.

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EVALUATION OF WHOLE BLOOD COLLECTION METHODS USING THE OG4C3 ELISA IN BANCROFTIAN FILARIASIS

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Venous blood samples were collected as part of the Papua New Guinean Filariasis Elimination Programme and the serum tested by the Trop-Ag W. bancrofti test kit for antigen. Before the blood clotted, whole blood was tested by the ICT and also added to filter paper where it was dried, transported back to Townsville, Australia and tested by the TropBio ELISA. The positive percent agreements (PPA), with the TropBio serum assay, of the ICT and filter paper technique were 63.6% (95% confidence interval [CI], 58.6%-68.4%) and 67.2% (95% CI, 62.1%-72.1%). The negative percent agreements were (NPA) 97.6% (95% CI, 95.7%-98.9%) and 99.2% (95% CI, 97.8%-99.8%) respectively. The sensitivity was 88.6% (95% CI, 82.0%-93.5%) and 88.4% (95% CI, 81.4%-93.5%) compared to microfilaraemia as determined by a 60 ul blood film. A sample of ICT tests had the filter paper removed and tested by the Trop-Ag W. bancrofti filter paper test. There was no significant difference (P=0.23) in the PPA between the ICT, blood collected onto filter paper and using filter paper from the ICT for this sample set. A fast friendly field (FFF) version of the Trop-Ag W. bancrofti test kit was trialled in Bougainville after it was shown that the boiling step was unnecessary in the procedure. The positive percentage agreement using 250 serum specimens compared to the boiled supernatant was 94.6% (CI, 88.7-98.0) and negative percentage agreement (NPA) 97.1% (CI, 92.8-99.2) with a significant correlation between optical densities (r=0.97, P<0.001). For the FFF assay, whole blood was added to an ELISA plate, incubated overnight and then processed in the field using reagents supplied in dropper bottles. Reactions were read by eye and compared to the TropBio assay. The PPA was 96.0% (95% CI, 79.7%-99.9%) and the NPA 98.4% (95% CI, 94.2%-99.8%). The FFF is suitable for use with capillary collection and is simple to perform unlike the filter paper collection which is labour intensive. The FFF could be a suitable field alternative for the ICT.

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DENSITY-DEPENDENT MORTALITY OF THE HUMAN HOST IN ONCHOCERCIASIS: RELATIONSHIPS BETWEEN MICROFILARIAL LOAD AND EXCESS MORTALITY

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The parasite *Onchocerca volvulus* has, until recently, been regarded as the cause of a chronic yet non-fatal condition. Recent analyses, however, have indicated that in addition to blindness, the parasite can also be directly associated with human mortality. Data from the Onchocerciasis Control Programme in West Africa (OCP) collected between 1975 and 2002 are used to determine functional relationships between microfilarial load and excess mortality of the human host. Either an exponential or a sigmoid model (both of which saturate beyond a certain microfilarial load, but at different rates) were found to fit the relationship satisfactorily, although it was not possible to distinguish statistically between these two possible functional forms. Incorporation of these functional relationships between microfilarial load and excess human mortality into mathematical models for the transmission and control of onchocerciasis will have important implications for our understanding of the parasite's population biology and of the projected benefits of control programmes for this disease in both human and economic terms.

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EFFECT OF SINGLE DOSE IVERMECTIN ON *ONCHOCERCA VOLVULUS*: A SYSTEMATIC REVIEW

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The broad spectrum antiparasitic drug ivermectin has been licensed for use against human onchocerciasis since 1987, yet the mechanisms by which it exerts a fast decrease and long-lasting suppression of *Onchocerca volvulus* microfilaridermia, and a prolonged inhibition of microfilarial release by female worms remain largely unknown. A better understanding of the effects of ivermectin on *O. volvulus* micro- and macrofilariae is crucial to improve our ability to predict quantitatively the long-term impact of treatment. We conducted a systematic evaluation of individual- and population-based ivermectin trials to provide estimates of the temporal dynamics of the drug's microfilaricidal and embryostatic efficacy following administration of a single standard dose (150 µg/kg). Meta-analyses were conducted on data from 26 microfilarial and 15 macrofilarial studies and results were linked by a mathematical model describing the dynamics of fertile female parasites and skin microfilariae. The model predicts that following treatment, microfilaridermia would be reduced by 70% after 24 h, 85% after 72 h, 92% after 1 wk, and 97-98% after 2 mo, with the latter also corresponding to the time-point the fraction of females harbouring live microfilariae is at its lowest (reduced by ~80% from baseline). Studies aimed at understanding the mechanisms by which microfilariae disappear from the dermis and are eventually eliminated should investigate long-term time series after treatment. Our results highlight the need for more comprehensive studies on the effects of ivermectin upon adult *O. volvulus*.

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FIVE YEARS OF MASS DRUG ADMINISTRATION (MDA) FOR FILARIASIS: REFLECTIONS ON THE SUCCESSES, CHALLENGES AND ASPECTS OF PROGRAM INTEGRATION

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The Mass Drug Administration (MDA) program for filariasis in a hyper-endemic area of Tanzania has been successful, however there are practical aspects that are forgotten or misunderstood in terms of the translation of program plans to actuality in the field; it is important to understand and accommodate these issues. Structured interviews, as well as parasitological and clinical assessment of over 1000 people in the treated areas were carried out, and their understandings and comments about the Program, their ICT status, the clinical changes over the period of the MDA provided insight into the characteristics of the program. The prevalence of infection was reduced from over 50% ICT positivity to less than 10% in all areas studied, and was reduced to 0% in certain locations. Marked reductions in clinical disease were recorded in all patients who participated in the MDA annual distribution of ivermectin and albendazole. Importantly, no new cases of lymphatic filariasis were found in any of the MDA areas. The MDA programs for the elimination of filariasis have focused on coverage and breaking transmission; those affected by the clinical disease have generally been peripheral to the main program. This approach, it now appears, was somewhat short sighted. The importance of focusing programs on the clinical disease and not purely on drug (tablet) distribution was shown by an analysis of the reasons behind the different degrees of coverage obtained in different villages. This presentation will also describe the key factors that have both enhanced the Filariasis Elimination Program to date and, in contrast, those factors that were difficult and obstructive to the overall goals; these factors are important for the planning of new programs and especially the need for financial and man-power issues. Some of these are misunderstood or over-looked by external agencies unfamiliar with the field situation. Aspects of integration will be discussed, both integration of the Program into the national health system as well the challenges faced with integration with the various control programs in place in the LF MDA areas.

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DEVELOPMENT OF A RAPID FLOW CYTOMETRIC ASSAY FOR THE MEASUREMENT OF MURINE BASOPHIL ACTIVATION

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Several recent studies suggest basophils may play a central role in the Th2 immune response to helminths. One of the main obstacles to studying basophils has been the lack of a simple and rapid assay to measure basophil activation in mice. To better understand basophil function in a murine model of filariasis, we sought to establish a novel and rapid technique for measuring murine basophil activation. As there is currently no murine basophil-specific marker, we initially stained mouse blood cells with various combinations of positive markers (IgE, IL-3R, FcεRI, CCR2, CD49b) and negative markers (CD4, CD11b, c-kit, Gr-1, CCR3, CD19, B220) for basophils. Using anti-IgE-FITC as a positive marker and anti-CD4-PERCP as a negative marker resulted in a well-separated basophil population that, when sorted, was >98% pure by May-Grunwald staining. Subsequent experiments demonstrated that CD200R, an inhibitory receptor of the Ig supergene family, serves as an excellent activation marker in basophils. CD200R expression on basophils increases from 2% to 65% after activation with anti-IgE. Time course experiments revealed that CD200R expression on murine basophils increases at 30 minutes, is maximal after 2 hours, and decreases dramatically after 5 hours of incubation with anti-IgE. Support for CD200R as a murine basophil activation marker comes from our findings that: 1) CD200R expression increases with increasing concentrations of activating anti-IgE antibody, 2) Most CD200R positive basophils also stain positively for IL-4, and; 3) *in vitro* parasite-antigen exposure increases CD200R expression in basophils of infected mice once they develop parasite-antigen specific IgE but not in basophils of uninfected mice. This novel technique for measuring murine basophil activation is straightforward and rapid, taking approximately half a day for obtainment of blood, *in vitro* culture, staining, and flow cytometric analysis.

(ACMCIP Abstract)

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P-GLYCOPROTEIN-LIKE PROTEIN, A PROMISING GENETIC MARKER TO FOLLOW POTENTIAL IVERMECTIN RESISTANCE IN *ONCHOCERCA VOLVULUS*

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Ivermectin (IVM) is the only safe drug for mass-treatment of onchocerciasis. IVM-resistance has been reported in gastrointestinal nematode parasites of animals. A reduction in response to IVM in *Onchocerca volvulus* could have significant consequences for the onchocerciasis control programs. Over the last few years, several studies have reported genetic selection or reduced responses to IVM in some *O. volvulus* populations. The risk of a recrudescence of the disease was even reported with the emergence of a resistant adult parasite population in Ghana. We have found evidence that repeated IVM treatment selects for specific alleles of p-glycoprotein-like protein (PLP), a half-sized ABC transporter, in *O. volvulus*. In this study, *O. volvulus* samples were derived from a clinical trial in Cameroon, in which patients were sampled before, and following three years (1994-1997) of IVM treatments. There were four treatment groups: 150µg/kg (1xp.a. or 4xp.a.) and 800µg/kg (1xp. a. or 4xp.a.). DNA from macrofilariae (367 adult worms collected pre-treatment in 1994 and 224 collected in 1997after treatment) was genotyped over a 476 bp region of the PLP gene and at two control genes. Of the six polymorphic positions found in the PLP amplicon, 3 of them showed significant selection after 4xp.a. treatment with IVM (total of 13 IVM treatments) in female worms, and 1 of the same single nucleotide polymorphisms (SNPs) showed significant selection in the male worms. One of the selected SNPs in the female worms caused an amino acid coding change in the protein. We found a clear selection of some genotypes, and a loss of polymorphism following 4xp.a. treatment with IVM. This PLP gene appears to be a promising DNA marker for IVM selection which may be important in the event that IVM resistance spreads.

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GENE EXPRESSION AND LOCALIZATION STUDIES OF THE FILARIAL DIAGNOSTIC ANTIGEN BM14

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Bm14 (also known as Bm-SXP1) is an immunodominant antigen that is useful for detecting antibodies to lymphatic dwelling filarial parasites.

Antibodies are detectible prior to the onset of microfilarial patency in infected humans and in animals. This 13kDa antigen has also been reported to induce partial immunity to *Brugia malayi* infection in jirds. The purpose of this study was to improve characterization of Bm14. Database searches show that Bm14 is a member of a gene family that is widely distributed in parasitic and free-living nematodes. There are no homologues outside of Nematoda, and no functional information is available for Bm14 or related genes; Nembase reports "no phenotype" associated with RNAi of homologues of Bm14 in C. elegans. Bm14 is encoded in 3 exons that are adjacent to 3 exons that encode the related protein Bm5. The latter protein contains a homeobox signature (not present in Bm14) that suggests that it may be a transcription factor. The 152 aa Bm14 sequence contains a putative signal sequence at the 5' end, 4 phosphorylation sites, 1 N-myristolation site, and a large globular domain. qRT-PCR studies show that the gene is highly expressed in all stages of the parasite (MF, L3, L4, immature and mature male and female worms). Relative expression levels (quantity of message relative to total mRNA) declined when L3 were maintained in culture. In situ hybridization studies showed that Bm14 is highly expressed in the lateral chords of adult *Brugia malayi* males and females and in developing sperm and embryos (especially the pretzel stage). Immunolocalization studies performed with mouse anti-Bm14 antibody showed that the protein is diffusely present in L2 and L3 larvae, in lateral chords and muscle of adult worms, and in reproductive tissue (sperm in males and oocytes and developing larvae in females). Bm14 protein was not detected in the cuticle or hypodermis of adult worms. The Bm14 localization pattern is different from that reported for the *Onchocerca volvulus* protein Ov17 (62% amino acid identity with Bm14), which was primarily localized to *O. volvulus* hypodermis. Although these results suggest that Bm14 is important in the developmental biology of *B. malayi*, additional studies will be needed to identify the biological function of this important diagnostic antigen.

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WHOLE GENOME AMPLIFICATION AND OLIGONUCLEOTIDE ARRAY HYBRIDIZATION FOR GENOMIC CHARACTERIZATION OF FILARIAL PARASITES

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Genetic characterization of field isolates and clinical specimens of filarial nematodes are often limited due to insufficient amounts of parasite DNA. To overcome this problem we evaluated a multiple displacement amplification (MDA) based method for amplifying the genome of the filarial parasite *Brugia malayi*. The quality of amplified DNA was examined using real-time PCR, conventional PCR of target sequences of up to 3.5 kb, genomic BAC library hybridization and microarray DNA hybridization. Amplification of 5.0 ng purified B. malayi DNA or of DNA isolated from a single microfilaria resulted in 6.3 µg and 4.2 µg of amplified DNA, respectively. Real-time PCR analysis showed that the amplified DNA product was remarkably similar to that of the genomic template, although for some templates or sequences two to fourfold less target DNA was detected for a given DNA concentration. Target sequences of up to 3.5 kb were amplified by conventional PCR from as little as 1 ng of amplified DNA. No bias in amplification was detected when amplified DNA was hybridized to a B. malayi BAC library or to an oligonucleotide microarray containing approximately 16,000 filarial gene sequences. Based on these results, we conclude that MDA is useful for whole genome amplification of parasite DNA from very small amounts of starting material. This technology will enable detailed molecular analyses of individual parasites or larvae that were not feasible in the past.

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LONG-TERM DOXYCYCLINE TREATMENT AFFECTS *WOLBACHIA* AND PARASITE GENE EXPRESSION IN ADULT FEMALE *BRUGIA MALAYI*

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Like most filarial species, *Brugia malayi* (Bm) contain intracellular bacteria (*Wolbachia*, *w*Bm) that are essential for their normal development, reproduction and survival. We have previously reported that short-term exposure to doxycycline (Doxy) significantly altered *w*Bm and Bm gene expression profiles as assessed by microarray and qRT-PCR. The purpose of this study was to assess changes in gene expression after long-term Doxy treatment. Microfilaremic jirds were treated with Doxy (100 mg/kg p.o. daily for 6 weeks). Doxy decreased microfilaria production and embryogenesis in female worms. qRT-PCR studies showed that treated female worms had decreased expression of *w*Bm surface protein (WSP) and cell-cycle ftsZ genes. Electron microscopy showed degenerating bacteria and embryos in treated female worms. Microarray studies were performed using the Version 2 Bm microarray to gain a broader understanding of the effects of long term Doxy on gene expression in adult worms and *w*Bm. cDNAs were labeled with a random priming protocol to enable efficient labeling of prokaryotic and eukaryotic transcripts. Genes with 2-fold differences in hybridization intensities between treated worms and control worms with $P < 0.01$ were considered to be differentially expressed. 200 *w*Bm genes showed differential expression after Doxy treatment, and all but 3 were downregulated. This is not surprising since wBm were greatly reduced after Doxy treatment. 563 Bm genes were differentially expressed after treatment. 462 were up-regulated including many novel or predicted genes with unknown biological functions. These changes may be due to direct effects of Doxy on Bm or indirect effects due to drug-induced changes in *w*Bm. 101 Bm genes were down-regulated after treatment. This list included genes that encode collagen, caveolin, embryonic fatty acid binding protein, and other novel or predicted genes. Ongoing KEGG analysis and InterPro domain mapping studies may provide clues regarding *w*Bm and Bm genes that are essential for parasite reproduction and survival.

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ANNOTATION AND FUNCTIONAL ANALYSIS OF GENDER-REGULATED GENE EXPRESSION IN ADULT *BRUGIA MALAYI*

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We have previously described gender-regulated gene expression in *Brugia malayi* based on results obtained with the Version 1 *B. malayi* microarray (3,569 elements) and qRT-PCR studies. Major gender differences in gene expression were related to reproductive functions. We have now extended this analysis with the V2 filarial array which has more than 18,000 oligonucleotide elements that represent expressed genes and predicted ORFs from *Brugia, Onchocerca, Wuchereria* and *Wolbachia*. The *B. malayi* elements cover ~85% of the genes in this species. Of 14,293 elements with significant hybridization signals, 1,467 (9.8%) were preferentially expressed by female worms and 1,323 (9.3%) by male worms, respectively, using the criteria of a > 2-fold differential hybridization signal with $P < 0.01$ (8 hybridizations per element). Approximately 86% of 112 genes identified by microarray were confirmed by qRT-PCR. Functional assignments of gender-regulated genes were performed using InterPro, KEGG (Kyoto Encyclopedia of Genes and Genomes), and GO (Gene Ontology) with online software and updated locally stored databases. Gender-altered pathways were identified using the hypergeometric test ($P < 0.02$). The most significant pathways assigned by KEGG analysis for female-upregulated genes were cell cycle and replication, protein

recombination and repair, and lipid pathways. Male upregulated pathways included signal transduction, pentose phosphate, and calcium signaling. GO analysis of the transcriptome suggested striking gender differences in biological processes and molecular functions. Genes related to cell adhesion, chromosome segregation, cell differentiation, gametogenesis, lipid binding and development were more prominent in females, while cell communication and transport were prominent in males. Functional annotation of filarial microarray data (a large work in progress) is an important step in the evolution of filarial genomics from raw sequence through descriptive studies of gene expression toward a deeper understanding of biological processes such as reproduction, development, and metabolism that may provide a foundation for future vaccines or rational drug design.

(ACMCIP Abstract)

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THE *WOLBACHIA* ENDOSYMBIONT OF FILARIAL PARASITES CONTAIN HEME BIOSYNTHESIS ENZYMES WHICH ARE POTENTIAL DRUG TARGETS

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Filarial parasites (*Brugia malayi*, *Onchocerca volvulus*, *Wuchereria bancrofi*, etc) are causative agents of elephantiasis and river blindness, which are among the most disabling tropical diseases. The current anti-filarial chemotherapy, e.g. diethylcarbamazine, ivermectin and albendazole can successfully interrupt transmission by killing the microfilariae, but are less effective on adult worms, which can live 10-15 years in humans. There is an urgent need to develop macrofilaricidal drugs. The obligate endosymbiotic alpha-proteobacteria *Wolbachia* has been identified in most human filarial pathogens. Antibiotic depletion of W*olbachia* leads to the death of adult worms and subsequently reduced microfilariae without causing severe adverse reactions, although current treatments are not practical due to the dosages and length of treatments which are required. Nevertheless, anti-*Wolbachia* targeting appears promising for filariasis control. There is evidence that heme-containing ecdysteroid-like hormones are critical for molting and reproduction in worm host. However, heme biosynthesis genes are absent in the *B. malayi* genome, but are readily identified in its *Wolbachia* endosymbiont genome (except for one, protoporphyrinogen oxidase, PPO). Phylogenetic analyses indicate some of the enzymes in the *Wolbachia* heme pathway are evolutionarily distant from their human orthologs and could be potential drug targets. A progress report on the recombinant cloning of the *Wolbachia* and human heme biosynthetic genes and their protein purification (for use in for downstream high throughput inhibition assays) will be presented.

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ULTRASTRUCTURAL STUDY OF *BRUGIA PAHANGI* : A RICH ANTIGENIC SOURCE

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Indirect immunofluorescent study of *Brugia pahangi* cuticle revealed the filarial parasite's a rich antigenic source. As revealed by TEM, the cuticle of the *B. pahangi* L_3 and adult is surrounded by a discontinuous trilaminate membrane-like epicuticle. This may be one of the richest sources of antigens because this structure directly contact with host immune system. The cuticle comprises the basal , middle,and cortical layers without distinct demarcation between each layers. All layers comprise fine filamentous structures arranged in several directions.These fibrils of the cuticle are believed to be collagen-like protein that comprised of finer fibrils. These cuticular proteins may slowly be turned over and released into the environment and act as a source of immunogen. The hypodermis shows cellular components in the lateral cords. Each cell bears organelle characteristics of highly synthetic activity and infolded plasma membranes at both apical and basal regions. Thus,in addition to its role insynthesizing cuticular proteins the infolded plasma membrane may play roles in controlling and facilitating the exchange of nutrient and waste materials through the cuticle. The excreted materials may also be another source of antigen.

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A RANDOMIZED DOUBLE-BLIND CONTROL TRIAL OF A SINGLE DOSE OF DIETHYLCARBAMAZINE IN COMBINATION WITH DOXYCYCLINE FOR TREATMENT OF *WUCHERERIA BANCROFTI* INFECTION

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Standard treatment of lymphatic filariasis with diethylcarbamazine (DEC) (either with or with out albendazole or ivermectin) is effective at killing microfilariae, but shows partial macrofilaricidal activity. Systemic inflammatory-mediated adverse reactions are commonly found in patients using DEC. These reactions are thought to be caused by the rapid release of antigens from microfilariae and *Wolbachia,* the endosymbiotic bacteria of filariae, into the blood. We assessed whether a single dose of doxycycline, in combination with DEC, would have superior efficacy to DEC alone, and reduce the severity of the adverse reactions. A total of 45 individuals (29 were infected with *Wuchereria bancrofti,* 16 were endemic normals) from Tak province, Thailand, were recruited to the randomized double-blind clinical trial study; 26 received 300 mg DEC in combination with placebo (placebo group), and 19 received 300 mg DEC in combination with 200 mg doxycycline (doxycycline group). The microfilaria level was rapidly reduced to zero within 24 hours after treatment in both treatment groups. Adult *W. bancrofti* antigen levels, measured by ELISA, were significantly lower in the doxycycline group than in the placebo group at 6 months after treatment ($P < 0.05$). Although there is no statistical significance, the incidence of adverse reactions was lower in the doxycycline group (31%) than the placebo group (46%). No severe adverse reactions were found in the doxycycline group, but occurred in the placebo group (3 of 25 subjects). Plasma IL-6 levels were associated with severity of adverse reactions. At 6h, 12h, 24h, and 48h after treatment, the IL-6 levels were significantly lower in the doxycycline group than the placebo group ($P < 0.05$). To resolve whether the single dose of 200 mg doxycycline can reduce the bacterial load enough to affect the host's immune response, we are during study on the level of *Wolbachia* bacteria in plasma after treatment. In conclusion, a single dose of doxycycline combined with DEC reduced severity of adverse reactions compared to using DEC alone for treatment of lymphatic filariasis caused by *W. bancrofti*. Our findings emphasize the use of doxycycline in combination with DEC to lymphatic filariasis control program.

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VARIANT SNPS OF THE IL-10 PROMOTER AT POSITIONS -854 AND -627 RESPONSIBLE FOR LOW IL-10 SECRETION ARE ASSOCIATED WITH LOWER LEVELS OF CIRCULATING *BRUGIA TIMORI* MICROFILARIAE BUT NOT WITH FILARIAL LYMPHEDEMA

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Lymphatic filariasis infections can present as clinically asymptomatic or have chronic pathology from mild to severe lymphedema. Studies have shown that pathology and whether a person has microfilaremia cluster in families. The immune response of patients with asymptomatic infections or chronic disease differs significantly. High levels of pro-inflammatory cytokines and no worms/microfilariae (MF, first stage larvae) characterize chronic pathology, while asymptomatic patients are characterized by immunosuppression, but presence of many worms/MF. IL-10, secreted by T-regulatory and other immune cells, plays a role in the immunosuppression seen in human and animal filarial infections. The IL-10 promoter single nucleotide polymorphisms (SNPs) at positions -1117, -854 and -627 (previously -1082, -819, -592) are associated with different levels of IL-10 secretion. In this case-control study, an isolated population infected with *Brugia timori* from Alor Island, Indonesia, was genotyped to elucidate if there was an association of the IL-10 SNPs with pathology or microfilaremia. While no association with pathology was seen, the SNPs at -854 and -627 were associated with the number of MF in patient blood. These results suggest that genetic factors which determine IL-10 secretion by immune cells are involved in the wide range of MF levels seen in human populations.

(ACMCIP Abstract)

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IDENTIFYING *TRYPANOSOMA CRUZI* INFECTION IN CHILDREN DURING A VECTOR CONTROL CAMPAIGN

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Millions of people are infected with *Trypanosoma cruzi*, the causative agent of Chagas disease, in Latin America. Anti-trypanosomal drug therapy can cure infected individuals, but treatment efficacy is highest early in infection. Vector control campaigns disrupt transmission of *T. cruzi*, but without timely diagnosis children infected prior to vector control often miss the window of opportunity for effective chemotherapy. We performed a serological survey in children 2-18 years old living in a peri-urban community of Arequipa, Peru, and linked the results to entomologic, spatial and census data gathered during a vector control campaign. Twenty-three of 433 (5.3% [95% CI 3.4-7.9]) children were confirmed seropositive for *T. cruzi* infection. Spatial analysis revealed that households with infected children were very tightly clustered within looser clusters of households with parasite-infected vectors. Bayesian hierarchical mixed models, which controlled for clustering of infection, showed that a child's risk of being seropositive increased by 20% per year of age and 4% per vector captured within the child's house. Receiver operator characteristic (ROC) plots of best-fit models suggest that more than 83% of infected children could be identified while testing only 22% of eligible children. In conclusion,we found evidence of spatially-focal vector-borne *T. cruzi* transmission in peri-urban Arequipa. Ongoing vector control campaigns, in addition to preventing further parasite transmission, facilitate the collection of data essential to identifying children at high risk of *T. cruzi* infection. Targeted screening strategies could make integration of diagnosis and treatment of children into Chagas disease control programs feasible for lower-resource countries.

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MULTI-SITE EPIDEMIOLOGIC STUDIES OF CHAGAS' DISEASE IN PREGNANT WOMEN FROM THREE LATIN AMERICAN COUNTRIES

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Mothers with Chagas' disease can transmit *Trypanosoma cruzi* to their fetuses, who are then at risk of developing severe cardiac disease later in the course of their lives. There is an urgent need to better understand the epidemiology of mother-to-child transmission of *T. cruzi*. International comparisons are often hampered by methodological differences from one study to another. This study was undertaken to determine the prevalence of antibodies to *T. cruzi* infection in pregnant women in maternal intravenous blood samples (IV) at birth and umbilical cord blood samples (UC) in three Latin American countries. The study was conducted from September 2006 to March 2007 in one hospital in Argentina, one in Honduras, and two in Mexico. At the time of delivery, maternal IV and UC blood were collected for analyses of maternal antibodies to *T. cruzi* by Chagas Stat-Pak (Chembio, New York). Recombinant Chagas ELISA Tests (Wiener, Argentina) will also be performed. The study enrolled 2008 women. The frequency of positive rapid IV tests was 6.0% (30/504) in Argentina, 10.0% (50/500) in Honduras, and 0.7% (7/1004) in Mexico. UC rapid tests were positive in 78.2% (68/87) of cases with positive IV rapid tests. In conclusion, we detected *T. cruzi* antibodies among pregnant women in all sites under study, and congenital transmission control is thus an issue across the region. The validation of the Chagas Stat-Pak against Chagas ELISA is in process.

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AN EPIDEMIOLOGICAL SURVEY FOR HUMAN AND CANINE LEISHMANIASIS IN AYDIN PROVINCE, TURKEY

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Human and canine visceral leishmaniasis caused by *Leishmania infantum* is mainly observed in the Aegean and Mediterranean Regions, while cutaneous leishmaniasis (CL) caused by *Leishmania tropica* is mainly seen in the Southeastern Region in Turkey. In last ten years, CL has been spreading to other regions. In Aydin province, both VL and CL has been diagnosing much more because of the new leishmaniasis center in the Parasitology Department of Adnan Menderes University. A total of 45 (14 male and 31 female, ages between 1-65 yrs) and 99 (58 female and 41 male, ages between 2-81 yrs) CL cases and were identified in 2004 and 2005, respectively. Additionally, 2 VL cases were identified in 2004. All cases were treated with antimonials after parasitological/serological diagnosis. A pilot study for the epidemiology of leishmaniasis including dogs and vectors and an active surveillance for CL cases were carried out mainly in the rural areas of Aydin province in 2002. Totally, 217 dogs were physically examined and bloods samples were taken. Anti-*Leishmania* antibodies were searched in the samples by IFA test and 12 of 217 (5.52%) were found to be seropositive at ≥128 titres. The rK39 dipstick test was performed to IFA positive samples and 10 out of 12 dogs were found to be seropositive. Lymph nodule aspiration was applied to 8 out of 10 seropositive dogs and the parasites were observed in all samples. In order to determine the vector species, 183 sand fly specimens were collected using CDC miniature light traps in two villages where CL is endemic and 8 species were identified. Two Larroussius species *Phlebotomus neglectus* (38.25%) and *Ph. tobbi* (30.60%) was found to be dominant species. The others were; *Ph. similis* (9.83%), *P. papatasi* (9.83%), *Ph. burneyi* (4.90%), *Ph. simici* (3.27%), *Ph. alexandri* (2.11%) and Ph. perfiliewi (1.09%). The pilot study results showed that the risk for humans and dogs to have leishmaniasis is high in the rural areas of the province. Because of this, an educational program together with Aydin Branch of Ministry of Health for the community leaders in the villages to explain the situation and controlling the diseases in the province. Additionally, a control program for fighting with vectors has been applied.

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SINGLE STRAND CONFORMATION POLYMORPHISM AND INFECTION IN MICE OF VISCERAL LEISHMANIASIS ISOLATED FROM NEPALESE PATIENTS

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Protozoan parasite *Leishmania donovani* is the causative agent of visceral leishmaniasis (VL) or kala-azar in Nepal. Ninety-seven were positive microscopically and twenty nine strains were successfully isolated and cultured out of 166 bone-marrow aspirates obtained from VL suspected patient in endemic area of Nepal which are border with endemic area of Bihar, state of India in 2003-2005. To compare them molecularly each other we did PCR-restriction fragment length polymorphism analysis (RFLP) of mini-exon and cysteine proteinase b gene and single strand conformation polymorphism (SSCP) analysis of ribosomal internal transcribed spacer (ITS). All 29 isolates were *L. donovani* and the RFLP and SSCP pattern of the Nepalese isolates corresponded to the standard Indian strain of *L. donovani* but differed from that of the Kenyan strain. Both RFLP and SSCP analysis showed that no genetic heterogeneity within those twenty nine Nepalese isolates. We inoculate all isolates into Nude and BALB/c mice to compare infectivity. Intraperitoneal inoculation with the promastigotes of all isolates resulted in amastigote proliferation in the spleen of twenty nude mice, of which ten isolates were highly infective and ten were moderately infective, and one BALB/c mouse. Of the twenty amastigotes isolated from the spleen of nude mice, only the ten highly infective isolates infected BALB/c mice, of which two isolates were low, three isolates were moderately and five isolates were highly infective. The ten isolates that were highly infective to nude mice could be classified into three degrees of infectivity in BALB/c mice, suggesting that diversity exists in the virulence of Nepalese isolates.

(ACMCIP Abstract)

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EPIDEMY OF CUTANEOUS LEISHMANIASIS IN OUAGADOUGOU, BURKINA FASO (WEST AFRICA): INVESTIGATIONS ON THE VECTORS AND THE RODENT RESERVOIR OF THE PARASITES

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An epidemic outbreak of cutaneous leishmaniasis occurred in the town of Ouagadougou in 1996. Since 1998 we carried out several studies which aimed to identify the parasite as *Leishmania major*. From October 2005 to October 2006 we undertook epidemiological investigations which objectives were to identify the species of the phlebotomes responsible for the transmission of the parasite, and to identify the species of the rodent reservoir. The vectors have been collected using the CDC traps, every week during 12 months in selected sites of the town where the prevalence of the cases were higher. *Phlebotomus* species were identified with the determination key developed by JC Gantier. The females were dissected to find out by microscopical examination, by culture in the NNN medium, and by PCR if they were infected by *Leishmania major.* The rodents were catched using the Sherman trap and the Montpellier trap, in the same selected sites as for the vectors collection. Their species were determined in Paris at the Museum of natural history. Their infection by the parasite was studied by microscopical examination of smears from spleen, and by PCR examination. Regarding the results of the vectors study, a total of 8204 phlebotomes were collected with a peak from July to November. The sub-genus *Phlebotomus* represented 6% and *Sergentomyia* which is not a vector represented 94%. Only the specy *P. duboscqi* was found in the *Phlebotomus* whereas *Sergentomyia* consisted in 17 species. We could not detect the parasite in *P. duboscqi* which is the vector found in other countries in West Africa. Concerning the animal reservoir, a total of 182 micromammals were captured, of whom 101 were rodents. The PCR examination allowed to find that *Mastomys* sp and *Taterillus* sp were infected by *L. major* and may be the reservoirs in Ouagadougou. The study is ongoing for confirming the infection of *P. duboscqi* and for finding out if there are other rodent species involved as reservoirs. In conclusion, a better understanding of the functionning of the epidemiological chain of cutaneous leishmaniasis in the town is a prerequisite for a successful control of the disease.

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IS PHLEBOTOMUS HALEPENSIS NATURAL VECTOR OF *LEISHMANIA TROPICA*? A PARASITOLOGICAL SURVEY IN A NEW CUTANEOUS LEISHMANIASIS FOCUS IN CENTRAL ANATOLIA OF TURKEY

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Anthroponotic cutaneous leishmaniasis (ACL) caused by *Leishmania tropica* is still keeping importance as a health problem in Turkey. The 6 villages belonging to Nigde province is located in southeast of Central Anatolia Region of Turkey, were chosen for field work according to the previously reported 126 cutaneous leishmaniasis cases. We aimed to perform a parasitological and entomological survey in Nigde province to obtain epidemiological data. For this purpose; (i) lesion aspiration fluids and blood samples from CL patients were taken; (ii) all primary and secondary schools in the area were visited to check children for CL scar

on their bodies; (iii) blood samples were taken from 43 dogs and they were examined for clinical signs. A total of 7 out of 43 dog's popliteal lymph node aspirates obtained; (iv) sand fly specimens were collected in three villages. Indirect Fluoresan Antibody Test and rK39 ELISA were used for human and dog sera. Standart PCR method was applied for lesion fluids and lymph node aspiration samples. A total of 10 patients among 50 were diagnosed as cutaneous leishmaniasis by direct examination and/or PCR method. Two *Leishmania* strains were isolated from two patients and identified as *Leishmania tropica*. No antibody response were detected using IFAT and rK39 ELISA. The CL scars were detected in 0.7% (16/2231) of the children. Totally, 3 (6.97%) out of 43 dogs were found to be seropositive. A total of 228 sand flies were collected and 5 species of Phlebotomus spp. were identified as *Phlebotomus halepensis, P. papatasi, P. simici, P. sergenti* and *P. syriacus. P. halepensis* was found to be dominant species in all three villages with the raitos between 65.21% and 84.68%. In conclusion, although this species of *Adlerius* has not yet been proven to transmit *L. major* or *L. tropica* in nature, under laboratory conditions were shown that the Jordan strain of P. *halepensis* is highly susceptible to both *L. major and L. tropica*. The present study was demonstrated the feasibility of *P. halepensis* being a CL vector in the nature.

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IMMUNO-EPIDEMIOLOGY OF VISCERAL LEISHMANIASIS IN A COHORT OF BRAZILIAN DOGS

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Dogs are problematic reservoirs of visceral leishmaniasis (VL) in the New World, but the immunological basis of resistance and susceptibility in dogs is not well defined. In a cohort of 115 dogs naturally infected with *Leishmania infantum* in north Brazil, Th1- and Th2-type responses over time were characterised by assessment of the relative titres of IFN-y and IL-10 cytokines in stimulated peripheral blood mononuclear cell supernatant samples. This provided a unique dataset from which associations between clinical outcome of infection, exposure to saliva of Lutzomyia longipalpis sandflies, and T-cell responses could be explored. The implications of this study will be discussed in the context of the transmission potential and control of VL.

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ACUTE CHAGAS DISEASE OUTBREAK ASSOCIATED TO AÇAÍ JUICE CONSUMPITION - PARÁ STATE/BRAZIL, 2006

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In Latin America an estimated 20 million persons are chronically infected with *Trypanosoma cruzi*, the agent of Chagas' disease. In Brazil oral transmission is an important cause of acute Chagas' disease (ACD), with most cases occurring in the Amazon region and a fatality rate of approximately 6% in the last two years. We report the results of an investigation of ACD outbreak in the municipality of Barcarena, Para State. A case-control study (1:3) matched for sex and age-group, a retrospective cohort study and an entomological survey were conducted in the affected area to evaluate disease-associated risk factors. A confirmed ACD case-patient was defined based on: *T. cruzi* identified by microscopy of thick blood smears; serum reactive for Chagas-specific IgM by indirect immunofluorescence. We identified 11 confirmed ACD case-patients, all (100%) based on microscopy. No deaths occurred. Median age of case-patients was 39 years (range7-70); 82% were female and 73% lived in urban area. Main symptoms included fever (100%), weakness (100%), facial edema (100%), myalgia (82%) and edema of lower extremities (82%). Risk factors associated with a higher odds of ACD included: drinking fresh açaí (palm tree fruit) juice (McNemar X^2=4.75; p=0.02) and drinking açaí juice in one specific area A (p<0.001). In the retrospective cohort, ACD was associated with consuming açaí (RR=4.5;95%CI=1.33-15.28) and drinking açaí in the Area A health clinic (RR=4.5;95%CI=1.33-15.28); ingesting frozen açaí juice was associated with lover risk (RR=0.13;95%CI=0.02-0.78). No other exposure(s) related to possible *T.cruzi* transmission was identified among case-patients. Inspections of case-patient residences and locations where juice produced did not identify any Triatome insects. We conclude that in this ACD outbreak the most likely risk factor for transmission was consumption of unpasteurized açaí juice, due to contamination of fruit with the parasite before production, however the specific mechanism of contamination was not identified.

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IMPACT OF HUMAN AFRICAN TRYPANOSOMIASIS IN A RURAL COMMUNITY IN DEMOCRATIC REPUBLIC OF CONGO

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Human African trypanosomiasis (HAT) causes the loss of some 1.5 millions of DALYs according to global burden of disease evaluations. This study describes the impact of HAT between 2000- 2002 in Buma, a rural community near Kinshasa in the Democratic Republic of Congo. HAT-related household cost and Disability Adjusted Life Years (DALYs) were estimated by retrospective questionnaire surveys. The HAT outbreak in Buma involved 57 HAT patients and affected 21% (47) of the community's households. The household cost was equivalent to five month's household income. The total number of HAT-related DALYs was 2145 and intervention measures to control HAT averted 1408 DALYs. The cost per DALY averted was US$ 17. HAT has a serious economic impact for households in the affected localities and intervening to control is very cost-effective. For these reasons, considering only global burden of disease rankings for resource allocation could lead to misguided priority setting if applied without caution in HAT-affected countries.

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IMPACT OF CLIMATE VARIABILITY ON CUTANEOUS LEISHMANIASIS IN VENEZUELA

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Background: Cutaneous leishmaniasis (CL), a tropical vector-borne disease caused by *Leishmania spp*, vectorized in Americas by species of *Lutzomyia*, could be influenced by climatic variability. This issue has been understudied in many places. For this reason we report possible impacts of climatic variability and El Niño events occurred during 1994-2003 on CL in 17 endemic states of Venezuela. Methods: Climatic data was obtained from remote sensing systems. Epidemiological data was obtained from Environmental health service (DGSACS), Ministry of Health. Climatic events classification was made according NOAA and the indexes SOI and ONI were used as main global climatic variability indicators. Comparisons of yearly variations and deviation from medians trends between CL incidence and climatic variability as well lineal regression models were made. Statistical analyses were made with SPSS 10.0 and GraphPad Prism 4.0, 95% of confidence. Results: During this period a considerable global climatic change was present, with strong El Niño events during

years 1994, 1997 and 2002, and strong La Niña events during 1995/1996 and 1998-2001. El Niño in eastern region of the country is expressed as drought periods and La Niña as increases in rainfall. During this period, in these states 17,589 cases of CL were registered (20.2% from Lara, 11.9% Miranda and 10.5% Trujillo), mean of 2281.1±443.8 cases/year. During years with El Niño a mean increase of 78.61% in CL incidence was observed (ranging 8.3 to 614%), whilst in La Niña a mean decrease of 9.78% was evidenced (ranging 8.3 to 614%). Comparisons in deviation according these seasons were significant 15 out of 17 states ($p<0.001$). Lineal regression models analysis, for Lara found that with a higher value of SOI (tending to La Niña) less incidence of CL is observed, although did not reached statistically significance ($r^2=0.3085$, $p=0.0955$); with higher values of ONI an increase in CL incidence was observed, being significant ($r^2=0.4254$, $p=0.0410$). Similar patterns were observed in other states. Conclusion: This evaluated data reflected importance of climatic variability on CL incidence and phlebotomine sandflies vectorial transmission, and open further investigations in the area to develop possible forecasting and monitoring systems with relevance in regional public health.

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IDENTIFICATION OF THE ETIOLOGIC AGENT OF THE EPIDEMIC OF CUTANEOUS LEISHMANIASIS IN TOLIMA, COLOMBIA

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American Cutaneous Leishmanaisis (ACL) has been traditionally characterized as a zoonotic disease, primarily affecting males occupationally exposed to the transmission cycle. Recently, peridomestic and domestic transmission caused by several species of the *Viannia* subgenus has been reported in countries in Central and South America. *Leishmania guyanensis* has not previously been associated with peridomestic transmission. This study sought to identify the etiologic agent of the ACL outbreak that began in 2003 in southern region of the department of Tolima. Methods: Lesion aspirates were obtained from patients consulting the Leishmaniasis Control Program in three of the municipalities affected by the outbreak. Species were identified using monoclonal antibodies and isoenzyme electrophoresis. The geographic distribution in Colombia of *L. guyanensis* isolates obtained prior to this epidemic was determined and the age and gender distribution of the corresponding cases was compared to that of cases diagnosed during the ongoing epidemic in Tolima. Isolates were obtained from a total of 57 patients. Fifty-four of the isolates were identified as *L. guyanensis* and three as *L. panamensis*. All of the *L. guyanensis* strains pertained to a single zymodeme. The geographic distribution of *L..guyanensis* strains (n=26) isolated prior to the epidemic was confined to the sub-andean or amazon regions of Colombia, ecosystems that differ from that of the epidemic in Tolima. Children accounted for 35,7% of cases infected with *L. guyanensis* in Tolima and 45,8% were females, in contrast with the exclusive prevalence in adult males among cases from other settings diagnosed prior to 2004. In conclusion, *L. guyanensis* is the probable causal agent of the largest ACL outbreak reported in the history of Colombia. This is the first report of *L. guyanensis* transmission in the peridomestic setting. *L. guyanensis* had not been previously reported in the Andean region of Colombia. The vector implicated in this epidemic remains to be identified.

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

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During the life cycle of *Plasmodium falciparum,* the most pathogenic of the human malaria parasites, sporozoites are infectious for both mosquito salivary glands and vertebrate tissue. Since this dual infectivity of sporozoites is critical to the survival and development of the parasite, the sporozoite presents an effective target for control by vaccines, drug therapy, or novel mosquito control methods. A *P. falciparum* sporozoite gene, known as *MAL13P1.319*, was identified by a search of the annotated *Plasmodium* genome database (PlasmoDB) using specific criteria. The alignment of PfMAL13P1.319 protein with proteins in other *Plasmodium* species shows segments of conserved sequences, which could potentially identify regions of conserved structure and function. In addition, according to PlasmoDB, *MAL13P1.319* is transcriptionally and translationally expressed in sporozoites, while other analyses such as TargetP and ProtComp predict the protein to have a signal peptide and to be extracellular. These data suggest that MAL13P1.319 could potentially function as a secretory molecule that aids sporozoite invasion of host tissue. Transcription of *MAL13P1.319* during the erythrocytic stages, which was reported in PlasmoDB, was confirmed by RT-PCR. Concomitantly, anti-MAL13P1.319 polyclonal antibodies are being produced and will be used to investigate MAL13P1.319 expression and localization patterns in various parasite stages via western blot analysis, immunofluorescent assays, and/or electron microscopy. In addition, we will study MAL13P1.319 function via gene disruption assays and MAL13P1.319 protein trafficking by constructing a MAL13P1.319-GFP fusion protein. These studies will be used to assess the role of *MAL13P1.319* in sporozoite biology and, more specifically, to determine if it has a role in host tissue invasion.

(ACMCIP Abstract)

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ACUTE LUNG INJURY IN A SEVERE MALARIA MODEL IS DEPENDENT ON TOTAL PARASITE BURDEN AND CD36-DEPENDENT LOCAL SEQUESTRATION IN THE LUNG

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Respiratory distress and acute respiratory distress syndrome (ARDS) are common features of severe malaria and embody a poor prognosis. However, little is known about the underlying molecular basis of acute lung injury (ALI) in malaria. Based on the hypothesis that ALI is dependent upon parasite burden, lung sequestration and associated inflammatory tissue injury, we investigated the development of ALI *in vivo* using the *Plasmodium berghei* ANKA (PbA) model of cerebral malaria (CM). PbA-infected susceptible mice develop a cytokine-associated encephalopathy by day 7-10 of infection. Previous work identified that PbA-infected mice also develop a septal pneumonitis and display increased vascular permeability in the lung. We show that bronchoalveolarlavage (BAL) fluid in PbA-infected C57BL/6 mice reveals a significant increase in IgM and total protein 1-2 days prior to the development of cerebral malaria symptoms, indicating pulmonary edema; however, the alveolar cell content remained constant with no infiltration of neutrophils, as is common with other types of acute lung injury. Histopathology confirmed the occurrence of septal inflammation with no cellular infiltrates in the alveoli. BAL fluid IgM and protein content were subsequently used as markers of ALI. Using a panel of inbred mice, we demonstrate that there is a spectrum of susceptibility to PbA-induced ALI. However of interest, ALI did not closely correlate with susceptibility to CM development - i.e. some CM-resistant mice (e.g. BALB/c) develop lung injury whereas others (e.g. AKR/J) do not. It was also observed that ALI was strongly positively correlated with peripheral blood parasitemia and parasite burden. Mice with decreased lung sequestration (e.g. *Cd36*$^{-/-}$ mice) were relatively protected against ALI. In summary, parasite burden and CD36-mediated localized sequestration in the lung are primary determinants of ALI in the PbA experimental model of severe malaria.

(ACMCIP Abstract)

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DEVELOPMENT OF A MOUSE MODEL FOR PREGNANCY-ASSOCIATED MALARIA STUDIES

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Pregnancy-associated malaria (PAM) caused by *Plasmodium falciparum* is characterized by sequestration of infected erythrocytes in the placenta that, together with maternal malaria induced anemia or hypoglycemia, lead to decreased fetal viability and infant low birth weight. Easily handled PAM mouse models will be very useful in overcoming some restrictions posed to PAM studies in humans. A mouse model reproducing the main characteristics of PAM has been developed. Murine PAM caused by Plasmodium berghei reveals similar features to those that occur in humans, such as higher disease susceptibility during pregnancy, evidences of intrauterine growth retardation and placental pathology. These models will allow addressing questions and running experiments that could not be performed in humans, which might be important for the understanding of disease pathogenesis mechanisms.

(ACMCIP Abstract)

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PLASMODIUM YOELII MAKES A FUNCTIONAL HOMOLOG OF THE MAMMALIAN MACROPHAGE MIGRATION INHIBITORY FACTOR

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A gene bearing similarity to mammalian cytokine macrophage migration inhibitory factor (MIF) was found in the genomes of *Plasmodium* parasites. MIF is an important immune mediator of inflammation causing the prolonged activation of the proinflammatory cytokine cascade by counter-regulating the immunosuppressive effects of glucocorticoids. In malaria, MIF has been shown to be overexpressed in cases of fatal falciparum malaria and in cases of placental malaria. Interestingly, MIF has been shown to play a role in malaria associated anemia by inhibiting erythroid differentiation and hemoglobin production. To study the role of the parasite protein in disease pathogenesis *in vivo*, we characterized the *P. yoelii* homolog of MIF, which is 30% identical to the mammalian MIF and 76% identical to the *P. falciparum* homolog. The *pymif* gene was shown to be transcribed during blood stage parasite development with peak expression in mid-trophozoite stages. A non-tagged recombinant *Py*MIF was expressed and purified from a soluble lysate of induced bacteria by preparative isoelectric focusing and hydrophobic interaction chromatography. Following passage over polymyxin B, endotoxin levels in purified protein were less than 0.1 E.U. per 1 µg of protein. The purified recombinant *Py*MIF was functionally active as shown by its ability to induce the chemotaxis of thioglycollate elicitated macrophages in a dose dependent manner. A high titer polyclonal antisera was raised against r*Py*MIF that showed no serological cross-reactivity with recombinant human MIF. By immunoprecipitation of metabolically labeled *P. yoelii* 17X blood-stage proteins, *Py*MIF was detected in parasitized erythrocytes and was present extracellularly in culture supernatants. Analysis of sera obtained from mice that recovered from *P. yoelii* malaria showed that mice produce antibodies to *Py*MIF and that the response is boosted by repeated infection. Mice immunized thrice with r*Py*MIF formulated with Quil A as adjuvant were challenged with *P. yoelii* 17X parasitized RBCs. Immunized mice showed no changes in mean peak parasitemia or in the magnitude of infection-associated anemia relative to adjuvant controls. However, immunized mice had a prolonged period of reduced hemoglobin levels and low levels of persistent parasitemia. Studies are in progress to further evaluate the contribution of parasite-derived MIF versus host-derived MIF in the pathogenesis of blood stage malaria.

(ACMCIP Abstract)

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GENE EXPRESSION ANALYSIS OF ENDOTHELIAL CELL ADHERENT VERSUS NON-ADHERENT RETICULOCYTES INFECTED WITH *PLASMODIUM YOELII* 17X

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Plasmodium falciparum invades both normocytes and reticulocytes and causes most malarial fatalities. However, *Plasmodium vivax* is infrequently fatal and preferentially invades reticulocytes. These host cell preferences can be studied in rodent models using lethal *Plasmodium yoelii* 17XL parasites which infect both mature and immature RBCs, or non-lethal *P. yoelii* 17X parasites which primarily invade reticulocytes. We have previously reported that in the presence of merozoite surface protein-8 specific antibodies *P. yoelii* 17XL parasites preferentially invaded immature rather than mature RBCs. Analysis of gene expression changes associated with this switch in host cell tropism revealed altered expression in sets of genes (*pyst-a*, *yir*, *pypmp*) predicted to be expressed on the surface of infected RBCs. We hypothesize that expression of these proteins promotes the localization of *P. yoelii* infected RBCs to reticulocyte-rich tissues such as bone marrow and spleen. This may limit exposure to merozoite neutralizing antibodies while providing access to target RBCs that are normally present in low numbers in circulation. In studies to test this hypothesis, we show that *P. yoelii* 17X-infected reticulocytes specifically adhere to bEnd.3 endothelial cells *in vitro*. Adherence was restricted to a low percentage of early trophozoite-stage parasites and was reversed by treatment with dextran sulfate. By panning on these cell monolayers, stage-separated *P. yoelii* 17X infected reticulocytes were fractionated into adherent and non-adherent populations. RNA was isolated from each subpopulation and transcription profiles were compared by DNA microarray analysis. Data analysis from replicate experiments allowed the identification of a gene set whose expression is significantly upregulated in adherent, *P. yoelii* 17X infected reticulocytes. A number of these genes remain uncharacterized but are predicted to contain signal sequences and/or transmembrane domains, have orthologues present in *P. falciparum* or *P. vivax* genomes, and/or are members of multi-gene families. Studies are ongoing to determine which of these *P. yoelii* genes are consistently expressed in association with endothelial cell adherence and which are variably expressed *in vivo*, in *P. yoelii* parasites under antibody-mediated immune pressure.

(ACMCIP Abstract)

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TRANSPLACENTAL TRANSFER OF MSP1$_{42}$ USING THE *IN VITRO* PLACENTAL PERFUSION MODEL

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In *Plasmodium falciparum* endemic areas 5-52% of newborn cord blood lymphocytes show immune reactivity to the abundant merozoite blood stage antigen, MSP1. MSP1 undergoes proteolytic cleavage during erythrocyte invasion releasing fragments into the serum that can be detected by an antigen capture assay. We hypothesized transplacental transfer of MSP1 occurs in the form immune complexes via the FcRn located in the syncitiotrophoblastic membrane and endothelium of vessels in the fetal villi. To test this hypothesis an *in vitro* perfusion model was used that dually perfuses a placental cotyledon with independent maternal and fetal circuits. The perfused cotyledon remained viable over

the 9 hour experiment as measured by oxygen and glucose consumption and production of hCG and lactate. Each experiment (N=4) had 3 phases: phase I added recombinant $MSP1_{42}$ alone into the maternal circuit, phase II added $rMSP1_{42}$ plus 25% Kenyan serum with anti-$MSP1_{42}$, and phase III added $rMSP1_{42}$ plus 10% Kenyan serum. During each phase maternal and fetal circulation was sampled every 30min for 2h. At the completion of the experiment the perfused cotyledon was fixed. There was no transplacental transfer of $MSP1_{42}$ in the absence of immune serum. In phase II-III $MSP1_{42}$ was detected in fetal circulation that peaked at 30min following addition of antigen and serum. Immunohistochemistry of fixed tissues localized $MSP1_{42}$ to the fetal villous stroma, primarily in probable Hofbauer cells, a fetal tissue macrophage. These data demonstrate transplacental transport of $MSP1_{42}$, likely in the form of immune complexes, and provides a valuable model to examine the impact of malaria on placental tissues.

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PLASMODIUM DERIVED FACTORS INDUCE APOPTOSIS IN HUMAN NEUROGLIA AND VASCULAR ENDOTHELIAL CELLS

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Cerebral malaria (CM) is the most severe neurological complication of infection with *Plasmodium falciprium* and is a major cause of acute non-traumatic encephalopathy associated with mortality rates of 10-40% and an estimated 1-2.5 million deaths in sub-Saharan Africa and Southeast Asia. CM associated mortality is multi factorial and difficult to assess in diverse environments. The severity and poor prognosis of CM has been tied to cytokines, cytoadherence of parasitized erythrocytes (PRBCs) in post-capillary venules, acidosis, ischemia, as well as apoptosis. These factors appear to be induced by parasite derived factors although their role in CM related deaths is unclear. We have previously demonstrated that *Plasmodium berghei* ANKA (PBA) derived molecules induce apoptosis in human brain vascular endothelial cells (HBVEC) without a requirement for physical interaction between PRBC's and HBVEC. We have also shown that high plasma and CSF concentration of IP-10 is a determinant of poor prognosis in human CM. In this study, we hypothesize that *P. falciparum* derived factors mediate apoptosis in HBVEC and neuroglia cells (NG) crucial to CM pathology. We evaluated the apoptotic effects of *P. falciparum* infected patient plasma from non-malaria (NM), cerebral malaria survivors (CMS) and cerebral malaria non-survivor (CMNS) on HBVEC and NG by TUNEL assay with or without antibodies targeting the IP-10/CXCR3 apoptotic pathway. Our data shows that CMNS plasma induced the highest apoptotic effects in HBVEC when compared to CMS and NM patients. Furthermore, treating these cells with anti-human IP-10 or CXCR3 mAb altered apoptotic effects. We conclude that *P. falciparum* derived factors mediate apoptosis in endothelial and neuroglia cells via IP-10/CXCR3 pathway without a need for physical contact between parasites and host cells and that targeting these factors in future vaccine strategy may be a way forward for preventing CM associated deaths.

(ACMCIP Abstract)

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PLASMODIUM BERGHEI ANKA IS ASSOCIATED WITH COGNITIVE DYSFUNCTION IN MICE

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Cerebral malaria (CM) causes significant morbidity throughout the developing world. Malaria is often complicated by neurological and cognitive sequelae that persist even with successful anti-parasitic treatment. C57BL/6 mice were infected with *Plasmodium berghei* ANKA (PbA). Six, 7 and 11 days post infection (PI) we investigated the effect of infection on cognitive function by using an object recognition test of working memory, and we compared them to uninfected matched control mice. Infected mice had a significant impairment in visual memory at day 7 that worsened by day 11 PI. At day 11 PI there was an observable decrease in the time spent exploring new objects. Impairments were seen in the absence of confounding effects of infection as the infected mice showed no alteration in locomotor activity, arousal, reflexes or motor coordination on a functional observation battery. The cognitive dysfunction correlated with immunostaining evidence for prominent microglial activity throughout the brain at 8 and 12 days PI, with hypertrophy of the microglia and retraction and thickening of the processes as compared with control mice. Astrocytes in the infected mice appeared to display increased affinity for the perivascular region. There was a disruption of the normal polarized distribution of Aquaporin-4 at the astrocytic end-feet in the brains of infected mice. In this murine model we demonstrate that PbA infection is associated with cognitive impairment which is associated with loss of integrity in brain microglial and astrocytic morphology and activity. CM should be considered a gliopathy as well as a vasculopathy. Further studies using this model may suggest testing strategies that could potentially identify subjects at risk for adverse cognitive outcome as well as elucidate underlying pathogenesis in CM.

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FUNCTIONAL ASSESSMENT OF A 72KDA PUTATIVE GLUCOSE REGULATED PROTEIN IN *PLASMODIUM KNOWLESI* BLOOD-STAGE PARASITES

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Plasmodium parasites synthesize and traffic proteins into various parasite-induced structural modifications of the infected red blood cell (RBC). A 72kDa *P. knowlesi* protein was immunoprecipitated using a cross-reactive *Plasmodium vivax* monoclonal antibody. Mass spectrometric analysis produced peptide hits that identified this protein as a member of the heat shock protein 70 (HSP70) family. Bioinformatic analysis revealed that the 72kDa protein has a predicted N-terminal signal sequence, three coiled-coil domains and a C-terminal SDEL motif, consistent with the KDEL motif in mammalian glucose-regulated proteins (GRPs), which are members of the HSP70 family. This motif may target the protein (henceforth referred to as PkGRP) to the endoplasmic reticulum (ER) of the intraerythrocytic parasite. PkGRP is encoded by a two-exon gene located on chromosome 7, and shows greater than 90% identity to its orthologs in *P. vivax*, *P. falciparum*, *P. berghei*, *P. chabaudi*, *P. yoelii* and *P. gallinaceum*. *P. knowlesi* cDNA representing full-length PkGRP was cloned into a pGEX *E. coli* expression vector and the recombinant protein expressed and purified from *E. coli* BL-21 cells for mouse and rabbit antisera production. Functions postulated for HSP70 family of proteins include protein folding and unfolding, thermal adaptation and post-translational translocation of proteins in the ER. Protein translocation studies, including PkGRP immunolocalization and site-directed mutagenesis of the putative ER-retention motif, will be discussed.

(ACMCIP Abstract)

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THE MURINE COMA AND BEHAVIOR SCORE: A RAPID ASSESSMENT TOOL FOR MURINE CEREBRAL MALARIA

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To quantitatively assess murine cerebral malaria (mCM), we developed a rapid murine coma and behavior score (MCBS), enabling us to tease apart

mice that are merely symptomatic due to malaria infection and those who demonstrate true CM. This score utilizes eleven neuro-behavioral parameters, each scored between 0 and 2; the lowest achievable score is 0, the highest is 22. We utilize a CM-susceptible mouse, C57BL/6, IP injected with $1x10^6$ *Plasmodium berghei*, ANKA strain. Mice who develop CNS dysfunction consistent with mCM demonstrate a MCBS of 10, or less, and succumb to the disease between 5-9 days of infection, compared to mice that do not develop CM but who ultimately succumb to severe anemia, within 14-21 days of infection. CM develops in 88% of our test mice, with a high level of histopathological findings (ie., cerebral hemorrhages) consistent with mCM. Chloroquine (10mg/kg, IP) cure successfully abates parasitemia, but 40-80% of the mice still succumb to mCM. This MCBS allows us to quantitatively, and consistently, follow the progress of disease in our mice, confidently label them with mCM, allowing us to competently pursue drug cure trials, RNA hybridization studies, and proteomic analysis.

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VARIANT SPECIFIC IMMUNITY TO MALARIA IN PREGNANCY: PROTECTION AGAINST ANAEMIA AND REINFECTION, AND EFFECTS OF IPTP ON DEVELOPMENT OF ANTIBODY

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Antibodies to variant surface antigens (VSA) expressed by infected erythrocytes (IE) associated with placental malaria have been correlated with improved pregnancy outcomes, but such responses have been measured in samples collected at delivery. To examine the predictive value of measures of immunity for better pregnancy outcomes, we studied changes in variant specific immunity over time, and the relationship between variant specific immunity at study enrolment and the development of malaria-associated complications in pregnancy, including maternal anaemia, low birth weight, and recurrent parasitemia. Investigations took place in association with two studies of antimalarial drugs in pregnant Malawian women. We measured total IgG to (VSA) expressed by two parasite lines, CS2 and HCS3, which both adhere to chondroitin sulphate A. Gender and gravidity-associated antibody responses were seen in each study, to each isolate. Responses were frequently (>80%) detected to each isolate, and were generally (~80%) concordant, but relative antibody levels were higher to CS2. In one study of 141 parasitemic pregnant women, levels of antibody to CS2 at enrolment were significantly higher in women who did not suffer further parasitemic episodes (p=0.012) and in women who were not anaemic at delivery (p=0.0011). In a second study, 67 pregnant women receiving regular doses of IPTp had antibody measured at enrolment, 3rd trimester, and 1, 3 and 6 months post partum. Antibody levels were dynamic, but showed an overall decline between enrolment and 1 month post partum. There was little change between 1 and 6 months post partum. Cluster analysis revealed that primigravid women were in the group which had persistent low or absent antibody responses throughout gestation, suggesting that IPTp may be preventing these women from acquiring pregnancy-specific immunity, and potentially increasing their risk of malaria in subsequent pregnancies. Antibody to VSA associated with malaria in pregnancy appears to protect from subsequent parasitemia and anemia, and IPTp may prevent these responses from developing. The established benefits of IPTp must be extended to women of all gravidities, to prevent complications of pregnancy malaria in women lacking appropriate immunity. Antibody measures at ANC booking may be a useful tool to predict women at risk of adverse pregnancy outcomes.

(ACMCIP Abstract)

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ENHANCED DETECTION OF GAMETOCYTES PREDICTS HIGHER POTENTIAL FOR *PLASMODIUM FALCIPARUM* TRANSMISSION

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Aggregated hemozoin crystals within malaria-infected erythrocytes confer susceptibility of parasitized cells to magnetic capture. This property was previously exploited to demonstrate that trophozoites, schizonts and gametocytes, and all four human malaria species are enriched on microscope slides exposed to a magnetic field. A trial to test the utility of this diagnostic method was conducted in a malaria-endemic region of Papua New Guinea (PNG). Individuals observed with malaria symptoms at local health centers were recruited to provide blood samples for conventional blood smear (CBS) and magnetic deposition microscopy (MDM) diagnosis. For MDM, blood samples (25 µL) were mixed with malaria cultivation media (1 mL) and applied to transparent mylar slides exposed to a high gradient magnetic field under constant flow (8.3 uL/minute). Light microscopic examination was performed following standard methanol fixation and Giemsa staining of slides prepared by CBS and MDM. It was observed that *Plasmodium falciparum* (Pf) parasitemia measured by CBS was significantly amplified by MDM. Of 29 infected individuals MDM detected stage 3, 4 and 5 gametocytes in 48.3% of study participants compared to 6.9 % by CBS. Moreover, comparing MDM with CBS we observed consistent fold-increased capture of gametocyte (61), mature schizont (40), early trophozoite (3.7) and ring (2.5) erythrocyte developmental stages, and a 2.7 fold (R2=0.911) increase in overall parasitemia. MDM increased detection sensitivity of Pf-infected, hemozoin-containing erythrocytes from infected humans. At approximately 50% gametocytemia, infection of mosquitoes by the human population is consistent with gametocyte prevalence predicted by RT-PCR-based methods and significantly higher than estimates based on detection of gametocytes by CBS. These findings suggest that a higher percentage of people are infectious to mosquitoes in malaria-endemic sites in PNG that previously predicted.

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EVALUATION OF REAL-TIME PCR PROTOCOLS FOR LABORATORY DIAGNOSIS OF MALARIA

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The clinical management of malaria depends on which of the four *Plasmodium* species (*P. falciparum, P. vivax, P. ovale, P. malariae*) is causing the infection. The gold standard for malaria diagnosis, microscopic examination of stained blood smears, can sometimes fail to correctly identify the infecting species, especially in samples of low parasitemia or in mixed infections. In such cases, a molecular PCR test is required to confirm microscopy results or resolve suspicious cases. A literature search identified five real-time PCR protocols reporting species-specific detection of malaria parasites. One assay used four species-specific TaqMan probes (designated the dual-duplex TaqMan), one detected three of the species (excluding *P. malariae*) with TaqMan probes (the triplex TaqMan), one was a LightCycler assay with melt curve analysis (the LC assay), and two were SYBR Green-based assays (the multiplex SYBR Green and the 2-primer SYBR Green, respectively). Using blood samples submitted to CDC for malaria reference

diagnostic testing, we evaluated these five real-time PCR assays and compared them to microscopy and a well-characterized conventional nested PCR assay. Two of the assays were excluded from the analysis at an early stage due to unsatisfactory results: the LC assay consistently failed to discriminate between *P. vivax* and *P. ovale*, and the triplex TaqMan failed to detect all genetic variants of *P. ovale*. The other three assays were further evaluated with blood infected with either *P. falciparum* (n=13), *P. vivax* (n=19), *P. ovale* (n=13) or *P. malariae* (n=1), one *Babesia microti*-infected blood, 14 uninfected blood samples, one blood sample from a concurrent infection with *P. falciparum* and *P. malariae*, and one brain tissue sample. The three real-time PCR assays all failed to detect the *P. malariae* component in the mixed sample, but otherwise produced results identical to the nested PCR and/or microscopy results. This highlights the importance of careful validation of real-time PCR assays before applying them as diagnostic tests.

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AVOIDING MISCLASSIFICATION OF RECURRENT *PLASMODIUM FALCIPARUM* PARASITEMIAS AFTER THERAPY

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In order to evaluate efficacy of drugs in treating falciparum malaria, it is necessary to distinguish reinfection from recrudescence. Currently, this is done by electrophoretically comparing PCR-amplified polymorphic genes from pre- and post-treatment parasite populations. However, the definition of a reinfection is controversial, especially whether the appearance of new variants (bands) in the recurrence signifies reinfection. In order to establish a criterion for reinfection, we conducted blinded analyses of samples from patients who participated in a Phase II clinical trial at the Hospital for Tropical Diseases in Bangkok, Thailand, many of whom never left the hospital and could not have been reinfected. Enrollment and recurrent parasite populations were evaluated by nested PCR of merozoite surface protein 1 and 2 (msp-1 and msp-2) and by heteroduplex tracking assay (HTA) of msp-1. Twenty-eight patients experienced recurrent falciparum malaria episodes within 28 days of treatment. Of these, 19 remained in the hospital or were discharged for <6 days at the time of recurrence and were not out of the hospital for enough time for their recrudescences to be patent reinfections. Using the criteria that a shared (but not highly prevalent) variant indicates recrudescence, all patients who were successfully amplified by HTA (23) or nested PCR (12) were classified as recrudescence. Using the criteria of the appearance of a new band in the post-treatment sample, 14 of 23 patients (61%) were classified as reinfections by HTA, of which only 4 were out of the hospital >5 days, and 3 of 12 patients (25%) were classified as reinfection by nested PCR, only one of whom was out of the hospital for >5 days. Thus, the shared band criterion would lead to the misclassification of >10/23 (43%) of HTA patients and >2/12 (17%) of patients by HTA and nested PCR, respectively. Thus, the appearance of a new band in a recrudescent sample is likely due to growth of a pre-existing minority variant and is not indicative of reinfection.

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ESTIMATION OF MALARIA PARASITE DENSITY IN URINE AND SALIVA SAMPLES USING REAL-TIME QUANTITATIVE PCR (QPCR) METHODS

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Presently all laboratory methods for malaria diagnosis and estimation of parasite density rely on blood sampling. Although single-point blood sampling of individuals with symptoms may not present problems of compliance, it is often difficult to recruit volunteers for studies that require repeated blood sampling over a short period of time. However, efficacy trials of anti-malaria interventions do necessarily require multiple blood sampling from the same individual, because time-to-blood infection and time-to-parasite clearance are important end points in such studies. Therefore, a non-invasive method of parasite detection and quantitation is needed in central laboratories in disease-endemic areas, where field and clinical trials are performed. Recently, it was shown that malaria parasite DNA could be detected in urine and saliva samples obtained from *Plasmodium falciparum*-infected patients but it is not known if parasite densities measured in blood, urine and saliva correlate. We report here a study to determine the degree to which parasite detection and density estimation using quantitative real-time PCR-amplification (qPCR) of parasite DNA in urine and saliva agree with that from blood-derived DNA and slide microscopy. A secondary endpoint was to determine patient's preference to give either a blood, urine or saliva sample for malaria parasite detection. Of 386 patients enrolled in the study, 14% (53) had *P. falciparum* infection detectable by microscopy compared to 17% (66) by nested PCR. Parasite density estimates obtained for equivalent volumes of blood, saliva, and urine indicated that blood samples yielded more parasite DNA than either saliva or urine samples. The threshold cycle at which a positive amplification reaction was measured (Ct value) in samples with blood parasite density greater than 5,000/µl (n=23) ranged from 15 - 23 cycles for blood; 24 -35 for saliva and 28-42 for urine samples. Our results show that malaria parasite DNA can be quantified in saliva and to a lesser extent in urine samples of infected individuals. Questionnaire responses from patients suggest that for repeated sampling, most preferred urine sampling to either blood or saliva. qPCR of parasite DNA in saliva samples of malaria patients could be a useful method for estimating parasitaemia but would require optimized DNA isolation methods in order to approach the level of sensitivity useful for routine application in clinical trials.

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SEVERE THROMBOCYTOPENIA: A CLUE IN A PATIENT WITH MALARIA

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Introduction Approximately 30,000 travelers from industrialized countries contract malaria each year. The relative risk of malaria for travelers who stay in Sub-Saharan Africa for one month and do not take chemoprophylaxis is approximately of 1:50. Patients returning from endemic areas with thrombocytopenia should have peripheral smears since there is a high predictive value; empiric treatment for malaria must be considered. A 65 years old Korean male in previously good health presented to the ER with lethargy, fever and malaise after returning from Guinea, Africa 5 days earlier. He was in Africa performing missionary work for one month. He did not take malaria prophylaxis, never had malaria or blood transfusions. Patient presented with thrombocytopenia of 8,000cells/mm^3; was admitted to the ICU where he became comatose, developed renal failure requiring dialysis, had respiratory distress and ARDS. *Plasmodium falciparum (PF)* was identified in over 50% of the RBC. Exchange transfusion was combined with intravenous quinidine gluconate and Doxycycline; after 48 hours, the level of parasitemia reached <5 %. Within days, the patient was extubated, consciousness and renal function improved and he was discharged home. Malaria is a true medical emergency that requires rapid diagnosis and treatment. The incidence of thrombocytopenia in malaria can range between 40-85%; a higher incidence is seen with *PF*. Possible causes of thrombocytopenia include a shortened life span of the platelet, immune destruction and splenic sequestration. In one study, all 40 cases of malaria had thrombocytopenia (<150,000 cells/mm^3) and cases with severe thrombocytopenia (< 50,000 cells/mm ^{3}) were more likely seen with *PF*. Unfortunately, in two thirds of tropical travelers who die of malaria, either treatment is delayed or the

diagnosis is simply missed. We stress the importance of thrombocytopenia as a supporting clue for the diagnosis of malaria and urge prompt therapy.

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COMPARISON OF BLOOD SMEAR MICROSCOPY AND PCR BASED METHODS IN THE DETECTION OF *PLASMODIUM FALCIPARUM* IN ACTIVE SURVEILLANCE FOR HIGHLAND MALARIA IN WESTERN KENYA

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A number of PCR-based methods have been used to detect *Plasmodium falciparum* infection in studies of malaria in areas of stable transmission, but the optimal method to use in areas of unstable transmission is not clear. In the present study, we compared the performance of blood smear microscopy and three PCR based methodologies in detecting *P. falciparum* parasite infection in epidemic-prone areas of Nandi Hills District, Kenya. Individuals presenting with malaria symptoms to the local health centers were recruited and finger prick samples collected. Thick and thin films were prepared for microscopy, and *P. falciparum* nested PCR, multiplex PCR ligase detection reaction (LDR) and merozoite surface protein-2 (MSP 2) PCR performed on DNA extracted from filter paper blood spots. Of the 379 samples tested, *P. falciparum* was detected in 95 (25%), 139 (37%), 156(41%) and 158 (42%) samples by blood smear microscopy, nested PCR, MSP-2 PCR and PCR-LDR respectively. *P. falciparum* PCR-LDR has detected lower levels of parasitemia than other PCR methods in previous studies, so PCR-LDR was taken as the gold standard for comparison between tests. As compared to PCR-LDR, sensitivities of 68%, 79% and 90%, and specificities of 95%, 91% and 94% were obtained for blood smear microscopy, nested PCR and MSP2 PCR, respectively. Concordance was highest between PCR-LDR and MSP2 PCR (92%) and lowest between blood smear microscopy and LDR-PCR (78%). The concordance between PCR-LDR and MSP2 PCR suggests that PCR-LDR was not detecting false positives. The multiplex PCR-LDR system also enabled us to assess for *P. vivax*, *P. ovale* and *P. malariae* infection from the same sample. We documented frequencies of infection of 0%, 0% and 0.8% for *P. vivax*, *P. ovale* and *P. malariae* infection respectively. In this area of unstable transmission, PCR-LDR appears to have sensitivity as good as or better than nested PCR or MSP2 PCR for detection of *P. falciparum* infection and allows detection of all 4 human malaria species from a single sample.

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SPECIATION OF ALL FOUR HUMAN MALARIA PARASITES IN A SINGLE, MULTIPLEX REAL-TIME PCR REACTION

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Real-time PCR has emerged as a highly sensitive and quantitative method for the detection and speciation of malaria parasites. While different PCR conditions have been reported, most of these require separate reactions to identify each of the four human *Plasmodium* species. As published previously, TaqMan probes were multiplexed for two of the four species at a time, reducing the number of speciation reactions by half. We have re-designed these probes with a new combination of fluorophores to multiplex all four probes in a single reaction. A region of the *Plasmodium* 18S rRNA gene was amplified using genus-specific primers, combined with the four species-specific probes, and PCR reactions run on the ABI Prism 7500. Plasmids containing each of the four species-specific sequences were reliably detected in the 4-plex reactions with a sensitivity of 10 gene copies per reaction. With a selective set of clinical samples positive for malaria, we detected *Plasmodium* DNA at a concentration of 22 gene copies/µl blood. No cross-reactivity between species was observed, suggesting a high level of probe specificity. Speciation of parasites using this assay, including detection of one mixed infection, was confirmed by conventional nested PCR. We are currently validating this new assay against a panel of clinical samples from patients positive and negative for malaria.

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DB75, A NOVEL DIAMIDINE, DEMONSTRATES A STAGE SPECIFIC KILLING ACTION AND UPREGULATES DNA PRIMASE EXPRESSION

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DB289 is a broad spectrum anti-parasitic compound which has been shown to be effective against both Human African Trypanosomiasis and malaria in recent clinical trials. DB75, 2,5-bis(4-amidinophenyl)furan is the active metabolite of this drug. Previous work by our group showed that DB75 localizes in the nucleus of the *Plasmodium falciparum* parasite. The objective of the current study was to determine the mechanism of action of DB75 in *P. falciparum*. Morphology and parasitemia counts of synchronized parasites with prolonged drug exposure were used to show DB75 kills parasites in a stage-specific manner. The ring stage parasites are more sensitive to drug than trophozoites. Once rings are exposed the parasite maturation arrests during the trophozoite stage and cell death follows. Also, synchronized rings were three times as sensitive to DB75 than synchronized trophozoites at 36 hours (IC_{50}'s=66nM vs 190nM), but equally sensitive at 96 hours (IC_{50}'s=4.0nM vs 5.5nM). To determine if DB75 affects gene transcription, real time PCR was used to monitor transcript levels of 6 developmentally expressed genes over time. Results suggest DB75 does not inhibit gene expression globally or developmentally. In fact, the drug up-regulates expression of DNA primase in both synchronized rings and trophozoites. DNA primase is an integral enzyme in the DNA replication process. Taken together, these results suggest that a mechanism of action of DB75 against *P. falciparum* may involve interruption of the nuclear DNA replication process.

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USING 3D-QSAR TO IDENTIFY NEW CHEMICAL CLASSES THAT SPECIFICALLY INHIBIT B-KETOACYL ACP SYNTHASE III (PFKASIII) IN *PLASMODIUM FALCIPARUM*

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In recent years, enzymes in the type II fatty acid synthesis pathway (FAS) found in *Plasmodium falciparum* have been very attractive targets for the discovery of antimalarial drugs that have host-parasite specificity, including β-Ketoacyl ACP Synthase III (PfKASIII). The combination of 3D-QSAR, a pharmacophore, and SAR searches have yielded well over 1000 PfKASIII-specific compounds to screen in our PfKASIII inhibitor assay. To date, we have found 95 compounds with low IC_{50} values, 69 of these have an IC50 of less than 10 micromolar, and 33 have an IC_{50} of less than 1 micromolar. These compounds have been sent to our *in vitro P. falciparum* assay, and 47 compounds had IC_{50}s of less than 10 micromolar in both the

W2 and D6 strains. Selectivity is a constant concern in target-based drug design. There is no human equivalent to PfKASIII, but *E. coli* β-ketoacyl-ACP-synthase III (EcFabH) is a homologous enzyme. When the homology model of PfKASIII was compared to the crystal structure of EcFabH, clear structural differences were found between their Coenzyme-A binding channels. The PfKASIII Coenzyme-A binding channel has N197, N262, and a long lysine side chain that contribute to surface differences and steric bulk. In contast, the EcFabH Coenzyme-A binding channel is occupied by two small aliphatic residues, G152 and A208. These dissimilarities could account for the disparity between PfKASIII and EcFabH IC50 values. Without a solved crystal structure for PfKASIII, we used 3D-QSAR to develop a molecular model for compound selection. A training set of 26 structurally diverse compounds was selected for 3D-QSAR computation. The compounds were organized into three activity classes with one order of magnitude distinguishing each class. This model is iteratively refined with new enzyme and parasite data, and is used to provide compounds that are specific to the PfKASIII enzyme. Five new chemotypes have been identified (bundt salts, sulfides, sulfanomides, sulfonyls, and sulfur-containing compounds) through these efforts.

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CONTRIBUTION OF POLYMORPHISM IN *PFCRT*, *PFMDR1* AND *PFNHE* GENES IN THE REVERSAL OF QUINOLINE RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Ten molecules were tested *in vitro* to assess their ability to reverse quinoline resistance on 27 distinct parasite strains or isolates of *Plasmodium falciparum*. Each strain was genotyped to observe polymorphism on quinoline resistance-associated genes such as *pfcrt*, *pfmdr1*, *pfmrp* and *pfnhe*. Three dihydroethanoanthracene (DEA) derivatives, verapamil, cyproheptadine and ketotifen increase the activity of chloroquine and monodesethylamodiaquine on resistant isolates. These effects seem to be associated with mutations on codon 74, 75, 76, 220 and 371 of *pfcrt* and with polymorphism on *pfnhe*. The same compounds decrease the IC_{50} significantly in quinine resistant strains. However, quinine IC_{50} in resistant parasites did not drop to IC_{50} susceptible strain levels. This effect on quinine seems to be not associated with polymorphism in *pfcrt*, *pfmdr1*, *pfmrp* or *pfnhe* genes. The four DEAs, verapamil, reserpine, cyproheptadine and nicardipine reverse significantly mefloquine resistance. This synergistic effect seems to be associated with mutations on codon 1034 and 1042 of *pfmdr1*. The quinoline resistances and their reversals involve multiple genetic determinants.

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THE RELATIONSHIP BETWEEN SUBSTITUTED 1,7-DIAMINOISOQUINOLINE STRUCTURE AND ANTIMALARIAL ACTIVITY

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7-Benzylated 1,7-diaminoisoquinolines have been shown to be powerful inhibitors of *Plasmodium falciparum*. This class of compound was designed to effect antimalarial activity via the novel mechanism employed by the chalcones. Recent exploration of the *in vitro* SAR in multiple strains of *P. falciparum* will be discussed, along the results of studies designed to determine the mechanism by which this class of compound mediates its effect. Additionally the results of *in vivo* efficacy testing for select compound(s) will be presented.

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ASSESSMENT OF MALARIA *IN VITRO* DRUG COMBINATION SCREENING AND MIXED STRAIN INFECTIONS USING THE SYBR GREEN FLUORESCENCE ASSAY

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The emergence of multidrug-resistant *Plasmodium* species has made decisions regarding malaria chemoprophylaxis and treatment more complicated. Several drug development strategies have been used to counter malaria drug resistance, including optimization of new anti-malarial drug combinations. While several *in vitro* drug sensitivity assays have been used to analyze anti-malarial drug interactions, the ability of the MSF (malaria SYBR Green I-based fluorescence) assay for this purpose has not been characterized. Therefore, the first goal of this study was to evaluate the MSF assay for its use in malaria *in vitro* drug combination screening. Drug combinations of previously published synergistic (atovaquone and proguanil), additive (chloroquine and azithromycin), and antagonistic (chloroquine and atovaquone) anti-malarial drug interactions were tested against *P. falciparum* strains D6 and W2 using the MSF assay. The IC_{50}s were calculated for each drug alone and in fixed ratio combinations relative to their individual IC_{50}s. Subsequent isobologram analysis and determination of fractional inhibitory concentrations demonstrated the expected drug interaction pattern for each combination tested. Additionally, the ability of the MSF assay to examine mixed parasite population dynamics has not been explored. Thus, the second goal of this study was to examine the capacity of the MSF assay to discern mixed populations from single ones. Fixed ratios of D6 and W2 strains were co-cultured with anti-malarial drugs and IC_{50}s were determined using the MSF assay. Dichotomous concentration curves were generated, indicating that the sensitive and resistant parasites composing the genetically heterogeneous population were detectable. Biphasic analysis was performed. In conclusion, the MSF assay allows for reliable anti-malarial drug combination screening and provides the sensitivity necessary to discern heterogeneous parasite populations seen in natural patient isolates.

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A COLORIMETRIC HIGH THROUGHPUT SCREEN FOR THE DETECTION OF HEME CRYSTALLIZATION INHIBITORS

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Malaria is a major public health concern, infecting up to 500 million people a year primarily in sub-Saharan Africa. Drug resistance is emerging as a major challenge in the fight against malaria, making the development of new antimalarial drugs an important research focus (WHO 2000). During the bloodstage of the malaria lifecycle the parasite digests hemoglobin releasing free heme, which is toxic. In a process unique to the parasite, heme is detoxified via a crystallization mechanism. The heme crystallization pathway is an ideal drug target as the molecule that interacts with the drug (heme) is host derived, therefore unlikely to mutate to support resistance. Further, the targeted mechanism, heme crystallization, is unique to the parasite and the pathway has previously been successfully targeted by drugs such as chloroquine. Here we describe the development of a 384-well plate screen to detect small molecule inhibitors of heme crystallization based on the Phiβ screen, asreported previously. The screen is parasite free and does not utilize radioactive material, making it amenable to use in both biology and chemistry laboratory settings. The screen was validated against a library of 2,500 known bioactive compounds, followed by a high throughput screen of over 18,000 compounds. Hits in the heme crystallization screen

were compared to hits from a live/dead *Plasmodium falciparum* screen conducted on the same library set, as reported previously. Chemotypes of interest killed the parasite and inhibited heme crystallization. Several of these chemotypes were chosen for focus library production based on a lack of toxicity against mammalian cell lines and novelty. Follow up experiments will determine heme-drug complex structures for several compounds of interest.

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NEW INSIGHT ON ORALLY-ACTIVE ACRIDONE ANTIMALARIALS: STRUCTURAL AND FUNCTIONAL DIVERSITY

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Innovation and diversity in drug development and treatment for malaria is needed in the present age of increasing multi-drug resistance. The unique chemistry of the acridone pharmacophore allowed us to expand the structural and functional diversity, and add a new chemotype to the antimalarial acridone family. The latest chemotype is efficacious as prophylaxis in a murine model of sporozoits-induced malaria, but shares the following features with two previously reported acridone chemotypes: 1) Potency against multi-drug resistant *Plasmodium falciparum* with low nanomolar IC_{50} values, 2) Efficacy *in vivo* against rodent malaria by either oral or parenteral administration in a three-day patent infection regimen, and 3) Strong heme-complexation. Each chemotype evolved from the acridone scaffold is distinct in important, clinically-relevant mechanistic aspects: one is simply a novel potent antimalarial candidate; another is a potent antimalarial but also demonstrates *in vitro* and *in vivo* synergism with quinine and other quinolines; and the third exhibits prophylactic activity in addition to blood stage efficacy. Whether on the basis of the nature of hemozoin inhibition, the property of shared intrinsic and synergistic potency, or the combination of liver and blood stage efficacy, each holds unique promise as novel antimalarials and offers new strategies to maximize drug potential and sustainability. Details of the design, chemistry, safety assessments, metabolic and mechanistic studies will be presented.

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IDENTIFYING NOVEL DRUG TARGETS FOR *PLASMODIUM FALCIPARUM* THYMIDYLATE SYNTHASE DIHYDROFOLATE REDUCTASE

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Plasmodium falciparum (Pf) thymidylate synthase-dihydrofolate reductase (TS-DHFR) is an essential enzyme in the folate biosynthesis pathway and a known, effective drug target in malaria. Because Pf TS and DHFR are highly homologous to their human counterparts, existing active-site antifolate drugs are associated with toxicity like hematologic complications. In humans, TS and DHFR are two separate proteins. In Pf, however, TS-DHFR is a bifunctional enzyme with both TS and DHFR active sites on a single protein. Consequently, Pf TS-DHFR contains two 'non-active' regions unique to the enzyme: 1) an N-terminal tail; and 2) a 'linker' region tethering DHFR to TS, and encoding a 'crossover helix' that forms critical electrostatic interactions with the DHFR active site. The role of these non-active sites, unique to the Pf enzyme, is unknown. We thus proposed to study these regions to enable the design of novel, more selective inhibitors against Pf TS-DHFR. Using mutational analysis, we disrupted the electrostatic interactions of the crossover helix, and also deleted part of the N-terminal tail. We expressed these mutant enzymes *in vitro*, and used an in-depth, pre-steady state kinetic analysis to understand the molecular mechanisms of these mutants. Our assay conditions can follow all three reactions individually: DHFR, TS, and the bifunctional TS-DHFR reactions. The single turnover DHFR, TS and TS-DHFR rates were similar in wildtype (WT) and helix mutants. TS was significantly slower than DHFR, and was the rate-limiting step in both WT and helix mutants. However, the N-terminal mutant slowed both the DHFR rate and the TS-DHFR rate by 2x. We then asked whether communication exists in this bifunctional enzyme - i.e. if TS is in an 'activated' conformation, is the DHFR rate enhanced? Our studies showed a 2x rate enhancement of DHFR if TS was liganded. This activation was not disrupted in helix and N-terminal mutants, although even the activated N-terminal mutant remained slower than WT. In summary, these studies show that TS is the rate-limiting step in the bifunctional Pf TS-DHFR reaction, and that communication exists between TS and DHFR domains. Interestingly, mutations to the N-terminal tail - although in a location remote to the active site - slowed both DHFR and bifunctional TS-DHFR reactions. Our studies suggest the N-terminal tail of Pf TS-DHFR is a highly selective, novel target for potential antifolate development in falciparum malaria.

(ACMCIP Abstract)

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DISCOVERY OF POTENT, SPECIES-SELECTIVE INHIBITORS OF *PLASMODIUM FALCIPARUM* DIHYDROOROTATE DEHYDROGENASE THAT POSSESS ANTIMALARIAL ACTIVITY

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Plasmodium falciparum is the causative agent for malaria and accounts for over one million annual fatalities. While the use of chloroquine and sulfadoxine-pyrimethamine has provided effective treatment against malaria for many years, the spread of resistance is greatly outpacing the discovery of new antimalarials. *P. falciparum* is unable to salvage pyrimidines and must rely on *de novo* biosynthesis for survival. Dihydroorotate dehydrogenase (DHODH) catalyzes the rate-limiting step of this pathway and is therefore an attractive chemotherapeutic target. Fifty potent, species-selective inhibitors of *Plasmodium* DHODH were identified by high-throughput screening of a chemical library containing approximately 250,000 compounds. These inhibitors represented a variety of chemical classes from which eight compounds afforded high activity *in vitro* against three strains (3D7, HB3, and Dd2) of *P. falciparum.*

(ACMCIP Abstract)

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NEW GENOME-BASED DRUG TARGET IDENTIFICATION PLATFORM FOR *PLASMODIUM*

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Malaria is caused by protozoan parasites of the genus *Plasmodium*. Four species of *Plasmodium* can cause the disease in its various forms. They include *P. falciparum*, *P. vivax*, *P. ovale* and *P. malaria. P. falciparum* is the most widespread and dangerous of the four because, if untreated, it can lead to fatal cerebral malaria. Drug resistance is a major problem in the treatment and prophylaxis of malaria because plasmodium parasites have developed resistance against many different types of drugs, such as the aminoquinolines and the antifolates. Furthermore, a variety of new malaria vaccines have been tested in animal models, yet no vaccines have been demonstrated to protect humans. The lack of an effective vaccine and the emergence of drug-resistant parasites as well as insecticide-resistant mosquitoes heighten the need for new strategies to combat this disease. Here, we report a high-throughput platform for identification, characterization and validation of genes and proteins that may serve as *Plasmodium* drug targets. The platform utilizes bioinformatics, genomics and proteomics for gene and protein identification, which also facilitates

screening customized cDNA libraries, construction of protein expression panels and application of web-based computational pipelines for high-throughput gene mining. Our high-throughput approach enables rapid discovery of molecular targets in *Plasmodium* for drug development by combining genome-based target identification with target-directed compound screening.

(ACMCIP Abstract)

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ANTIMALARIAL PYRIDONES: *IN VITRO* PHARMACODYNAMIC STUDIES

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4(1H)-pyridone derivatives are a family of potent antimalarials that act as selective inhibitors of the *Plasmodium* mitochondrial function. Representative compounds GW308678 and GW844520 showed potent inhibition of *Plasmodium falciparum in vitro* and were highly effective in animal models of infection. Pharmacodynamic parameters are important for determining optimal dosing schedules in patients. We have previously demonstrated by time-killing methodology that pyridones displayed a time-dependent pattern of inhibition, that is, the extent of the activity seems to be primarily dependent on the duration of exposure. The purpose of the current study was to determine the minimum concentration and exposure time required to eradicate *Plasmodium falciparum in vitro*, as part of Pharmacokinetic/Pharmacodynamic (PK/PD) preclinical studies. Cultures of *P. falciparum* were exposed to Pyridones in a range of 6 concentrations (from IC50 to 1000XIC50) for three different periods of incubation, 2, 3, and 6 days. After the corresponding time of incubation, the compounds were removed, and the cultures were incubated in standard culture media. Giemsa thin-blood films were made daily to count the parasitemia. Efficacy was assessed by determining the reappearance of *P. falciparum* parasites up to 28 days post-exposure. Parasites exposed to compound GW844520 at a concentration of 1 µg ml^{-1} for 3 days did not reappear after 28 days of culture and were thus most probably no longer viable. When cultures were exposed to 1 µg ml^{-1} of GW308678 for 3 days the inhibition lasted for 19 days. Recrudescence was prevented when parasites were exposed to 0.5 µg ml^{-1} of GW308678 for 6 days. Further *in vivo* PK/PD studies are required to confirm the minimum dose and pattern of treatment to avoid *in vivo* recrudescence.

(ACMCIP Abstract)

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HAPLOTYPE PROFILING OF SP-RESISTANT STRAINS OF *PLASMODIUM FALCIPARUM* FROM KILIFI, KENYA, 1987-2006

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Resistance of *Plasmodium falciparum* to antifolates such as Fansidar (sulfadoxine-pyrimethamine, SP) is primarily 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; "triple-mutants") 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. Moreover, it is possible that other triple-mutants have arisen locally in the years since the Asian strain arrived. To investigate this question, we analyzed over 300 blood samples collected in Kilifi, Kenya, from 1987 to 2006, a period spanning the first use of SP. We genotyped each sample at *dhfr* and at several microsatellite loci near *dhfr.* Results indicate that the Asian triple-mutant arrived in Africa before any widespread use of SP, and has persisted for decades.

(ACMCIP Abstract)

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LONGITUDINAL SURVEY OF ANTIMALARIAL RESISTANCE IN KILIFI, KENYA, 1987-2006

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Resistance of *Plasmodium falciparum* to sulfadoxine-pyrimethamine (SP) is due to point mutations in two genes: dihydrofolate reductase (*dhfr*), which encodes the target of pyrimethamine, and dihydropteroate synthase (*dhps*), which encodes the target of sulfadoxine. Resistance to chloroquine depends principally on point mutations in the *pfcrt* gene. To track the evolution of alleles in these three genes we analyzed blood samples collected in Kilifi, Kenya, between 1987 and 2006. This period spans a switch in drug use from chloroquine to SP, allowing us to study the effect of drug use on the frequencies of drug resistance alleles. At all time points, a majority of samples carried alleles conferring resistance to chloroquine, even after chloroquine was replaced with SP. Moreover, alleles conferring resistance to SP were found prior to any widespread use of SP, indicating that current drug usage is not the only factor determining the presence of drug resistance alleles in a given population.

(ACMCIP Abstract)

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THE IMPACT OF INCOMPLETE WITHDRAWL OF CHLOROQUINE USE ON THE RATE OF DECLINE IN CHLOROQUINE RESISTANT *PLASMODIUM FALCIPARUM* PARASITES UNDER DIFFERENT TRANSMISSION CONDITIONS

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Several studies have reported a decline in the prevalence of chloroquine resistant (CQR) *Plasmodium falciparum* parasites following changes in malaria treatment policies that replace chloroquine (CQ) as a first-line therapy. Further it appears that the decline in the prevalence of CQR parasites is due to the re-emergence of CQ sensitive parasites and not a back-mutation. However, studies of the molecular epidemiology of *P. falciparum* in Hainan Island, China indicate that the rate of decline in CQR parasites is dramatically slower than that reported for Malawi, Africa; a reduction of approximately 37% over 20 years in Hainan versus 72% over 8 years in Malawi. Several reasons have been hypothesized for this data. The two most obvious differences between the study sites is the intensity of transmission and the *Plasmodium* species composition. *P. vivax* parasites are present in Hainan and CQ is still used for the treatment for vivax infections. As a consequence of this, cessation of *P. falciparum* exposure to CQ has not been complete due to residual drug levels following treatment and possible misdiagnosis of falciparum infections. While both of these factors are likely to contribute to rate of reduction in CQR parasites, it is difficult to ascertain if these explanations alone are sufficient to explain the reported difference, and if so, which factor is the most critical in determining the rate of CQR parasite decline. We have used a stochastic simulation model that incorporates the detailed within-host dynamics of *P. falciparum* infections and parasite transmission to investigate the effect transmission conditions and incomplete withdrawal of CQ treatment has on the decline of CQR parasites. The results highlight conditions that

accelerate the reduction in CQR parasites and provide valuable information about the impact of residual CQ exposure. These issues are important for policy decisions regarding national malaria treatment programs.

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EFFICACY OF INTERMITTENT TREATMENT WITH SULFADOXINE-PYRIMETHAMINE ALONE OR SULFADOXINE-PYRIMETHAMINE PLUS ARTESUNATE FOR PREVENTION OF PLACENTAL MALARIA IN TANZANIA

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Intermittent preventive treatment in pregnancy (IPTp) regimens using sulfadoxine-pyrimethamine (SP) are efficacious in decreasing placental malaria in areas with chloroquine-resistant *P. falciparum*. Growing resistance to SP for treating malaria illness is raising concerns that SP alone will soon fail for IPTp. A combination of SP plus artesunate (SP+AS) has been shown to be highly efficacious for treating malaria illness in Tanzania. There are no data supporting its use in the prevention of placental malaria. Methods. Upon enrollment at two antenatal care (ANC) centers in Kilombero District 1201 primi and secundigravid women were randomized to receive IPTp with 2-dose SP, monthly SP, or monthly SP+AS. The women were followed in the ANC and at delivery. Data were also collected on an additional group of 413 women delivering with no history of having received IPTp. Reported bednet use (88.7%) was comparable in the three treatment arms and the untreated group (p=0.86). Fewer than two percent (1.8%) of women in the 2-dose SP group had placental parasitemia at delivery compared with 1.2 % of those receiving monthly SP, 1.5 % receiving monthly SP+AS and 8% of women who took no IPTp. Nineteen percent of women in the 2-dose SP group, 17% of women taking monthly SP,18% of women receiving monthly SP+AS, and 15.3% in the non-IPTp of those with no IPTp delivered infants of low birth weight. There were no significant differences potential adverse effects such as vomiting, rash, weakness, seizures, or difficulty walking among the groups. In conclusion, all three IPTp regimens were efficacious for the prevention of placental parasitemia in primi and secundigravid women in Southern Tanzania. Though high bednet coverage has reduced the burden of malaria in pregnancy, all three IPTp regimens significantly decreased placental parasitemia. Artemisinin combination therapy was safe and efficacious and its use may suggest an alternative to IPTp in areas with increasing failure rates to SP alone.

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PFNHE POLYMORPHISM IN WILD ISOLATES OF *PLASMODIUM FALCIPARUM* FROM DIFFERENT CONTINENTS WITH A LOW SENSITIVITY TO QUININE

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Quinine remains a drug of choice to treat severe and chloroquine-resistant malaria. However its mechanism of action is poorly understood. Like mefloquine and chloroquine, sensitivity of parasites to quinine appears to be modulated by proteins such as Pgh1 and PfCRT expressed in the food vacuole but havenot been clearly associated with a lower sensitivity to quinine. Recently, QTL analysis pointed out that 3 loci in chromosomes 5, 7 and 13 are involved in the control of quinine sensitivity. Results from the field and *in vitro* studies supported the role of pfnhe, a gene located in this area and coding for a Na+/H+ exchanger, in modulation of the IC_{50} to quinine. Studies in Mali highlight again association between the length of the microsatellite ms4760 located in the coding frame of pfnhe with the quantity of quinine used in the villages. To further characterize pfnhe association with quinine response, we studied 19 wild isolates of *Plasmodium falciparum* with high IC_{50} for quinine (>500nM), collected among patients suffering from mild malaria attack in Africa and Asia. Five isolates fully sensitive to quinine and 3D7 were used as controls. We first analysed the copy number of the gene present in the genome of the parasites by real-time PCR using β-tubuline and 3D7 as reference and calibrator. For all the isolates, only one copy of pfnhe was found. In a second time we compared expression of the gene by RT-qPCR, in field isolates obtained from patients and cultured *in vitro* for 40h, under or not pressure of quinine. A dose effect of quinine was observed on mRNA production, in the same time as a retardation of the cell cycle of the parasite. To analyse polymorphism of the gene, a 1500 bp fragment including ms4760 was amplified by PCR for all the samples and sequenced. Length variation of the microsatellite was found. Using heteroduplex approach, the full sequence of pfnhe (5915 pb) is under analysis for all the isolates to detect presence of SNP all over the gene. Associations between pfnhe polymorphisms and quinine sensitivity will be presented and discussed.

(ACMCIP Abstract)

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EXAMINATION OF MOLECULAR MARKERS OF RESISTANCE IN ARTEMISININ COMBINATION THERAPY (ACT) FAILURES FOUND ALONG THE THAI/CAMBODIAN BORDER

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Resistance to antimalarial drugs is a world wide public health problem. Drug-resistant *Plasmodium falciparum* is particularly problematic in Southeast Asia where strains are typically resistant to chloroquine, anti-folates, and mefloquine. Using classical and reverse genetics and molecular epidemiology, a number of genes have been identified in *Plasmodium falciparum* associated with susceptibility to antimalarial drugs. The relative contribution of these resistance factors is not well understood, but one notable factor with plausible association to mefloquine and artesunate resistance is *Plasmodium falciparum* multi-drug resistance gene 1 (*Pfmdr1*). To better understand the relationship between *Pfmdr1* and artemisinin combination therapy (ACT) failures along the Thai/Cambodian border (Trat, Thailand), we measured PfMDR1 copy number and presence/absence of PfMDR1 single nucleotide polymorphisms (SNPs), N86Y, Y184F, S1034C, and N1042D, in samples collected during a ACT trial. Out of 9 failures, 6 were found to be recrudescence via PCR correction. 5 of 6 recrudescent samples and 1 of 52 ACPRs were found to have PfMDR1 copy numbers >3.

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CHLOROQUINE - RESISTANCE MOLECULAR MARKERS (*PFCRT* T76 AND *PFMDR-1* Y86) AND AMODIAQUINE RESISTANCE IN BURKINA FASO

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The relationship between the two main markers for CQ resistance (*Pfcrt* T76 and *Pfmdr-1* Y86) and the clinical efficacy of Amodiaquine (AQ) was investigated in Burkina Faso. AQ efficacy was determined with a 28-day follow-up in 195 children aged between 6 months and 15 years. The PCR-corrected treatment failure was 6% (12/195). The presence of *Pfcrt* T76 and *Pfmdr-1* Y86 mutations was determined before and after treatment.

Before treatment the prevalence of both *Pfcrt* T76 and *Pfmdr-1* Y86 mutations was significantly higher among the PCR-confirmed recrudescent samples compared to the adequate clinical and parasitological responses' (ACPR) samples ($p<0.05$). Infections carrying both the *Pfcrt* T76 and *Pfmdr-1* Y86 mutations were significantly more frequent among treatment failures [66.7% (8/12)] than in patients classified as ACPR [20.7% (35/169) n/N)] ($p = 0.001$). Patients who experienced a treatment failure had similar parasite genotypes before and after treatment, with very similar prevalence of the *Pfcrt* T76 and *Pfmdr-1* Y86 mutations. In conclusion, the 2 molecular markers linked to CQ resistance seem to be linked also to AQ resistance. Therefore, they could be useful in monitoring amodiaquine resistance, particularly in countries where this drug is used in combination with artesunate as first or second line treatment.

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STABLE RELATIONSHIP BETWEEN MOLECULAR MARKERS FOR SULFADOXINE-PYRIMETHAMINE RESISTANCE AND CLINICAL OUTCOMES AS EFFICACY DECLINES

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Sulfadoxine-pyrimethamine (SP) is still the first-line treatment for falciparum malaria in Malawi. We previously defined the genotype failure index (GFI) as the rate ratio of drug resistant genotypes to drug treatment failure. In Malawi, the GFI for SP was approximately 1 each year from 2000-2002. Infections from children treated with SP underwent molecular analyses to explore the genetic basis of declining in SP efficacy. Malaria episodes in children treated with SP in a 28-day drug efficacy trial comparing SP with chloroquine were analyzed. Treatment failure was defined as early or late treatment failure or late parasitological failure. PCR allele-specific restriction analysis was used to determine the genotype of codons dihydrofolate reductase (DHFR) 59 and 164 and dihydropteroate synthase (DHPS) 540. Infections with only resistant parasites at DHFR 59 and DHPS 540 were designated "SP-resistant". Recrudescent and new infections were distinguished by MSP-2 genotyping. The GFI was calculated as the prevalence of SP-resistant infections divided by the incidence of treatment failure. Among 86 children in the SP arm who completed the study, 71 (83%) experienced treatment failure. Among 50 cases of late clinical and parasitological failure that were successfully genotyped, 68% (28/41) were recrudescent infections. The prevalence of SP-resistant infections among pre-treatment samples was 84% (71/85). No infections with the DHFR 164 mutation were identified in any pre-treatment or post-treatment infections. The GFI in this population was approximately 1 (0.84/0.83). The relationship between prevalence of parasites carrying the SP resistance-conferring mutations at DHFR codon 59 and DHPS codon 540 and treatment outcomes has remained stable in Malawi as SP efficacy has fallen over the past five years. The increase in SP treatment failure is not due to the spread of the highly-resistant DHFR mutation Leu-164. SP is ineffective in Malawi due to increased resistance and a change in treatment policy is urgently required.

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IN VITRO ANTIMALARIAL DRUG RESPONSE OF FRESH *PLASMODIUM FALCIPARUM* ISOLATES FROM MALI

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Little is known on the *in vitro* efficacy of antimalarial drugs on field isolates of Mali. In the context of training and development of the capacity for *in vitro* antimalarial drug efficacy testing at the Malaria Research and Training Center, we assessed the *in vitro* sensitivity of 48 *Plasmodium falciparum* isolates collected in Bougoula-Hameau, Sikasso from August to December of 2004. Chloroquine (CQ), artemisinine (AR) amodiaquine (AQ) quinine (Q) mefloquine (MQ), and halofantrine (HF), were included in the assays. The standard ^{3}H hypoxanthine-based micro test was used. The media was supplemented with 10% human serum; hematocrit was set at 1.5% and parasitemia at 0.1 to 1%. The candle jar environment was used. For logistic reasons approximately half of the isolates had to be stored at 4ºC for up to 48 hours prior to drug testing. Overall 88 isolates were assayed but 23 (26.1%) failed to grow. Of the remaining 65 samples, 48, 48, 31, 15, 6 and 6 isolates gave interpretable results with CQ, AR, AQ, Q, MQ and HF, respectively. Mean IC50s were 229.9nM/L (range: 20.3 to 915), 2nM (range: 1.5 to 4.5), 6.31nM/L (2-17.5), 18.7nM/L (1-25), 2nM/L (1.7-3) and 3.6nM/L (2.4-6.6) for CQ, AR, AQ, Q, MQ and HF, respectively. We show that 70.8 % (n=34) of isolates were resistant to CQ while one of the 6 isolates successfully tested for HF was resistant to that drug. In this setting all isolates successfully tested with AR, AQ or MQ were sensitive to the respective drugs. These results confirm high level chloroquine resistance in the area but high *in vitro* efficacy of local *P. falciparum* isolated to artemisinine and its potential partner drugs.

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AN ABCG HOMOLOGUE GENE IN MULTI-DRUG RESISTANT *PLASMODIUM YOELII*

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Malaria drug resistance poses a formidable challenge to public health systems worldwide. Multi-drug resistance is often mediated by membrane proteins belonging to the ATP-Binding Cassette (ABC) superfamily of transporter, which translocates substrates across cell membranes. One of such groups of transporters belongs to the ABCG subfamily, which play a critical role in drug resistance in neoplastic cancer cells. We identified the *Plasmodium yoelii* ABCG homologue gene (*pybcrp*) in PlasmoDB 5X coverage (Contig 56). Computed topology predictions revealed a structure typical of half transporters, consisting of an ABC and a transmembrane domain composed of six transmembrane helices (TM). The characteristics Walker A, glutamine loop, ABC signature, Walker B and histidine loop motifs were identified in the ABC domain. The ABCG homologue gene shares 85% and 57% identity at the amino acid level with the homologue genes in *P. berghei* and *P. falciparum*, respectively. To ascertain if point mutations were present in the drug resistance lines of *P. yoelii*, the complete open reading frame of the gene was PCR amplified and sequenced in *P. yoelii* NS (chloroquine selected), NS/1100 (mefloquine selected) and ART (artemisinin selected) lines. Preliminary results revealed four amino acid substitutions in NS/1100 and two in ART, as compared to the NS parental line. In addition, expression on the gene was confirmed in intraerythrocytic stages of the parasite by RT-PCR. Currently, we are performing additional experiments to measure gene copy number and expression levels of the *P. yoelii* ABCG homologue.

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COLOMBIAN NETWORK FOR SURVEILLANCE OF *PLASMODIUM FALCIPARUM IN VITRO* SUSCEPTIBILITY TO ANTIMALARIAL DRUGS

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To contribute with the surveillance of antimalarial drug efficacy to treat *Plasmodium falciparum* malaria in Colombia, *in vitro* susceptibility methods were implemented in three sentinel sites: Tumaco-Nariño, Quibdo-Chocó and Leticia-Amazonas. Personnel from the public health laboratories in the three sentinel sites were trained in the *in vitro* ELISA-HRP2 and schizont maturation methods to assess the susceptibility to CQ, AQ, DEA, MQ, and DHA. Supervision and quality control was conducted on site by CIDEIM staff and indirectly on the slides of the modified schizont maturation tests. Assays were done with fresh *P. falciparum*-only infected blood samples from consenting individuals ≥7 years old, with a parasitemia between 500-100,000 asexual forms/µL, and with a chloroquine negative urine test. Drug plates were prepared and their quality assessed at CIDEIM and sent refrigerated to each site. IC_{50}s for each drug were calculated using the HN-NonLin program. Median and ranges of IC_{50}s were obtained for each drug per site. A total of 41 isolates were tested in 2006 (17 in Quibdo, 10 in Tumaco, and 14 in Leticia) from which 15 showed reliable HRP2 results, 2 were contaminated, 6 suffered technical problems, and 9 were not interpretable as protein increase was less than expected. The other 9 samples were not detected by the HRP2 antibodies but three of them that were tested with ELISA-pLDH produced interpretable results. Median IC_{50}s were 314.7nM to CQ, 17.1nM to AQ, 140.9nM to DAQ, 30.1nM to MQ, and 0.53 to DHA. In conclusion, we showed the technical challenges and lessons learned during the first year of the network. Time restrictions in lab staff in the sentinel sites, unforseen events and methodological constrains such as the variability in the HRP2 sequence and the potential effect of atmosphere culture conditions in HRP2 production may explain the results. Sequencing of the HRP2 gene in samples from Leticia-Amazonas is undergoing. Updated information for the second year of the network will be presented at the meeting

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DISTINCTION OF RECRUDESCENCE AND RE-INFECTION BY MSP2 GENOTYPING: AN EMPIRICAL STANDARDIZATION OF CLASSIFICATION CRITERIA

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Molecular genotyping is widely used to distinguish recrudescence from re-infection in antimalarial drug efficacy monitoring programmes. However, agarose gel DNA measurements have limited precision and the tolerance limits for classing different-sized bands as same or new infection are often subjective without empirical data. *Plasmodium falciparum* field samples from 161 volunteers were genotyped by nested PCR using polymorphic MSP2 family specific primers. Amplicon from each sample was loaded in randomized duplicate lanes. The mean paired difference (95% CI) in band size between identical alleles was 9.8bp (1.48 - 18.16bp, p = 0.022) for 3D7 and 2.54 (-3.04 - 8.11bp, p = 0.368) for FC27. Allele detection sensitivity was highest with 13µl compared to 20µl or 30µl loading volumes. Based on these findings, pre- and post-treatment samples showing less than 18bp and 11bp difference can be classified as recrudescence, for 3D7 and FC27 alleles respectively, greater difference indicating new infection.

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ASSESSMENT OF EXPRESSION OF THE *PLASMODIUM FALCIPARUM* CHLOROQUINE RESISTANCE TRANSPORTER GENE (PFCRT) IN THE ASEXUAL SATGES OF MALARIA PARASITES USING REAL-TIME PCR

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The *pfcrt* gene which is approximately 3.1 kb in size with 13 exons, encodes a transmembrane protein PfCRT (424 amino acids, 48.6 kDa) with 10 predicted transmembrane domains that localizes at the parasites' acidic digestive vacuole. Chloroquine resistance (CQR) in *Plasmodium falciparum* is associated with mutations in this protein. In order to study gene expression, we used Real-Time PCR (BioRad iCycler Real-Time PCR Detection System) in both chloroquine resistant (CQR) and chloroquine susceptible (CQS) parasites as controls. Total nucleic acids were extracted from Haiti 135 and Indochina I strains which were maintained under standard culturing conditions, as reported previously. Highly synchronized populations of parasites were obtained by standard synchronization with 5 % sorbitol for stage specific sampling to quantify relative differences in gene expression between the different lifecycle stages. Primers were designed using Beacon Designer software (Premier Biosoft International) and spanned exon borders 5-10 and 4-5 to ensure cDNA specificity of the amplification reaction. PfCRT expression was normalized to endogenous housekeeping reference genes, which display stable and non-inducible expression. Real - Time PCR is a useful tool in the study of stage specific transcription profiles in genes such as the PfCRT gene which is hypothesized to achieve greatest expression during the trophozoite stage when the parasite's acidic digestive vacuole and site of chloroquine action is most active.

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GENETIC DIVERSITY OF MEROZOITE SURFACE PROTEIN-1 GENE OF THE KOREAN ISOLATES OF *PLASMODIUM VIVAX*

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A major concern in malaria vaccine development is the polymorphism observed among different *Plasmodium* isolates in different geographical areas across the globe. The merozoite surface protein 1 (MSP-1) is a leading vaccine candidate antigen against asexual blood stages of malaria parasite. To date, little is known about the extent of sequence variation in the *Plasmodium vivax* MSP-1 gene (PvMSP-1) among Korean isolates. Since *P. vivax* malaria has been endemic in the Republic of Korea, it is essential to know the PvMSP-1 gene variability in this country to sustain it as a vaccine candidate. The extent of polymorphism in PvMSP-1 gene among Korean isolates is described. The sequence variation in the region encompassing the 10 inter-species conserved blocks (ICBs) of PvMSP-1 gene was examined. PCR was carried out to amplify the polymorphic region of PvMSP-1 and the PCR products from twenty nine (twenty five Korean and four imported cases) isolates were sequenced and aligned with Belem and Salvador-1 sequences. PCR amplification of DNA fragment encompassing 10 blocks (ICB1-ICB10) of the PvMSP-1 gene revealed size variation among Korean isolates and imported cases, ranging from 430 to 925 base pairs on each block. Two genotypes, SKOR type I and type II, were identified from the basis of nucleotide sequences on each block of Korean isolates. It was also shown that 25 Korean isolates contained two different allele combinations (association types) in PvMSP-1. The association type A of PvMSP-1 was consisted of genotype I in all variable blocks and present in the 18 Korean isolates. The association type B of

PvMSP-1 was consisted of genotype I of 4 variable blocks (CB3-ICB4, ICB5-ICB6, ICB6-CB7, and CB9-ICB10) and genotype II of three variable blocks (ICB2-CB3, ICB4-ICB5 and ICB8-CB9), and present in the 7 Korean isolates. The association type C was consisted of all genotype III (imported genotype) in all variable blocks and present in the 3 imported cases from Southeast Asian countries. The association type D was consisted of genotype III and genotype II (ICB6-CB7), and present in the one imported case. In the amplified fragment of PvMSP-1, allelic frequency of type I (68%) was higher than that of type II (32%) in each variable block. The analysis of 29 PvMSP-1 association types revealed two association types (types A and B) from Korean isolates and two types (types C and D) in imported cases.

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ZAMBIAN INDOOR RESIDUAL SPRAYING (IRS) PROGRAM: A GEOGRAPHIC INFORMATION SYSTEM (GIS) TO SUPPORT IRS PLANNING AND MANAGEMENT

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Zambia is aggressively scaling up malaria interventions to meet national, coverage targets. This includes the use of indoor residual spraying (IRS) in >85% of urban and peri-urban houses in the 15 most urban districts. We describe efforts to optimally plan, finance, implement, and monitor our IRS program through systematic assessment of IRS needs and costs and the development of a personal digital assistant (PDA)-based information system for mapping, enumerating and monitoring district IRS work. Data from successive malaria season IRS program efforts were used to quantify annual needs (quantities and costs) for insecticide, storage, spray equipment, staff, training, and IRS implementation. We used pre-programmed PDAs with global positioning system (GPS) units and worked at district level to identify and geo-code all targeted spray structures. We compared across successive seasons for improvement in planning, timely conduct, and efficiency of the IRS program; and we projected the expanded needs and costs for our planned transition to include 22 districts for IRS. Because commodity costs (e.g., insecticide, spray equipment, protective clothing) are relatively standard, we focused on district implementation costs (e.g., training, transport, salaries, allowances). The 15 targeted districts include 33% of the national population and the mapping of IRS-eligible structures identified approximately 80% (range 65%-90%) of district households as eligible for IRS. Thus, the 15-district IRS program covers ~740,000 HH (estimated at 34% of national HHs). The summary annual district implementation cost can be expressed as US$1.8 per HH structure sprayed or US$0.46 per person living in IRS HH. PDAs are an effective way to address the information needs for planning and implementing district-driven IRS program management. With the GPS attached to the PDA, it is possible initially map and then quantify the amount of insecticides used and where these insecticides have been used. In conclusion, results support the feasibility of planning, implementing, and financing a growing IRS program at national level and allow for clear assessment of costs and benefits to be compared.

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ESTABLISHMENT AND STRENGTHENING OF SITES FOR MALARIA VACCINE TRIALS IN KOROGWE DISTRICT, TANGA REGION, NORTH-EAST TANZANIA: DSS, MALARIA SURVEILLANCE, MALARIA EPIDEMIOLOGY AND HUMAN IMMUNE RESPONSES TO MSP3

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NIMR-AMANET project operating in 14 villages in Korogwe, Tanzania, is preparing a site for testing malaria vaccines. Villages are located in highlands and lowlands. Lowlands comprises semi-urban and rural areas. Main study activities are: Demographic Surveillance System (DSS), passive case detection (PCD) of malaria, malaria surveys, and human immune responses to malaria vaccine candidates. Four of 14 villages (lowland rural =2, highlands =2) are involved in PCD using Community Resource Persons (CORPs). Three cross-sectional malaria surveys have been conducted in 12 villages during short and long rains since November 2005. Actual DSS was implemented in January 2006. PCD of malaria through CORPs was introduced in January 2006 in 4 villages. CORPs treat all cases with first line anti-malarial and paracetamol after completion of questionnaire and taking blood smear. Cross-sectional malaria surveys in 12 villages involved individuals aged 0-19 years. Standardised ELISA was employed in assessment of human immune responses to MSP3 from villages with PCD. By December 2006, population was 28,978. Total fertility rate was 3.56. Infant mortality rate was 47.3. Over 90% of under-fives had received at least one type of EPI vaccine. Bed-net use in highlands was significantly lower than in rural lowland by 44% (95% CI 35%-52%, p=0.001). Around 3,499 cases visited CORPs between January and December 2006. Fever prevalence was 27.0% in highlands and 55.5% in lowlands. There was sustained decrease in malaria prevalence in 2 highland villages with CORPs but not other 2 in lowlands. Malaria parasite prevalence in cross-sectional surveys were significantly higher in rural lowland (OR=4.8, 95%CI 3.9-5.8) and in non bed-net users (OR= 2.13 95%CI 1.82-2.48). Parasite prevalences and densities in semi-urban lowland were similar to the highlands. There was a trend of an increase in ratio of cytophilic to non-cytophilic antibodies to MSP3 by transmission intensity (p=0.08). More data on IgM, IgG and IgG subclasses as well as clinical malaria incidence will be presented. Low malaria parasite prevalence in semi-urban lowland might be due to better socio-economic status or high bed-net coverage. The site has reached required GCP level and will conduct phase 1b malaria vaccine trial of MSP3 in children.

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USE OF PREVENTIVE MEASURES FOR MALARIA AMONG WOMEN DELIVERING IN A RURAL DISTRICT HOSPITAL IN NORTH-EASTERN TANZANIA

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Pregnant mothers and their offspring in Sub-Saharan Africa belong to the most vulnerable group of the life-threatening *Plasmodium falciparum*. Despite widely used preventive measures, yet malaria burden is still increasing since *P. falciparum* has developed multi-drug resistance to antimalarials, which has led countries to change to artemesinin based combination therapy. However, still sulphadoxine-pyrimethamine (SP)

continues to be the drug of choice for intermittent presumptive treatment in pregnancy (IPTp). Pregnant mothers attending with labour pain at term in Korogwe District Hospital in north-eastern Tanzania were enrolled into a study on malaria morbidity and immunity. Information collected from the mother included age, parity, place of residence, level of education and use of malaria preventive measures (IPTp and/or bed nets) between February 2005 and August 2006. 803 among 850 enrolees (94.5%) used SP in the IPTp, whereas 716 (84.2%) pregnant women used bed nets during pregnancy. 361 (42.5%) were primigravidae, 167 (19.6%) were secundigravidae, whereas the rest had three or more previous deliveries. Peripheral *P. falciparum* parasitaemia among pregnant women at delivery were detected in 85 (10%), and the parasite density was 1638 rings per µl (95% confidence interval: 986 - 2726), whereas eleven out of 153 (7.2%) placentas collected had *P. falciparum* malaria. 63 out of 780 (8.1%) among the single tone deliveries had low birth weight. Despite high resistance of *P. falciparum* to SP, it is still national policy in Tanzania to use SP in the IPTp. Whether this drug of choice remains effective in reducing the risk of malaria in pregnancy, and low birth weight in newborn, should be studied further.

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OPTIMIZING MALARIA CONTROL SUPPLY SYSTEM PLANNING AND MANAGEMENT FOR SCALING UP NATION-WIDE ITN DISTRIBUTION

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As national programs scale up malaria interventions, timely distribution of commodities is critical. Modern transport systems, when applied to insecticide treated net (ITN) distribution, offer new opportunities for efficient delivery. We describe nation-wide scale up of ITNs in Zambia to achieve an average of ~3 ITNs per household and compare different methods and results for two mass ITN distribution efforts. Between 2005 and 2007, the Government of Zambia and its partners distributed ~4.5 million ITNs through various methods including a "standard method" of ordering, receipt, and storage in central locations, and distribution to districts for delivery using local channels. Modern container shipment technologies allow secure distribution directly to district levels with potential reduced cost, quicker availability. Employing newer technologies requires prior planning and increased data collection to optimize efficient distribution patterns. Planning needs, in-country receipt, storage and distribution costs and timeliness of delivery were compared for the "standard method" (2005-6 season) and an "updated method" (2006-7 season) distributing directly as low in the distribution chain as possible. The total information and system needs for the two methods were generally similar and included population, population growth rates, average household size, number of ITNs needed per household, coverage targets, rural/urban population variations, existing net stocks, age of existing net stocks, partnership maps, transport capabilities, local storage capability, local education and communication needs, and local staff and responsibilities mapping. However, more up-front quantification and planning were needed for the "updated method". Largely because of reduced need for central receipt and redistribution costs to districts, the "updated method" showed a per-net distribution costs savings of 38% or U.S. $0.49 per ITN distributed. The amount of time required for nets to be available for use in homes following arrival in country was reduced by 75% from an average of 8 weeks to a maximum of 2 weeks. In conclusion, optimizing distribution channels using population-based and qualitative data combined with modern container shipping technologies can increase efficiency of malaria control scale up efforts with ITNs.

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THE COUNTER-INTUITIVE INFLUENCE OF SOCIAL ECONOMIC STATUS AND EDUCATION LEVEL UPON MALARIA PREVALENCE: ARE RICHER PEOPLE AT GREATER RISK OF INFECTION?

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Although half of the African population will live in cities or towns by 2015, little is known about the epidemiology of urban malaria. As part of the urban malaria control programme (UMCP) in Dar es Salaam, Tanzania, household surveys of socio-economic status, education level, house characteristics, protection measures against malaria and prevalence have been collected since 2004 and data on mosquito densities were measured since 2005. Using a logistic regression model, education level and age seem to be the main determinants of malaria prevalence. Education level is furthermore strongly correlated with socio economic status and usage of essentially all malaria-prevention measures. Surprisingly, the more educated people are, the higher their risk of malaria infection, differing clearly from other cities in West Africa. In-depth analysis of the underlying ecological reason for this observation is presented and the importance of assessing malaria risk patterns in cities is highlighted as an essential precursor to successful urban malaria control.

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THE AGE-RELATED PATTERN OF INFECTIOUSNESS WITH *PLASMODIUM FALCIPARUM* ASSESSED BY MEMBRANE FEEDING ASSAYS: ASSOCIATION WITH SEXUAL STAGE-SPECIFIC ANTIBODIES OF POPULATIONS LIVING UNDER NATURAL MALARIA TRANSMISSION PRESSURE IN BURKINA FASO

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Transmission of *Plasmodium falciparum* requires the presence of infectious gametocytes in the human host. Experimental studies have demonstrated the capacity of monoclonal and human Pfs48/45- and Pfs230- sexual stage antigen-specific antibodies to block transmission to mosquitoes. However, the ability of such antibodies to reduce malaria transmission under natural conditions is less well explored. Defining these parameters in endemic areas is important for the development of transmission reducing strategies and particularly of transmission blocking vaccines. Blood samples of 80 children aged 3 to 13 years living in rural villages of Burkina Faso were used in a direct membrane feeding assay to infect locally reared *Anopheles gambiae*. Since sub-microscopical levels of gametocytes are sufficient for transmission, a very sensitive molecular technique (QT-NASBA) was used for gametocyte detection to complement microscopy and improve interpretation of the results. The anopheline infection rate increased with age in the whole group (OR=2.16; 95%IC, 1.1-4.1; p<0.02), but also in those found either to be gametocyte carriers by microscopy (odds ratio: OR=9.0; 95%IC, 1.1-73.4), or not (OR=1.75; 95%IC, 0.8-3.83). The NASBA technique gave estimates of gametocyte density 3 fold higher than microscopy and revealed the presence of gametocytes in infectious samples considered negative by microscopy. The levels of Pfs230-specific

antibodies level showed a non-significant inverse association with age (β= -0.035, β(se)=0.019) in contrast to Pfs48/45-specific antibodies (β= 0.011, β(se)=0.007). This study thus revealed an age-dependent pattern of increasing gametocyte infectiousness in residents of an endemic area. These findings need to be complemented with data from a bigger sample population including adults of all ages.

(ACMCIP Abstract)

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USING DENATURING HPLC TO GENOTYPE *PLASMODIUM FALCIPARUM* GENES - APPLICATION TO THE VACCINE CANDIDATE PFMSP3

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The spread of drug-resistant *Plasmodium falciparum* is making the need for a subunit-based malaria vaccine increasingly urgent. Unfortunately, widespread sequence variation in candidate antigens continues to plague vaccine development. Given the increasing cost of vaccine clinical trials, a comprehensive understanding of candidate antigen variability, both at a population and individual level, is a critical step in the vaccine development pipeline. In order to generate such data, we have developed a rapid, inexpensive and efficient method of genotyping a leading vaccine candidate antigen, *P. falciparum* Merozoite Surface Protein-3 (PfMSP3). This new assay relies on denaturing high performance liquid chromatography (dHPLC) to detect variation. In this technique, the PfMSP3 N-terminal region, which contains the majority of observed polymorphism, is amplified from *P. falciparum* patient isolate DNA and then mixed with an amplicon from a reference *P. falciparum* strain of known PfMSP3 sequence. The mixture is heated and cooled to allow for heteroduplex formation, bound to a WAVE column (Transgenomic) and eluted with a linear acetonitrile gradient. If the test sample has a different sequence to the reference sample, the heteroduplex will elute at a different concentration than the homoduplexes, resulting in detection of an additional elution peak. Detected mutants are then sequenced to identify the mutation. This method is sensitive enough to detect a single nucleotide difference between the unknown and reference samples. We have applied this technique to samples from an ongoing longitudinal cohort study in Iquitos, Peru, in order to characterize the extent of PfMSP3 variation both at a population level between consecutive transmission seasons, and at an individual level between consecutive *P. falciparum* infections. Using dHPLC as a genotyping tool has allowed us to rapidly screen hundreds of *P. falciparum* isolates in this population. Both major *PfMSP3* allele types are present in our study population and while results to date indicate that there is limited intra-allele sequence variation, the frequencies of the two alleles appear to be changing over the course of multiple transmissions seasons, perhaps indicating selection pressure. This work lays the groundwork for rapid, cost effective analysis of vaccine candidate genetic diversity in the endemic setting.

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THE DAR ES SALAAM URBAN MALARIA CONTROL PROGRAMME: EARLY LESSONS AFTER ONE YEAR OF SYSTEMATIC LARVICIDING

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The Urban Malaria Control Programme of Dar es Salaam in Tanzania serves 614,000 people, in 15 of the 73 wards that comprise the city. Initial evaluation of the first year of larviciding in the 3 wards of Mikocheni, Buguruni and Kurasini, has shown that larviciding reduced malaria transmission and enhanced the general downward trend in malaria prevalence across the city. Despite substantial challenges during this first year of implementation, the first areas covered with larviciding experienced a 20% reduction of transmission and a 33% greater reduction in malaria prevalence over the first year (37% reduction in non-intervention areas versus 49% in intervention areas, P=0.083). The structure, scalability and potential for improvement of this programme is discussed in detail.

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CAN RIVER BLINDNESS VILLAGE WORKERS IMPROVE THE QUALITY OF LLIN DISTRIBUTION? A MOSQUITO NET COVERAGE AND MALARIA PREVALENCE IN OROMIYA AND SNNP REGIONS OF ETHIOPIA, 2006-2007

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Ethiopia has seasonal malaria transmission and many of the malarious areas overlap with endemic areas for onchocerciasis (river blindness). The Carter Center has aided in ivermectin distribution for river blindness (RB) in Ethiopia since 2000, and together with the National Malaria Control Program is now distributing long lasting insecticidal nets (LLIN) to areas at malaria risk in and around RB areas. Within RB areas, there is an established network of community ivermectin distributors (CDs) who will assist with LLIN distribution and promote proper net use after distribution. In order to assess how CDs might help LLIN distribution and use, we conducted a baseline net coverage and malaria prevalence survey in and outside RB control areas via a large household survey conducted January 2007 in Oromiya and SNNPR Regions. A total of 64 clusters of 25 households each were sampled in 8 quadrants of these 2 regions (1607 households total) with one quadrant of each region being the RB areas. All individuals in even numbered households were surveyed for malaria prevalence (N=4106). All areas surveyed were targeted for LLIN distribution by the regional malaria programs. Preliminary analysis indicated that overall 47.8% (95% confidence interval (CI) 45.3 to 50.3%) of households had at least one net, and 30.3% (95% CI: 28.1 to 32.6%) had at least one LLIN. The median LLIN per house was 0 and the mean number of LLIN was 0.31 (SD=0.49) per house. The malaria prevalence by slide was 3.2% (95% CI: 2.7 to 3.8%) for both regions combined. However, in RB areas, 22.5% (95% CI: 18.5 to 26.9%) of households had at least one LLIN, significantly lower than non-RB areas where 36.7% (95% CI: 34.0 to 39.5%) had at least one LLIN. We were unable to determine why there was a lower LLIN coverage in RB areas at baseline. A repeat survey will be conducted after completion of nationwide LLIN distribution to determine whether RB and non RB areas both achieved the national target of 100% coverage with LLIN of households. The use of CDs to monitor use of LLINs and provide health education for malaria, as well as provide annual ivermectin, will also be assessed in future studies.

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WITHIN-HOST AND POPULATION-LEVEL GENETIC DIVERSITY OF *PLASMODIUM VIVAX* IN PERU

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Despite near-eradication from South America, *Plasmodium vivax* re-emerged in the Peruvian Amazon in the 1990's and has continued in seasonal hypoendemic transmission form since 1994. Many infections are expected to show mixed genotypes since the infection rate in an endemic community (Zungarococha) has been measured as high as two *P. vivax* infections per person per year. Thus, determining genetic diversity both within a population and within a given infection has the potential to elucidate both the frequency of 'superinfection' and/or infection caused by a mosquito carrying more than one *P. vivax* parasite clone. We have developed a longitudinal study site in Zungarococha, a collection of villages near Iquitos, Peru. In 1,093 individuals undergoing active blood sampling during 2003 and 2004, we detected 450 *P. vivax* infections. Of these infections, we randomly selected 200 for genetic analysis to determine the population-level and within-host level genetic diversity of the *P. vivax* infections. We determined genetic diversity first using restriction fragment length polymorphism (RFLP) using the Merozoite Surface Protein 3 α (MSP-3α) gene, and subsequently using five neutral microsatellite markers at defined regions in the *P. vivax* genome. The population-level diversity (PLD) (#alleles discovered / #infections analyzed) and frequency of within-host complex infection(s) (CI) (#infections having >1 allele / #infections) were determined. Using the RFLP method, 123 *P. vivax* infections were analyzed. There were 86 distinct MSP-3α patterns (PLD=69.9%), of which 68 were only seen in one *P. vivax* infection. Despite this high PLD, only 25.2% of the *P. vivax* infections could be explained by a potential CI. For each infection, the corresponding microsatellite pattern was evaluated. The genetic diversity seen in the MSP-3α gene as well as the microsatellites suggests that there was considerable genetic diversity present in the population of *P. vivax* parasites entering Iquitos in the 1990's. The data suggest that although there is considerable genetic diversity in the population, the potential for multiple genetic types recombining and rapidly creating new allelic types is limited due the low frequency of mixed (complex) infections.

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SPATIAL ASPECTS OF MALARIA CONTROL WITH LARVICIDES AND ENVIRONMENTAL MANAGEMENT IN DAR ES SALAAM, TANZANIA

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The paper presents an initial assessment of the use of biological larvicides after one year of intervention in Dar es Salaam, Tanzania. The outcome variable is the prevalence of *Plasmodium falciparum* infection obtained from parasitological surveys. Overall results indicate that areas under larval control had a 63% (95% CI 53-71%) decline in the odds of infection after 1-year of intervention. Potential spatial effects impacting the magnitude of this impact are addressed at different levels of scale. In addition, the opportunity for incorporating environmental management in urban malaria control activities is detailed based on inventory surveys of drains recently conducted in Dar es Salaam. Results show that drains are a productive source for mosquito breeding, given the lack of maintenance and inadequate waste collection in the city.

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CIRCUMSPOROZOITE AND MSP1 POLYMORPHISM AMONG *PLASMODIUM VIVAX* ISOLATES FROM SOUTHERN MEXICO

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The genetic characteristics of the *Plasmodium vivax* parasites that persist in an affected region of Southern Mexico were determined. After informed consent of patients, *P. vivax* infected bloods were obtained and DNA extracted. The CSP gene and the MSP1 polymorphic block ICB5-6 were amplified, cloned and sequenced. Most isolates showed hybrids of the Sal 1 and Belem strains for the MSP1 fragment: Isolates with the CSPVk210 genotype showed MSP1 of Sal I type, Bel/Sal 1 (type A) or Sal 1 /Bel hybrids. Parasites having the hybrid Bel/Sal 1 (type B) were CSPVk247 and hybrids Sal 1/Bel/Sal 1 were either CSPVk210 or Vk247. Also isolates with the CSPVk210 genotype showed 20, 17 or 11 repeat units, these repeats initiate just next to the KLKQP sequence, while in CSPVk247 showed 18 central repeat units and an insertion of two amino acids ED between region I and the repeats. For the carboxyl region, in isolates CSPVk210 of 20 repeats showed as Sal I strain, the amino acid sequence GGNAG next to conserved GQGQ *P. vivax* block, other isolates with 11 or 17 central repeats showed an additional insertion of 29 amino acids, sharing with the isolates CSPVK247 the sequence GAGGQAAGGNAANKKAGDA, repeated twice in the later. The CSPVk210 sequences showed a limited polymorphism while the CSPVK247 ones were conserved. Comparison among isolates of America and other continents will be discussed.

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GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN SITES WITH VARYING TRANSMISSION PATTERNS IN A WESTERN KENYA HIGHLAND

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Malaria in highland areas of East Africa is characterized by unstable transmission and many highland areas experience epidemics. There is a need to understand the biology and genetics of parasite populations for targeted control of malaria in epidemic areas. Here, we report genetic diversity of parasite populations from Kipsamoite and Kapsisiywa in the highlands of western Kenya experiencing sporadic and seasonal malaria transmission respectively. Individuals with malaria symptoms were recruited and tested for infection with blood smear microscopy and finger prick filter paper samples obtained. *Plasmodium falciparum* infection was confirmed by nested polymerase chain reaction (PCR) and genotyping done using three antigen loci; merozoite surface protein 1 and 2 (msp-1 and 2), glutamate rich protein (glurp), and a panel of 9 microsatellite loci. Statistical analyses were done to test for differences in complexity of infection, population genetic diversity, genetic differentiation and microsatellite linkage disequilibrium of the parasite populations between the two sites. There was no significant difference in complexity of infection between the two sites, whether assessed by antigen or microsatellite loci ($P > 0.05$ for all loci), but multiple infections were frequent in both sites (78% and 74% for microsatellite loci, and 72% and 62% for antigen loci for Kapsisiywa and Kipsamoite, respectively). There was significant linkage disequilibrium of parasite populations for the two sites, consistent with documented low malaria transmission in highland areas. Genetic diversity measured as mean number of alleles detected and expected heterozygozity were also similar in the two sites for the antigen and microsatellite loci, except for the microsatellite loci, Poly-α

and TA81, which detected a significant genetic differentiation index of 0.021 (P = 0.03) and 0.019 (P = 0.04), respectively between the two sites. Microsatellite loci are valuable in detecting small differences in diversity between the parasite populations in areas of unstable, low transmission. Low parasite diversity between sites suggests that similar drugs or vaccine approaches in controlling highland malaria across different sites, though these findings require duplication in other sites. However, the high frequencies of multiple infections pose a challenge as they offer potential for increased parasite recombination and clone expansion.

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IMPLICATIONS OF GENOME WIDE ALLELIC DIVERSITY SCANS FOR HIGH RESOLUTION GENETIC MAPPING IN *PLASMODIUM FALCIPARUM*

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An increasing need for control measures against emergence of multidrug resistance in *Plasmodium falciparum* has led to the development of genome-wide initiatives to identify origins and mechanisms of drug resistance. Genetic linkage mapping studies relying on the current linkage map of 901 microsatellite (MS) markers spaced at an average distance of 17 kb/cM in 35 progeny of the HB3 × Dd2 cross have led to the chromosomal localization of monogenic as well as multigenic determinants of drug sensitivities. New ultra-high resolution tools promise to reveal the potential causal DNA variants linked to these traits, identify previously unknown crossovers, and precisely located gene conversion events. High density oligonucleotide arrays provide an unbiased, high-throughput genome-wide view of the plethora of polymorphisms present within the genome. Here we describe the segregating allelic variation identified by comparative genome hybridization (CGH) in the progeny of the genetic cross. Parents and progeny DNA were co-hybridized on a reference 3D7 array of 385,585 oligonucleotide probes spaced at a median 21 bps. Polymorphisms within the probe sequences affect the hybridization signal of the probe-target interaction that can be tracked across parent and progeny DNAs. Hybridization differences can serve as new genetic markers at a much higher resolution, which when integrated into the MS linkage map will provide a powerful tool for trait mapping. Markers were chosen for their exhibition of a bimodal distribution in parents and progeny. Polymorphisms such as MS, minisatellites, SNPs, INDELS, and amplifications are recognized by CGH. We use these polymorphisms to revise and refine the linkage map, to pinpoint crossover locations, to investigate putative gene conversion events, and to highlight hot- and cold-spots of recombination throughout the genome.

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CHARACTERISATION OF THE MITOCHONDRIAL ATP SYNTHASE/HYDROLASE COMPLEX OF THE MODEL PROTIST *TETRAHYMENA THERMOPHILA*

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Tetrahymena are members of the Ciliate class of protozoa within the Alveolate kingdom. Apicomplexa, which includes the malaria parasites, form another branch of the Alveolates. *T. pyriformis* mitochondria were found previously to have an active electron transport chain and supported oxidative phosphorylation. The ATP synthase/hydrolase complex of *T. pyriformis* was reported to be highly resistant to the normal FoF1 ATPase inhibitors. We have found that the ATP hydrolase activity of *T. thermophila* is also largely resistant to the classical inhibitors azide and oligomycin. Similar resistance to inhibitors has been observed for the ATP hydrolase in the mitochondria of the malaria parasite (see poster by Mather, et al). At present, the ability of mitochondria in malaria parasites to carry out ATP synthesis by oxidative phosphorylation is controversial, although it is clear that the major source of ATP in the parasites is cytosolic glycolysis. Further doubt on the ability of malaria mitochondrial F1Fo complex to synthesize ATP resulted from the apparent absence of genes for 2 key subunits, 'a and b' of the Fo subcomplex, in the genome of *P. falciparum*, and indeed of all Apicomplexan species sequenced to date. While ATP synthesis in the related *T. pyriformis* is well established, the recently published genome sequence of *T. thermophila* also failed to reveal candidate genes for the a or b subunits of the mitochondrial F1Fo ATP synthase. Therefore, we have initiated a proteomic study of the ATP synthase/hydrolase in *T. thermophila*. Initially, we have identified bands displaying in-gel ATP hydrolase activity in Clear Native (CN) PAGE of digitonin solubilized mitochondrial samples isolated from *T. thermophila*. Further work is underway to identify the subunits of the ATP synthase/hydrolase of *T. thermophila* and their component subunits which should provide critical insights into its mode of functioning; this may also shed light on the mystery of the missing 'a and b' subunits in *Tetrahymena* as well as in apicomplexan parasites.

(ACMCIP Abstract)

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PROSPECTIVE STUDIES OF CHILDREN WITH ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN MISSIRA, MALI: GENETIC HETEROGENEITY REVEALED BY SEQUENCING

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We performed a prospective study in the village of Missira in Mali to test the hypothesis that the development of disease in children with asymptomatic *Plasmodium falciparum* infection is related to either the acquisition of new parasite genotypes or substantial increases in the copy numbers of preexisting parasite genotypes (≥ 50%). The results reported here used the TA vector to clone and sequence amplicons from Block 2 of *msp1* in order to test for differences in parasite genotypes which would be missed using real-time PCR and capillary electrophoresis. Based on studies of parasites from a limited numbers of subjects, we have found point mutations and deletions which were not detectable with PCR and capillary electrophoresis. These results provide evidence for genetic diversity that is not detected using standard methods, which may affect parasite biology (non-synonymous mutations, deletions). However, similar studies in control subjects who did not develop disease will be necessary to determine whether this variability is likely to be causally related to the development of disease.

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MITOCHONDRIAL ATPASE ACTIVITY IN INTRAERYTHROCYTIC MALARIA PARASITES

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Early measurements of carbon flux in infected erythrocytes indicated that glycolysis is the major energy producing pathway in *Plasmodium*, suggesting that classical mitochondrial oxidative phosphorylation is at best a minor contributor. Nevertheless, uncertainty and debate concerning the presence and significance of classical mitochondrial activities in the malaria parasite mitochondrion has continued. We have recently shown that the sole essential function of the mitochondrial electron transport chain in the erythrocytic stages of *Plasmodium falciparum* appears to be to maintain turnover of dihydroorotate dehydrogenase, a key enzyme in the pyrimidine biosynthetic pathway, as reported previously. Here we present preliminary results of our work to characterize the ATP hydrolase activity (reverse reaction of ATP synthase) present in mitochondrial preparations from *P. falciparum* and *P. yoelii*. The level of ATPase specific activity present is very much lower than that found in mitochondria from previously studied

species. The ATPase activity is also largely insensitive to known inhibitors of ATP synthases/hydrolases, such as oligomycin and azide, similar to previous results reported for *Tetrahymena* ATP synthase (*Tetrahymena* is a ciliate; ciliates are a sister clade of *Apicomplexa* within the Alveolate kingdom). Interestingly, both apicomplexans and ciliates lack the genes for the classical, and essential, a and b subunits of the Fo subcomplex found in other known ATP synthases. We are attempting to identify the subunits of the mitochondrial ATP synthase/hydrolase complex, using Native and SDS polyacrylamide gel electrophoresis and proteomic analyses.

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ASSESSING THE ROLE OF MITOCHONDRIAL ELECTRON TRANSPORT IN *IN VIVO* SURVIVAL OF *PLASMODIUM BERGHEI*

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Although the mitochondrial electron transport chain (ETC) is coupled to the synthesis of ATP in most eukaryotes, mitochondria also serve many additional critical functions. Mitochondria in the blood stages of malaria parasites are not a major source of ATP, yet are validated targets for antimalarial drugs such as atovaquone. Recent studies conducted in our laboratory have shown that the sole essential function of the mitochondrial ETC in the erythrocytic stages of the *Plasmodium falciparum* is to regenerate the oxidative co-substrate (ubiquinone) of dihydroorotate dehydrogenase (DHODH), an essential enzyme required for the de novo synthesis of pyrimidines, as reported previously. Cultures of transgenic *P. falciparum* expressing a yeast DHODH were found to be resistant to all mitochondrial ETC inhibitors. To assess the role of the mitochondrial ETC for *in vivo* growth of malaria parasites, we have generated transgenic *P. berghei* parasites expressing the yeast DHODH. When tested for ^{3}H-hypoxanthine incorporation *ex vivo* under drug pressure, the transgenic *P. berghei* were found to be resistant to atovaquone in a manner similar to the transgenic *P. falciparum*. Interestingly, atovaquone treatment of mice infected with the transgenic *P. berghei* resulted in clearance of the parasites; however, the rate of clearance appeared to be slower than for the non-transgenic parasites. Further experiments are underway to gain insights into this apparent discrepancy between the necessity of the mitochondrial ETC for *in vivo* and *in vitro* growth of the rodent malaria parasites.

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CAN THE MITOCHONDRIAL GENOME IN ERYTHROCYTIC *PLASMODIUM FALCIPARUM* BE DEPLETED?

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In addition to the nuclear genome, malaria parasites possess mitochondrial and plastid genomes. These organelles are proven and potential targets for antimalarial drugs. The 6 kb *Plasmodium* mitochondrial DNA (mtDNA) is the smallest genome known in eukaryotes. It encodes only three proteins of the electron transfer chain (cytochrome b, subunits I and III of cytochrome c oxidase) and ribosomal RNA in a fragmentary, scrambled arrangement. A predicted mitochondrial RNA polymerase (mtRNAP) is likely to be essential for the transcription of mtDNA, and possibly for providing primers during mtDNA replication. Hence, the mtRNAP gene can be expected to be refractory to disruption. On the other hand, by removing the requirement for the electron transfer chain, it may be possible to render the mtRNAP function non-essential. We have recently shown that erythrocytic stages of transgenic *P. falciparum* expressing *Saccharomyces cerevisiae* dihydroorotate dehydrogenase are completely resistant to inhibitors of mitochondrial electron transport, as reported previously. We are therefore transfecting these transgenic, as well as the parental, *P. falciparum* strains with an mtRNAP positive-negative selection knock-out construct in an attempt to generate an mtRNAP knock-out. In addition to providing insights into mtRNAP functions in malaria parasites, these knockout parasites may tend to lose the mtDNA, thereby generating rho^{-} parasites. Such parasites could be very useful in further elucidation of mitochondrial physiology in malaria parasites.

(ACMCIP Abstract)

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COMPARATIVE STUDY OF THE GENETIC DIVERSITY OF THE RHOPTRY-ASSOCIATED PROTEIN 1 (RAP-1) IN *PLASMODIUM* SPP.

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We investigated the genetic diversity of orthologous genes encoding the rhoptry -associated protein 1 (RAP-1), one of the rhoptry proteins involved in the red blood cell invasion, across *Plasmodium* species. We studied complete sequences of *P. falciparum* (n= 34) and *P. vivax* (n= 6), as well as its orthologs in non-human primate malarial parasites (*P. cynomolgi*, *P. inui*, and *P. knowlesi*). In contrast with genes encoding antigens expressed in the surface of the sporozoite and merozoite, the RAP-1 gene exhibits low levels of genetic polymorphism. We estimated $\varepsilon = 0.0057$ for *P. falciparum* and $\varepsilon = 0.0026$ in *P. vivax*; approximately 10% of the polymorphism observed in non-repetitive regions of merozoite and sporozoite surface antigens. The same pattern was observed in non-human primate parasites. There were not significant differences in the synonymous and non-synonymous substitutions rates within the samples of *P. falciparum* and *P. vivax* alleles, indicating that the observed polymorphisms in these two human parasites are compatible with the expectations under neutrality. However, we found an excess of synonymous versus non-synonymous polymorphism in the non-human primate parasites (*P. cynomolgi*, *P. inui*, and *P. knowlesi*) indicating that these genes may be the subject of purifying selection. Our results suggest that immune selection may be acting differently on this antigen in non-human primate *Plasmodium* when compared with human malarial parasites.

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CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* SPOROZOITE GENE PFA0490W

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Human malaria is becoming increasingly difficult to control due, in part, to the rapid emergence of drug resistant parasites. There is a need to identify novel *Plasmodium*-specific genes which could serve as new targets for drug and vaccine development. PFA0490w is one such gene that has been detected only in sporozoites of *P. falciparum* by mass spectroscopy and shares approximately 40% identity to other *Plasmodium* orthologs, none of which have any homology to known proteins. PFA0490w is predicted, by several protein localization programs, to have a signal peptide and an extracellular location. Proteins secreted by or located on the surface of sporozoites are thought to be involved in sporozoite-host interactions and/or immune evasion and hence serve as good intervention targets. Polyclonal antibodies against purified recombinant PFA0490w did not recognize any protein in western blots of *P. falciparum* 3D7A asexual and gametocyte parasite extracts. The same antiserum did not label asexual or gametocyte parasites following immunofluorescence assays, but labeled salivary gland sporozoites. Currently, efforts to tag PFA0490w

with the green fluorescent protein (GFP) via transfection are underway in an attempt to follow protein expression in real time, especially in the mosquito stages of the parasite's life cycle. Though PFA0490w protein was not present in intraerythrocytic parasites, transcripts of PFA0490w were detected by reverse-transcriptase PCR (RT-PCR) in *P. falciparum* 3D7A parasite cultures. PFA0490w disrupted parasites have been generated and integration at the targeted gene confirmed by PCR analysis of genomic DNA extracted from mutant parasites using integration-specific primers. No PCR product was detected when primers designed to amplify the intact PFA0490w were used, suggesting that the target gene was indeed disrupted and is therefore not essential for normal intraerythrocytic asexual parasite growth. However, PFA0490w disrupted parasites appear to have a significant decrease in their ability to form gametocytes compared to controls. Efforts are underway to generate sufficient gametocytes from the PFA0490w disrupted parasites to infect *Anopheles stephensi* and characterize the development of PFA0490w mutant parasites within the mosquito.

(ACMCIP Abstract)

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STRUCTURE AND FUNCTION OF *PLASMODIUM FALCIPARUM* MALATE DEHYDROGENASE: ANALYSIS OF N-TERMINAL DINUCLEOTIDE BINDING FOLD BY SITE-DIRECTED MUTAGENESIS

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It is known that *Plasmodium falciparum* cells have mitochondria but do not operate fully functional Tricarboxylic Acid (TCA) cycle to generate ATP. Malaria parasite thrives on anaerobic fermentation of glucose for energy. Earlier, we have shown that inhibition of lactate dehydrogenase (PfLDH) *ex vivo* in *P. falciparum* culture leads to overexpression of a cytosolic malate dehydrogenase (PfMDH), which may complement NADH/NAD^+ coupling functions of PfLDH. Further, a highly refined model of PfMDH shows striking structural similarities to PfLDH. Interestingly, both the malarial enzymes efficiently utilize 3-acetylpyridineadininedinucleotide (APADH), a synthetic analog of NADH. Based on this knowledge and conserved domains among prokaryotic and eukaryotic MDH, the critical amino acids in PfMDH were changed by site-directed mutagenesis. The active site amino acid residues involved in substrate binding are conserved in PfMDH but the N-terminal glycine motif, the characteristic Rossman dinucleotide-binding fold, is similar to GlyXGlyXXGly signature sequence found in PfLDH and other α-proteobacterial MDHs. Insertion of Ala at 9^{th} position in PfMDH, which converts the N-terminal GlyXGlyXXGly motif (characteristic of PfLDH and proteobacterial MDH) to GlyXXGlyXXGly (as in bacterial and eukaryotic MDH) detached regulation of the enzyme through substrate inhibition. Distinct affinity of PfMDH to APADH is not the characteristic of GlyXGlyXXGly motif since Ala9 and Ala10 insertion mutants still utilized APADH. The Gln11Met mutation, which converts this signature motif in PfMDH to same as in PfLDH, did not change the enzyme function. However, Gln11Gly mutation caused 5 fold increase in catalytic activity of the enzyme and higher susceptibility to inhibition with gossypol. These studies provide critical insights into distinct molecular characteristics of PfMDH co-substrate pocket and have helped in designing of selective PfMDH and PfLDH inhibitors as potential antimalarials.

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POLYMORPHISMS OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN IN ISOLATES FROM THAI PATIENTS

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Interaction of the *Plasmodium vivax* Duffy binding protein (DBP) with its erythrocyte receptor, duffy antigen receptor for chemokines (DARC), is necessary for maintaining malaria blood-stage infection, making DBP an attractive vaccine candidate. However, high rates of nonsynonymous polymorphisms have been observed in DBP at region II and may have arisen to avoid host immunity. METHODS: Using molecular methods, we examined the allelic diversity of *P. vivax* Duffy binding protein gene at region II (DBPII) as well as merozoite surface protein-3α (MSP-3α) in Thai *Plasmodium vivax* malaria isolates. Both DBPII and MSP-3α were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). RESULTS: MSP-3α exhibited more polymorphism than the DBPII gene. PCR-RFLP revealed one DBPII locus with 2 alleles and 3 MSP-3α loci with 13 alleles. RFLP genotyping of the MSP-3α and DBPII genes showed 54% and 41% of patients had multiple infections, respectively. To further characterize variability, DBPII was cloned and sequenced from 32 isolates using Sal I as a reference strain. Two genotypes, TA-1 and TA-2, were identified among these Thai isolates on the basis of amino acid mutations. Of the total 330 amino acids of DBPII gene, one synonymous and 18 nonsynonymous mutations were demonstrated corresponding to a low polymorphism rate (5.7%) in this region. Among 18 nonsynonymous mutations, 5 amino acids revealed significant changes in charge or polarity. The functional significance of these amino acid polymorphisms is unclear. Phylogenetic analysis and measurement of nucleotide diversity in the conserved region of DBPII gene of 32 Thai isolates displayed four Clades, I, II, III, and IV. Variation among isolates was mainly due to nucleotide substitution, however Clade II contained 13 unique sequences never reported previously. CONCLUSION: Overall, region II of DBP was found to have a low level of polymorphism within our sample population. However, the polymorphisms present represented areas of the protein important for DARC binding, possibly due to selection pressure by the immune system. This work emphasizes areas of region II DBP that may provide effective and/or ineffective targets for vaccine development.

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FIRST REPORT ON POPULATION STRUCTURE FOR THE *LEISHMANIA MAJOR* VECTOR, *PHLEBOTOMUS PAPATASI* SANDFLY USING MICROSATELLITE LOCI

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Although many methods have been applied so far to characterize populations of *Phlebotomus papatasi* sandfly, still their genetic structures are not well understood. This widely distributed sandfly species is the principal vector of *Leishmania major*, which causes cutaneous leishmaniasis in the Old World. Multilocus microsatellite typing (MLMT) was used to infer population structure of these sandflies and assign individuals to populations. This approach used five microsatellite loci and 188 P. papatasi individuals originating from 35 populations distributed in 15 countries. Unique microsatellite signatures were observed for some populations analyzed and 114 different genotypes were found in total. Analysis of the data set showed comparable results using model and distance-based methods. Individual-based analyses split the data set into two distinct genetic clusters; 'A' and 'B' with further sub-structuring among each. Within 'A' group, five sub-groups had geographical correlations with large numbers of alleles. The genetic differentiation (FST)

of the five sub- groups within 'A' cluster ranged from 0.816 to 0.403. The degree of genetic isolation was relatively high and statistically significant ($P < 0.005$). There was no correlation between linearized genetic distance and geographic distance as a whole. Understanding the genetic structure of P. papatasi populations is important for the implementation of efficient measures for sandfly control.

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INCREASED TOLERANCE TO PYRETHROIDS IN *ANOPHELES ARABIENSIS* DURING COTTON SPRAYING IN NORTH CAMEROON: EVIDENCE FOR CONSTITUTIVE OVER-EXPRESSION OF ANTIOXIDANT DEFENSES

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The use of insecticides and pesticides in agriculture may select for insecticide resistance in field mosquito populations, jeopardizing the efficacy of insecticide based vector control programs. We explored temporal variation in insecticide resistance levels in adult *Anopheles gambiae s.l.* sampled from within and outside an area of extensive cotton cultivation in North Cameroon. Mosquitoes were collected as larvae before, midway through and at the end of the cotton spraying program in 2005. Unfed females were then exposed in batches of 20 to pyrethroids, DDT, organophosphates and carbamates and their susceptibility was assessed using standard WHO assays. *A. gambiae s.l.* populations (85% *A. arabiensis*) were fully susceptible to carbamates and organophosphates, but showed increased tolerance to pyrethroids and DDT, with lower mortality rates in cotton-growing areas. Increased tolerance was also manifested in prolonged time to knockdown (KdT50R<2). With the exception of two *A. gambiae s.s.* specimens, all survivors (N=195) screened for the East and West Kdr mutations were wild type, suggesting alternative mechanisms conferring the observed increase in pyrethroids and DDT tolerance. To explore alternative, metabolic resistance mechanisms, RNA was extracted from the F1 progeny of field collected *A. arabiensis* females and hybridized to the *A. gambiae* detox chip. Microarray analysis revealed a set of constitutively over-expressed genes with antioxidant properties in the progeny of mosquitoes collected during the spraying program. These included the superoxide dismutases *SOD2* and *SOD3*, the glutathione transferase *GSTS1* and the thioredoxin-dependent peroxidase *TPX4*. In addition to their neurotoxic effect, organocholrines and pyrethroids may induce oxidative stress and lipid peroxidation in insects. Hence, our results suggest that, besides target-site insensitivity and insecticide-specific detoxification mechanisms, enhanced resilience to oxidative stress might provide the basis for mosquito tolerance to these compounds.

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MOLECULAR DIFFERENTIATION OF ANOPHELINE SPECIES FROM NORTHEAST PERU

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Anopheles mosquitoes of the subgenus *Nyssorhynchus* have been described as the principal vectors of malaria in endemic regions of South America. Species identification of the primary and secondary vectors in any region is the most basic requirement for ecological, behavioral, or vector competence studies, and is necessary before control measures can be designed or implemented. Identification of adult females with traditional keys has often proved difficult due to morphological similarity among species. High intraspecies variability and overlapping dichotomous features has yielded erroneous data on mosquito vectors in Colombia, Peru, and Brazil. It is suggested that identification be based on morphology of immature life stages and male genitalia, although these samples are often unavailable in human landing catches and other field studies. In such cases, molecular methods of identification have proven successful. The purpose of this study was to assess the usefulness of molecular methods as a means of identifying species of *Anopheles* commonly reported in the Northeast region of Peru. The following species of *Anopheles*, implicated as human plasmodium vectors in South America, were used for molecular analysis: *An. (Nys.) benarrochi*, *An. (Nys.) darlingi*, *An. (Nys.) nuneztovari, An. (Nys.) oswaldoi, An. (Nys.) rangeli,* and *An. (Nys.) triannulatus*. In addition, three available species of the subgenus *Anopheles*, *An. (Ano.) forattini*, *An. (Ano.) mattogrossensis*, and *An. (Ano.) peryassui* were included for testing. After extraction of DNA, a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to adult female specimens and immature life stages. Following amplification the ITS2 fragment via PCR, the product was submitted to a double digest RFLP analysis using pre-determined restriction endonucleases. Each of the 9 species tested in this study resulted in a distinct banding pattern after digestion of the PCR product. The PCR-RFLP method was successful in identifying all life stages, with only small fractions of the sample being required. The assay is rapid and can be applied as an unbiased confirmatory method for identification of morphological variants and disputed samples. In particular, this method will be useful for identification of wild caught females, larval samples, and imperfect specimens that cannot be accurately identified using traditional morphological keys.

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GENETIC RELATIONSHIPS AMONG *AEDES AEGYPTI* COLLECTIONS IN VENEZUELA AS DETERMINED BY SINGLE NUCLEOTIDE AND DELETION-INSERTION POLYMORPHISMS

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Genetic relationships were analyzed among nine *Aedes aegypti* collections from six geographic regions of Venezuela. We developed 10 Single Nucleotide Polymorphisms (SNPs) and 1 Deletion-Insertion polymorphisms (DIPs) genetic markers, based on melting curve PCR analysis. Variation in allele frequencies were compared among six geographic regions, among collections within regions, among mosquitoes in collections, and within mosquitoes. Highly significant variation was detected at all levels. In contrast with our previous results using mitochondrial DNA (mtDNA), an isolation by distance pattern was not detected with SNP markers. Cluster analysis of pairwise linearized F_{ST} values among collections were similar between SNP and mtDNA. Implications for the Health Public are discussed.

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DENSITY AND SPECIES COMPOSITION OF *ANOPHELES GAMBIAE S.L* IN BANAMBANI, MALI

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Villages in the Sudan savana can experience mosquito vector densities more than 100 times greater during the rainy season as compared to the dry season, but these changes in population size do not occur simultaneously in all species and sub species of vectors. Three chromosomal forms of *Anopheles gambiae* are present in Banambani, a village in the Sudan savanna of Mali: Bamako, Savana (S Molecular form) and Mopti (M molecular form), as well as *An. arabiensis*, another member of the sensu lato group. To assess the environmental factors involved in the change of *An. gambiae s.l* species complex members that are present over a year in Banambani, an entomological study was carried out from January 2005 to January 2006. Male and female mosquitoes were collected biweekly through pyrethrum spray catch in a constant set of 20 houses selected randomly. Molecular diagnostics were used to infer species and molecular form composition over the sampling period. Climate data from a weather station installed at Banambani were used to compare with the sample sizes and species composition data. The results show a peak of population size from about June to November. *An arabiensis* was present through most the year and outnumbered by *An. gambiae s.s* during January and November. The M molecular form of *An. gambiae* was present throughout the year but was less numerous than the S form when population sizes increased. After comparison with climate data indicates we were able to detect a succession of species and forms in Banambani. During the cold dry season (January February) most the individuals captured were *An. arabiensis* (95%) with few M form *An. gambiae* (5%). As humidity begins to increase the M form is the only type found in the sample (March and April). In May when wetness increases, the S form appears, increasing in frequency to be the most numerous during the wettest part of the year (August). This succession reflects the known adaptation of *An. arabiensis* and the M form to aridity compared to the Savana and Bamako chromosomal forms. Physiological reasons for this variation are described and the overall evolutionary implications of the massive increase and crash in population sizes observed here are also explored.

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A PHYSICAL MAP FOR THE ASIAN MALARIA VECTOR *ANOPHELES STEPHENSI*

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Anopheles stephensi is an important vector of malaria in Asia and a convenient model system for studying mosquito--parasite interactions. It can be readily bred in a laboratory environment and efficiently infected with both human and rodent *Plasmodium* species. In addition, stable genetic germ-line transformation and high quality polytene chromosome maps are available for this species. However, the lack of sufficient genomic information for *A. stephensi* has prevented researchers from taking full advantage of this mosquito as a model system. A physical genome map could serve as a useful tool for localizing Single Nucleotide Polymorphisms (SNPs) and identifying quantitative trait loci (QTL) that confer insecticide resistance or vector competence. Also, it will complement a genome sequencing project by facilitating the genome assembly. We used a standard Indian wild type laboratory colony to develop a physical genome map of *A. stephensi*. We have mapped 206 *A. stephensi*, *A. gambiae*, and *A. funestus* cDNA and BAC clones to 239 chromosome sites. If the size of euchromatic part of the genome is 230.5 Mb (as evident form the *A. gambiae* assembly), the resolution of the current map is about 1 Mb. This makes *A. stephensi* second only to *A. gambiae* in density of a physical map among malaria mosquitoes. Of 206 probes 179 had unique locations on chromosome arms and 27 probes had multiple locations. 23 cDNA clones from a library made of the A. stephensi midguts were species specific. Of these 23 clones, 8 (35%) hybridized to multiple locations on various chromosome arms and 7 (30%) hybridized to X chromosome. Two of the *A. stephensi* specific cDNA clones were mapped to heterochromatin. Of 12 *A. stephensi* BAC clones, one was species specific and it was mapped to heterochromatin as well. The *A. gambiae* cDNAs of A.Gam.ad.cDNA1 and A.Gam.ad.cDNA.blood1 libraries and the *A. gambiae* BAC clones of NotreDame1 and ND-TAM libraries were obtained from the MR4. We thank Nora Besansky and Frank Collins for providing the genomic inserts from the *A. funestus* SMART cDNA library and *A. funestus* BAC clones, respectively. We thank Abraham Eappen and Marcelo Jacobs-Lorena for providing some of the *A. stephensi* cDNA clones.

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GENETIC LINKAGE MAPPING IN THE WEST NILE VIRUS VECTOR *CULEX TARSALIS*

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Culex tarsalis is an important North American vector of West Nile Virus, Western Equine Encephalitis Virus and St. Louis Encephalitis Virus. Molecular tools have recently become available to study genetic variability, population structure and quantitative trait loci (QTL) in *Cx. tarsalis*. We have constructed the first linkage map of the *Cx. tarsalis* genome using microsatellites, cDNA markers, and ISSR (inter-simple sequence repeat) markers. 35 of 55 microsatellite loci (61%) segregated in an F2 intercross between 2 colonies from Coachella and Kern counties, CA. Eight cDNA markers, adapted from previously identified loci in *Aedes aegypti* and *Culex pipiens*, were scored using SSCP analysis. Six ISSR primers produced 12 segregating markers which were scored on agarose gels and validated by sequencing. Our linkage map provides the foundation for QTL analyses to identify loci associated with virus transmission, autogeny and other phenotypes of interest in this widespread arboviral vector. Additionally, delineation of linkage groups allows for the selection of markers covering all chromosomal arms for use in further characterizing population genetic structure.

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PRELIMINARY DATA ON INSERTION POLYMORPHISMS OF SINE200 ALONG THE 2L CHROMOSOMAL ARM IN *ANOPHELES GAMBIAE.*

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Polymorphic insertions of short interspersed transposable elements (SINEs) are considered homoplasy-free and easy-to-use PCR-amplified co-dominant genetic markers for population and genetic mapping studies. SINEs have been widely exploited to assess diversity in human populations (e.g. *Alu*), but the potentiality of these markers has been rarely explored in population genomics of other organisms, due to difficulties in obtaining flanking regions for primer design. In the major afro-tropical malaria vector *Anopheles gambiae* this information was gained by means of TE display technique, a sensitive and efficient experimental method to detect TE insertions. Here we report the first data on SINE200 polymorphisms in *A. gambiae* s.s. The rate of fixation and evolution at 12 loci were evaluated along the left arm of chromosome 2 (2L) on 180 karyotyped individuals. Specimens from three field populations (Cameroon, Burkina Faso and Angola) polymorphic for alternative arrangements on the 2L chromosomal arm ($+^a/+^a$, $a/+^a$ and a/a) were analyzed. To complement field data, heterokaryotypic and homokaryotypic backcross progenies from laboratory colonies were also investigated. Results are providing preliminary information about the insertion polymorphism of SINE200 inside/outside the 2La inversion and at different distances from the inversion breakpoints, where different recombination rates are expected. These data shed light on the evolutionary dynamics of SINE in *An. gambiae*, and allow to speculate on the possible advantages of using transposable elements