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PHASE I STUDY OF THE SAFETY AND IMMUNOGENICITY OF RDEN4Δ30-200,201 A LIVE ATTENUATED VIRUS VACCINE CANDIDATE FOR DENGUE SEROTYPE 4

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The Laboratory of Infectious Diseases at the NIH is committed to the development of a live attenuated tetravalent (LATV) DEN vaccine. To this end, we have evaluated several monovalent DEN vaccines in clinical trials. rDEN4Δ 30 was the first monovalent candidate we tested in humans. In that study, 5/20 volunteers developed mild or moderate alanine aminotransferase (ALT) elevations following vaccination with 100,000 PFU. To study if it was possible to abrogate this ALT elevation, a more attenuated DEN4Î"30 candidate vaccine was developed. This candidate, rDENΔ 30-200,201 was created using paired charge-to-alanine mutagenesis to introduce mutations at amino acids 200, 201 of the NS5 gene. rDEN4Δ30-200,201 was highly attenuated in animal models. This study was undertaken to determine the safety and immunogenicity of the rDEN4Δ30-200,201 vaccine virus in healthy volunteers in a double-blind phase I study. 28 healthy volunteers, all seronegative to flaviviruses, were randomized to receive vaccine (20) or placebo (8). 20 vaccinees received 100,000 PFU of rDEN4Δ30-200,201 as a single injection and 8 volunteers received placebo. Volunteers were evaluated every other day for 16 days, and again on study days 21, 28, 42, and 180. Blood was collected for safety labs and virus titer through study day 16 and on study days 28, 42, and 180 for serologic assays. Although all vaccinees seroconverted to DEN4, virus was not recovered from the blood of any vaccinee. The vaccine virus was well tolerated and no volunteer experienced an ALT elevation above the laboratory-defined upper limit of normal. In conclusion, the rDEN4Δ30-200,201 was found to be safe and immunogenic. The attenuation phenotype of reduced replication in SCID mice bearing HuH7 mice bearing HuH7 human liver cells was reproduced in humans as evidenced by abrogation of ALT elevation. Based on these results, rDEN4Δ30-200,201 could be integral in the development of a safe and cost-effective LATV DEN vaccine. Further evaluation of this vaccine candidate or a lower dose of rDEN4Δ30 in a LATV formulation is warranted.

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PREVALENCE OF DENGUE VIRUS NUCLEIC ACID IN BLOOD PRODUCTS DONATED IN PUERTO RICO

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Case reports of transfusion-associated transmission of dengue virus are limited but this problem may be more widespread than documented. In endemic areas such as Puerto Rico, donated blood components may be affected because many dengue infections are asymptomatic. This study attempts to determine the prevalence of dengue virus among blood donors to the American Red Cross (ARC) in Puerto Rico. Samples from all blood donations to ARC sites in Puerto Rico from September 20, 2005 to December 4, 2005, just after the peak of dengue season, were tested for the presence of dengue viral RNA by a dengue virus-specific nucleic acid amplification test (NAT). A laboratory-positive case was defined as one having two repeatedly-reactive NAT test results, while a laboratory-negative case was defined as one that was not initially- or repeatedly-reactive. Supplemental testing was conducted on laboratory-positive specimens to identify infecting dengue serotype(s). The prevalence of laboratory-positive cases was determined. Associations between donor demographic characteristics and laboratory-positivity were assessed using exact methods. There were 16,521 blood samples donated during the study period. The mean donor age was 38 years (range:13-85). Most (64.5%) donors were male, and 40.1% were from the San Juan metropolitan area. Of all the samples donated, 12 (0.07%) were found to be laboratory-positive. Dengue serotypes 2 and 3 were identified as the infecting viruses. Occurrence of laboratory-positive cases did not vary over time nor did they cluster by donation site. No donor demographic characteristics were associated with laboratory-positivity. In conclusion, he finding that nearly 1 in 1000 donor blood samples contain dengue virus underscores the potential risk of transfusion-associated dengue transmission. Screening for dengue virus should be considered as part of the blood donor screening regimen in endemic areas.

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THE IMPACT OF INTERACTION BETWEEN SEROTYPES, AGE STRUCTURE AND SEASONALITY ON THE TRANSMISSION DYNAMICS OF DENGUE: A FOUR SEROTYPE MODEL

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Several models of dengue transmission have been proposed but none combines the 4 following factors that are potentially key in the dynamics of this disease: host-vector interaction, interaction between the 4 serotypes, seasonality and age-structure of the population. The objective of this study was to develop a model of dengue transmission, to explore its ability to reproduce the observed disease dynamics and to assess with a well validated model the potential impact of candidate vaccines, currently in development. A set of ordinary differential equations is used to represent dengue transmission dynamics in humans and vectors. The model extending Bartley et al. (2002) enables to differentiate primary from subsequent cases and to consider both cross-protection and cross-interference either in isolation or sequentially. Cross-interference refers here to the possibility of an increased susceptibility after a first dengue infection and, in case of a secondary infection, to an increased infectiousness and an increased risk of severe outcome. Seasonality is generated by varying the vector birth rate. The model was used to reproduce Dengue transmission dynamics using parameters appropriate for Thailand and Vietnam. Transmission-related parameters were fitted using previously estimated force of infection in the case of Thailand and seroprevalence data and case notification data in the case of Vietnam. In the case of Thailand, when seasonality and transient cross-protection between serotypes is included, dengue incidence exhibits multiyear cycles with a period of 8 to 22 years depending on serotype. Similar oscillations are seen in the case of Vietnam but with different periods. Transient cross-interference tends to extend the duration of each cycle. Under a wide range of parameter values, vaccination can interrupt dengue transmission. Serotype interactions and seasonality play a key role in generating interannual cycles that are characteristics of dengue transmission. The model also shows the potentially dramatic impact of vaccination on disease control.

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ANNUAL TARGETED LARVICIDING CAMPAIGNS IN CAMBODIA AGAINST THE DENGUE VECTOR *AEDES AEGYPTI*: ARE THEY COST-EFFECTIVE?

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This study determined the cost-effectiveness of annual targeted larviciding campaigns against the dengue vector *Aedes aegypti* in two urban areas of Cambodia carried out from 2001 through 2005. Annual larviciding campaigns targeted medium to large water storage containers in households and other premises in two cities- Phnom Penh and Kandal- with a population of 2.9 million people. The cost-effectiveness (CE) analysis compared the annual campaigns against the limited routine vector control activities carried out by the public health authorities prior to the intervention or in elsewhere in Cambodia. The study calculated the ratio of disability adjusted life years (DALYs) saved to the net cost of the intervention (in 2005 US dollars) by year as the cost-effectiveness indicator. To explore the range of study parameters, a sensitivity analysis was performed. Dengue cases and deaths were reduced by 53% by the annual larviciding campaigns. On average, 2,980 dengue hospitalizations, 11,921 dengue ambulatory cases, and 23 dengue deaths were averted each year. This benefit resulted in a saving of 997 DALYs per year. The annual campaigns had a gross cost of US$567,800 per year, or US$0.20 per person covered. Because use of extensive (but mostly privately funded) medical care was avoided, the net cost of the intervention was US$312,214 from a public sector perspective and US$37,137 from a societal perspective. Therefore, the resulting cost-effectiveness ratios from the public and societal perspectives were: US$313/DALY gained and US$37/DALY gained, respectively. Under the most conservative assumption, the intervention still remained cost effective from both perspectives. In conclusion, annual targeted larviciding campaigns proved to be effective and cost-effective medium-term interventions to reduce the epidemiologic and economic burden of dengue in urban areas of Cambodia.

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CLINICAL DIAGNOSIS OF UNCOMPLICATED MALARIA IN OLDER CHILDREN AND ADULTS IN KENYA: AN EVIDENCE BASE FOR NEWLY INTRODUCED GUIDELINES

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Clinical guidelines for the rational diagnosis and treatment of malaria should be evidence-based. In April 2006, along with the introduction of artemether-lumefantrine, Kenya introduced new clinical guidelines for diagnosis and treatment of uncomplicated malaria. For older children (5-15 years) and adults (>15 years), the guidelines recommend that individuals with "obvious other causes of fever" such as soft tissue infection, ear infection, or urinary tract infection, receive treatment for those infections and not be tested and treated for malaria. In the context of an ongoing malaria study, we examined the validity of this approach. As part of a trial of malaria rapid diagnostic tests (RDTs), we collected detailed clinical information on older children and adults presenting for initial outpatient consultation in 60 health facilities in three districts in western Kenya (two districts with holoendemic and one with seasonal malaria transmission) from July-September, 2006, during the high transmission season. We assessed the sensitivity and specificity of clinical signs and symptoms of "obvious causes of fever" in the new guidelines to predict malaria parasitemia. We collected information on 1827 outpatient older children and adults. Overall, reported fever was sensitive (83%), but not specific (45%) for parasitemia. Among those with reported fever, the absence of a single "obvious other cause of fever" was highly specific for parasitemia; these included soft tissue infection on exam (99%), urinary tract infection symptoms (87%), ear infection symptoms (89%), and reported symptoms of genital infection (97%). However, excluding all of these patients with "obvious other causes of fever" reduced the sensitivity (60%) and increased the specificity (60%) of the guidelines for malaria parasitemia. The guidelines performed similarly in low versus high transmission areas and in older children versus adults. In conclusion, among older children and adults, reported fever is sensitive, but not specific for malaria parasitemia. Inclusion of additional symptoms to help rule out other "obvious causes of fever" reduces sensitivity and thus increases missed diagnoses of malaria. Though patients should be assessed and treated for other "obvious causes of fever", all older children and adults with reported fever should be tested for malaria with an RDT or microscopy, and treated according to test results.

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PREDICTORS OF ANTICONVULSANT TREATMENT FAILURE AMONG CHILDREN WITH SEVERE MALARIA

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Convulsions continuing for five minutes or longer are common in children in sub-Saharan Africa. A single blind randomized clinical trial was conducted in the pediatric emergency unit of Mulago Hospital, Kampala, Uganda's national referral hospital. We randomized 330 children aged 3 months to 12 years who presented with a convulsion of greater than five minutes to receive buccal midazolam (0.5 mg/kg) plus rectal placebo or rectal diazepam (0.5 mg/kg) plus buccal placebo. Treatment success was defined as cessation of visible seizure activity within 10 minutes, without recurrence in the subsequent hour. Malaria was the most common underlying diagnosis (67.3%). Treatment failures occurred in 71 of 165 patients who received rectal diazepam (43.0%) compared to 50 of 165 patients who received buccal midazolam (30.3%; RR 1.42, 95% CI 1.06 -1.90; p = 0.016). Treatment failure rates were similar for convulsions associated with malaria; rectal diazepam (35.8%) compared to buccal midazolam (31.8%; RR 1.12, 95% CI 0.78 - 1.62; p=0.534). For children without malaria buccal midazolam was superior; failure rates (55.9% vs. 26.5%; RR 2.11, 95% CI 1.26 - 3.54, p=0.002). Upon multivariate analysis of the 221 children with malaria, presenting with focal convulsions (OR = 3.22, 95% CI =1.41 - 7.39) or blood sugar $\geq$ 200mg/dl (OR 2.64, 95% CI = 1.10 - 6.35) were independent predictors for treatment failure. Additionally, children who had cerebral malaria were more likely than those with other forms of severe malaria to fail treatment (OR = 2.32, 95% CI =1.12 - 4.78) or experience seizure recurrence in the subsequent 24hrs after initial control (hazard ratio 2.95; 95% CI 1.77 - 4.93). Buccal midazolam offers a promising alternative to rectal diazepam for the treatment of seizures in malaria endemic areas. Identifiable predictors of treatment failure, including cerebral malaria, can identify patients who need additional therapy.

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CLINICAL PRESENTATION OF SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN

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Severe malarial anemia (SMA, Hb<6.0 g/dL) is a common complication of *Plasmodium falciparum* (Pf) malaria in holoendemic areas resulting in mortality rates that may exceed 30%. To determine the distinct

clinical factors associated with SMA in a pediatric population exposed to holoendemic *P. falciparum* transmission in western Kenya, Pf(+) children (aged 3 to 36 mos) were recruited at their first hospital presentation for childhood illness. Since HIV-1 and bacteremia also promote severe anemia, children with these co-morbidities, were excluded from analyses. Patients with evidence of cerebral malaria were also excluded. Children with *falciparum* malaria (n=374) were stratified into three groups according to their Hb level: SMA (Hb <6.0 g/dL), mild-to-moderate MA (MMA, Hb=6.0-10.9 g/dL), and uncomplicated malaria (UM, Hb≥11). Complete demographic, clinical, and hematological measures were collected. Children presenting with SMA (median age=8 mos) were younger than those with either MMA or UM (median age=10 and 11 mos, respectively, p<0.01). Palmar pallor was detected in 63% of the children with SMA, as opposed to 20% of children with MMA (p<0.01), and 0% of those with UM (p<0.01). Splenomegaly was detected in 30% of the children with SMA, 13% of those with MMA (p<0.01), and 0% in children with UM (p<0.01). Jaundice was also more common in children with SMA (3% vs. 0% in both the MMA and UM groups, p<0.01). Respiratory distress was also higher in the SMA group (7% vs. 0.5% in the MMA and 0% in the UM groups, p<0.01). Although there was a higher proportion of children with SMA meeting the WHO criteria for wasting and underweight-for-age than the other groups, differences were not significant. Dehydration and gastrointestinal illnesses were also not significantly different across the groups. In summary, children with SMA were significantly younger and more often presented with palmor pallor, splenomegaly, jaundice, and respiratory distress. Recognition of these primary clinical signs in children residing in resource-poor settings in which complete hematological indices are not available may help guide the identification and management of childhood SMA in holoendemic regions.

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PLASMODIUM VIVAX INFECTION: A MAJOR DETERMINANT OF SEVERE ANAEMIA IN PAPUA, INDONESIA

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This study was undertaken to determine the contribution of malaria infection to anaemia in Timika, Papua, Indonesia, an area where drug resistant *Plasmodium falciparum* (Pf) and *P.vivax* (Pv) are highly prevalent. Haematology and malariometric data were collected from 19,751 patients admitted to hospital (June 2004 and February 2007), from 1640 patients attending and outpatient clinic with malaria and from 5255 participants in a house to house survey cross sectional school surveys. Results: In the prevalence survey the risk of anaemia (Hb<10g/dl) was similar for subjects with malaria irrespective of the species of infection (overall 50% 267/538) but significantly higher than that in people without malaria (28% 915/3256); OR=2.5 [95%CI 2.1-3.1]; p<0.001. Hospitalized patients were at greater risk of being anaemic than those interviewed at home (OR=4.1 [3.8-4.4]; p<0.001), with 14% (2752/19751) graded as severely anaemic (Hb<5g/dl). In total 11% (1221/11363) of hospitalized patients without malaria had severe anaemia compared to 16% (833/5236) patients with *P. falciparum* infection and 21% (467/2258) with *P. vivax* infection (p<0.001). *P. vivax* infection was the dominant species for malaria infections in infants (49% 300/616) but less important in adults (23% 1039/4597); p<0.001. The population attributable risk of severe anaemia associated with malaria was greatest (62%) in the first year of life with 34% of cases due to *P. vivax,* 20% to *P. falciparum* and 7.9% to mixed infections. Conclusions: Although often regarded as a benign infection *P. vivax* is a cause of considerable morbidity in Papua, Indonesia, and a major determinant of severe anaemia, particularly in young children. The co-factors associated with severe anaemia in this region will be presented.

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AN ASSESSMENT OF THE MANAGEMENT OF SEVERE MALARIA IN ZAMBIAN HEALTH FACILITIES

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Malaria is the leading cause of death and morbidity, especially in children and pregnant women in tropical countries. Zambia Health Management Information Systems (HMIS 2005) estimates a country average incidence at 373/1000 population and hospital case fatality rate of 49/1000 admissions. Access to clinical care is poor especially in the most rural areas where malaria transmission is most intense. Severe malaria is a medical emergency and if untreated, mortality is estimated to be 100%. Death from severe malaria often occurs within hours of admission to hospitals or clinics. It is essential that prompt diagnosis is made and appropriate treatment administered correctly. The purpose of this assessment was to provide comprehensive evidence to facilitate formulation of guidelines and capacity building for the management of severe malaria. The study was a cross section evaluation of the management of severe malaria in selected health facilities of Zambia using a multi-stage sampling method. The assessment employed survey instruments that were adapted from the World Health Organization: Hospital care assessment tool (WHO, 2002). The tools evaluated three aspects namely case management, patient records and available basic requirements so that the basic information recommended as "good practice" by World Health Organization was captured, as well as to describe in greater depth severe malaria related treatment practices. Severe malaria was correctly diagnosed in 49% of the cases. Only 45% were found to have received supportive care, 40% received treatment for concurrent conditions and relevant laboratory investigations were performed in 29% of the cases. Further, appropriate antimalarials (for severe malaria) were administered in 70% of the cases. Results varied by level of health care with the lower levels performing poorer than the higher levels of care. Malaria deaths among total severe admissions ranged from 0 to 30%. More than 80% of the facilities had drug stocks below the required minimum balance.

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WHY DOES IMPORTED MALARIA STILL KILL? 20 YEARS OF MALARIA DEATHS IN THE UNITED KINGDOM

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The Malaria Reference Laboratory collects information on all malaria cases reported in the UK. We reviewed the last 20 years of malaria deaths, comparing them with surviving cases to identify factors associated with death. Initial data shows there were 38881 cases of malaria and 189 deaths, 97% from *P. falciparum*, which accounted for 25053 cases. Mortality rate was 0.8%. Mortality increased substantially with age; comparing those aged >65y with those aged 20-35y OR for death was 8.6 (95%CI 4.7-15.9, p<0.001 adjusted for birth in malaria-endemic countries). Mortality also varied markedly depending on country of birth and reason for travel. Most deaths occurred in tourists, and most non-fatal cases in individuals visiting friends and relatives in their country of origin (VFRs). Odds ratio for death from malaria (adjusted for age) for those born in non-endemic areas was 6.4 (95%CI 4.2-9.9, p<0.001) compared to those born in endemic areas. West Africa illustrates this; case fatality

rates varied from 3.5% in cases from the Gambia, where 57% were holiday makers, to 0.4% in those from the rest of West Africa, where VFRs accounted for 60% cases. The odds ratio of death for an individual visiting the Gambia versus other countries in West Africa was 6.9 (95%CI 4.2-11.1, p<0.001, adjusted for age). Case fatality rates were higher in other areas of Africa (1.4% in southern and 1.7% in East Africa), and Asia (1.1%) compared to West Africa (0.43% overall). There was considerable seasonal variation in mortality rate, with a peak of 2.6% in December (49 deaths). The odds ratio of dying in December compared to other months was 4.5 (3.2-6.3). This contrasted with peaks in imported malaria cases in January and September. 73% (n=136) malaria deaths occurred in hospital, with 3% dying in an ambulance, 18% at home and 3% in an aeroplane or airport. Median time from onset of symptoms to diagnosis of malaria was 5 days (IQ range 3-8). We will discuss these findings compared with the North American experience.

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CLINICAL DEVELOPMENT OF NEW PROPHYLACTIC ANTIMALARIAL DRUGS AFTER THE 5TH AMENDMENT TO THE DECLARATION OF HELSINKI

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Malaria in non-immune populations is a growing concern. While a number of drugs are approved for malaria prevention (mefloquine, doxyxcline, chloroquine and atovaquone-progunail), each has weaknesses, and the future development of resistance will likely render them less useful. Maintenance of a full pipeline of compounds in clinical and preclinical development is important to ensure that safe and effective drugs will be available in the future. Traditionally, antimalarial drugs have been developed as agents for dual indications (treatment and prophylaxis). Studies conducted in semi-immune volunteers have traditionally comprised a key component of the clinical development paradigm. However, since 2000 and the 5th Amendment to the Declaration of Helsinki, development of prophylaxis drugs in this manner has effectively been stalled. Major pharmaceutical companies have interpreted the fifth Amendment to mean that the traditional development paradigm is unethical because some of the study populations from which study subjects are drawn have no realistic prospect of benefiting directly from a chemoprophylactic drug (Article 19). The 5th Amendment has also been interpreted to impose on a sponsor the burden of provision of the study drug at a reasonable cost to the host country (Article 30). In this presentation we will argue that the traditional development plan remains ethical and compliant with the 5th Amendment under certain circumstances. We will also present two alternative clinical development paradigms that may potentially provide a more attractive pathway forward for potential sponsors. The first of these involves the testing of investigational drugs only in populations who unequivocally have the potential to benefit from malaria prophylaxis - non-immune travelers and military in endemic and non-endemic countries. The second involves the co-development of new drugs for intermittent prophylactic treatment in endemic countries and intermittent prophylactic treatment for travelers (IPTt). More nuanced application of the traditional development paradigm, or its appropriate modification, should once again pave the way for the development of new drugs for malaria prevention.

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FAMILIAL AGGREGATION OF ACUTE LYMPHATIC FILARIASIS IN PAPUA NEW GUINEA

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Acute lymphatic filariasis is a temporary but debilitating condition characterized by lymphangitis or lymphadenitis. Incidence of acute lymphatic filariasis was observed weekly in a population of more than 3,000 people during a five year annual mass drug administration field trial in Papua New Guinea. Despite a marked reduction in acute disease events (0.38 per person-year prior to treatment to 0.18 after four consecutive annual mass drug administrations), disease appeared stable among a sub-set of individuals. This study was designed to estimate the amount of disease that may be attributable to heritable components. Self-reported family relationships were used to create pedigrees. The ASSOC program from S.A.G.E. software, version 5.3 was used to partition the observed variation of acute disease events attributable to familial relationships and environment. The database included 2,837 individuals with disease and family relationship data. The disease phenotype of any acute events during the follow-up provided a heritability estimate of 0.46. When controlling for potentially confounding variables (age and chronic disease status), the heritability estimate increased to 0.53. This study provides evidence of a heritable component of acute disease in this population. Although this analysis assumed polygenic inheritance, segregation analysis may be used to further describe phenotypic inheritance. This information may be used to improve and evaluate the ongoing effort to eliminate lymphatic filariasis as a public health problem.

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A RAPID HEALTH IMPACT ASSESSMENT OF THE AFRICAN PROGRAMME FOR ONCHOCERCIASIS CONTROL (APOC)

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Onchocerciasis is an important cause of visual impairment, blindness, skin lesions and severe itching. The African Programme for Onchocerciasis Control (APOC, 1995-present) operates in 15 countries, aiming to eliminate onchocerciasis as public health problem by yearly mass treatment with ivermectin. We assessed the impact of APOC on the burden of onchocerciasis-related disease by 2005 and explored expected trends for the future. We categorized the population in the APOC area according to type of onchocerciasis, pre-treatment endemicity level, and number of treatment rounds provided by 2005. Based on literature data on the association between infection and disease, we estimated the pre-APOC prevalence of various clinical manifestations and the annual number of DALYs lost in each category. Computer simulation was used to estimate the decline in the various indicators since the start of APOC. All reported numbers are standardized to the size of the population in 2005. Before the start of APOC, respectively 25% and 75% of the 88.5 million target population lived in areas with savannah or forest/mixed type of onchocerciasis; 5%, 30%, 33% and 31% respectively lived in non-, hypo-, meso- and hyperendemic villages; 27%, 17%, 16% and 41% respectively had 0, 1-3, 4-6 or 7-9 rounds of CDTI. Overall, the prevalence of troublesome itch, blindness and low vision were about 15.3%, 0.4% and 1.1%. By the end of 2005, these numbers were reduced by about 50%, 23%, and 12% respectively. The annual DALY-loss was 1.7 million before APOC, but was about halved by 2005. With continuation of APOC and especially by inclusion of the 27% APOC population not yet treated in 2005, the annual DALY loss could be reduced to less than 15% of its pre-APOC level by 2015. In conclusion, APOC has strongly reduced the burden of onchocerciasis-related disease. The strongest impact is seen on itch, which is directly related to the presence or absence of infection. The immediate impact on blindness and low vision are lower, because of the irreversibility of these clinical manifestations. A strong further reduction is expected, if the programme continues successfully in the next 10 years. Limited data availability and high variability between studies hampered accurate estimation of the pre-APOC prevalence of clinical manifestations and their decline during mass treatment. Field studies are required to validate the results of this rapid impact assessment.

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HAS EGYPT ELIMINATED LYMPHATIC FILARIASIS?

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Egypt was one of the first countries to complete 5 annual rounds of mass drug administration (MDA) with DEC plus albendazole in a national program to eliminate lymphatic filariasis (LF). We initiated surveys in sentinel villages and towns with a panel of surveillance tools to detect residual infections and recent transmission. The panel includes: A survey for circulating filarial antigenemia (CFA, ICT card tests in randomly selected households, 450 people per village); Blood microfilaremia (Mf, filter and thick smear) testing is limited to subjects with positive antigen tests; Antibody tests (Bm14 IgG4 ELISA) are performed on 350 first year primary school children per village to detect recent infections or exposure to W. bancrofti; Molecular xenomonitoring (MX) surveys detect W. bancrofti DNA in mosquitoes collected with gravid traps. We test 200 pools per village of Culex pipiens gravid females (15 mosquitoes/pool) for W. bancrofti DNA by LDR-qPCR. We established provisional elimination criteria prior to initiation of the study as follows: CFA rate < 2%, Mf rate (by 50 µl smear) < 0.5%, antibody rate in first year primary school children <2%, and mosquito infection rate estimated by Poolscreen <0.25%. This program will eventually cover 44 of 183 formerly endemic localities in Egypt. Selected localities include those with the highest baseline infection rates in all 27 LF-endemic districts in the country. We have completed 9 village surveys for CFA and Mf to date. Filarial antigenemia was detected in 3 of 9 villages with rates of 0.2%, 3.0% and 3.6%. One Mf carrier was identified in the village with the highest CFA rate (Mf rate 0.2%). No Mf carriers were detected in the other 8 villages. School antibody surveys have been completed in 12 villages. No positives were detected in 10 villages, and antibody rates were 0.3 in two villages. MX studies have been completed in 16 villages. Positive mosquitoes were detected in 8 villages with Poolscreen infection rates from 0.03% to 0.37%. The village with the highest mosquito infection rate was the village with the Mf carrier and the highest CFA rate. These data suggest that MX and CFA testing are more sensitive monitoring tools than blood Mf surveys. These preliminary data also suggest that 5 rounds of MDA rounds were effective for eliminating LF in most endemic localities in Egypt.

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GRAVID TRAP COLLECTIONS OF *CULEX PIPIENS* FOR MOLECULAR XENOMONITORING NATIONAL PROGRAMS FOR ELIMINATION OF LYMPHATIC FILARIASIS

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Molecular xenomonitoring (MX) uses PCR to detect filarial DNA in wild-caught mosquitoes as a means of evaluating the success of filariasis elimination programs based on mass drug administration (MDA). We have previously reported results from a pilot study that used MX with indoor-resting blood-fed or gravid Culex pipiens aspirated in houses to assess the impact of MDA in sentinel Egyptian villages. Large numbers of mosquitoes are needed to detect low-level persistence of filarial infections after MDA, and indoor mosquito collection is not practical for evaluation of national filariasis elimination programs. Therefore, we compared the sampling efficiency of gravid traps and indoor-resting collection for MX in two villages known to have residual infections following MDA. Gravid traps caught 6 times more mosquitoes per trap-night than the number recovered per house-night by aspiration. Parasite infection rates (estimated by Poolscreen) for indoor-resting mosquitoes were significantly higher than those in gravid trap mosquitoes. Thus, larger mosquito samples are needed to demonstrate the absence of filarial infections by MX with gravid traps than with indoor-resting mosquitoes. We are now using gravid trap MX to assess LF endemicity in 44 Egyptian localities after 5 rounds of MDA. Our protocol calls for testing 200 pools of 15 gravid mosquitoes for filarial DNA per locality. The mosquitoes are collected with 50 gravid traps placed for 2 nights per locality. We found important seasonal variability in gravid trap yields in terms of the quantity and quality of mosquitoes captured; gravid mosquitoes were relatively uncommon in the early weeks of the mosquito season in Egypt (May), but we were often able to collect more gravid mosquitoes per locality than we needed in a single night during July and August. We now have MX results and microfilaria (MF, based on 450 people per locality) rates for 9 localities that were LF endemic prior to MDA. MX and MF results were completely negative in 8 localities. However, filarial DNA was detected in 11 of 200 mosquito pools tested in one village that had MF and filarial antigenemia (card test) rates of 0.2% and 3.6%, respectively. This study has shown that MX with gravid trap mosquitoes is practical for large scale assessment of filariasis elimination programs and that MX is likely to be more sensitive than MF testing for detecting residual filarial infections in communities following MDA.

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DIFFERENTIAL EUKARYOTIC GENE EXPRESSION IN *ONCHOCERCA VOLVULUS* (WITH A *WOLBACHIA* ENDOSYMBIONT) AND *O. FLEXUOSA* (*WOLBACHIA* NEGATIVE)

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Gene expression was studied in two sibling *Onchocerca* species, namely the *Wolbachia* dependent human parasite *O. volvulus* (O.v.) and the Wolbachia-free deer parasite *O. flexuosa* (O.f.). Adult worm total RNA was transcribed into cDNA using an oligo dT protocol and labeled with Cy3 or Cy5. Samples were hybridized to a high density oligonucleotide array containing 15412 *B. malayi*, 1016 *O. volvulus*, 872 *W. bancrofti* and 803 *Wolbachia* (B.m.) sequences (all in duplicate) that were designed to uniquely represent filarial gene clusters. We tested two biological and two technical replicates (4 arrays) and followed MIAME guidelines. O.v. cDNA hybridized to 11% of the *B. malayi* oligos, 80% of the O.v. oligos, 19% of the W. bancrofti oligos, and 6% of the *Wolbachia* oligos, while O.f. cDNA hybridized to 13%, 65%, 18% and 0.6% of these oligos, respectively. 306 clusters were at least 2-fold up-regulated in O.v., and 240 clusters were up-regulated in O.f. Approximately equal percentages (1 to 2%) of *B. malayi* and *W. bancrofti* target sequences appeared to be up-regulated in each of the *Onchocerca* species. However, 14% of the O.v. clusters appeared to be up-regulated in O.v. while only 2% of the O.v. clusters were up-regulated in O.f. This difference may be more apparent than real and reflect sequence differences between the species rather than differences in gene expression. Analysis of KEGG orthology revealed a significant up-regulation of genes involved in energy metabolism in O.f. relative to O.v. (17 vs. 8). Mitochondria are believed to have evolved from an α-proteobacterial ancestor. Analysis of the full mitochondrial sequences revealed no major differences between the two *Onchocerca* species, but minor differences were observed. We hypothesize that *Wolbachia* (an α-proteobacterium) contributes to energy metabolism in filarial species with *Wolbachia* and that these functions are provided by products of chromosomal or mitochondrial genes in *Wolbachia*-free species such as *O. flexuosa*.

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CIS ACTING ELEMENTS NECESSARY FOR TRANS-SPLICING IN TRANSIENTLY TRANSFECTED *BRUGIA MALAYI*

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Many genes in parasitic nematodes are both cis- and trans-spliced. Previous studies have demonstrated that a 7nt element encoded in the first intron of the *Brugia malayi* 70 kDa heat shock protein (BmHSP70) gene was necessary to permit trans-splicing of transgenic mRNAs in embryos transfected with constructs encoding portions of the BmHSP70 gene driving the expression of a luciferase reporter gene. We now report evidence that suggests that this 7nt conserved sequence motif (designated the *B. malayi* HSP70 trans-splicing motif, or BmHSP70 TSM) is both necessary and sufficient to direct trans-splicing of transgenic mRNAs derived from the HSP70 gene. Mutations introduced into any position of the BmHSP70 TSM eliminated its ability to direct trans-splicing. Transfer of the BmHSP70 TSM into the first intron of the *B. malayi* 12 kDa small subunit ribosomal protein (BmRPS12) gene rendered a construct containing a portion of this gene competent for trans-splicing. In contrast, insertion of the BmHSP70 TSM into the single intron of the *B. malayi* RNA binding protein (BmRBP1) gene did not render this sequence trans-splicing competent. However, tagged constructs of the full-length *BmRBP1* gene were trans-splicing competent. These data together demonstrate that while the BmHSP70 TSM is necessary and sufficient to direct trans-splicing in some genomic contexts, independent trans-splicing signals are employed by other genes.

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PLASMODIUM SPOROZOITES LACKING AN ASPARAGINE RICH PROTEIN FAIL TO ESTABLISH LIVER STAGE INFECTION AND ELICIT STERILE IMMUNITY AGAINST MALARIA

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Plasmodium sp. DNA is characterized by its high A/T nucleotide content. This is reflected by the unique abundance of low complexity domains (LCD) among *Plasmodium* proteins, which is the highest among all eukaryotes. Although it was suggested that LCDs might play a role in the virulence of some pathogenic bacteria, including a number of human pathogens, nothing is known about the role or function of these protein domains in the biology of infection. We identified a gene encoding a low complexity protein that was shown to be highly upregulated in sporozoites and early liver stages, sporozoite asparagine rich protein1 (*SARP1*). Targeted deletion of *SARP1* in *P. yoelii* did not affect asexual blood stage growth and mosquito stages development. Interestingly, *Pysarp1(-)* salivary glands sporozoites were capable of entering hepatocytes but failed to establish liver stage infection and did not cause blood stage infection *in vivo*. Moreover, immunization of mice with *Pysarp1(-)* sporozoites led to a sterile protection against wild type sporozoites challenge. This study demonstrates for the first time a vital function of low complexity protein in the infectivity of a pathogenic organism and provides a new live-attenuated vaccine candidate against malaria.

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TO LIVE OR DIE: INVESTIGATING THE ROLE OF *PLASMODIUM FALCIPARUM* MITOCHONDRIA IN THE FACE OF ELECTRON TRANSPORT INHIBITION

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The role of the mitochondria in the life and death decisions of the cell has been well established in metazoa and some protozoa. Although mitochondrial electron transport is a well established anti-malarial drug target, molecular details underlying parasite demise have not been elucidated. We have recently shown that the essential function of mitochondrial electron transport during the blood-stages of *Plasmodium falciparum* is *de no*vo pyrimidine synthesis and that disrupting the flow of electrons does not abolish the mitochondrial membrane potential, as reported previously. We are exploring effects of atovaquone, alone as well as in combination with its synergistic partner, proguanil, on mitochondrial physiology and its contributions to parasite survival. Our results suggest that, in *P. falciparum,* the effects of atovaquone and atovaquone/proguanil are dependent upon the erythrocytic stage of the parasites exposed to the drugs. We found that ring-stage parasites are the most resilient to drug treatment and can survive for up to 48 h, with a small fraction remaining viable even after 96 h of treatment. The survival of the parasites seems to depend on their ability to exist in a 'static' phase; once treatment ceases, the parasites "re-enter" the erythrocytic development cycle. To further understand this static state, we have analyzed the global transcription profile of treated parasites to determine the mRNA stability and transcriptional changes that may accompany the drug treatment. These studies begin to provide information on the physiological state of the drug-inhibited parasites when mutations in mitochondrial DNA could arise that lead to drug resistance.

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CHARACTERIZATION OF MOLECULAR EVENTS OF AUTOPHAGY IN *PLASMODIUM FALCIPARUM*

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Malaria is one of three major infectious diseases in the world, with about 3 billion people living at risk, 500 million new cases every year and between 1 and 3 million deaths annually. Human mortality is caused mainly by *Plasmodium falciparum*, an apicomplexan protozoan parasite. During all stages of the lifecycle, malaria parasites undergo massive intracellular remodeling and restructuring. In other organisms, this remodeling is partly accomplished by autophagy processes. Currently, the autophagy pathway has not been investigated in *P. falciparum*. Autophagy has two major forms; macrophagy and microphagy. Macrophagy is used to recycle amino acids by transporting unnecessary proteins to lysosomes via double membrane vacuoles (autophagosomes). Microphagy functions to directly import cell cytosol into the lysosomes. Genetic manipulation in yeast and other eukaryotes revealed that *ATG* (*Au*to*p*ha*G*y) genes are used in macrophagy. *ATG* genes are essential in the normal development of *Drosophila melanogaster*, *Caenorhabditis elegans*, *Mus musculus*, and parasites such as *Leishmania major, L. mexicana* and *Trypanosoma brucei*. We have identified autophagy-related genes in the *P. falciparum* genome. Similar to *Leishmania* and *Trypanosoma*, *P. falciparum* lacks homologs of Atg5 and Atg12 but does contain a homolog of Atg8, and a small Rab7-like GTPase involved in vesicle conjugation and fusion. We treated *P. falciparum* infected erythrocytes with aluminum tetrafluoride (AlF_4^-), a small GTPase activator and a compound that interferes with membrane fusion. Electron microscopic analysis of treated parasites showed double membrane vesicles adjacent to the food vacuole and cytosolic invaginations starting at the surface of the food vacuole. These structures are morphologically analogous to autophagosomes and microphagic membranes, respectively, observed in mammalian cells and yeast. Microarray analysis of AlF_4^- treated and non-treated cells was performed, but no autophagy related genes were observed to be upregulated. These results suggest that *P. falciparum* does perform autophagy and that AlF_4^- inhibits autophagosome fusion to the food vacuole. To investigate autophagy in *P. falciparum*, the distribution of PfAtg8 and PfRab7 in infected erythrocytes subjected to environmental stress and other perturbations is being investigated.

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HOST LIPOATE IS REQUIRED FOR MALARIA SURVIVAL

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Lipoate is a fatty acid-derived molecule acting both as a prosthetic group for enzymes of oxidative metabolism and as an antioxidant. The *Plasmodium falciparum* genome encodes the enzymes required for *de novo* biosynthesis of lipoate, and these enzymes appear to be active in a variety of functional assays. These results suggest that malaria parasites can synthesize lipoate and that host-derived lipoate should not be required for parasite growth and survival. We show that erythrocytic stage parasites do import lipoate from the external environment and that scavenged lipoate is attached to specific parasite proteins. Disruption of lipoate scavenging leads to morphological abnormalities and cell death. Lipoate scavenging appears to be essential due to the partitioning of key metabolites between two organelles in the parasite - the apicoplast and the mitochondrion. This arrangement of lipoate metabolic pathways, results in a previously unknown nutrient requirement, and exposes vulnerabilities that could be exploited by therapeutics.

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GENOTYPING ANALYSIS OF *PLASMODIUM FALCIPARUM* REVEALS GREATER GENETIC DIVERSITY IN SENEGAL COMPARED TO THAILAND AND POPULATION DIFFERENCES ENRICHED FOR AMINO ACID SUBSTITUTIONS

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Genetic variation in *Plasmodium falciparum* reflects natural selection from pressures including antimalarial drug use and host immunity. Patterns of genetic diversity reveal genomic regions under positive selection, allowing us to understand mechanisms of evasion and identify new targets for drug and vaccine strategies. We developed a genome-wide map of genetic diversity, identifying over 93,000 single nucleotide polymorphisms (SNPs) that serve as markers across the parasite genome. Further investigation of population structure and work toward association studies requires a high-throughput genotyping method. A pilot study using Affymetrix technology was undertaken to better understand the global distribution of these SNPs both within and between parasite populations in Africa, America and Asia. This array tests 3,000 SNPs representing a set of 2200 from chromosome 7 and 800 from the rest of the genome. It performs very well, with call rates of 77% and shows 95% concordance with sequence data across 16 samples. In addition to identifying a previously known selective sweep on chromosome 7 surrounding the *pfcrt* locus, strong structure between Senegal and Thailand parasites was observed (Fst = 0.37, p<0.0001). Diversity was 49% higher among Senegal parasites (π = 0.1659) relative to isolates from Thailand (π = 0.1116), as expected. Among the SNPs, 52 individual SNPs differ significantly in frequency between Senegal and Thailand samples (FET p < 0.05 after Bonferroni correction). Finally, non-synonymous SNPs are significantly enriched among those SNPs that differ significantly in frequency between Senegal and Thailand as 47% (839 out of 1794) testable SNPs were non-synonymous, and 73% (38 out of 52) of SNPs significantly different between Senegal and Thailand were non-synonymous (χ^2 = 14.0, p<0.001). With collectively over 112,000 SNPs now identified in the *P. falciparum* genome, the ability to perform high throughput genotyping is a powerful tool to determine population structure, estimate linkage disequilibrium, and identify polymorphisms for subsequent association studies to identify genes that mediate drug resistance or virulence. Finally, such a tool enables the identification of specific loci subject to natural selection and provides the basis for tracing the historical and future spread of malaria worldwide.

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DISTINCT PHYSIOLOGICAL STATES OF THE PARASITE *PLASMODIUM FALCIPARUM* IN MALARIA INFECTED PATIENTS

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Infection with the malaria parasite *Plasmodium falciparum* leads to widely different clinical conditions in children - ranging from mild flu-like symptoms to coma and death. Despite the immense medical implications, the genetic and molecular basis of this diversity remains largely unknown. Studies of *in vitro* gene expression have found few transcriptional differences among different parasite strains. Here, we present a large study of *in vivo* expression profiles of parasites derived directly from infected patient blood samples. The *in vivo* expression profiles define three distinct transcriptional states. The biological basis of these states can be interpreted by comparison with an extensive compendium of expression data in the yeast, *Saccharomyces cerevisiae*. The three states *in vivo* closely resemble (i) active growth based on glycolytic metabolism; (ii) a starvation response accompanied by oxidative phosphorylation; and (iii) an environmental stress response. The glycolytic state is highly similar to the known profile of the ring stage *in vitro*, but the other states have not been observed *in vitro*. The results reveal a previously unknown physiological diversity in the *in vivo* biology of the malaria parasite, in particular, evidence for a functional mitochondria in the asexual stage parasite, and point to *in vivo* and *in vitro* studies to determine how this variation may impact disease manifestations and treatment. We will also present two additional data sets: 1) ten matched parasite expression data obtained after twenty four hours on antimalarials 2) matched host expression profiles.

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COMPREHENSIVE PROTEOMIC ANALYSIS OF ZYGOTE AND OOKINETE STAGES OF AVIAN *PLASMODIUM* REVEALS ORTHLOGS OF THE UNKNOWN EARLY MOSQUITO STAGES OF *PLASMODIUM FALCIPARUM*

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The transition from gametes to zygote and then to ookinete is the weakest link in the lifecycle of *Plasmodium.* Hence understanding of the zygote and ookinete proteome will facilitate the development of new tools to prevent malaria transmission, which are not yet available for most lethal human parasite *P. falciparum*. *P. gallinaceum*, an avian malaria parasite for which zygotes, ookinetes and secreted/released proteins in axenic medium

are readily obtainable, is one of the most relevant animal models of the *P. falciparum* sexual stages. Based on circumsporozoite protein, chitinase, cytochrome b and ribosomal RNA genes sequence analysis of the bird malaria parasite, *P. gallinaceum* has been suggested to be evolutionarily closer to *P. falciparum*. *P. gallinaceum* zygotes and ookinetes were culture *in-vitro* using serum free media, which has not been possible with *P. falciparum* and has not been done with the rodent malaria parasites. Here, we present a proteomic analysis of *P. gallinaceum* zygote, ookinete and ookinete secreted/released proteins using Multi-dimensional Protein Identification Technology (MudPIT). Of 19,869 open reading frames (ORFs) identified in the *P. gallinaceum* zygote, ookinete, secreted proteins proteome, 33% were found to have orthologues in the *P. falciparum* genome, while only 9.9% had orthologues in the *P. berghei* genome. A total of 1617 *P. falciparum* and 766 *P. berghei* orthologous ORFs were identified; just over half were found to be hypothetical proteins (51%, *P. falciparum,* 54% *P. berghei*). A majority of proteins with predicted secretory signal peptides or transmembrane domains were hypothetical proteins. Proteomic analysis of *P. gallinaceum* zygote, ookinete, and secreted proteins provides a more comprehensive view of the hitherto unknown proteome of the early mosquito midgut stages of *P. falciparum*. The results underpin more robust study of *Plasmodium*-mosquito midgut interactions, fundamental to the development of novel strategies of blocking malaria transmission.

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NATURAL HISTORY OF HYDATID INFECTION AND DISEASE

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Cystic echinococcosis, a zoonotic disease caused by larval stage of *Echinococcus granulosus*, is distributed throughout the world. The most frequently involved organs are the liver and the lung. Although it is known that a proportion of cases will spontaneously resolve, there is only limited information on the natural evolution of human infection and disease. We re-visited a series of 11 asymptomatic individuals with either positive serology only (n=3) or cystic hydatid disease (n=11) detected in surveys of household members and neighbors of patients with CHD performed. These individuals had been given their diagnostic information and referred for treatment when detected. One to three years later, one of the patients had died from unrelated causes, and two refused to participate in the follow up survey. Only two of the eight participating individuals had seek treatment (surgical in both cases). From the six not treated individuals, two had had lung cysts and one had a liver cyst. On re-evaluation, the two patients with pulmonary CHD had had no changes on imaging status (similar characteristics), or serology (one continued to be seropositive and one continued to be seronegative). The liver lesion in the other patient showed a small increase in size from 69 to 74 mm in its maximal diameter. From the three seropositive, imaging negative individuals, one had now a detectable liver cystic image of approx. 30 mm. One of the other two continued to be seropositive but without detectable lesion, and the remaining one seroreverted to negative. All four patients were referred to the local health center for treatment. Despite the small size of this cohort, evolution of serum antibody reactions and imaging progression of lesions showed to be highly variable.

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STRAIN CHARACTERISATION OF HUMAN HYDATIDOSIS IN SUDAN

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Cystic echinococcosis (hydatidosis) is caused by various species or genotypes of the *Echinococcus granulosus* complex. In Sudan, several surveys have been conducted on hydatidosis in animals, but only few reports mentioned the situation in humans. Prevalence estimates are high for dogs, camels and cattle in most surveyed regions, indicating a high risk for the human population. The latter, however, is not supported by hospital records. Preliminary observations indicate a severe underreporting in rural areas, where traditional life styles facilitate transmission of hydatidosis. In addition, there is mounting evidence that the genotype (or species) of the pathogen, which is predominant in the region, may influence the incidence of the human disease. Published and unpublished data indicate, that the majority of isolates from animal sources in Sudan belong to the "camel strain" (G6) of *E. granulosus*. While - world wide - few patients were diagnosed as carrying this particular genotype, no information is available on the causative agent of human hydatidosis in Sudan. In this study we characterized Echinococcus isolates from seven patients from western and southern Sudan, using genotype specific PCR and sequencing. Five isolates could be characterized as camel strain (G6) whereas, the other isolates were identified as the sheep strain (G1) of *E. granulosus.*This corresponds well with the epidemiological sitation in animals, and constitutes the first record of the strains of E.granulosus affecting humans in Sudan.

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PAIR IN BULGARIA: A FIVE-YEAR EXPERIENCE

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This study was undertaken to report our 5-year experience with PAIR in the treatment of cystic echinococcosis (CE) in Bulgaria. PAIR with sonographic guidance was used to treat 190 patients (85 m, 105 f) with 347 CE1 and CE3 WHO type hepatic and extrahepatic echinococcal cysts (mean size 7.2 cm, range 4 - 20). PAIR was performed according to the WHO recommendations. Scolecidal agents were 95% alcohol and 25% NaCl. Standard and modified PAIR techniques, including catheter drainage, were performed. After 6 months, a reduction in size > 50% was obtained in 90.7% of the cysts, 64.8% showed complete or partial solid cyst content, 11 % disappeared. Residual fluid content was observed in 27.6%. At 12-month follow-up, different amounts of heterogeneous "solid" content were obtained in 85.3%, while no solidification was seen in 5.5%. Reduction in size or sonographic changes were not observed in 9.2% of the cysts, all of which were CE3. Percutaneous drainage with Seldinger approach was performed in 15 residual cavities (4.3%). Complications occurred in 23.7% of procedures, with severe anaphylactic reaction in two of them (1.05%). In conclusion, experience in Bulgaria confirms that PAIR is an effective treatment for selected hepatic and extrahepatic echinococcal cysts. Failures seem to be related to cyst type as shown in recent series from other countries. To the best of our knowledge, this is the first report on the use of PAIR in this country, where CE is endemic and represents a major public health problem.

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LONG-TERM SONOGRAPHIC AND SEROLOGICAL FOLLOW-UP OF INACTIVE ECHINOCOCCAL CYSTS OF THE LIVER

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Recent expert opinion recommends that uncomplicated, inactive echinococcal cysts of the liver (CE4 and CE5 in the WHO - IWGE Standardized Sonographic Classification) should be left untreated and monitored only. To support this recommendation, we report our experience with long-term sonographic and serological monitoring of CE4 and CE5 cysts of the liver. From August 1994 through February 2007, 22 patients with 27 CE4 and CE5 liver cysts at the time of diagnosis, were monitored in our clinic. No active of transitional cysts were present. All patients, followed-up at 6 or 12-month intervals on ultrasound and serology, were retrospectively evaluated. The mean time of follow-up was 53 months (range 24-216). Serological tests were negative in 17 and 15 patients by IHA and ELISA respectively; they became negative in 2 and 1, respectively; and remained positive in 5 and 4, respectively, during follow-up. Patients with positive serological tests were also investigated by CT scan (chest and abdomen) to rule out cysts located elsewhere. No reactivation or complication occurred during the time of monitoring. In conclusion, our findings confirm the importance of a stage-specific approach to the management of Cystic Echinococosis, support a conservative approach to inactive, uncomplicated cyst, and confirm that serology has only a complementary function in the clinical management of these patients, compared with ultrasound and other imaging techniques. The implications of these findings for clinical management and natural history of cystic echinococcosis will be discussed.

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CLOSE CORRELATION OF CLINICAL REGRESSION AND SPECIFIC SEROLOGY IN THE FOLLOW-UP OF PATIENTS WITH ALVEOLAR ECHINOCOCCOSIS

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Alveolar echinococcosis (AE) caused by the fox tapeworm *Echinococcus multilocularis* is a serious parasitic disease. Presently, results of imaging studies guide the management and follow-up of patients with AE. Serology was generally rated as unreliable for follow-up purposes, but has recently shown promising results. We tested four ELISAs in parallel on sera of 25 patients with different clinical stages of AE according to the WHO-PMN-staging system. The sequential antibody responses against two recombinant antigens (Em10, Em18), an affinity purified antigen combined with a recombinant antigen (Em2 plus), and a crude antigen extract were measured in a total of 125 sera and compared. In general, there were good correlations between the antibody responses against all antigens employed. Patients in a more progressive stage of AE, i.e. with a longer duration of the disease, had a tendency to have higher antibody indices compared to patients in a less advanced stage of AE. In patients with unresectable lesions who received albendazole treatment, antibody indices decreased only slowly but remained clearly above the cut-off level. In contrast, patients who underwent curative surgery had a drop of antibody-indices below the cut-off level, indicating that sustained antibody responses are dependent on the presence of the parasite's tissue. There were no differences in antibody kinetics between patients of distinct PMN stages. Antibodies directed against the crude antigen extract and the recombinant Em18 antigen had the highest indices measured in all patient groups. In patients who underwent curative surgery the antibody response against Em18 demonstrated the fastest kinetics.

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CHARACTERIZATION OF THE *IN VITRO* ACTIVITIES OF ARTEMISININ-DERIVATIVES AGAINST *ECHINOCOCCUS MULTILOCULARIS* METACESTODES

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The larval (metacestode) stage of *Echinococcus multilocularis*, the small fox tapeworm, represents the causative agent of alveolar echinococcosis (AE). Metacestodes infect mainly the liver tissue, and proliferate by exogenous budding, resulting in severe organ damage. Current chemotherapeutical treatment of AE relies on the use of benzimidazoles (albendazole, mebendazole), but these drugs act parasitostatic rather than parasitocidal, and in case of side effects such as liver toxicity, patients are left without valuable alternatives. Artesunate and arthemeter are derivatives of artemisinin, which is widely used for the treatment of malaria. A recent study using a rat model, as reported previously, has demonstrated the good *in vitro* and *in vivo* efficacies of artesunate and artemether against *Fasciola hepatica,* a trematode causing hepatic lesions, fibrosis and chronic inflammation in the bile duct. This has prompted us to investigate the potential of artemisinin derivatives for the treatment of AE. Artesunate and dihydroartemisinin, but not artemisinin and artemether, were effective against *in vitro* cultured *E. multilocularis* metacestodes at a concentration of 30 µM and above. Morphological alterations were visible latest after 10-14 days of treatment, similar to the destruction seen in albendazole-treated metacestodes, and this correlated with a rise in alkaline phosphatase activity in medium supernatants and vesicle fluids. Further examination of culture medium demonstrated increased leakage of parasite proteins into the culture medium. Ultrastructural studies by SEM and TEM revealed dramatic breakdown of the structural integrity of the parasite germinal layer occurring in a dose-dependent manner. Bioassays in mice using metacestodes treated with artesunate, dishydroartemisinin, albendazole, and a combination of compounds, are being currently carried out to investigate whether these drug-induced alterations exhibit parasitocidal or parasitostatic potential.

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MICROSATELLITE POLYMORPHISM AS A TOOL TO STUDY THE SPATIAL DISTRIBUTION OF *ECHINOCOCCUS MULTILOCULARIS*

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Alveolar Echinococcosis (AE) (infection with *Echinococcus multilocularis*) is an important zoonotic disease occurring in many parts of the Northern hemisphere. So far, classical nuclear and mitochondrial targets of the parasite genome to genotype *E. multilocularis* isolates provided a marked genetic homogeneity. We addressed microsatellite sequences to explore the E. multilocularis genetic diversity at a higher level of discriminating sensitivity. Thus four microsatellite targets (EmsJ, EmsK, EmsB, all three designed in our laboratory, and NAK1, derived from literature) were tested on a panel of 76 E. multilocularis samples. These consisted of larval and adult worm stages geographically obtained from Alaska, Europe, Japan and China. Genetic diversity for each target was shaken after fluorescent PCR with the help of labelled primer and analysed by size polymorphism. For the EmsJ and EmsK targets, 2 alleles for each of these loci yielded 2 and 3 genotypes, respectively, which enabled us to discriminate European isolates from isolates of the North American - Asian group. With NAK1, 5 alleles were found, which allowed to cluster the samples into 7 genotypes, including peculiar genotypes for Tibetan and Alaskan isolates. The EmsB

locus provided a complex pattern, as defined by its tandem repeated multi-locus microsatellite origin, and allowed to highlight 17 alleles. Cladistic analysis with R software was performed with EmsB results. A genetic threshold was built and yielded 24 clusters. EmsB targets provided a higher sensitivity level than the other targets, including the major part of polymorphism information. The complex EmsB-pattern associated with focal parasite population characteristics, while avoiding distinction between individuals of the same microfocus. As a consequence, we will use this tool to study the spatial and temporal dynamics of *E. multilocularis* on the prevalent regions of the European continent.

(ACMCIP Abstract)

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COMBINATION LIPOSOMAL AMPHOTERICIN B (AMBISOME®, AMB) AND MILTEFOSINE (MF) FOR THE TREATMENT OF VISCERAL LEISHMANIASIS (VL) IN NORTHERN BIHAR, INDIA

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India, Bangladesh and Nepal have agreed on a program for the elimination of VL. Parasite resistance has made antimony ineffective in Bihar. Cost-effective, practical treatments are needed. In India, single-dose AmBisome (AmB) at 5 mg/kg is 90% effective; oral miltefosine (MF) for 28 days is 94% effective. Combining drugs can protect against developing resistance, improve effectiveness and shorten duration of therapy, thereby improving compliance and reducing costs. A randomized, open-label Phase II study with a group-sequential (triangular test) design, parallel-arm, non-comparative (Step 1 for treatment selection) followed by a future comparative Step 2 for superiority. Data were analysed every 5 patients. Non-pregnant adults and children with a positive spleen aspirate were recruited and treated with: (a) AmB 5 mg/kg single dose; (b) AmB 5 mg/kg followed by MF for 14d; (c) AmB 3.75 mg/kg + MF for 14d; (d) AmB 5 mg/kg + MF for 10d; and (e) AmB 5mg/kg + MF for 7d. Initial cure was based on spleen aspirate on day 16. Definitive cure was at 6-month follow-up. The study was IRB approved and registered. Step 1 is completed -- all five treatment groups (n = 45 per group) were initially cured on day 16 and all regimens were well-tolerated. Final cure rates will be available and presented. Step 2 will start with at least one combination vs. single-dose AmB 5 mg/kg. In conclusion, simple, short course combinations, such as AmB plus MF, need to be effective and feasible with no in-patient stay. AmB is given as an out-patient and MF is taken orally at home. Weekly patient visits will be needed. Because of MF, pregnancy must be avoided during treatment plus 2 months. Larger scale studies are needed including other combinations (e.g. with paromomycin). Efficacy, safety and cost-effectiveness considerations will inform the choice of candidate treatments.

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WHAT BASELINE PATIENT CHARACTERISTICS PREDICT TOXICITY DURING SODIUM STIBOGLUCONATE TREATMENT?

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Pentavalent antimonials are used for the treatment of leishmaniasis with good efficacy but some predictable toxicity including pancreatitis, myalgias, arthralgias, elevated liver associated enzymes (ALT, AST), and fatigue. We sought to identify patient baseline characteristics associated with common subsequent toxicities. Data were collected from an ongoing protocol using Pentostam™ (SSG, sodium stibogluconate, Glaxo Smith Kline, United Kingdom) for treatment of cutaneous leishmaniasis. SSG was intravenously administered at a dosage of 20 mg/kg/day for 10 or 20 days duration (assignment to duration was not random). Baseline characteristics included age, race, duration of illness, body mass index (BMI), body surface area (BSA), number and total area of lesions, laboratory values, daily dose and cumulative dose. Studied outcomes were selected adverse events (musculoskeletal, elevated ALT, fatigue, symptomatic pancreatitis, cytopenias), severe toxic events, and persistent symptoms. A univariate analysis identified six toxicities which were evaluated using multivariate logistic regression models. 399/ 403 enrolled subjects were included in this analysis. There were 143 persons prescribed 10 days and 256 prescribed/extended to 20 days SSG. Over 93% of each group reported at least one of the evaluated toxicities. Baseline variables associated with at least one toxicity outcome in univariate analysis included age, BMI, BSA, ALT/AST levels, renal function, pancreatic enzymes, number and total area of lesions, white blood count, hematocrit, platelets, lymphocytes, and eosinophils. Multivariable logistic models (unadjusted for multiplicity, $p< 0.05$) suggested severe musculoskeletal toxicity in 20 day SSG was associated with total area of lesions (Odds Ratio (OR) 0.94/100 mm^2) while BSA (OR 2.14/0.5 m^2) and eosinophils (OR 1.34/ percent) were related to fatigue. Symptomatic pancreatitis was associated with number of lesions (OR 1.92/five lesions) in 10 days SSG arm and creatinine (OR 0.60/ 0.1mg/dl) and glomerular filtration rate (OR 0.93/ml/min/1.73 m^2) in the 20 day SSG arm. In conclusio, no single factor was consistently related to all toxicities studied. While dosage was normalized by total weight, it appears that high BSA and decreased baseline renal function (in this fairly normal population) showed some association with several toxicities.

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TREATMENT OF AMERICAN CUTANEOUS LEISHMANIASIS PREVIOUS TO ULCER DEVELOPMENT IS ASSOCIATED WITH HIGH RATE OF FAILURE IN NORTHWEST BRAZIL

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Cure rates for American Cutaneous Leishmaniasis (ACL) range between 60-90%; however, early evidence suggests lower cure rates for early ACL before the development of the classic ulceration. We evaluate clinical and immunologic risk factors for treatment failure in patients with early and classic ulcerative ACL caused by *Leishmania braziliensis.* This is a prospective cohort study of patients in an endemic area. Cases are age 13-60 years with lesions of 15-90 days duration confirmed by intradermal skin test and/or culture. Those with previous or non-cutaneous leishmaniasis or chronic conditions were excluded. Serial stools and peripheral blood were collected at presentation to determine heminth co-infection and immune cytokine levels, respectively. All participants were treated with antimony (20mg/kg/day x 20 days). Helminth co-infection was treated at day 60. Follow-up was at 30, 60 and 90 days. The primary outcome was lesion cure by 90 days without recurrence. A total of 137 patients were enrolled in the study between 2004-2006. There were 120 patients with classic ulcerative lesions and 17 patients with early non-classic lesions, including papules, nodules, plaques, and superficial ulcerations. Patients with classic ulcerative lesions had a longer duration, larger lesion and intradermal response to skin testing, and higher levels of IFN-γ (all $p<0.01$). There was no diffrenence in helminth co-infection. Ulcerative lesions were associated

with lower treatment failure compared to early non-classic lesions (25.8% versus 70.6%, p<0.01). In a multivariate Cox model, lesion ulceration is the strongest predictor of lesion healing (HR 3.74 [95% CI 1.49-9.42]); helminth co-infection prolonged healing (0.36 [0.20-0.65]). Intradermal immune response was also associated with lesion healing (1.17 [1.02-1.35]), while the level of IFN-γ cytokine was not (p=0.12). There was no difference in time to healing by sex, age, number of lesions or lesion duration (all >0.30). In conclusion, early treatment of ACL does not prevent lesion ulceration and is associated with higher rates of treatment failure. Lesion ulceration was the strongest predictor of lesion healing. This research suggests the importance of the *in situ* immune reponse in tissue damage and healing in patients with ACL and the potential for local or systemic immune-modulating adjunctive treatment.

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SEROPREVALENCE OF TOXOCARIASIS IN SCHOOLCHILDREN IN SAN JUAN DE LURIGANCHO, LIMA, PERU

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Toxocariasis is a worldwide health problem, though more frequent in tropical areas. It affects mainly children and is associated with the presence of *Toxocara* eggs in the environment. Located in Lima, Peru, San Juan de Lurigancho (SJL) is the district that holds the largest population with 803, 911 inhabitants. It is geographically divided into two areas, the lower altitude is urban and the higher altitude is rural. Of all the parks in the district, 70.6% are contaminated by *T. canis* eggs. We aimed to determine the seroprevalence of toxocariasis in schoolchildren from SJL and to determine the factors associated with this infection. A cross-sectional study was performed from May 2006 to September 2006. Schoolchildren from 2 to 15 years old were randomly selected in SJL district. ELISA IgG anti-*T. canis* with excretor-secretor antigen titres ≥ 1:200 determined seropositivity. Age, sex, geophagia, pet ownership, contact with puppies, socioeconomic and educational level of the parents, school location (lower or higher altitude), chronic cough, wheezing, abdominal pain, urticaria and eosinophilia were registered. Chi-square test and Odds Ratio were used for statistical analysis. Results: A total of 301 children were included. Mean age was 8.3 years (±3.7 years), 161 (53.5%) were female. The toxocariasis seroprevalence was 46.5 %. There was no association among age and sex with *T. canis* infection. However, children under 5 years had higher titres (≥1/800) (p=0.045). Attending a school in the lower altitude was the strongest factor associated with *Toxocara* seropositivity (OR:2,76; CI:1.62-4.69, p:0.001). Parents living in the lower altitude had a higher socioeconomic level (p<0.001) and educational level (p<0.001). There were no significant relationships among geophagia, pet ownership, contact with puppies, symptoms, eosinophilia and positive serology. Conclusions: Seroprevalence of toxocariasis in schoolchildren from SJL district is high. Location of the school was the main risk factor. Further studies should be done to explain the relation between geography and *T. canis* infection.

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THE PREVALENCE OF RHEUMATIC HEART DISEASE AMONG CHILDREN IN BAMAKO, MALI

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Rheumatic heart disease (RHD) is a disabling illness resulting from untreated Group A streptococcal (GAS) pharyngitis. In developing countries there is a paucity of data on the epidemiology of RHD involving representative samples and standardized case definitions that can be used to design strategies for disease prevention. This ongoing study aims to systematically describe the prevalence of RHD in Malian children. A sample of 3,600 children 5-16 years old was randomly selected from a census population living in a poor urban quartier of Bamako, Mali. Demographic and clinical data are collected. A cardiologist performs a screening echocardiogram on each child, and if abnormal, a complete study. Definite, probable, and possible RHD is diagnosed by a jury of cardiologists using World Health Organization criteria. Since all studies have not yet been read, we preliminarily estimated the prevalence of RHD using a modified denominator ([number of echocardiograms read/total number of echocardiograms)] X the total children enrolled). From February 15 to May 7, 2007, 809 eligible children were offered enrollment and 782 (96.7%) were included (27 refused). Among those enrolled, 49% are female; age distribution is: 5-8 yrs (n=331, 42%), 9-12 yrs (n=220, 28%), and 13-16 yrs (n=231, 30%). Sixty children with abnormal screening echocardiogram underwent a complete study; 42 have been reviewed by a cardiologist and 29 were diagnosed with RHD as follows: 15 (36%) definite, 8 (19%) probable, and 6 (14%) possible. Sixteen of the 29 children had a pathologic murmur. Using the modified denominator (n=547), the prevalence of RHD per 100,000 population is 5302 (95% CI 3579-7526), definite RHD 2742 (1543-4483), probable 1463 (633-2861), and possible 1097 (404-2372). In conclusion, these results suggest that RHD is an unrecognized major health concern among Malian children. Further elucidation of the epidemiology and clinical outcome of this illness is needed.

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VIRAL HEPATITIS IN NEWLY ARRIVED IMMIGRANTS AND REFUGEES

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Recent data shows that immigrants have increased mortality from viral hepatitis and hepatoceullar carcinoma as compared to their Canadian counterparts. This is likely due to chronic hepatitis B and C infections since >70% of all Canadian immigrants in the past 30 years have originated from countries highly endemic for hepatitis B and C. We performed a seroprevalence study to determine the burden and risk factors for these diseases in the immigrant population. A total of 1309 foreign-born adults (≥18 years), having lived ≤5 years in Canada were recruited from 2 hospitals and 3 clinics in Montreal, between October 2002 and December 2004. Socio-demographic information was collected via a questionnaire. Hepatitis BsAg, HepBcAb, and Hepatitis C Ab were measured by a commercial ELISA method. Hepatitis C infection was confirmed with a western blot and plasma virus was assayed by a commercial qualitative PCR method. The mean age was 32± 9 years and 67% were female. The prevalence of Hepatitis B carriers (HepBSAg +) and Hepatitis C infection overall was [%, (95% CI)] [3.5% (3.3-3.8); 1.8% (1.6-2.0)] respectively. The prevalence of Hepatitis B Carriers and Hepatitis C infection by region of origin was: SubSaharan Africa [7.2% (3.6-10.8); 5.1% (2.0-8.2)], N. Africa/Mediterranean [4.3% (0.9- 7.7); 0.7% (0-2.1)], South Asia [0.7% (0-1.6); 2.0% (0.4-3.6)], South East Asia [6.7% (3.2-10.2); 0.52% (0-1.5)], Latin America/Caribbean [0.6% (0-1.4); 0.6% (0-1.4)] and Eastern Europe [6% (2.2-9.8); 2.7% (0.1-5.3)] respectively. In multivariate analysis Hepatitis B carriers, as compared to non-carriers, were more likely to be <35 years [odds ratio, (95%CI); [2.8 (1.2-6.5)], and to have originated from certain high risk regions, after controlling for these variables, gender, immigrant status, and household crowding. Those with hepatitis C

infection were more likely to be >40 years [3.7 (1.5-9.3)], male [3.0 (1.5-9.3)], to have been a refugee [4.2 (1.2-14.9)], and to have originated from certain high risk regions after controlling for these variables and household crowding. In conclusion, the burden of Hepatitis B carriage and Hepatitis C infection in immigrants is high and likely reflects the seroprevalence of their countries of origin. Strategies to detect and prevent viral hepatitis in the immigrant population need to be developed to decrease the health burden, morbidity, and mortality due to these infections.

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A PHASE II VACCINE TRIAL OF MENINGOCOCCAL A CONJUGATE VACCINE (PSATT) IN AFRICAN TODDLERS

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Recurrent severe epidemics of meningococcal disease strike the countries in the meningitis belt extending from Senegal to Ethiopia. A vaccine, inducing long-lasting protection and herd immunity, is urgently needed. Under the leadership of the Meningitis Vaccine Project, a new MenA conjugate vaccine, manufactured by Serum Institute of India, Limited (PsA-TT; 0.5 ml contains 10µg Ps, 10-20µg TT, and adjuvant [AlPO4]) was safe and immunogenic in a phase I study in Indian adults. A pivotal Phase II, observer-blind, randomized, controlled study to assess safety, immunogenicity, induction of immune memory and antibody persistence of PsA-TT toddlers aged 2 to 23 months is underway in Mali and in the Gambia. After parental informed consent, each participant received a single intramuscular injection of PsA-TT, meningococcal quadrivalent polysaccharide (PsACWY) vaccine or *Haemophilus influenzae* type b (Hib) vaccine. Blood samples were obtained at baseline and at 4 weeks for serum bactericidal antibody (SBA) and anti-polysaccharide group A (anti-PsA) IgG levels. Participants were followed for local and systemic reactions until 4 days after vaccination and for adverse events for 4 weeks. From September to November 2006, 601 participants were enrolled (300 in Mali and 301 in the Gambia); 201 received PsA-TT, 200 PsACWY and 200 Hib. SBA geometric mean titers at baseline were 14, 16 and 13 and rose to 6234, 365 and 61, respectively, at week 4. Four-fold rise in SBA titers were observed in 96% (95%CI 92-98), 64% (57-71) and 36% (29-43) of participants from each group. Local reactions were reported by 13%, 5% and 10% of participants (13% vs 5%, p<0.05) and 17%, 16% and 16% reported systemic reactions. Adverse events were reported in 38%, 33% and 30% of study participants. Four serious adverse events occurred, all unrelated to vaccines administered. In conclusion, these early data demonstrate that PsATT is indeed immunogenic and safe in African toddlers. By the end of the study, the vaccine's ability to induce memory and provide long-lasting protection will also be known.

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MODELLING THE SPREAD OF ANTHELMINTIC RESISTANCE

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In recent years there has been a dramatic increase in the use of mass drug administration for the control of helminth infections of humans, increasing the likelihood that anthelmintic resistance may become a major public health concern of the future. Mathematical models are used to investigate how the relaxation of density-dependent processes that regulate parasite population growth following chemotherapy may increase the rate of spread of anthelmintic resistance. Using human onchocerciasis as an example we show how the complex pharmacodynamics of drug treatment and the population dynamics of *Onchocerca volvulus* may cause the resistance allele frequency to vary substantially across different parasite life-stages and over time after the start of control. Mathematical models are used to illustrate how the presence of drug resistant parasites may not prevent a species from being eliminated if mass chemotherapy has pushed the parasite population size beneath its breakpoint density. The prospect that anthelmintic resistance may be already present should be considered before mass drug administration control programmes are halted, as their premature termination may render the drug ineffective for future efforts.

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PHENOTYPIC EVIDENCE OF EMERGING IVERMECTIN RESISTANCE IN SOME POPULATION OF *ONCHOCERCA VOLVULUS*, THE CAUSATIVE AGENT OF ONCHOCERCASIS

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Ivermectin (IVM) has been used for onchocerciasis control in Ghana and other areas of West Africa since 1987. We have investigated the responses of *Onchocerca volvulus* to IVM treatment by carrying out a 22 month longitudinal study to assess the microfilaricidal effect of ivermectin and its impact on adult female worm reproduction by determining skin microfilaria (mf) repopulation in subjects, from 9 communities that had received 6-19 annual rounds of IVM and an IVM-naïve community. We also carried out embryograms of adult female worms recovered after nodulectomy and a parallel vector studies. Mf clearance of > 99% was observed 1 month after treatment. However, Mf repopulation at day 90 posttreatment were < 3% of initial mf count in six communities, however 4 other communities had significantly higher mf repopulation of 7-21% of pre-treatment counts, rising up to 53.9% by day 180. One year post treatment assessment showed six communities having <100% of pretreatment counts while four communities had >100% of pretreatment counts and significantly higher (p<0.01) viable worms and female worms per nodule. Embryogramme results also showed these four communities having significant higher (p< 0.01) female worms having viable mf in the uterus.Mf repopulation at days 90, 180 and 365 post treatment and embryograms showed evidence of a lack of a sustained response of *O. volvulus* to IVM in 4 communities, manifested as a rapid return to fertility after IVM treatment. The rapid repopulation of skin mf, fertility in female worms, 90 days after treatment and increasing microfilarial prevalence rates in some communities, despite ongoing IVM treatment, indicate that IVM resistance is emerging, manifested as a loss of effect of IVM on suppression of parasite reproduction.

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THE ALTERED STATE OF ONCHOCERCAL NODULES AFTER TREATMENT WITH IVERMECTIN; INSIGHTS INTO MECHANISMS OF CHEMOTHERAPEUTIC ACTION

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Onchocerca nodules have long been used as measure of parasite status in onchocerciasis patients, including as an indicator of the effects of chemotherapeutic agents such as ivermectin. However, these are also sites of active response by the host to this parasite and they reflect changes in

the host's inflammatory and immune responses to the worm in different situations such as after chemotherapy. A detailed re-examination of the microstructure has proved new information about the interactions between worm, host and drug. The basic immunohisto-anatomy of the nodule reveals it to be an organized, compartmentalized, entity with a definitive vascular component, and a varyinf immuno-pathological cellular response during chemotherapy. This present study examined the changes in anatomic structure, in inflammatory status, in immunoprofile and in parasitic profile of 532 nodules from different African and Latin American onchocerciasis endemic foci where mass drug administration has taken place. Ivermectin-treated nodules show an increased inflammatory profile and varied immuno-cytochemical profiles when compared to untreated nodules. These differences include responses to microfilariae, to degenerating adult worm components, including the Wolbachia endosymbionts and the characteristics of the previously described "embryonic neoplasms". A lack of effect of ivermectin on male *O. volvulus* worms was seen in this study and differs from current published data. The importance of standardizing the approaches to sampling of onchocercal nodules for examination, especially when seeking changes induced by chemotherapy, will be characterized and emphasized.

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TARGETING ENDOSYMBIOTIC *WOLBACHIA* IN *WUCHERERIA BANCROFTI* REDUCES PLASMA VEGF-A AND IMPROVES CONDITION OF HYDROCELE PATIENTS

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Hydrocele, caused by *Wuchereria bancrofti* results from accumulation of fluid in the tunica vaginalis of affected individuals. It is a disease of considerable socio-economic burden in the tropics. While there is ample evidence suggesting that pathology of filarial diseases have genetic propensity, very little work has been done to address it. Vascular endothelial growth factors (VEGF) are a major mediator of vascular permeability and angiogenesis and play a pivotal role in mediating the development and progression of many diseases. VEGF-A promotes extravasation of fluid and plasma proteins from the blood vessels into surrounding tissues, making them a candidate for hydrocele development. The treatment of choice of hydrocele at the moment is surgery-hydrocelectomy, which is expensive and sometimes unsafe. Therefore, a drug which could halt or improve this condition would be greatly welcome to replace or supplement hydrocelectomy. Targeting *Wolbachia* endosymbionts in W. *bancrofti* leads to reduction of pro-inflammatory cytokines which in turn leads to reduction of VEGF molecules. To assess the effect of *Wolbachia* depletion on hydrocele, 47 hydrocele patients of filarial origin took part in a double blind, placebo-controlled trial of a six-week regimen of 200 mg/day doxycycline in Ghana. Four months after doxycycline treatment, all patients received 150µg/kg ivermectin and 400mg albendazole used for mass chemotherapy. Patients were monitored for *Wolbachia* and microfilaria loads, antigenaemia, plasma levels of VEGF-A and the size of the hydrocele. Prior to the doxycycline treatment, the role of VEGF-A genetic polymorphisms in hydrocele development was assessed in a cohort of 221 lymphatic filariasis patients. Of three VEGF-A promoter polymorphisms examined, the C/C genotype at -460 was significantly higher in the 47 hydrocele patients (P=0.0004, OR=3.9 (95% CI=1.8-8.2)) as were plasma levels of VEGF-A. Furthermore, a positive correlation (R^2=0.412, P=0.026) was observed between plasma VEGF-A and stage of hydrocele. The C polymorphism at -460 is, therefore, a novel genetic risk factor for hydrocele development in lymphatic filariasis. Following doxycycline treatment, *Wolbachia,* microfilaria and antigenaemia levels were reduced significantly up to 24 months in the doxycycline group compared to the placebo group with doxycycline showing a macrofilaricidal activity. Preceding clinical improvement, the mean plasma levels of VEGF-A decreased significantly at 12 months in patients treated with doxycycline, resulting in the reduction of the size of hydrocele in doxycycline-treated patients whilst the condition deteriorated in the placebo patients

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WOLBACHIA ENDOBACTERIA DEPLETION BY DOXYCYCLINE AS ANTIFILARIAL THERAPY IS MACROFILARIDICAL IN ONCHOCERCIASIS

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Onchocerciasis (river blindness) caused by the filaria *Onchocerca volvulus* is a devastating filarial infection of 37-40 million people in the tropics, which often leads to skin disease and blindness. The major drug currently in use for treatment is ivermectin, which acts mainly on the larval stages, the microfilariae, but has only limited long-term sterilizing effects on adult female worms and does not show a relevant macrofilaricidal activity. The only available macrofilaricidal drug, suramin, is too toxic for broad use. The search for a macrofilaricidal drug has therefore been a high priority for decades. Many filarial species, including *O. volvulus*, contain essential *Wolbachia* endobacteria, which can be depleted by tetracycline antibiotics. A randomized, placebo-controlled trial was conducted in an endemic area in Ghana where 44 onchocerciasis patients were given 200 mg/day of doxycycline or matching placebo for six weeks, followed by a single dose of 150 µg/kg ivermectin six months after the beginning of the study. At 6, 20 and 27 months after study onset, patients underwent extirpation of their onchocercomas. These were processed for qPCR to determine *Wolbachia* loads and for immunohistology to determine presence or absence of *Wolbachia*, embryogenesis / fertility of female worms and the proportions of live and dead adult worms. Doxycycline was well tolerated, with no study participant requiring interruption of treatment due to adverse reactions. In the doxycycline group, *Wolbachia* depletion was already extensive at 6 months, and at 20 and 27 months endobacteria were found only in young worms, probably acquired after the doxycycline therapy. Doxycycline resulted in complete sterilization of living female adult worms and reduction of insemination of female filariae. Significantly, this regimen resulted in a macrofilaricidal activity of more than 60% of the female worms. The results allow the prediction that after less than three years the majority of worms exposed to doxycycline treatment will have died. Since the only available macrofilaricidal drug, suramin, is too toxic for broad use and requires i.v. application, the doxycycline regimen in this study can be considered the first and currently only one of its kind with low toxicity, which has strong macrofilaricidal effects and can be administered orally. This regimen opens up a new possibility for macrofilaricidal chemotherapy of onchocerciasis patients.

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EOSINOPHILS ARE NOT REQUIRED FOR DEC-MEDIATED CLEARANCE OF MICROFILAREMIA

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Diethylcarbamazine (DEC) has a rapid microfilaricidal effect and has been used for more than 50 years in the treatment of human filarial infections. Although the mechanism of action of DEC is poorly understood, it is known that components of the immune system are required for DEC-mediated parasite killing. Eosinophils have been theorized to play a role in DEC-mediated filarial killing at several levels. Not only does DEC enhance the adherence of eosinophils to microfilariae (mf) *in vitro*, but eosinophils are a source of inducible nitric oxide (iNOS), shown recently to be essential for DEC-mediated mf clearance *in vivo*.The purpose of the current study was to determine whether eosinophils are required for DEC-mediated clearance of mf *in vivo*. Twenty-four Balb/c mice were injected intravenously with *Brugia malayi* mf. After two weeks, mice were treated with a single dose of monoclonal antibody to interleukin-5 (TRFK-5; 5 mg/kg iv) (n=10), isotype control (n=10), or no antibody (n=4). Subsets of these mice were then treated with DEC (6 mg/kg po daily for 3 days), and mf levels were followed weekly by Nuclepore filtration of 100 microliters of blood. The geometric mean (GM) microfilarial level prior to DEC treatment was 147 mf/ml (range 30-440), and there were no significant differences among any of the groups. At 2 weeks post-DEC treatment, mf levels in the DEC treated mice had fallen to a GM of 7 mf/ml (range 0-90), and 6 mice had no detectable circulating mf. GM mf levels in the untreated mice decreased to 45 mf/ml (range 0-170; p=0.03 compared to the DEC treated mice), and only 1 mouse had no detectable mf. There were no differences in GM mf levels or the rate of mf clearance between the mice treated with DEC+anti-IL5 and those who received DEC+isotype control (p=NS). These results suggest that eosinophils are not required for DEC-mediated clearance of microfilariae in a murine model of *Brugia malayi* microfilaremia. Confirmatory experiments using two strains of eosinophil-deficient mice are currently underway.

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LUTZOMYIA LONGIPALPIS SALIVARY RECOMBINANT PROTEINS RECOGNIZED BY HUMAN, DOG AND FOX ANTIBODIES

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Saliva from sand fly has a wide range of components that are able to induce and modulate antibody and cellular immune responses. This response against saliva has already been used as an epidemiologic marker of exposure to vectors. Using recombinant soluble proteins produced on mammalian cells from transcripts coding for the most abundant salivary proteins from the sand fly *Lutzomyia longipalpis*, we investigated the presence of anti-saliva antibodies in human, dog and fox sera that had been exposed to sand flies bites. The sera were tested against the recombinant proteins LJM17, a 45 kDa protein that belongs to the yellow related family of proteins, LJM11, a 44 kDa protein from the yellow family of proteins, LJM111, a 43 kDa protein that belong to the yellow family of proteins, and LJL23, a 34 kDa apyrase. Antibodies against LJM17 recombinant protein were detected in human, dog and fox sera. However, the two other members of the yellow-related family of proteins (LJM11 and LJM111) were not recognized by these sera. LJL23, the recombinant salivary apyrase, was recognized only by dog antibodies. Because of the relationship between the vertebrate host immune response to sand fly salivary proteins and protection to parasite infection these results suggest that salivary recombinant proteins could be useful markers to identify the level of exposure to the vector in the natural *Leishmania* reservoirs such as dogs and foxes and in the accidental hosts such as humans and be attractive targets for vaccine development to control *Leishmania chagasi* infection.

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DETERMINANTS OF *TRIATOMA INFESTANS* INFESTATION CLUSTERING IN RURAL COMMUNITIES OF MORENO DEPARTMENT, NORTHWESTERN ARGENTINA

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Chagas disease remains a serious health and economic concern in Latin America, despite declining prevalence and incidence. Vector-borne transmission of Chagas disease still persists in the Gran Chaco region of northern Argentina, Paraguay and Bolivia, where *Triatoma infestans* is the main vector. Vector control activities in this area are limited due to economic and logistic constraints, consisting largely of sporadic spraying with pyrethroid insecticides. As a consequence, reinfestation rates are high in the rural communities. The objective of the present study was to identify environmental, demographic and vector control factors associated with the clustering of house infestation by *T. infestans* in the Department of Moreno, one of the poorest and most endemic areas for Chagas disease in Argentina. Control history data for each of the 275 communities in Moreno was obtained from the Argentinean National Vector Control Program; environmental data (elevation, landscape cover type, and land surface temperature) were derived from satellite imagery. Clustering of domestic infestation by *T. infestans* was positively associated with density of rural houses and negatively associated with distance to the nearest infested community. Inclusion of environmental factors increased the model's predictive value; maximum temperature, percentage of degraded and of deforested landscape (positively) and elevation (negatively) were also associated with membership in a cluster. The resulting model was then used to construct a map of Moreno showing the risk for *T. infestans* infestation clustering. Field validation and extrapolation of this model to the Gran Chaco will be useful for identifying areas at high risk for reinfestation, where targeted control actions should be performed. Given the limited resources available for surveillance and control, these finding will contribute to improved allocation of resources for the ultimate goal of reducing the burden of Chagas disease in this region.

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IMMUNOMODULATORY EFFECTS OF SALIVARY GLAND EXTRACT OF BLACK FLY, *SIMULIUM VITTATUM* (DIPTERA: SIMULIIDAE) ON MOUSE SPLENOCYTES

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Black flies are known vectors of *Onchocerca volvulus*, which causes river blindness in humans, and vesicular stomatitis virus in livestock. Earlier studies established that salivary gland extract (SGE) contains components with anti-hemostatic and immunomodulatory functions. Salivary gland components of other vectors have been shown to modulate the host immune system in a manner that favors transmission of pathogens. As a prelude to examining such a role for black fly saliva, we have begun to reexamine the immunomodulatory effects of Simulium vittatum SGE on mouse splenocytes. Black fly SGE decreased proliferation of splenocytes stimulated by concanavalin A. Flow cytometric analyses imply that this inhibition is due to a combination of cell cycle arrest and induction of apoptosis, particularly affecting CD4+ T rather than CD8+ T cells. Further differential induction of cell cycle arrest and induction of apoptosis by black fly SGE will be addressed.

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HIDDEN SYLVATIC FOCI OF *TRIATOMA INFESTANS* IN THE ARGENTINE CHACO: A THREAT TO THE VECTOR ELIMINATION CAMPAIGN?

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Disease eradication programs depend on a time-limited intensive campaign and are likely to fail if resistance to insecticides or drugs (malaria) or sylvatic cycles occur (yellow fever). Chagas disease is the most important vector-borne disease in Latin America, with an estimated 10-18 million people infected. *Triatoma infestans* is the main vector in the southern cone of South America, and considered to be limited to domestic and peridomestic habitats throughout most of its range. It is s the target of an intergovernmental elimination program started in 1991. Only limited success was achieved in the Gran Chaco region due to repeated reinfestations, even in areas subject to intensive vector control efforts. In 2005 and 2006, we found in northern Argentina sylvatic colonies of *T. infestans* up to1900 m from the nearest house, and at 870-2230 m from the nearest infestation detected during the previous 18 months after full coverage residual insecticide spraying. We have also detected adult *T. infestans* and recently established small bug colonies of unknown origin in domestic and peridomestic habitats. Based on mitochondrial and microsatellite markers and on wing geometric morphometry, we conclude that the *T. infestans* found in sylvatic habitats are probably derived from domestic or peridomestic populations. Whether the sylvatic habitats provide a refuge for domestic/peridomestic populations following spraying and whether they can serve as a source for reinfestation remains to be determined. The occurrence of sylvatic foci of *T. infestans* in the Gran Chaco may pose a threat to ongoing vector elimination efforts.

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HUMAN IMMUNE RESPONSES AGAINST *PHLEBOTOMUS PAPATASI* SALIVA

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Hematophagous insects, including Phlebotomine sand flies, inject a variety of compounds during blood feeding to combat the host's haemostatic response. These molecules can enhance pathogen transmission in naïve individuals, but also are extremely immunogenic. Interestingly, the immune response elicited after repeated exposure to the antigens found in sand fly saliva protects against *Leishmania* infection in animal models, suggesting that these antigens could serve as vaccines against leishmanial disease. The underlying hypothesis of anti-saliva vaccine development is that upon repeated exposure, conserved sand fly salivary components will elicit delayed type hypersensitivity reactions in the host that will influence the outcome of *Leishmania* infection. If sand fly saliva as a vaccine component for use in humans is to be realized, it is necessary to first understand the variability of salivary genes and human immune responses to sand fly saliva. The focus of our work here is on elucidating the immune responses of individuals exposed to sand fly bites. To explore the early events of anti-saliva human immune responses monocyte derived dendritic cells and macrophages were generated from naïve individuals and the effect of sand fly salivary gland homogenate (SGH) on co-stimulatory molecule expression and cytokine secretion was assessed. Blood samples also were collected from US soldiers deployed to Iraq and compared to US soldiers that have never been deployed to *P. papatasi* endemic regions. As patients exposed to *Leishmania* parasites have been shown to alter their antibody responses to sand fly saliva, the experimental group was divided into those individuals that had been diagnosed with cutaneous leishmaniais and those that exhibited no clinical signs. Salivary antigens recognized by the sera of exposed individuals were identified by western blot analysis of *P. papatasi* SGH. Peripheral blood mononuclear cells were assessed for anti-SGH proliferative capacity and cytokine secretion.

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MOLECULAR BASIS OF SPECIFICITY AND CROSS REACTIVITY IN DELAYED-TYPE-HYPERSENSITIVITY REACTIONS TO BITES OF SAND FLIES AND IMPLICATION FOR PROTECTION AGAINST *LEISHMANIA* INFECTION

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Saliva of phlebotomine sand flies contains immunogenic proteins that elicit strong Delayed-Type-Hypersensitivity (DTH) reactions that may be highly protective against *Leishmania* infection. In previous molecular studies we demonstrated a high degree of conservancy in sand fly proteins from two populations of *Phlebotmus dubsocqi* from Mali and Kenya and predicted that they share similar MHC class II T-cell epitopes. The current study was carried out to investigate the specificity and cross reactivity of DTH reactions induced by cross biting between different populations, species, subgenera and genera of sand flies. Using colonies of *Lutzomyia longipalpis* from Brazil, *P. duboscqi* from Kenya, *P. duboscqi* from Mali and *P. papatasi* from Turkey, groups of C57BL/6 mice were pre-sensitized on the left ear by repeated biting from one population of sand flies and then challenged on the right ear by bites of females from either the same population or a different population, species or subgenus. Skin measurements and histological sections, taken at 24 and 48 h later, from the bite site showed cross reactivity of DTH reactions resulting from cross biting between different populations of the same species or two species from the same genus (*P. papatasi* and *P. duboscqi*), but not between species from different genera. Examination of immunohistochemistry sections indicated that the DTH reactions were characterized by high degree of infiltration of macrophages, eosinophils and neutrophils. Work was also done to determine degree of protective anti-*Leishmania* immunity conferred by DTH resulting form cross biting between different populations and species of sand flies. Results are discussed in relation to the epidemiology of leishmaniasis and the prospect of developing universal vaccines based on sand fly salivary proteins.

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PHLEBOTOMUS PAPATASI SALIVARY GLAND SEQUENCE VARIABILITY AND IMPACT ON DEFINING VACCINE CANDIDATES

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Hematophagous vectors are not simply tools that inoculate pathogens, rather they are capable of inoculating molecules that have profound effects on the host immune system. Recent studies indicate that repeated exposure to sand fly saliva creates an inhospitable environment for the establishment of *Leishmania* infection, leading to attenuated disease in rodent models. These data suggest that the incorporation of salivary molecules in multi-component vaccines may be a viable strategy for the development of anti-*Leishmania* vaccines. If sand fly saliva as a vaccine component is to be realized, it is necessary to understand the variability of salivary genes and human immune responses to such variability. Here we investigated amino acid sequence variability of salivary gland proteins from field populations of *P. papatasi* sand flies from the Middle East. Salivary gland cDNAs encoding secreted proteins were PCR amplified, sequenced and the results were compiled using various bioinformatics tools. For each protein, predicted MHC class II T-cell epitopes were obtained and compared to areas of amino acid sequence variability. Ten salivary gland proteins from *P. papatasi* were analyzed. Our results indicate greater sequence variability than was previously suggested and we have identified additional MHC class II T-cell epitopes. Mapping the amino acid variability and MHC class II epitope sites in sand fly salivary gland proteins is a crucial step in defining saliva-based vaccine candidates.

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TEMPORAL AND SPATIAL VARIATION IN ABUNDANCE OF THE MALARIA VECTOR *ANOPHELES* (*ANOPHELES*) *PSEUDOPUNCTIPENNIS* IN NORTHERN ARGENTINA

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Anopheles pseudopunctipennis is an important vector of malaria from Mexico to Argentina, yet very little is known about factors that control its distribution and abundance. Patterns of abundance of *An. pseudopunctipennis* and their relationship to climate variables were examined from monthly collections for four years in six rainforest locations in northwest Argentina, where this species is the principal malaria vector. A total of 5006 specimens was collected, the majority (98.3 %) from northern locations. Peaks in abundance occurred during spring in both northern and southern locations. Among climatic variables, maximum and minimum temperatures, average minimum humidity and accumulated precipitation were significant predictors of abundance of this species in north locations. In the south, only accumulated precipitation accounted for significant variation in abundance. A strong relationship was observed between abundance and latitude, *An. pseudopunctipennis* densities decreasing with increasing latitude. These relationships between geography, anopheline abundance and climatic variables contribute to a better understanding of the life cycle of this species in nature and, therefore, the improvement of local malaria control strategies.

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AN UNUSUAL CASE OF ALIMENTARY CANAL INFESTATION BY THE MILLIPEDE *BRACHYIULUS LUSITANUS* (DIPLOPODA) IN A FIVE-YEAR-OLD BOY: A CASE REPORT

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We report here the infestation of the alimentary canal of a five-year old boy by the millipede *Brachyiulus lusitanus*, a free-living organism which commonly feeds on organic matter. The boy expelled an estimated 150-200 millipedes over a period of 4-5 months without major pathologic effects. His parents' claims had previously been rejected as delusions by some physicians. The millipedes were frequently encountered in the family's surroundings and commonly entered their house, but the route by which they reached the boy's alimentary canal and their survival for such a long period of time remains enigmatic. This case is evidence that some individuals may truly have an undiscovered affliction which poses a problem, and that reports of unusual infestations should not be disregarded.

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SELECTIVE DELTAMETHRIN SPRAYING OF TRIATOMINE INFESTED HOUSES FOR THE CONTROL OF CHAGAS DISEASE IN SOUTHERN ECUADOR

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Chagas disease is endemic in ~70% of Ecuador. *Rhodnius ecuadoriensis* and *Triatoma carrioni* are the primary vectors of Chagas disease in Southern Ecuador. This study tested the effectiveness of selective domiciliary unit (DU) deltamethrin spraying of triatomine infested houses, coupled with community education activities and a community-based surveillance system. Eight communities with triatomine infestation rates (I)>7% and two uninfested communities were selected in Loja Province. A total of 466 DUs were visited by two-man field entomology teams that conducted 1 man/hour triatomine searches of the intradomicile (ID) and peridomicile (PD). Of these, 5.6% were infested with *R. ecuadoriensis (Density [D]* = 4 bugs/DUs searched*, Crowding [CR]* = 71 bugs/infested house*, Colonization Index [CI]* = 77% infested DU with nymphs*),* 8% with *T. carrioni* (*D* = 0.6, *CR* = 7, *CI* = 64%), 1.5% with *Panstrongylus rufotuberculatus* (*D* = 0.1, *CR* = 74, *CI* = 71%) and 0.6% with *P. chinai* (*D* = 0.02, *CR* = 2.7, *CI* = 0%). Infested DUs were sprayed (ID and PD) with 25 mg a.i./m^2 deltamethrin. Fifteen min educational talks were conducted in every DU and workshops for school children were organized. Subsequent visits were conducted 1, 6 and 12 mo. after spraying. At each time point new entomological searches were carried out in all DUs. All entomological indexes dropped significantly for the primary vector species one year after the initial intervention (*R. ecuadoriensis: I* = 2%, *D* = 0.1*, CR = 7, CI* = 100%; *T. carrioni: I* = 1.6%, *D* = 0.1, *CR* = 5.5, *CI* = 50%*)*. However, 7 and 2 previously uninfested DUs, and 3 and 6 previously sprayed DUs were found infested with adults and/or nymphs of *R. ecuadoriensis* and *T. carrioni,* respectively, at 6 and 12 months. Sylvatic populations of *R. ecuadoriensis* were found in one community, associated with 16% of 43 squirrel nests (*Sciurus stramineus*) examined (45 bugs). These data shows that the proposed control strategy is effective. However, there is a high risk of DU reinfestation, possibly from sylvatic habitats or other sources, that highlights the need for continuous surveillance and targeted vector control.

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EVALUATION OF NOVEL LONG-LASTING, INSECTICIDE-IMPREGNATED BED NETS TO CONTROL ADULT SAND FLIES (DIPTERA: PHLEBOTOMINAE) IN HUMAN LANDING STUDIES IN KENYA AND EGYPT

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Currently there are no vaccines or prophylactic drugs to protect military personnel against leishmaniasis. The only method available is to prevent bites from infected phlebotomine sand flies. An aggressive prevention and control program at Tallil Air Base, Iraq, included the use of permethrin-treated bed nets and DEET repellent, but had minimal impact on sand fly populations and did little to protect soldiers from the disease. The goal of our study was to evaluate the efficacy of long-lasting, insecticide-impregnated bed nets to control adult sand flies. Field studies were conducted in Kenya and Egypt using deltamethrin- or permethrin-treated nets, compared with non-treated nets. Human volunteers sitting inside bed nets caught sand flies landing on their legs, or provided bait to attract sand flies inside a double net, to approximate biting rates. These results are compared with previous results of light trap catches inside bed nets at the same locations. The results of these studies are discussed in the context of novel control methods, e.g. long-lasting, insecticide-impregnated bed nets, to prevent bites from sand flies.

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FAST-ID: FLIGHT SIGNATURE RECORDINGS OF *AEDES* AND *CULEX* MOSQUITOES FOR AUTOMATED SPECIES IDENTIFICATION AND VECTOR SURVEILLANCE

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APTIV Inc's FAST-ID™ is a novel emerging technology platform that efficiently integrates sensors, information system and wireless network technology to deliver real-time automatic unattended vector surveillance. FAST-ID is designed to count and identify individual flying insects. When an insect flies between a light source and photosensor, a flight signature can be captured by a datalogger. Individual insects are automatically identified by comparing their flight signature with a library of signatures using a statistical classifier or an artificial neural network. The integrated unit is compact, light-weight and field proven. APTIV built 24 units for 2004 research on moths and mosquitoes, and made >1000 hours of insect recordings in the field and laboratory, building a classification library of 56,784 mosquito signatures (15 species) and 4,702 moth signatures (7 species). The FAST-ID system will correctly identify seven different mosquito species (*Aedes aegypti, Ae. albopictus, Culex quinquefasciatus, Cx, pipiens, Cx. salinarius, Cx. tarsalis, Cx. nigripalpus*) in flight in the laboratory, including accurate discrimination of two sibling species, *Cx. quinquefasciatus* and *Cx. pipiens* in flight, which are currently only separated by intensive molecular techniques in specialized laboratories. The system will discriminate sex, age and physiological condition of flying mosquitoes. When fully engineered, this system will address a need in vector surveillance that was identified in surveys of the rural mosquito management community.

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COMPARISON OF ECOSYSTEMIC AND TRADITIONAL METHODS FOR THE LONG TERM CONTROL OF THE CHAGAS' VECTOR *TRIATOMA DIMIDIATA* IN JUTIAPA, GUATEMALA

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Triatoma dimidiata is the most important Chagas' disease vector in Guatemala. After a nationwide spraying campaing against this vector, control efforts are now focusing in the prevention of reinfestation. To test if the management of key environmental elements of the ecosystem could help assure the long term protection of the people, two different approaches to controlling this vector were compared in Jutiapa province. These were (a) "ecosystemic", based on a transdisciplinary effort aimed to identify key elements for environmental management, and (b) "traditional", based mostly on insecticide spraying. Here we summarize the main discoveries about ecosystem elements that were risk factors for intradomestic infestation, and give an overview of the impact that the control approaches had to reduce vector infestation and prevent reinfestation. 644 houses in 4 villages were surveyed for bugs and 17 variables observed in the houses were studied as risk factors. Significant associations were found between vector presence and house sanitary and construction conditions (which facilitate shelter for the bugs). Regardless of house age the odds of vector presence in clean and good quality houses were lower than for poorer, generally less hygienic houses. The odds of vector presence were lower in houses with completely plastered walls than in those with no or low quality plastering. In the ecosystemic villages, researchers and community members worked together to improve plastering formulations and hygienic practices, that were later implemented. In the other two villages, the population was informed about the disease and how they could protect themselves by cleaning and improving their houses. All four villages were sprayed with insecticides to reduce vector populations. Both approaches achieved a reduction in vector intradomestic infestation, but only the ecosystemic villages achieved high levels of house improvement (20% and 39% increase in better sanitation in houses and 13% and 20% increase in good quality wall plastering in the two villages, respectively) that could ultimately prevent reinfestation by the vector.

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FEASIBILITY, ACCEPTABILITY AND SAFETY OF ARTEMETHER-LUMEFANTRINE IN HOME MANAGEMENT OF UNCOMPLICATED MALARIA IN SOUTHWEST NIGERIA

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Artemisinin containing therapy (ACT) was recently adopted as first line drug for the treatment of Malaria in Nigeria. Apart from deploying artemether-lumefantrine (AL) at health facilities, the Federal Ministry of Health, Nigeria is committed to introducing artemether-lumefantrine at the community level using the home management of malaria (HMM) strategy. It thus becomes imperative that the use of this expensive and effective drug be safe guarded to prolong its usefulness and reduce the risk of emergence of parasites resistant to the drug. A quasi-experimental study designed to determine the degree of: compliance, acceptability, accessibility and safety that can be achieved using AL unsupervised was evaluated in 40 communities selected using multistage random sampling technique from Ona-Ara Local Government (LGA), Oyo State

in Nigeria. Sixty Community Medicine Distributors (CMDs) selected by these communities were trained. The CMDs trained and mobilised their community members, distributed treatment guidelines and free AL to caregivers with under-five febrile children. The study was evaluated a year after commencement of drug distribution using CMDs clients' records, two week fever recall survey and qualitative methods. CMDs' clients' records revealed that 1101 febrile children with mean age of 31.3 (16.3) months were enrolled. Proportion of caregivers to which correct dose of AL was dispensed and who reported CMDs gave instructions on drug use was 97.9%. The drop out rate of CMDs was 10.0%. The survey showed that 354/697 (50.8%) caregivers reported using AL and 89.4% of these used AL correctly. AL was perceived to be effective by 96.2% caregivers and 99.3% reported their children got well after using AL. Only 10/285 (3.5%) reported adverse events, which included body rash and vomiting worms. AL was commenced within 24 hours of noticing fever in child by 98.8% of respondents. The median time it took to walk to CMDs' place was 5 mins and 85.3% of caregivers reported receiving AL at first visit to CMD. The responses from the qualitative study corroborate findings at household survey. AL was found to be acceptable, very effective and safe. The CMDs were judged to be easily accessible, committed and efficient. The level of coverage and compliance achieved is encouraging for a newly introduced drug and demonstrated the feasibility and acceptability of using AL in the context of HMM.

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FEVER AND THE RECENT INTERNATIONAL TRAVELER PRESENTING TO THE EMERGENCY DEPARTMENT

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Last year alone there were 842 million international travelers with more than 50 million of those people traveling from the industrialized world to the developing world. Of these, nearly three percent reported a fever that required immediate medical attention. Emergency Departments are often a common place where travelers seek medical attention. St. Luke's Roosevelt Hospital Center is a large urban tertiary medical center located on the Upper West Side of Manhattan. A retrospective chart review of the electronic medical record of all patients presenting to the emergency department at the St. Luke's Division was performed. The data was collected between April and December of 2006. All patients presenting for triage to the emergency department were noted if they had a documented fever of greater than 100.4 and were asked the following questions: • Have you traveled outside of the United States within the last 7 days? • Have you had any contact with someone how as been traveling internationally within the last seven days? • Have you had any contact with birds within the last seven days? A total of 75,869 patient medical records were reviewed with the following results: • 4,956 patients presented with a fever as defined above 100.4 • 286 (5.7% of patients presenting with fever) admitted to having traveled internationally within the previous seven days or having had contact with someone who had traveled • 13 (0.26% of the fever population) patients had had recent contact with birds. In conclusion, it is not uncommon for febrile travelers to initially present at an emergency department to seek medical care. It is important that emergency medicine professionals are aware of this and identify a recent travel history, which will in turn allow them to consider an appropriate broader differential diagnosis. This may allow for a more rapid and accurate diagnosis of a number of tropical and emerging infectious diseases, and the recognition of a possible pandemic influenza outbreak.

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ASSESSMENT OF A TREATMENT GUIDELINE TO IMPROVE HOME MANAGEMENT OF MALARIA

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A quasi-experimental study was carried out in three rural health districts in Ona-ara Local Governent of Oyo State, Nigeria to determine the effectiveness of a treatment guideline in the treatment of febrile children ≤10 years using chloroquine. Baseline and post-intervention household survey, focus group discussion (FGD) and key informant interview (KII) were conducted. The intervention strategy was based on training a core group of mothers in selected communities and distributing a newly developed treatment guideline to each household. Mothers were expected to purchase chloroquine from their usual sources. Knowledge and awareness of cause, prevention and treatment of malaria increased with the intervention. Many, 70.4% of the respondents mentioned they used the guideline each time a child was treated for malaria. Evaluation of the impact of the guideline showed a significant overall increase in the correctness of use of chloroquine from 3.2% at baseline to 37.1% after intervention [OR=0.06; 95% CI 0.02-0.13, p<0.001]. The highest increase was among those who treated children at home in the intervention arm, 2.6% to 52.3% [OR=0.02, 95% CI 0.00-0.008, p<0.001]. The correctness of use was significantly associated with use of the guideline [OR=44.63, 95% CI 7.63-433.4, p<0.001]. The timeliness of commencing treatment was significantly much earlier in those who treated at home using chloroquine than those who took their children to the chemist or health facility (p<0.005). Majority, 91.6% mentioned that the guideline provided knowledge on correct use of chloroquine. FGD and KII corroborated these findings and showed that community members considered the guideline to be explicit and useful; mother trainers to be effective and acceptable. In conclusion, the use of the guideline with adequate training significantly improved correctness of malaria treatment with chloroquine at home. Adoption of this mode of intervention is recommended to improve compliance with drug use at home.

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USING "MOTHER TRAINERS" FOR MALARIA CONTROL: THE NIGERIAN EXPERIENCE

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Studies of care seeking in Nigeria show that a tremendous amount of treatment for malaria takes place at home and in most instances, such treatments are incorrect. This deficiency is attributed to caregivers' poor knowledge of treatment. This study was designed to empower households to treat malaria correctly in partnership with community members. Selected mothers from study communities were trained as "mother trainers" and were expected to train other members of their communities using a treatment protocol. "Mother trainers" were acceptable to most communities and judged to be effective. They were enthusiastic and their participation in the study boosted their ego and status in the community. Drop-out-rate of "mother trainers" was 24.2%. A few limitations to the use of mothers as trainers that were identified are discussed. It is concluded that mothers have good potential to effectively carry out health education activities in the community if appropriately selected, trained and supervised.

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COMMON INFECTIOUS AND NON-INFECTIOUS DISEASES AMONG RECENT IMMIGRANTS AT THE SOUTH BRONX'S REFUGEE AND IMMIGRANT CLINIC

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The Refugee and Immigrant Clinic at Montefiore Medical Center in south Bronx has been evaluating and supporting immigrants and refugees since 2004. We evaluated history, clinical presentations, and result of laboratory analysis of the 80 recent immigrants in our inner city clinic to assess the need for screening strategies in this population. Mean age was 22.4 (18-89 yrs) mostly from central and south America and west Africa. 50 % were men. Mean years in the U.S was 2 year. 22% were only Spanish speaking, 12 % French, and 7% Arabic. 26% had no job. 64% had no insurance. 26 % had high blood pressure at exam but only 6 with history of Hypertension. 5% were current smoker and 5% had Asthma. Two had history of Malaria, one Typhoid, and one with history of TB. 6% had been tortured in the past, and 5% had Depression. Laboratory analysis showed no positive syphilis test, one positive HIV and one positive HBSAg. 46% were exposed to hepatitis B, and 32 % had positive HBS Ab (immune to Hepatitis B after exposure). 7% had abnormal liver enzymes. 15% had Eosinophilia in blood. 12 had Ova/parasite in stool but only 2 with Eosinophilia. Out of 12 exposed to TB, only 2 had abnormal chest x-ray findings consistent with old TB. In conclusion, our findings suggest the need for screening strategies for infectious and chronic diseases as well as better social services.

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THERMOTHERAPY VERSUS MEGLUMINE ANTIMONIATE IN THE TREATMENT OF CUTANEAOUS LEISHMANIASIS IN MALI

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In Mali, the so called "bouton d'Orient" (ulcero crusted lesion) is the most common presentation of cutaneous leishmaniasis. Intralesional meglumine antimoniate (Glucantime®) is the first line drug against cutaneous leishmaiasis and can be effective against *Leishmania major* or *Leishmania tropica*. However, its cost, accessibility and related adverse effects, pose serious problem in developing countries. This study was aimed to assess the effectiveness of thermotherapy in the treatment of cutaneaous leishmaniasis in Mali. Two groups of patients were treated either with Glucantime® or with thermotherapy once a week. During the first 6 months of the study, we treated all cases of cutaneous leishmaniasis with Glucantime®. Thermotherapy was used from the 6th and the 12th month. Glucantime was used intra-lesionally at the dosage of 20mg/kg/week. Each lesion was injected at three points surrounding the lesion. Thermotherapy using a thermomachine® consisted in application of 50°C for 30 seconds on three points surrounding each lesion. Patients were follow-up once a week for treatment and evaluation of skin lesion. The evaluation was graded as follows: Marked improvement if all lesions were healed and without any wounds, fair improvement if some lesions healed or residual wounds seen and no improvement. Results: Overall, 112 patients were included in the study: 47 patients were treated with Glucantime®, and 65 patients by thermotherapy. The sex ratio was 1,4 with 58 % female. The mean age was 23 years (varying from 1 to 75 years old). Rapid complete re-epithelialization was observed with patients who received thermotherapy (% within 4 weeks) than those with glucantime (5 weeks). ($p<0.001$) No adverse event was observed with patients who received both thermotherapy or Glucantime. However, the Meglumine Antimoniate injection was reported more painful in the children than in adult as compared to thermotherapy. In conclusion, thermotherapy is an effective treatment of *L. major* cutaneous leishmaniasis. It is well-tolerated, and provides rapid healing of CL lesions and it should be promoted in endemic areas where access to drug is very difficult.

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CLINICAL PROFILE OF DENGUE OUTBREAK IN A DEVELOPING NATION (2003-2005)

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This study was undertaken to determine the clinical profile of patients presenting with Dengue fever during outbreaks in a developing nation. All adult HCW with short duration of fever, satisfying WHO dengue diagnostic criteria, found positive for Ig M anti-dengue antibody on capture ELISA (PanBio, Brisbane Australia) were included in the study. Patients with pneumonia, malaria or other identified cause of fever, and primary hematological disorders were excluded. This observational study was conducted in the emergency department of an academic urban hospital from July 2003 to March 2005. All patients underwent detailed clinical evaluation including laboratory and radiological investigations towards symptoms of dengue. Statistical analysis was done using SPSS version 10. Out of 375 eligible subjects with fever were screened for the study; 294 cases were defined as DF 145 (49.4%), DHF 123 (41.8%) and DSS in 26 (8.8%) cases. The predominant presentations were fever (100%), morbiliform rash (26%), abdominal pain (17%), seizures (2%), retrorbital pain (1%) and bleeding manifestations (49%). Bleeding manifestations were petechiae (48%), positive Hess test (14%), and epistaxis (11%). Den 2 serotype was identified on PCR. In DF, DHF and DSS; the mean (range) serum bilirubin [0.81 (0.3-1.8), 0.88 (0.4-1.8), 0.91 (0.6-1.8)] mg/dl ($p=0.5$), AST [121(23-445), 121(23-509), 119(67-498)] ($p=0.06$), ALT [110 (33-330), 111(30-452), 107(48-376)] ($p=0.04$) and serum alkaline phosphatase (SAP) [135(60-340), 126(56-280), 152(108-180)] ($p=0.2$),TLC [5(2-13), 4.7(1.3-10), 4.9 (2,5-11)] X 103 cells/cumm and mean Platelet count (Coulter method) [50(11-160), 46(7-157), 42(10-99)] X 103 cells/cumm were noticed. All patients except three (1%), who succumbed to excessive bleeding, recovered without sequelae. In conclusion, fever, rash, thrombocytopenia, elevated transaminases and decreased mortality rate were the predominant manifestations. Statistically significant elevation of transaminases along with normal serum bilirubin and SAP was observed in all categories (DF, DHF, and DSS). Liver dysfunction was reported irrespective of the severity of disease. Effective preventive measures controlling the regular outbreaks of Dengue are warranted to minimize the mortality and morbidity

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CASE REPORT OF A TRAVELER WITH *LEISHMANIA PANAMENSIS* TREATED WITH MILTEFOSINE

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While studying organic farming techniques in various locations in Ecuador for three months, a 19 year old woman developed multiple ulcers on her shoulder, face, ear, wrist and hand. She was treated locally with Meglumine antimonate for presumed leishmania, but she stopped treatment due to side effects of local pain at the injection site, nausea, myalgia and fatigue. Upon return to the United States, evaluation of the ulcers, including punch biopsies for culture, touch preps, and aspiration of fluid confirmed the presence of *Leishmania panamensis*. Treatment was delayed due to continued travel by the patient, during which the lesions became larger and superinfected with *Staphylococcus aureus*. In light of recent reports of the efficacy of Miltefosine against American Cutaneous Leishmania due to *L. panamensis* and the side effects already experienced with Meglumine, the patient was offered treatment with Miltefosine. She was treated with a dose of 2.5 mg/kg/day for 28 days. The patient exhibited rapid and steady clearing of the ulcers over a 6 to 8 week period. There were no changes in hematologic, liver or renal function

during treatment. Side effects included mild nausea and intermittent disequilibrium. The role of Miltefosine in patients with *L. panamensis* is discussed, especially in regard to the possibility of late relapse and development of mucocutaneous leishmania.

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REPORT OF THE UNITED STATES - EUROPEAN COMMISSION WORKSHOP "ADVANCES IN IMMUNOLOGY AND VACCINE DISCOVERY"

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On 12-14 December, 2006, more than eighty scientists from the European Union and from the United States, representing the fields of immunology and vaccinology, gathered at the National Animal Disease Center in Ames, Iowa, to discuss, design and prioritize initiatives for vaccine research. The workshop was organized under the auspices of the US- EC Task Force on Biotechnology Research. The goal was to bring together US and European experts to address the large class of infectious agents that have proven refractory to classic vaccine approaches, including parasitic infections, many chronically-infecting bacteria, fungi and viruses, and many additional infectious agents for which vaccines do not exist or are otherwise less than optimal. Tropical infections represent one of the largest categories of diseases where the vaccine armamentarium is inadequate. Workshop organizers envisioned that expanding knowledge in the field of immunology could be tapped to conceive of novel approaches and strategies for progress in vaccine development against these agents. In an effort to broaden the workshop's perspective, the organizers juxtaposed state-of-the art presentations in six diverse "focus areas" where new discoveries could potentially impact vaccine design: immune evasion, innate immunity, mucosal immunity, immunogenetics, comparative immunology, and genomics. The workshop examined the premise that research in these areas will generate novel ideas for vaccine design, and that these ideas could be advanced by immunologic and genomic tools. Furthermore, the workshop promoted cross-fertilization between veterinary and human medical research by pairing presentations in the human and veterinary fields, with the intent to further catalyzing progress in vaccine discovery. The proceedings of the workshop are now available in hard copy and will soon be posted on the web. Highlights of the workshop and the research priorities identified by the participants will be presented, focusing on those most relevant to tropical medicine.

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THE RELATIONSHIP BETWEEN MALARIA MORBIDITY AND AVAILABILITY OF HEALTHCARE FACILITY IN PARTS OF THE IMO RIVER BASIN, SOUTHEASTERN NIGERIA

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This study was carried out to determine malaria morbidity rates and patterns in relation to the availability of health care facilities in 6 communities in the Lower Imo river Basin, Southeastern Nigeria. A total of 1219 children (age <5 years) were examined for malaria parasitemia using the thin and thick blood smear methods. Malaria specific signs and symptoms among children were determined using structured questionnaires and key informant discussions. Ethical clearance was obtained from the Institutional Review Board of Imo State University, Owerri, Nigeria while informed oral consent was obtained from parents of the children involved in the study. The results showed that the average malaria prevalence rate among children was 25.8%. Oboama (60.7%) and Aboh (29.3%) communities recorded the highest and lowest morbidity rates respectively. Mean anemia, enlarged spleen and cerebral malaria prevalence rates were 24.5%, 9.7% and 9.1% respectively and were higher in communities without health care facilities than those with health care facilities. This study indicates that accessibility to health care service is an important determinant of malaria morbidity among rural communities in Southeastern Nigeria.

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RISING TREND OF CARDIOVASCULAR DISEASES AMONG SOUTH WESTERN NIGERIAN FEMALE PATIENTS

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Developing world though faced with increasing burden of cardiovsacular disease has the least contribution in articles published on cardiovascular research. There is particularly paucity of report on the trend of cardiovascular diseases. The aim of this study is to determine the trend of cardiovascular diseases and the gender effect. A retrospecive analysis of medical admission in a public, secondary healh center over a 5-year period (1997-2001) was carried out. Of the 2474 patients, 37.0%, with a mean age of 54.9(14.6) years had cardiovascular diseases, 51.3% were females. Mean duration of hospital stay was 11.8(9.1) days, range 1-90days. The was a significant steady rise in the trend of cardiovascular disease which was higher among females (p=0.003). Hypertension and hypertension related complications constituted the bulk of cardiovascular diseases. Overall mortality was 155 (17%) for cardiovascular diseases although not significantly different gender wise, was higher in males (86, 55.5%), p=0.063. In conclusion, targetted research and control of cardiovascular diseases among women may enhance control of the menace of cardiovascular diseases.

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DOXYCYLINE NONCOMPLIANCE RESULTS IN SEVERE AND COMPLICATED FALCIPARUM MALARIA IMPORTED FROM CHAD

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Methodology required to describe antimalarial drug resistance demands adherence to rigorous clinical and parasitological criteria. Bona fide Doxycyline (Doxy) resistant falciparum malaria has yet to be described: the drug is partially active against liver stages, there are no established molecular markers for blood stage resistance, the drug has a short half-life (drug levels are of little value), and there is stage and time dependent drug activity with significant variability in concentration response. The DoD GEIS program provides unique opportunities to exploit our capabilities for elucidating the mechanisms of resistance to better predict emerging drug resistance in the field. Clinicians providing post-deployment treatment of fever-producing illnesses returning from malaria-endemic countries are asked to consider falciparum malaria in their differential diagnosis. Soldiers with suspect fevers are evaluated by asking the following questions: Are they taking antimalarials and what was the date of last dose? Has the blood slide been read by a competent microscopist to confirm diagnosis? Is travel (exposure) history clear cut (i.e., has the service member also been in additional malaria endemic areas such as Africa, Iraq, Afghanistan, Korea, Asia or South America during the preceding 12-24 months)? ETDA purple top specimens (usually the admission CBC specimen) from suspect prophylaxis failures are aseptically collected before therapy and shipped on

wet ice (not frozen) to the WRAIR for evaluation. In the fall of 2006, two marines returned to Camp Lejeune, NC, and presented with severe malaria following six weeks deployment to Chad and admitted missing several doses of daily Doxy during and after the deployment. Parasites were markedly susceptible to chloroquine, desthylchloroquine, quinine and quinidine. WRAIR/Sigma Tau's new drug for severe malaria (artesunate) was up to 20 fold more potent than quinidine. The Doxy response was 2-4 less than control clones from Sierra Leone or Indochina. This dose response mimics that of the 1981 NF54 airport malaria strain with West African origins, a simple wild type strain that is highly susceptible to all known drugs, yet is potentially lethal to unprotected travelers. Efforts are currently underway to evaluate alternative salts of Doxy with less gastrointestinal side effects to improve compliance.

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CLINICAL, LABORATORY, AND MOLECULAR DETERMINANTS OF EPIDEMIC SEVERE AND COMPLICATED MALARIA (SCM) IN THE PERUVIAN AMAZON

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Seventy-six patients (47 adults, 21 children, 8 pregnant women) were entered into a hospital-based severe and complicated malaria (SCM) study in Iquitos, Peru in 1998-1999. All patients received IV quinine, and had daily clinical, lab, and parasitemia evaluations. Clinical symptoms/signs of fever, chills, headache, and vomiting were present in a majority of patients, while nausea, hepatomegaly, and splenomegaly were present in a minority. All patients had a clinical and most (74/76 - 97%) were slide positive for *Plasmodium falciparum*. PCR for speciation and drug susceptibility markers were obtained from 66 of 76 patients (87%); 61 were PCR-positive for *Pf* alone and 5 were co-infected with *Pf/P. vivax*. Genotyping of *Pf dhfr/dhps* showed that all 66 *Pf* isolates had the 108 mutation. One sample had a multi-clonal infection with mixed *dhfr/dhps* genotypes. Of the remaining 65 samples giving clean sequence data, 9 had a just single mutation at 108 and 56 had between 4M and 8M indicating high level of resistance to SP, which was the first line therapy for non-complicated *Pf* during this time. The mortality rate of study patients was 19.1% (9/47) for adults, 25% for pregnant women (2/8), and 9.5% (2/21) for children. Nine of 13 patients that died had *Pf* isolates with 7M in *dhfr/dhps* compared to 1/13 having just 108. No samples were available from the other three but they were diagnosed with clinical SCM. Clinical predictors of death were a Pf parasitemia ≥ 200,000, and a hospital length of stay ≤ 3 days.

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CORRECTION OF CENTRAL NERVOUS SYSTEM METABOLIC ABNORMALITIES, DEFICITS IN EXECUTIVE COGNITIVE FUNCTIONING AND ELEVATED C4A: A CLINICAL TRIAL USING LOW DOSE ERYTHROPOIETIN IN PATIENTS SICKENED BY EXPOSURE TO WATER-DAMAGED BUILDINGS (WDB)

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Recent studies demonstrate that erythropoietin (epo) is a neuroprotective agent for central nervous system (CNS) that prevents apoptosis of glial cells, improves capillary hypoperfusion and lowers elevated lactate in CNS. Previous studies of patients made ill solely by exposure to WDB ("mold illness") with refractory executive cognitive symptoms and elevated levels of C4a, a product of activation of complement, using epo safely lowered C4a and reduced neurocognitive symptoms. A prospective clinical trial was performed to (1) assess safety of epo in mold illness patients with elevated C4a; (2) efficacy of epo to improve symptoms, reduce C4a and correct abnormalities in metabolites measured by magnetic resonance spectroscopy (MRS); (3) provide data that supports a testable hypothesis of the inflammatory origin of systemic and CNS symptoms. 32 patients with mold illness provided informed consent for an IRB-approved study. Symptoms of executive cognitive function, C4a and MRS of 1 cubic cm areas of frontal lobes and hippocampus before and after treatment with 5 doses of 8000 units of epo given by the study physician over 2 weeks were compared to known controls. Symptoms, C4a and safety parameters were recorded at each visit. After 5 doses of epo, repeat MRS was performed. Epo use did not cause adverse effects: No adverse effects of clotting, elevation of blood pressure, polycythemia or development of iron deficiency anemia occurred. Symptoms of executive cognitive function were reduced in cases after treatment, though still exceeding controls. C4a was reduced beginning after the second dose of epo, achieving values equal to controls in 91% of cases. MRS-determined values of n-acetyl acetate; creatine; choline and myoinositol did not change in cases and equaled controls. Lactate was elevated in all patients, with reduction after epo to controls in 88%. Ratios of glutamate to glutamine were abnormal in all cases, with reduction to controls achieved in 75%. Use of low dose epo in mold illness patients is safe and effective to improve symptoms, C4a and CNS markers of abnormal capillary hypoperfusion (lactate); and excitatory neurotransmission (glutamate/glutamine). These results suggest that the systemic inflammation in mold illness caused by elevated C4a may be treated using epo and that the CNS correlates of cognitive dysfunction has an inflammatory basis. A double blinded, placebo controlled trial is planned.

(ACMCIP Abstract)

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DEFINING MOLD ILLNESS IN CHILDREN: A CHRONIC INFLAMMATORY ILLNESS WITH DISTINCTIVE BIOMARKERS

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Study of illness acquired following exposure of children to water-damaged buildings (WDB) has been hampered by absence of a case definition. Adults are defined by a two-tier model that includes (1) potential for exposure, presence of multiple symptoms from multiple organ systems and absence of confounders; and (2) presence of three of six objective parameters including reduced level of α melanocyte stimulating hormone (MSH); presence of a particular HLA DR haplotype; elevated MMP9; presence of a particular deficit in visual contrast testing (VCS); and dysregulation of ACTH/cortisol or ADH/osmolality. Tier 1 also applies to children. Tier 2 criteria required modification, as children may be unable to perform VCS testing and hypothalamic/pituitary axis immaturity may be present. We surveyed symptoms and lab results from known cases and controls from one practice to identify factors to correctly classify all cases and controls. 144 known pediatric cases with illness and 47 control patients were analyzed by chart review. Significant differences in symptoms, MSH, HLA DR by PCR and MMP9 were identified. Significant differences in incidence of antibodies (IgA and IgG) to gliadin as well as autoantibodies to cardiolipin (IgA, IgM and IgG) were identified. Cases were stratified by ability to perform VCS testing, as before age 8 most children couldn't perform VCS consistently. By age 8, nearly all children could perform VCS. Levels of C4a, a split product of complement activation were significantly different. Higher than 2830 ng/ml also were significantly different in cases compared to controls. Symptoms were analyzed by logistic regression, correctly classifying 189 of 191 patients. VCS deficits (N=110) correctly classified 102 of 110 patients. MSH levels < 35 pg/ml were found in 127 of 133 cases and 2 of 23 controls. MMP9 levels > 332 ng/ml were present in 100 of 105 cases and in 5 of 20 controls. Gliadin antibodies were found in 58% of cases and in no controls; autoantibodies to cardiolipin were found in 27% of cases and in no controls. C4a > 2830 was found in 33/33 cases and in 1/8 controls. Using HLA, MSH, antibodies to gliadin or cardiolipin, MMP9, VCS, all

cases were identified and all controls were identified correctly (N=110). For those unable to perform VCS, presence of 2 or 5 criteria identified all cases and controls correctly (N=71). These Tier 2 requirements will possibly be enhanced by adding C4a values in those unable to perform VCS testing.

(ACMCIP Abstract)

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SEQUENTIAL UPREGULATION OF INNATE IMMUNE RESPONSES DURING ACUTE ACQUISITION OF ILLNESS IN PATIENTS EXPOSED PROSPECTIVELY TO WATER-DAMAGED BUILDINGS (WDB)

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Previous data demonstrated a pattern of innate immune inflammatory responses following re-exposure of patients made ill previously by exposure to a given WDB with evidence of amplified growth or toxigenic organisms, including fungi. This report expands those observations, using a prospective model that confirms causation of illness by exposure marked by upregulation of innate response elements measured daily following re-exposure including complement activation product C4a, leptin, MMP9, vascular endothelial growth factor (VEGF) and coagulation factors. Following consent, 60 patients known to have a chronic biotoxin illness caused by exposure to a WDB followed a five step process: assessments of (i) symptoms (ii) VCS (iii) C4a (iv) leptin (v) MMP9 (vi) VEGF (vii) Factor VIII (viii) vWF (ix) vWF Ag were carried out at (1) baseline; (2) after first therapy with cholestyramine (CSM) (3) off CSM, without re-exposure for three days (4) after each of three days following re-exposure to suspected WDB (5) after second CSM treatment. Results were compared to known controls. In patients (N=38) with illness recrudescence, upregulation of innate immune elements was observed: C4a increased after 24 hours; leptin increased after 24 hours; MMP9 increased after 48 hours; VEGF initially increased after 24 hours, falling after 72 hours. Factor VIII fell concomitantly with the rise in C4a; vWF fell after 72 hours. Episodes of epistaxis or hemoptysis were observed in 6 patients, coinciding with fall of vWF. Symptoms and VCS decline increased daily during re-exposure, reaching baseline levels after three days. Patients (N=22) without recrudescence showed no changes and equaled controls. Buildings with repeat illness patients continued to have evidence of ongoing water intrusion; sites without reacquisition had no evidence of ongoing water intrusion. Re-exposure to WDB causes illness that can be identified by sequential changes in symptoms, VCS and innate immune responses. Use of sequential observation of symptoms, visual contrast sensitivity (VCS) and inflammatory responses following re-exposure to WDB can not only supports a model of disease mechanisms but can rapidly determine safety for re-occupancy.

(ACMCIP Abstract)

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CLINICAL AND EPIDEMIOLOGICAL STUDIES OF ANTERIOR SEGMENT DISORDERS DUE TO ONCHOCERCIASIS IN IMO STATE, NIGERIA

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A clinical and epidemiological assessment of Ocular Onchoceriasis with respect to Anterior Segment Diseases (ASDs) was carried out in 7348 consenting persons living in 38 rural communities in the Imo River Basin of Nigeria between March 2002 and September 2004. The results showed that 40.0% (2934/7348) of the subjects had one form of ASDs. Blurring of vision (10.1%) was the most prevalent disorder followed by anterior uveitis (8.5%) while the least was blindness (with no light perception) (0.2%). ASDs were absent in the 0-9 year group but increased in relation to microfilarial density (mfd) in the older age groups. Sex-prevalences of ASLs showed insignificant borderline differences in males and females ($p>0.05$). Blindness was weakly associated with mfd ($r=0.32$; $p>0.038$), while sclerosing keratits ($r=0.67$; $p>0.001$), punctuate opacity ($r=0.53$; $p<0.001$) and anterior uveities ($r=0.64$; $p<0.001$) were strongly associated with mfd. The results show that Ocular Onchocerciasis is a significant public health problem in the rainforest zone of Nigeria. This finding underscores the need for sustenance of control efforts in the area using Community-Directed Treatment with Ivermectin (CTDI).

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IMPACT OF REPEATED ADMINISTRATION OF ACTS ON SAFETY, EFFICACY AND INCIDENCE OF UNCOMPLICATED MALARIA IN MALI

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Most African countries have now changed their first line treatments for malaria from monotherapies to artemisinin-based combination therapies (ACTs). ACT efficacy measured at Day 14 or 28 may not adequately reflect true public health impact. Therefore, it is important to assess the efficacy and safety of repetitive administration of these new combinations in the African context in longitudinal trials. In randomized controlled Phase IV longitudinal trial in Bougoula-Hameau, Mali, we compared the impact of repeated administration of AS/AQ, AS/SP and AR-L for the treatment of consecutive episodes of uncomplicated malaria on the incidence density of uncomplicated falciparum malaria. The long-term efficacy of each of these regimens in this context of repeated administration was measured. Laboratory and clinical adverse events were recorded. Patients aged 6 months and above were eligible. To date, we have screened 3850 subjects and included 780 patients (260 per arm) who have experienced a total of 1833 cases of uncomplicated malaria over 18 months. The incidence density was similar between AS/SP and AS/AQ but significantly higher in the AR-L group (21.2; 21.6 and 28.4 per 100 persons -months for AS/SP, AS/AQ and AR-L, respectively; $p< 0.001$). The three groups were comparable with regard to PCR-corrected day 28 efficacy (ACPR >98%). Non-PCR corrected treatment efficacy was stable over time in the AS/SP and AS/AQ arms. However, in the AR-L arm, adequate clinical and parasitological responses were significantly lower during the peak of malaria transmission ($p < 0.05$). Safety analyses are underway and results will be presented at the meeting. The implications of these observations for large-scale ACT deployment in Africa will be discussed.

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COST ANALYSIS FOR *SCHISTOSOMIASIS* CONTROL IN PLATEAU AND NASARAWA STATES, NIGERIA

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Schistosomiasis is a parasitic disease affecting 200 million people worldwide. Urinary (*Schistosoma hematobium,* SH) and intestinal (*S. mansoni,* SM) schistosomiasis are coendemic in Nigeria. Since 1999 the ministries of health of Plateau and Nasarawa States, assisted by The Carter Center, have provided mass drug administration (MDA) with praziquantel (PZQ) for SH in villages with microhematuria in >20% of school-aged children (46% of villages) following 1993 WHO guidelines. In a limited survey for SM in 30 villages not qualifying for MDA for SH,

we found 57% warranted MDA for SM (for >10% prevalence, using 2002 WHO guidelines). A total of 81% of all villages required treatment for either SH or SM (2/3 of villages required school-aged MDA while 1/3 required community-wide MDA for prevalence of infection>50%). We subsequently performed an analysis, based on data from that study, of the relative costs of screening for SH, SH + SM, or treating without screening. We postulated a model health district with a total population of 30,000 in 60 villages. 400 people/village, including 100 school-aged children, were eligible for PZQ if MDA was given. We applied the overall SH and SM prevalence determined in our previous studies to this model. We calculated the cost of screening a 30 child sample in each village for SH (urine dipstick), SH and SM (urine dipstick and Kato-Katz fecal exam), and for presumptive treatment with PZQ, using field costs from our previous study. We used $0.20 for an average treatment of 2.5 tabs as the cost of PZQ. We calculated both year one 'assessment' costs, and 2 additional years' 'running costs' after baseline community diagnostics for four scenarios: SH screening and treatment, SH +SM screening and treatment, presumptive treatment of all school aged children, and presumptive treatment of all eligible persons. First year assessment costs were greatest for SM/SH (US$20,498) due to high costs for Kato-Katz, almost four times that needed for SH screening ($5,260). The cost of 3 years of treatment (including assessment costs) were $38,800 for presumptive community-wide MDA, $29,041for SH + SM, $10,714 to screen and treat for SH, $9,300 for presumptive MDA for school-aged children. Costs of implementing different WHO guidelines given over the past decade will be presented. We conclude that presumptive MDA of PZQ to all school-aged children is the most cost-effective method for controlling schistosomiasis in Plateau and Nasarawa States.

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RANDOMIZED COMPARISON OF AMODIAQUINE-SULFADOXINE/PYRIMETHAMINE, ARTEMETHER-LUMEFANTRINE, AND DIHYDROARTEMISININ-PIPERAQUINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN BURKINA FASO

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Drug resistance necessitates alternatives therapies for malaria in Africa. Artemisinin-based combination regimens are widely advocated, but they are expensive and in limited supply. We compared the efficacies of amodiaquine + sulfadoxine-pyrimethamine (AQ+SP), artemether-lumefantrine (AL), and dihydroartemisinin-piperaquine (DP) to treat uncomplicated malaria in Bobo-Dioulasso, Burkina Faso.
We carried out an open-label randomized clinical trial. Patients aged 6 months or greater with uncomplicated malaria were enrolled and followed up for 42 days based on WHO guidelines. Patients were randomized to receive standard doses of the study regimens over 3 days. Primary endpoints were the 28 and 42-day risks of treatment failure unadjusted and adjusted by genotyping to distinguish recrudescence from new infection. A total of 519 (92.5%) of enrolled patients completed the 42-day study. Early treatment failures were seen in 5 patients treated with AQ+SP and two each with the other regimens. The day 28 risk of recurrent parasitemia, unadjusted by genotyping, was significantly higher with AL than with AQ+SP (20.1% vs. 6.2%, RD 13.8%, 95% CI 7.0-20.7%) or DP (20.1% vs. 2.2%, RD 17.9%, 95% CI 11.6-24.1%). Similar differences were seen when considering only children under 5 years of age (54% of study population) and when outcomes were extended to 42 days. Significant differences were not seen between outcomes with AQ+SP and DP. Recrudescences were uncommon (<5%) in all treatment groups. Fever clearance was slower with AL than with the other two regimens. Parasite clearance was slower with AQ+SP. Gametocytes appeared uncommonly (≤5% of subjects) in all groups. Hemoglobin rose after therapy in all groups, but on the last day of follow-up was significantly lower with AL. In summary, all regimens were highly efficacious in clearing infections, but considering risks of recurrent malaria after therapy, AQ+SP and DP were more efficacious than AL, the new national regimen, for the treatment of uncomplicated falciparum malaria.

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EFFICACY AND SAFETY OF AMODIAQUINE-ARTESUNATE AND ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN BURKINA FASO

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The extension of chloroquine resistance prompted the National Malaria Control Program in Burkina Faso to move to artemisinin based combination therapy Artemether lumefantrine and artesunate + amodiaquine. We assessed the efficacies of Artemether Lumefantrine (Coartem®) and that of Amodiaquine (Flavoquine®) + Artesunate (Arsumax®) for uncomplicated malaria treatment in Burkina Faso.
We conducted an open-label randomized clinical trial where patients aged 6 months or greater with uncomplicated malaria were enrolled and followed up for 28 days based on WHO guidelines. We randomized patients to receive standard doses of the study regimens over 3 days. The 28 risk of treatment failure unadjusted and adjusted by genotyping to distinguish recrudescence from new infection was the primary endpoint Of 119 enrolled patients, 107 (89.9%) completed the 28-day study. Two patients treated with Artemether Lumefantrine and one with Amodiaquine + Artesunate experienced early treatment failure. The day 28 risk of recurrent malaria, unadjusted by genotyping analyzed in per protocol, was similar in the two groups Artemether Lumefantrine and Amodiaquine + Artesunate (15.2% versus 16.5% RD 1.3% 95% CI [-12.6%-15.2%]) Similar differences were seen when considering only children under 5 years of age. The analysis of the adjusted result in intention to treat revealed that Amodiaquine + Artesunate was less effective than Artemether Lumefantrine (13.1% versus 8.6% RD 4.5%, 95% CI [-8%-17%]. Fever clearance was slower with AL than with the other two regimens. Gametocytes carriage was rare in all groups. All regimen were well tolerated. In summary, all regimens were efficacious in clearing infections, but they efficacy should be monitored.

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ANTIMALARIAL IGG AND IGG SUBCLASS RESPONSES IN CAMEROONIAN CHILDREN WITH SEVERE AND UNCOMPLICATED MALARIA

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Plasmodium falciparum is a major cause of childhood morbidity and mortality in sub-Saharan Africa. Protection from severe disease is thought to be mediated by cytophilic IgG antibodies. We therefore hypothesized that the subclasses of IgG produced against key malarial antigens would correlate with severity of disease in children. Accordingly, we measure levels of IgG (total), IgG1, IgG2, IgG3 and IgG4 to 11 vaccine-candidate antigens using a multiplex Luminex-based assay. Antigens included recombinant Merozoite Surface Protein (MSP)-1_{42}, MSP-2, MSP-3, Apical Membrane Antigen (AMA)-1, Erythrocyte Binding Antigen (EBA)-175,

and synthetic peptides from the Circumsporozoite Protein, Liver-Stage Antigen, and Ring Erythrocyte Surface Antigen. Sera were tested from well-defined groups of children including healthy controls (n= 43), those with mild disease (n= 55), and severe infections (n= 114). Increased levels of the antimalarial IgG subclasses were found in infected children compared to uninfected controls. Children with uncomplicated malaria had the same IgG subclass profiles as those with severe malaria, but mean antibody levels were generally higher in children with severe disease. Most children had mixed IgG1/IgG3 responses. Highest antibody levels were found to MSP-1_{42}, AMA-1 and EBA-175 with IgG1 being predominate with relatively low levels of IgG3. MSA-2 and MSP-3 induced nearly equal amounts of IgG1 and IgG3. Very few children produced IgG2 and IgG4 antibodies. In conclusion, our results suggest that antimalarial IgG subclasses to these antigens do not correlate with severity of malaria in Cameroonian children and that young children may have a reduced ability to produce IgG3 as previously suggested.

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MICROSPORIDIOSIS AND MALNUTRITION IN UGANDAN CHILDREN WITH PERSISTENT DIARRHEA

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The microsporidian fungus, *Enterocytozoon bieneusi*, causes significant morbidity in HIV positive adults due to persistent diarrhea, intestinal malabsorption and wasting. Although infection is common in children, the impact of microsporidiosis on the nutritional health of this vulnerable population has not been thoroughly examined. In this cross-sectional study, we investigated the effect of microsporidiosis on growth rates of Ugandan children aged <60 months with persistent diarrhea. Simple and multiple linear regression was employed to test whether the rate of change in weight or height differed in children with and without microsporidiosis. After simultaneously adjusting for the impact of sex, HIV and concurrent *Cryptosporidium* infection, microsporidiosis was independently associated with reduced weight gain but not linear growth, (p=0.014 and p=0.151, respectively). The predicted growth trajectory of children with microsporidiosis was such that, by age 5, these children were approximately 1.3kg lighter than children without microsporidiosis. While a causal role of *E. bieneusi* cannot be implied from this cross-sectional data, we present evidence that microsporidiosis is associated with reduced weight gain in children with persistent diarrhea. Further longitudinal studies are required to establish the direction of this association and to determine whether these sub-normal growth rates are followed by catch-up growth.

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AN IMPORTED CASE OF PEDIATRIC MELIOIDOSIS: SOMETIMES HOOFBEATS ARE ZEBRAS!

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Melioidosis is an infection endemic to Southeast Asia and Northern Australia. The causative pathogen is *Burkholderia pseudomallei*, a gram negative bacterium commonly found in water and soil; transmitted by cutaneous or respiratory routes. Classically, it presents with rapidly progressive pneumonia and sepsis in adults with underlying diseases, such as diabetes. It may also present with localized abscesses, which can be confused with *Mycobacterium tuberculosis*. Pediatric infections are less common, and more likely to be skin and lymph node abscesses, including acute suppurative parotitis. Children are less likely than adults to have underlying disease, but more frequently have recent history of viral illness such as dengue or influenza A. Prolonged therapy is often needed, as up to 10% of patients will still relapse after 20 wks antimicrobial therapy. Cases of disease in the returning traveler are rare, requiring a high index of suspicion to make the diagnosis. We present a case of retropharyngeal melioidosis in a pediatric patient with an extensive travel history. This previously well 13 year old girl presented to a Canadian pediatric tertiary care center with 6 weeks of fever, weight loss and new erythema nodosum. She had previously been evaluated in hospitals in two other countries, and had received eight courses of antibiotics. Features of the case will be highlighted in order to review the presentation of melioidosis in the pediatric patient, along with an overview of current existing literature regarding management. If unrecognized or misdiagnosed, melioidosis can be a progressive disease with a high mortality. Intrinsic resistance of *B. pseudomallei* to a number of antimicrobials, as well as the prolonged course of treatment needed for adequate therapy make diagnostic confirmation essential. Limited awareness of the disease and the potential for confusion with TB can create diagnostic pitfalls for the clinician. Increasing world travel has placed the impetus on physicians to be familiar with the common presentations, in order to include this pathogen in the differential of a previously well child with skin, organ or lymph node abscess.

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EOSINOPHILIA AS A DIAGNOSTIC TOOL FOR ANGIOSTRONGYLIASIS

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Angiostrongyliasis, caused by *Angiostrongylus cantonensis*, has three main clinical manifestations: eosinophilic meningitis, eosinophilic meningoencephalitis and ocular angiostrongyliasis. The diagnosis is made by identifying the worm or the presence of $\geq$ 10% eosinophils in the cerebrospinal fluid. Lumbar puncture, an invasive procedure, is the effective diagnostic tool. However, in some suspected cases experienced this painful procedure with the negative results. More than half of the cases showed some degree of eosinophilia. Therefore, We did an observational study at Srinagarind hospital, Thailand to demonstrate the likelihood of using eosinophilia as a diagnostic tool for angiostrongyliasis. Between Aug 1, 05 and Jul 31, 06, we observed 120 cases of meningitic form (105 adults and 15 children), 14 cases of adult meningoencephalitic form, 5 ocular cases, 14,481 adult controls and 4,172 child controls. The controls were the patients who visited outpatient department (Internal Medicine and Pediatric) and had the total white blood cell count between 4,000-20,000 cells/mm^3 during the same period. The peripheral eosinophilia was significantly different from the controls in meningitic (both adults and children; p value < 0.000001) and meningoencephalitic form (p value < 0.001). In adult meningitic form, the 704.5 eosinophils yielded sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and prevalence of 82.9%, 87.5%, 95.4%, 99.9%, 72%, respectively. The area under the reciever operating characteristic (ROC) curve was 0.91. In children, the 780.5 eosinophils provided sensitivity, specificity, PPV, NPV and prevalence of 93.3%, 90.3%, 96.6%, 100%, 36%, respectively with the area under an ROC curve of 0.97. In the meningoencephalitic form, the 713 eosinophils gave sensitivity, specificity, PPV, NPV and prevelence of 64.3%, 87.7%, 99.5%, 100%, 10%, respectively. The area under an ROC curve was 0.75. Therefore, the peripheral eosinophil level is a non-invasive diagnostic tool for meningitic and encephalitic angiostrongyliasis.

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LEPTOSPIROSIS IN NEPAL

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The geographic distribution of leptospirosis includes rural and urban areas, and it is thought that Nepal has in circulation human pathogenic leptospira. To date no national surveillance program in Nepal exists to establish the incidence of leptospirosis and the disease burden is poorly defined. This study reports the prevalence of hospitalized leptospirosis in military personnel participating in an efficacy trial of a hepatitis E virus (HEV) vaccine in Nepal. We screened 61 patients with IgM antibodies (Leptospira ELISA IgM, PanBio, Brisbane) with confirmatory microscopic agglutination testing (MAT) using a battery of 24 serovars from 20 serogroups. Overall prevalence of leptospirosis confirmed by MAT in this cohort was 10%. Prevalence of leptospirosis was 9.1% among clinical hepatitis cases and 10.8% among all febrile cases. The serovars most reactive by MAT were Bratislava, Autumnalis, Icterohaemorrhagiae, and Sejroe. Further studies including epidemiological studies and culturing clinical isolates are needed to establish the specific risk factors for developing disease and circulating serovars. In Nepal febrile illnesses as well as icteric syndromes should be screened or empirically treated for leptospirosis as prompt treatment can improve outcome.

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DEVELOPMENT OF A LEPTOSPIROSIS REAL TIME PCR BASED ON LIPL32

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Leptospirosis is a zoonotic disease with global dispersal caused by the spirochete *Leptospira interrogans*. Clinical manifestations of leptospirosis present a diverse range of conditions, but have been shown to include pulmonary hemorrhage, uveitis, and acute renal failure. Current diagnostic "gold standards", microscopic agglutination test (MAT), enzyme linked immuno-sorbent assay (ELISA), and culture growth often require long incubation periods, paired sera, and expensive, complex equipment. Real time polymerase chain reaction (RTPCR) diagnostic assays offer a quick and easily achievable test that can confirm the presence of *Leptospira* in patient samples. Using publicly available sequence data, we designed a primer/probe set capable of amplifying LipL32, a leptospire outer membrane protein closely associated with pathogenic strains of *Leptospirosis*. PCR conditions were optimized ([MgCl2], [Probe], and [Primer]) limit of detection/limit of quantification (LOD/LOQ), and specificity were examined against cultured pathogenic and non-pathogenic strains. Our limit of detection was 0.488pg of DNA from cultured serovar Bangkok/Australis. We found our primer/probe set only amplified our cultured pathogenic serovars (n=13) and none of our non-pathogenic serovars (n=3).

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INVESTIGATION OF POSSIBLE ROLE OF TOXOPLASMOSIS IN PATIENTS WITH FIRST EPISODE SCHIZOPHRENIA

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Specific aims of the study were to determine relationship and prevalence of toxoplasmosis in patients with first- episode schizophrenia (FES). A total of 113 subjects with FES and healthy individuals were enrolled in the study. Based on serologic tests, serum samples from 32 (43.8%) and 25 (34.2%) of 73 individuals were seropositive for *Toxoplasma gondii* in the sampled population by ELISA IgG and Sabin-Feldman Dye test (SFDT), respectively. Only one subject had low IgG avidity. Forty additional serum samples from control subjects who recruited from healthy individuals having without psychiatric diseases were obtained. Of them, 13 (32.5%) and 15 (37.5%) serum samples were seropositive for *T. gondii* by ELISA IgG and SFDT, respectively. In analysis of patients with FES, 17 (68%) of 25 SFDT positive serum samples had also positive by ELISA IgG test while 15 negative serum had positive by ELISA IgG. In control group, 11 (73.3%) of 15 SFDT positive serum samples were also positive by ELISA IgG test while two negative serum samples were positive by ELISA IgG. Of the 73 subjects, only one was found to have low avidity by ELISA IgG avidity test. Our subjects presented rarely (9.6%) history of toxoplasmosis-like symptoms (sub febrile/fever, myalgia, dizziness, headache) with or without lympadenopathy. Having history toxoplasmosis-like disease (χ^2= 4.089; p=0.050) and having cat (χ^2= 7.62; p=0.006) were statistically significant factors. Ratios of consumption of uncooked or raw meat in study and control groups were 35.0% and 7.5%, respectively (χ^2 = 10.7; p=0.001). Consumption ratios of uncooked goat milk / eggs in controls were found 0%, while 19.0% in the study group. The difference between these two groups was statistically significant (χ^2= 8.76; p=0.002). Generally, contacting with soil rate was 2.5% and 56.2% in the control group and the study groups, respectively (χ^2= 31.86; p<0.001) was found significant between study and control groups. In analysis of patients with FES, 25 (34.2%) of 73 was found seropositive by reference test (SFDT) although there is not significant difference between control group and study group (χ^2 = 0.12; p=0.729). Our results implied that the latent toxoplasmosis might be in fact a very serious and greatly negligible public health problem. We need to perform more studies about association between *T. gondii* infection and patients suffered from schizophrenia.

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DIAGNOSIS OF ACUTE FEBRILE ENCEPHALOPATHY IN ADULTS: WHAT REALLY HELPS THE CLINICIAN?

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This study was undertaken to find the most useful tool to establish the diagnosis in febrile comatose adult patient. In this prospective study, we enrolled 71 patients presenting to the medical emergency with fever of less than 15 days duration with altered sensorium. After detailed history and examination all the patients were subjected to routine investigations, detailed CSF analysis, CT scan (NCCT followed by CECT) and MRI brain. Final diagnosis was reached after taking into consideration of the clinical findings, CSF analysis imaging results and response to therapy. The yield of clinical examination, CSF analysis and radiological investigations were compared against the final diagnosis. CNS infections were the most common cause seen in 74.6% patients. 29.6% patients had pyogenic meningitis, 18.3% had encephalitis, 19.7% had evidence of meningoencephalitis. In 11.3% patients' final diagnosis could not be made. The rest 21% were combined together in the miscellaneous group. Clinical examination, it was not helpful in as many as 55% patients. It failed in more than 50% in identifying meningitis, meningoencephalitis and encephalitis. CSF analysis was helpful in 55% patients. It picked up pyogenic meningitis in as many as 17/21 (81%) and meningoencephalitis in 9/14 (64.3%) patients. It was also helpful in diagnosing 7/13(53.84%) patients with encephalitis and 100% cases of tubercular meningitis. The CT scan was not useful in 56/71 (79%) patients in which it was performed. It picked up meningial enhancement in only 28% patients with pyogenic meningitis, however it picked up hydrocephalous in all three patients with tubercular meningitis and also brain abscess in one

patient. MRI was the most helpful radiological investigation. It was helpful in 64% cases. It picked up 100% cases of encephalitis and 90% with meningoencephalitis. It was able to help in diagnosis of 9/21 (42.8%) patients with pyogenic meningitis, all three cases of tubercular meningitis and one case of brain abscess. In conclusion, cerebrospinal fluid analysis was of maximum diagnostic help in a febrile, comatose adult patient and MRI was better than CT scan in the radiological evaluation in our study.

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DEVELOPMENT OF MOLECULAR AND ENZYMATIC ASSAYS TO SURVEY FOR PERMETHRIN RESISTANCE IN SCABIES MITES

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Recent evidence has demonstrated that scabies mites are becoming increasingly resistant to topical permethrin and oral ivermectin. We have investigated two possible mechanisms of permethrin resistance: 1) target alteration via specific mutations in the voltage sensitive sodium channel [*Vssc*] gene, and 2) increase in pesticide degradation by enzymatic activity. We have developed a real time PCR-based SNP genotyping assay following the partial sequencing of the *Vssc* gene in *Sarcoptes scabiei* and identification of a G to A SNP (G1516D amino acid change) in the *Vssc* gene of permethrin-resistant *S. scabiei* var *canis* mites. To survey for possible acaricide degradation as a mechanism of resistance, we have also developed enzyme activity assays for esterase, GST and cytochrome P450. We will present results of the application of these tests on clinical isolates of scabies mites.

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PROTECTIVE EFFICACY OF THE 56 KDA ANTIGEN FROM KARP AND KATO STRAINS OF *ORIENTIA TSUTSUGAMUSHI* IN HOMOLOGUS AND HETEROLOGOUS CHALLENGED MOUSE MODEL

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Scrub typhus is an acute, febrile disease caused by infection with *Orientia tsutsugamushi*. At the present time there is no vaccine for scrub typhus. Western blot analysis of whole cell antigen with naturally infected patient sera revealed several potential antigens, including 56 kDa, 47 kDa and 110 kDa proteins. The 56 kDa protein appeared to be the most immunodominant and a highly expressed protein which amounts to about 15% of the total amount of expressed proteins, making it one of the vaccine candidates. We have successfully cloned, expressed, purified and refolded the truncated form of the 56 kDa protein from both Karp (rKp56) and Kato (rKt56) prototype strains of *O. tsutsugamushi*. These expressed proteins were used individually or in combination to evaluate their protective efficacy in our mouse model against challenge with both homologous and heterologous strains. Swiss CD-1 outbred female mice of 7-8 weeks old were immunized twice at 4 week intervals with rKp56 or rKt56, or the combination of these two, at different doses subcutaneously using Montanide and CpG as adjuvants. Four weeks after immunization, mice were challenged with Karp, Kato, TA763, TH1812, TH1814 and Citrano strains of *O. tsutsugamushi*. The morbidity and mortality were monitored daily for 21 days post-challenge. Very good homologous protection was observed with either rKp56 or rKt56 alone when challenged with homologous strain (86% and 71% protection for Karp and Kato, respectively). The combination of rKp56 and rKt56 provided better protection against parent strain challenge (up to 100%), indicating that a combination of r56 from different strains will provide broader protection than the parent r56 only. Better protection against heterologous challenge was observed using the combination of rKp56 and rKt56 than by rKp56 or rKt56 alone, suggesting that broader protection was provided by the multivalent approach. These results prove the principle of the concept that a combination of r56 from different strains will provide broader protection than the parent r56 only.

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TEN YEARS OF TICKS SUBMITTED TO THE HUMAN TICK TEST KIT PROGRAM OF THE U.S. ARMY CENTER FOR HEALTH PROMOTION AND PREVENTIVE MEDICINE

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The Tick-Borne Disease Laboratory of the Entomological Sciences Program of the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), Aberdeen Proving Ground, MD, provides a tick identification and testing service (the Department of Defense [DOD] Human Tick Test Kit Program) for ticks removed in the continental U.S. (CONUS) from military personnel, military dependents and DOD civilian employees. The service was initiated in 1989 in response to the threat of Lyme disease, and then offered only immunofluorescent antibody (IFA) testing of ticks for *Borrelia burgdorferi*, the etiological agent of Lyme disease. The method of pathogen analysis was changed to polymerase chain reaction (PCR) in 1997, and the list of target pathogens has expanded to include in 2007 *Anaplasma phagocytophilum*, the agent of human granulocytic anaplasmosis, *Babesia microti,* an agent of human babesiosis, *Borrelia lonestari*, the agent of southern tick-associated rash illness (STARI), *Ehrlichia chaffeensis*, the etiological agent of human monocytic ehrlichiosis (HME), *Ehrlichia ewingii*, spotted fever group (SFG) rickettsiae, specifically, *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever and *Rickettsia parkeri*. Most of the ticks received are *Amblyomma americanum*, *Dermacentor varibilis* and *Ixodes scapularis* from installations in the mid-Atlantic, south, northeast and upper midwest regions, but infrequently *Rhipicephalus sanguineus*, *Amblyomma maculatum*, *Ixodes pacificus*, *Dermacentor andersoni*, *Dermacentor albopictus*, *Ixodes cookei* are also submitted. This review was undertaken to examine ten years' (1997-2007) accumulated data in order to compare pathogen infection prevalences and relative tick species abundances, and to describe the changes in the laboratory's methods of tick analysis over the time period.

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SERO-DIAGNOSIS OF MURINE TYPHUS USING RECOMBINANT OMPB FRAGMENTS IN ELISA

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Rickettsia typhi, a Gram-negative, obligate, intracellular bacterium, is the causative agent of murine typhus. Outer membrane protein B (OmpB), a heavily methylated and immunodominant antigen is responsible for serological reactions and capable of eliciting protective immune responses. Three recombinant fragments of OmpB, Fragment A (aa 33-273), AN (aa 33-744) and K (aa 745-1353), were expressed, purified and refolded. The intention was to replace the rickettsial derived antigens, either the whole cell extract or the native OmpB preparation, currently used in sero-diagnosis. The sero-reactivity of these recombinant antigens, either individually or in combinations, were evaluated in ELISA with 48 patient sera. Preliminary results have revealed that patient antibody responses to OmpB fragments were not identical. Some patient sera reacted with the AN fragments but not the K fragment. Others reacted with the K

fragment but not the AN fragments. Although the fragment A is part of the fragment AN, the reactivity of some patient sera with the fragment A can be detected but the reactivity of the same sera with the fragment AN was not detectable, and *vice versa*. The combination of both AN and K, which encompass the full length of matured OmpB, were recognized by almost all positive patient sera. These data suggested that the combination of fragment AN and K has the potential to replace the rickettsial derived antigens for murine typhus diagnosis.

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INTERSPECIFIC HYBRIDIZATION YIELDS NOVEL FILARIASIS VECTOR ELIMINATION APPROACH

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Lymphatic filariasis (LF) is the leading cause of disability in South Pacific regions, where >96% of the population are at risk. As part of the current global campaign, Mass Drug Administration (MDA) has reduced LF prevalence, but vector biology can complicate the MDA strategy. Obligate vector mosquitoes provide additional targets to break LF transmission, but existing methods are ineffective for controlling the primary vector throughout much of the South Pacific: Aedes polynesiensis. We demonstrate that interspecific hybridization and introgression results in an A. polynesiensis strain ('CP' strain) that is stably infected with endosymbiotic Wolbachia bacteria from Aedes riversi. The CP strain is bi-directionally incompatible with naturally infected mosquitoes, resulting in female sterility. CP males are equally competitive, resulting in population elimination when CP males are introduced into wild type A. polynesiensis lab populations. The results support the continued development of a vector elimination strategy to supplement ongoing MDA efforts.

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SEROPREVALENCE TO RICKETTSIOSES IN U.S. MILITARY FORCES DEPLOYED TO KOREA

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Many soldiers in the U.S. Forces Korea (USFK), especially those assigned to infantry units, work and train in field conditions conducive to the transmission of rickettsial diseases by arthropod vectors. The causative agents for murine typhus, scrub typhus and spotted fever have been reported in Korean soldiers and civilians; some have been identified in vectors or reservoirs at USFK training facilities. Currently there is little data on the risk for these rickettsioses in USFK. These three diseases may become more widespread should hostilities and hence deteriorating living conditions return to the Korean peninsula. Active surveillance was used to better understand this potentially significant risk. Matching sera, pre- and post-deployment, of 10,000 U.S. military personnel stationed in South Korea between 1990 and 1995 were obtained from the DoD Serum Repository. The study group consisted of males in combat-related job specialties who were deployed to Korea on active duty continuously for at least one year. Post-deployment sera were assessed for antibodies to *Rickettsia conorii* (representative of spotted fever group rickettsiae [SFGR]), *R. typhi* (typhus group [TG] member) and *Orientia tsutsugamushi* (scrub typhus) using group-specific ELISAs. So far, ~6000 samples have been tested for antibodies to each of the three rickettsiae. The seroprevalence of antibodies to SFGR, TG and *O. tsutsugamushi* as determined by preliminary screening was 643/6105 (10.5%), 93/5877 (1.6%) and 97/6426 (1.5%). Further testing will determine the titer of the antibodies in each screen-positive serum. Side-by-side comparison with the matched pre-deployment sera will allow for the detection of deployment-associated rickettsial infections. Interestingly, the seroprevalence to SFGR is higher than that found in DoD personnel in general (6.0%), but similar to that found in the ground combat specialty (9.5%) in a separate study in our laboratory. When completed, these will be the largest serosurveys of any population, military or civilian, for these three diseases.

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ASSESSING THE POTENTIAL IMPACT OF VACCINATION ON PREVENTION OF ROTAVIRUS DEATHS AMONG CHILDREN IN RURAL GHANA

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Rotavirus is the leading cause of diarrheal morbidity and mortality in young children worldwide. The impact of new, effective rotavirus vaccines on diarrheal deaths will depend on timing and coverage of immunization. We used 3-dose diphtheria, tetanus and pertussis (DTP) vaccine as a proxy to examine if children who died of diarrhea would be as likely to be vaccinated against rotavirus as those who did not, and to predict the impact of routine rotavirus vaccination in a population in rural Ghana. All acute diarrheal deaths among children <5 years of age in the Kassena Nankana District, Ghana from 1998-2005 were identified using verbal autopsy data. The timing and coverage of DTP immunizations were compared between children who died of diarrhea at ≥12 months of age, and 3 controls, matched by date of birth and gender who did not die of diarrhea. We estimated the proportion of diarrheal deaths attributable to rotavirus, and the impact of a routine 3-dose rotavirus immunization program. Of 391 diarrheal deaths among children <5 years of age during the study period, we estimated that 136 (35%) were attributable to rotavirus. Of these deaths, 15 (11%) occurred prior to the median age of DTP3 receipt (22.4 weeks). The coverage of DTP3 at 12 months of age was lower among children who went on to die from diarrhea (104/222: 47%) than in controls (374/674, 55%) (P-value<0.025). We estimate 49% of rotavirus deaths would have been averted if rotavirus vaccine had been administered routinely with DTP. If vaccine coverage of children who died of diarrhea was the same as children who did not, 59% of rotavirus-attributable deaths would be averted. In conclusion, in this population in rural Ghana, children who died of diarrhea were less likely to be vaccinated than other children. To maximize the life-saving impact of rotavirus vaccines, efforts are needed to ensure immunization as early in life as possible, and to develop strategies to improve vaccination coverage, especially among children at greatest risk of mortality from rotavirus disease.

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NEW CLY-A VACCINES SHOW POTENTIAL PROTECTION AGAINST *CRYPTOSPORIDIUM* INFECTION

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Cryptosporidium is a chlorine-resistant, low-infectious dose waterborne parasite for which no specific treatment is available. It causes potentially incapacitating, relapsing diarrhea in normal hosts and debilitating, protracted, cholera-like diarrhea in immune-compromized hosts that could lead to death. This parasite is endemic in many parts of the developing world but also affects many individuals in the United States living with AIDS. *Cryptosporidium* infects the small intestine, causing mucosal

disruption, and significant damage, as well as malabsorption leading to malnutrition. Protection against and eradication of *Cryptosporidium* requires activation of the cell-mediated immune response. We have investigated protection against the parasite afforded by new candidate vaccines developed in a live attenuated *Salmanella typhii* vector. Preliminary results suggest important differences in protective efficacy between these new Cly-A cryptosporidial vaccines. Neonatal mice were innoculated intranasally with 1 of 5 constructs, or PBS control at 8 days old, then orally infected at 12 days old with 10^6 *C. parvum* and measured daily for growth with stool collection. Mice were sacrificed at different ages after infection to determine *Cryptosporidium* burden change and repair within the intestine. Cly-A vaccine candidates showing the most promise in neonatal mice were those containing profillin and Cp15 protein constructs. Profillin containing vectors showed protection against weight loss in well nourished mice and Cp15 containing vectors showed protection against weight loss in malnourished mice. In conclusion, these new vaccine candidates hold hope for many children in the developing world suffereing from cryptosporidial induced malnutrition as a result of mucosal damage as well as AIDS patients failing HAART therapy due to malabsorption by preventing infection of the intestinal mucosa or enhancing function of the host immune system.

(ACMCIP Abstract)

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DEVELOPING RNAI-BASED RESISTANCE TO MULTIPLE DENGUE VIRUS SEROTYPES IN MOSQUITOES (*AEDES AEGYPTI*)

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Diseases caused by arthropod-borne viruses represent a significant public health problem, and novel methods are needed to control their transmission. Our hypothesis is that the genetic manipulation of *Aedes aegypti* mosquitoes can profoundly and permanently reduce mosquito competence to transmit dengue viruses (DENV) to human hosts. Our objective is to use the RNA interference (RNAi) pathway triggered by expression of virus-specific, double stranded RNAs (dsRNAs) to block DENV replication and transmission. The NS5 gene (2700 nt) which encodes the largest and most conserved protein of dengue viruses has shown great potential as a trigger for RNAi in mosquitoes. Previous work showed that dsRNAs (300 nt) targeting the RNA-dependent RNA polymerase (RdRP) encoding region (NS5-5 to 9) are effective inhibitors of DENV2 but not DENV4 in Higgs White Eye mosquitoes (HWE, Puerto Rico Rexville D). We developed a hybrid molecule bearing fragments of the NS-5 RdRP domain from DENV1-4 to address the need for specific effectors for each serotype. As a first step, HWE mosquitoes were injected with dsRNAs derived from the NS5-5 to 8 fragments from DENV3 (H87) and DENV4 (H241) and challenged against viral infection two days later. Viral titers at 7 and 12 days post-infection showed that the four fragments confer similar levels of protection against the homologous serotype. We designed and built a chimeric molecule bearing sequential regions from each serotype (NS5-5 DENV3/NS5-6 DENV4/NS5-7 DENV1/NS5-8 DENV2) by fusion PCR. We tested the effect of this molecule on DENV infection by injecting HWE mosquitoes with the derived 1167 bp long dsRNA and challenging them against each serotype. Our final aim is to transform HWE mosquitoes using the non-autonomous mariner MosI system to express an inverted repeat of this hybrid molecule under the control of the *A. aegypti* carboxypeptidase A promoter. Engineering transgenic *A. aegypti* with high levels of resistance against multiple DENV serotypes will provide a powerful tool for population replacement strategies to control transmission of dengue viruses.

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PLATELIA™ DENGUE NS1 ANTIGEN ASSAY: INNOVATIVE ASSAY FOR EARLY DIAGNOSIS OF ACUTE DENGUE INFECTION

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In tropical and subtropical region, endemic dengue disease is the most important arbovirosis in terms of morbidity and mortality. To implement appropriate treatment, there is a need for a direct, rapid and specific detection test during the acute phase of the infection. For that purpose, viral culture or nucleic acid detection (performed mainly in reference laboratories) require specialized environment but sometimes with delays to report results. Dengue specific antibodies detection is commonly used even if these antibodies appear several days after onset of symptoms. The objective of the study is to evaluate the performances of Platelia™ Dengue NS1 Ag (Bio-Rad Laboratories) for early diagnosis of acute dengue infection. Platelia™ Dengue NS1 Ag is a one step sandwich ELISA assay for qualitative detection of Dengue virus NS1 antigen in human serum or plasma. Specificity of the assay was determined on sera from 547 healthy blood donors and on 202 samples with potential cross-reactions. Sensitivity was studied on sera from 157 dengue virus infected patients confirmed positive by RT-PCR. Clinical information including delay after onset was available for 89 of them. Dengue infections belonged to type 1 (n=72), type 2 (n=31), type 3 (n=24) or type 4 (n=30). Serological status was determined using Dengue IgG Indirect and Dengue IgM Capture Panbio assays. All 749 samples from blood donors and cross-reactant panels were found negative for NS1 giving a specificity of 100%. Sensitivity of NS1 was 91.0% on the 157 PCR positive samples. No significant difference (Chi^2, p=0.3198) was observed between the 4 dengue virus serotypes. NS1 sensitivity was significantly higher in PCR+/IgG- patients (98.5%) compared to PCR+/IgG+ patients (85,6%) (Chi^2, $p<0.05$). During the first 5 days after clinical onset, detection rates ranged from 100.0 to 87.0% for NS1 Ag and from 0.0 to 30.8% for IgM serology. NS1 sensitivity was not significantly higher for IgM negative (91.2%) than for IgM positive (90.3%) samples (Chi^2, p=0.8462). Platelia™ Dengue NS1 Ag assay is an easy-to-use ELISA for dengue antigen detection. The assay allows early and specific diagnosis of dengue acute infections. This new test must be considered in diagnostic algorithms to improve patient care monitoring.

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ROLE OF A NS1 AG ELISA IN THE VIROLOGICAL DIAGNOSIS OF DENGUE INFECTION AND ITS VALUE FOR DENGUE VACCINE TRIALS

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Diagnosis of dengue infection in suspected cases is based on serological and virological tests as clinical symptoms are often too nonspecific. Dengue IgM and/or increased IgG identified by ELISA, are insufficiently reliable and convalescent samples are not always available. Virological tests used on acute samples include virus culture (VC), molecular-based methods (PCR), and detection of circulating dengue antigen. A new tool for the virological diagnosis of dengue infection is based on the detection of dengue NS1 antigen in serum samples. The Platelia™ Dengue NS1 Ag ELISA test kit from Bio-Rad Laboratories (NS1 ELISA) was evaluated in comparison with conventional VC and serotype-specific dengue NS5 qRT-PCR. Acute samples collected between day 0 and 5 after fever onset from a subset of hospitalized children in a 2003-2004 prospective clinico-epidemiological study in Vietnam were tested with all 3 methods. 236 of 276 clinically suspected cases, had serological evidence of dengue

infection. Ongoing acute infection was virologically confirmed in 147 cases with NS1-ELISA (62.3%), 57 with VC (24.2%), 83 with PCR (35.2%), and 103 by either VC or PCR (43.6%). 15/86 NS1-negative samples, were positive via VC and/or PCR. 3 samples displayed an NS1 ELISA result in the doubtful range, and all of them were culture and PCR negative. Dengue infection was detected with the NS1 ELISA in 93.0% of VC-positive cases, 84.3% of PCR positive cases, and 85.4% of the samples positive with VC and/or PCR. This ELISA also confirmed ongoing dengue infection in 44.4% of clinically and serologically suspected dengue cases, that were both VC- and PCR-negative. These data suggest the possible use of the dengue NS1-ELISA as the initial test for the virological confirmation of dengue infection in epidemiological and future dengue vaccine efficacy studies. Dengue serotyping and viremia quantitation could then be done by PCR on the NS1 positive samples

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EVALUATION OF A DUAL-USE, FIELD-DEPLOYABLE, DRY-FORMAT, QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (QRT-PCR) ASSAY FOR DETECTION OF DENGUE VIRUS IN MOSQUITOES AND HUMANS

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Dengue virus is transmitted by species of *Aedes* mosquitoes, and threatens nearly 2.5 billion people living in tropical climates. Approximately 25,000 people die every year from the more severe forms of dengue, for which there are no preventative vaccines or drugs available for treatment. An early and accurate diagnosis of dengue infection can help health care professionals take appropriate precautions to reduce patient suffering and save lives. In addition, vector avoidance remains the best method of prevention, and vector surveillance for the presence of dengue virus is a critical need. Thus, in order to serve the dual purpose of vector surveillance and human diagnosis, a dry-format, quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) based assay has been developed for use with the "ruggedized" advanced pathogen identification device (R.A.P.I.D) thermocycler (Idaho Technology). The assay demonstrated 100% analytical specificity for dengue using an extensive cross-reactivity panel including related flaviviruses. The assay was capable of detecting as low as 7 armored RNA genomic equivalents of dengue. The assay also detected as few as 10 plaque forming units (PFU)/ml of viral stocks diluted in water. The assay was able to successfully detect dengue in a panel of infected mosquito samples (n=40), establishing its efficacy for vector surveillance. Using a panel of human viremic and negative clinical samples, the assay demonstrated high sensitivity and specificity in identifying patients with dengue infection, when compared to viral isolation as a gold standard. Thus the assay shows promise both for vector surveillance and clinical diagnosis. In addition, the reagents are dried-down and the R.A.P.I.D platform is rugged. Thus, the assay enables dengue detection in mobile labs in the field where reliable power and refrigeration may not be available.

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INTERFERON-α IMMUNE MODULATION IN A DENDRITIC CELL - T LYMPHOCYTE MODEL OF SEVERE DENGUE DISEASE

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Blocking dendritic cell (DC) IFN-α will blunt $CD3^+$ T cells response in the setting of Dengue Virus (DV) infection. Developing an IFN-α immune modulation model will allow investigation into the origins and treatment of DHF. DV-2 infected and uninfected DC were cultured with and without IFN-α blocking agents: anti-IFN-α ab; anti-IFN-α ab + anti-Fc receptor, PMA, 2-AP, and B18R, a vaccinia virus-encoded protein receptor with high specificity for human type 1 interferons. Cells and supernatants were assessed for DC infectivity, DC activation, apoptosis/necrosis and IFN-α production. Anti-IFN-α antibodies resulted in suppression of IFN-α levels, but non-specifically activated DC. Combination with anti-Fc antibodies had the same limitation. PMA effectively suppressed IFN-α but was cytotoxic to DC. 2-AP suppressed IFN-α without affecting infectivity, activation, or apoptosis, however problems encountered with maintaining 2-AP in solution posed operational difficulties that potentially limit its utility in a laboratory model. B18R at 0.1μg/ml effectively suppressed IFN-α without altering infectivity, activation, or apoptosis and was used to assess T cell responses in co-cultures of DV-2 infected DCs and $CD3^+$ T cells from an immune donor with heterotypic infection. B18R effectively suppressed IFN-α in the immune DC-T cell co-culture model despite high levels of production in positive controls. T cell IFN-γ responses and apoptosis in the presence and absence of IFN-α are described. In conclusion, B18R is the only known agent to successfully produce a DC-DV infection IFN-α immune modulation model. Further development of this model will allow us to define the role DC derived IFN-α plays in stimulating cytokine responses and will provide novel insight into the cellular origins of DHF.

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MOLECULAR CHARACTERIZATION OF DENGUE VIRUSES ISOLATED IN MEDELLIN, COLOMBIA AND SURROUNDING AREAS

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We are studying the molecular epidemiology of dengue viruses (DENV) in the Metropolitan area of Medellín, Colombia. This region is inhabited by approximately three million people. It has been endemic for dengue since late eighties and dengue hemorrhagic fever (DHF) was first diagnosed in 1995. Surveillance efforts show that DENV types 1, 2 and 4 have been present at least since 1992 and DENV-3 was introduced in 2002. To study the origin, evolution, dispersion and persistence of DENV serotypes in this region, a sample of 35 isolates from a collection spanning 14 years (1993-2006) was sequenced in the E gene and phylogenetically analysed. Preliminary results show that for all serotypes a single genotype had been present. However the isolates in each serotypes are not monophyletic suggesting that DENV have been repeatedly introduced. In some cases the newly introduced lineage seems to have displaced the prevailing strain but in some others both strains have persisted for several years. Two different lineages of DENV-3 seem to have been introduced about the same time and have persisted. Some of the recently appearing strains are temporally related to increases in the incidence of dengue but the arising of DHF could not be clearly attributed to single strain introduction episode. In summary, dengue infection is characterized by frequent traffic of DENV between Medellin and surrounding areas and this region meets the conditions for simultaneous circulation and long term persistence of multiple DENV strains.

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FACTORS INFLUENCING THE TEMPORAL PATTERN OF CLASSIC DENGUE FEVER IN SINGAPORE

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In Singapore, the incidence of dengue fever has been on the rise, despite sustained and effective control of its primary mosquito vector.

Paradoxically, it has been proposed that vector control itself has been at least partly responsible for this increase in clinical disease, as over the long term, the success of control has not only lowered herd immunity by decreasing viral transmission, but has also raised the average age of infection to include a higher proportion of adults who are more prone to developing classic dengue fever following primary DENV infection. Contrary to this, some have argued that factors that increase, not decrease, viral transmission, are responsible for the disease pattern observed in Singapore, however, empirical data have never been used to quantify these relationships. The aim of this study was to use Box-Jenkins time series techniques to determine if the recent rise in DF was influenced by factors that have the potential to cause a short-term increase in DENV transmission or if the observed pattern can be explained primarily by the long-term decrease in transmission, driven by vector control. Dengue fever incidence, Aedes premise index and the force of DENV infection, for the years 1960-2006, served as primary dependent time series. Independent, extrinsic time series included various entomological, environmental and socioeconomic data. Cross-correlograms indicated that there was a significant relationship between DF incidence in Singapore and several of the extrinsic series. There was little temporal correlation, however, between climatic factors and DF incidence or *Aedes* PI over the interannual period. ARIMA transfer function models support these findings. Results suggest that yearly variation in DF incidence in Singapore is, in part, influenced by short-term fluctuations in transmission intensity caused by extrinsic factors. The fact that there is now evidence the recent increase in DF incidence in Singapore is related to both a decrease and an increase in DENV transmission intensity will be discussed, as well as how these findings from Singapore have implications for controlling dengue fever worldwide.

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LOST IN FRENCH POLYNESIA: WHICH STRATEGIES FOR A DENGUE VIRUS TO SPREAD?

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More than a hundred years after the first report on a dengue-like illness epidemic in Tahiti, dengue is still a public health concern in French Polynesia (FP) as well as in all the tropical and subtropical regions. FP has experienced epidemics caused by all the four dengue virus serotypes. Most of these epidemics emerged following the introduction of a new viral strain originating from the Americas, South East Asia or the Pacific: DEN-1 (1944, 1975, 1988, 2001), DEN-2 (1971, 1996), DEN-3 (1964, 1989), DEN-4 (1979). Although multiple serotypes of dengue virus co-circulate in most of the endemic countries, persistent co-circulation of multiple serotypes has never been observed in FP. The active transmission of a new serotype seems to block the endemisation process of the previous one, since the "endemic serotype" is quickly replaced by the new "epidemic serotype". Another particular feature of dengue epidemiology in FP is the "re-emergence phenomenon". On three occasions, FP experienced epidemics due to the viral serotype and genotype that had been involved in the previous outbreak: DEN-3 re-emerged in 1969, DEN-4 in 1985 and DEN-1 in 2006. The re-emergence always occurred five to six years after the previous epidemic, which could correspond to the necessary period for a sufficient increase of the susceptible population. This specific epidemiological pattern observed in FP, i.e. absence of co-circulation and dynamic of dengue epidemics, could be related to (i) insularity, (ii) relatively stable human flows (new residents, births, students, tourists) leading to a cyclic renewal of the susceptible population, (iii) a particular ecosystem, for instance a relatively constant climate. We will use the most recent epidemics of DEN-1 in 2001 and 2006, as a model to improve the understanding of dengue dynamic in FP. This should contribute to a better definition of predictive factors and to the development of new means of prevention of dengue.

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INHIBITION OF VIRAL REPLICATION IN HUMAN LIVER CELLS BY SHORT INTERFERING RNA DIRECTED TO 5'-NONCODING REGION OF DENGUE-2 VIRUS

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Dengue viruses belong to the genus *Flavivirus*, family *Flaviviridae*, and are classified into four antigenically related serotypes. They cause a disease (dengue) whose clinical spectrum ranges from an asymptomatic infection to a severe disease called dengue hemorrhagic fever/dengue shock syndrome. Currently, no specific intervention exists for dengue treatment or prevention. RNA interference (RNAi) is a sequence-specific gene-silencing mechanism commonly observed in eukaryotic cells that is believed to function as a defense mechanism against several pathogens, including viral infections. The goal of this project was to construct a double-stranded RNA directed to the 5'-noncoding region (5'-NCR) of dengue-2 virus (DEN2V), aiming at the inhibition of viral replication. The 3'-NCR and the 5'-NCR of the flavivirus genome are involved in interactions necessary for RNA replication. Another function of the 5'-NCR probably resides on the complementary region of the negative strand, serving as a site of initiation for the positive-strand synthesis during RNA replication. To accomplish this goal, plasmids expressing a double-stranded RNA, specific for the 5'-NCR of DEN2V (DEN2V 5'-NCR RNAi) and a double-strand RNA not directed to DEN2V (RNA-negative) were synthesized *in vitro*, and transfected into HepG2 cells. HepG2 cell clones constitutively expressing the DEN2V 5'-NCR RNAi were selected with geneticin. HepG2 cells and HepG2 cell clones constitutively expressing either DEN2V 5'-NCR RNAi or RNAi-negative plasmids were infected with DEN2V at a multiplicity of infection of 0.1, and cell supernatants were collected on days 1, 3, and 5 after infection. Viral RNA was quantitated by reverse-transcriptase Real Time-PCR using primers and serotype-specific probe. Three HepG2 cell clones expressing DEN2V 5'-NCR RNAi were able to completely inhibit virus replication when compared to controls, with the highest inhibition on day 5 after infection. Based on these results, it is clear that RNAis specific for the 5'-NCR region of the flavivirus genome are able to inhibit virus replication. Thus, the possibility of attenuating dengue virus infections using RNAi is now possible, although there have been few studies using this strategy.

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PROSPECTIVE STUDY OF DENGUE INFECTION IN SCHOOL CHILDREN IN LONG XUYEN, VIETNAM

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A dynamic cohort of 3-14 year-olds was established in a southern province of Vietnam in 2003 to provide background epidemiological data to prepare for a dengue vaccine efficacy trial. School children are followed all year-round to detect laboratory-confirmed dengue cases using active monitoring of febrile episodes by identifying absenteeism during school-term and home visits during holidays. Acute and convalescent serum samples are collected from children with clinically-suspected dengue or other viral infection. Additionally, sub-clinical dengue infections are identified annually by ELISA. The size of the cohort was 2,190 in 2004, 3,235 in 2005 and 3,079 in 2006. 205 clinically-suspected dengue episodes were reported in 2004, 153 in 2005 and 167 in 2006. Of these, 35.6% (73) were laboratory-confirmed in 2004, 33.3% (51) in 2005 and 46.7% (78) in 2006. The incidence rate of laboratory-confirmed dengue

cases was 36.5 per 1,000 person-year in 2004 and 17.0/1,000 in 2005 (2006 data analysis ongoing). In 2004, all but 1 of the virologically-confirmed cases were due to DEN-2. This serotype remained predominant in 2005, whereas in 2006 the majority of cases were caused by DEN-1 (though all four serotypes were isolated in 2005 and 2006). The annual primary dengue infection rate was 13.9% in 2004 and 12.2% in 2005. Study withdrawal rates were used as an indicator of the feasibility of conducting an efficacy trial in this population. Most withdrawals were children who moved to non-participating schools in the same area. The corrected withdrawal rate (excluding children who changed schools) was low: 4.8% (104/2,190) in 2004 and 5.9% (191/3,235) in 2005. These results confirm the high transmission of dengue in children in Long Xuyen and demonstrate the high potential of this study site for a large scale efficacy trial.

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IMMUNOHISTOCHEMISTRY ON SKIN BIOPSIES FROM VIETNAMESE PATIENTS WITH DENGUE SHOCK SYNDROME

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Dengue is the most widely distributed mosquito-borne viral infection of humans and is increasingly recognised as a major burden on global public health. Infection with any of the four viral serotypes may cause a variety of clinical disease manifestations ranging from asymptomatic infection to life-threatening dengue shock syndrome (DSS). DSS is characterised by increased vascular permeability, thombocytopenia and altered haemostasis, resulting respectively in capillary leak syndrome and haemorrhage. The mechanisms underlying these complications remain poorly understood and there is little information regarding structural or functional changes in the microvasculature during infection. With informed consent we performed small skin punch biopsies on 30 children and 12 young adults shortly after admission with DSS (when clinically stable, using local anaesthetic) and on 15 control children undergoing elective surgery - all patients recovered well with supportive care and there were no complications at the biopsy sites. Routine light microscopy showed no obvious structural changes apart from mild dilation of the lymphatic vessels in comparison to control biopsies, confirmed on immunohistochemistry with specific lymphatic markers. Results of immunohistochemistry for fibrinogen and important endothelial regulators of haemostasis will be presented, together with plasma studies of relevant coagulation proteins; the results suggest that the low fibrinogen levels normally present in patients with DSS arise through leakage rather than by consumption in a process of disseminated intravascular coagulation. Preliminary data on localisation of various dengue antigens in the skin microvasculature, as well as IgG and complement, will also be presented.

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ANALYSIS OF AGREEMENT BETWEEN INDIVIDUAL AND COMBINED CLINICAL AND LABORATORY FINDINGS AND AN EXPERT PHYSICIAN'S DIAGNOSIS OF DENGUE HEMORRHAGIC FEVER

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Guidelines for classification of dengue illness as dengue fever (DF) or dengue hemorrhagic fever (DHF) have been promulgated by the World Health Organization (WHO) criteria; however, these criteria have been a subject of recent controversy. The DHF Project is a prospective study of Thai children aged 6 months to 14 years who presented to Bangkok Children's Hospital or Kamphaeng Phet Provincial Hospital with fever onset <72 hours prior to entry and oral temperature >=38°C. Clinical variables collected daily included: patient's maximum values of alt, ast, percent change in hemoconcentration, pleural effusion index (PEI), tourniquet test, and minimum values of albumin and platelet count. Patients with laboratory-confirmed dengue received an expert physician diagnosis guided by WHO criteria. Univariate logistic regression was performed on each variable with physician's diagnosis (DHF vs. DF). Kappa coefficients (κ) were used to assess the extent of agreement between the physician diagnosis and WHO criteria diagnosis using different indicators for plasma leakage. Multiple logistic regression models were constructed to assess the predictive values of specific clinical criteria to the physician's diagnosis. Of 699 patients enrolled from 1994-97, 318 had dengue (177 DF, 141 DHF). There was 86% agreement between physician diagnosis and WHO criteria when PEI was used (κ=0.71); moderate agreement with 75% when percent change in hemoconcentration was used (κ=0.47); and 86% agreement when either was used (κ=0.72). In univariate analysis each of the clinical variables listed above was associated with a clinician's diagnosis of DHF vs. DF. Among multivariable models, the best model when PEI was included had an area under the curve (AUC) of 98%, 91% sensitivity and 98% specificity. Without PEI and using percent change in hemoconcentration, the best model had an AUC of 86%, 75% sensitivity and 83% specificity. Moderate to good agreement between physician's diagnosis of DHF and WHO classification was observed in this study. Predictive models using components of the established WHO criteria and other clinical variables could be constructed to improve the agreement with the clinician's diagnosis. In-patient observation with early medical intervention in this study may limit the generalizability of the findings, however. Clinical studies such as this are needed to critically evaluate the criteria for classification of dengue illness.

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PROSPECTIVE STUDY OF SUB-CLINICAL DENGUE INFECTION MEASURED BY NEUTRALIZING ANTIBODIES IN VIETNAMESE SCHOOL CHILDREN

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A cohort of 3,000 children aged 3-14 years was established in a southern province of Vietnam in 2003 to provide background epidemiological data to prepare for a dengue vaccine efficacy trial. School children are followed year-round to detect laboratory-confirmed dengue cases by actively monitoring febrile episodes. Additionally serum is sampled annually to assess dengue antibody levels. During the first year of the study, neutralizing antibodies were measured in a randomized subset of 200 4-11 year-olds to estimate the prevalence and the annual dengue infection rate. Plaque Reduction Neutralizing Test ($PRNT_{50}$) was chosen as the only available test that could provide serotype-specific results and differentiation between first, second and further dengue infections. However there were no published standardized criteria for the interpretation of PRNT results that can be complicated by the possible cross-reactivity between serotypes. We proposed criteria for the definition of immunological status at baseline: Negative: $PRNT_{50}$ <10 to all dengue viruses Monotypic: $PRNT_{50}$ ≥10 for one serotype or $PRNT_{50}$ ≥10 to more than one serotype with a high ($PRNT_{50}$ ≥80) and predominant (>5 fold the

other serotypes) response to only one serotype; Multitypic: $PRNT_{50} \geq 10$ to more than one serotype with more than one high serotype or without a single predominant serotype. First, second and further dengue infections were then identified when seroconversion occurred in previously negative, monotypic and multitypic children. On study admission, the prevalence rate was 34.4%. DEN-4 was the predominant serotype, followed by DEN-1, DEN-3, then DEN-2. 43 children seroconverted over one year, giving an overall infection rate of 22.8% (primary 21% and secondary and further 26.2%). We established criteria for the estimation of first and further infections. More data are being collected to further describe serotype-specific infection rates and the ratio of symptomatic/asymptomatic dengue infection in this high transmission area.

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STUDY OF DENV INTERACTIONS WITH RNAI PATHWAY IN THE MOSQUITO

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Dengue viruses (DENVs) are the most medically important arthropod-borne viral pathogens affecting humans. *Aedes aegypti* mosquitoes are the principal vectors of DENV serotypes 1-4 during urban outbreaks. DENVs enter the mosquito midgut when the adult female takes a blood meal from a viremic individual. RNA interference (RNAi) may be an important pathway that mosquitoes use to modulate DENV replication. We have tested if DENV could trigger formation of a class of RNAs that are identical in size with the siRNAs commonly associated with RNAi. Low molecular weight RNAs from C6/36 cells and HWE mosquitoes infected with DENV2 were analyzed further in a ribonuclease protection assay (mirVana miRNa Dectection Kit), using a labeled sense or antisense DENV RNA probes derived from 22 nt the DENV2 prM-encoding region. A 22 nt signal was detected following infection of C6/36 cells and mosquitoes, this result confirms that DENV is recognized by the RNAi machinery. In this study, we describe the effects of the RNAi pathway components AGO2, R2D2 or DICER2 on DENV2 infections of *A.aegypti*. Four day old female mosquitoes from the DENV-resistant transgenic family, Carb77 (as reported previously), and HWE were intrathoracically injected with 1 µg of dsRNA derived from the genomic DNA region encoding AGO2, R2D2 or DICER2. Two days later they were given an infective bloodmeal containing ε10^6 to 10^7 pfu/ml of DENV2 Jamaica 1409. Virus titer was assessed at 7 and 14 days post-infectious bloodmeal. Depletion of specific mRNA was confirmed by Northern blot analysis. Transient silencing of *ago2, r2d2,* and *dcr2* in mosquitoes (Carb 77 and HWE) significantly increase DENV2 viral titers, indicating a role for these components in anti-viral activity in mosquitoes. Transmission assays were conducted at 7, 10, 12 and 14 dpi using HWE mosquitoes injected with dsRNA (*ago2, r2d2*, and *dcr2*) and challenged with DENV2 two days later, to see if impairment of RNAi leads to shorter extrinsic incubation period (EIP) and higher virus load in saliva for transmission

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EVALUATION OF THE PLATELIA™ DENGUE NS1 AG ELISA KIT IN EARLY DIAGNOSIS OF DENGUE INFECTION

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Dengue fever/Dengue haemorrhagic fever/Dengue shock syndrome is an acute viral disease transmitted by mosquitoes and caused by one of four serotypes of dengue virus and become the most important arthropod-borne human viral disease in tropical regions. In Vietnamese national program for dengue control, dengue cases are confirmed by virological tests (including viral isolation, RT-PCR) and/or serological tests (MAC-ELISA) from acute samples. However, this strategy has not been fully successful to implement the outbreak intervention. In a comparative study using Platelia™ Dengue NS1 Ag (Bio-Rad Laboratories) for detecting the dengue NS1 antigen in acute samples, 98 acute sera collected between days 0 and 3 after fever onset from dengue patients were tested. Using virus isolation test as the gold standard, we have found an overall sensitivity of 91.8% (90/98) by RT-PCR, 88.8% (87/98) by Platelia™ Dengue NS1 Ag and 11.2% (11/98) by MAC-ELISA. The Platelia™ Dengue NS1 Ag assay was also evaluated in 122 acute serum samples (Day 0 - Day 3) from dengue suspected patients. Of 82 dengue patients confirmed by virus isolation and/or IgM antibody, the overall sensitivity of 59.8% (49/82) by Platelia™ Dengue NS1 Ag compared to 48.8% (40/82) by virus isolation, 47.6% (39/82) by RT-PCR and 46.3% (38/82) by MAC-ELISA. Of 40 dengue suspected patients which were neither confirmed by virus isolation or IgM antibody, Platelia™ Dengue NS1 Ag could detect 20% (8/40) more cases compared to 27.5% (11/40) by RT-PCR from acute serum samples. These results shown that the Platelia™ Dengue NS1 Ag assay will be useful not only for early diagnosis of dengue infection in the hospital but also for starting as soon as possible the outbreak intervention measures in dengue control.

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DENGUE VIRUS CROSS-REACTIVE MOUSE OR HUMANIZED CHIMPANZEE MONOCLONAL ANTIBODIES FAVOR ENHANCED DENGUE VIRUS IMMUNE COMPLEX INFECTIVITY IN ENGINEERED HUMAN FCγ RECEPTOR CD64 OR CD32-EXPRESSING CELLS

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Antibody-dependent enhancement (ADE) of viral replication has been widely studied in cultured monocyte/macrophages which simultaneously display multiple Fc receptor classes. We have examined the interaction between dengue virus (DENV) immune complexes and human Fcγ receptors (FcgR), individually, and have found that FcγRIIA (CD32) is strikingly more efficient at mediating ADE than is FcγRIA (CD64), as reported previously. Our results suggested that different mechanisms operate between FcγR classes to determine the fate of infectious DENV immune complexes following FcgR engagement. To identify specific IgG antibody characteristics that favor DENV immune complex neutralization vs. infectivity, we have engineered native CD64 linked to its accessory γ-chain, and CD32 constitutively into CV-1 fibroblasts, cells which are used in DENV neutralization assays. The stable CV-1/FcγR-expressing cell lines displayed comparable surface abundance of the respective FcγR types and exhibited predicted IgG subclass binding profiles. We observed markedly increased infectivity of immune complexes formed with DENV serotype cross-reactive mouse (IgG2α) or humanized chimpanzee (h-c) (IgG1) MAbs in FcγR-expressing cells (CD32 > CD64), as reported previously. In contrast, immune complexes formed with DENV serotype-specific mouse or h-c MAbs were more efficiently neutralized in either CD32 or CD64 integrates; introduction of a 9-amino acid Fc piece deletion into the h-c MAbs further favored neutralization. Our experimental results point to characteristics desired of antibodies to be stimulated by a safe and effective future DENV vaccine.

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COMMUNITY PARTICIPATION PROJECT FOR DENGUE PREVENTION AND CONTROL IN PUERTO RICO: KNOWLEDGE, ATTITUDES, AND PRACTICES IN 2005-2006

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Dengue is a viral disease transmitted most often by *Aedes aegypti* mosquitoes. Research in Puerto Rico has shown that even with high levels

of awareness about dengue, changes in larval indices are limited. The goal of this project was to implement a novel approach to community-based dengue prevention by developing a model that involves the active participation of residents in conducting activities to reduce *Aedes aegypti*. The specific objective for the two-year project (2005 to 2007) was to increase knowledge, attitudes and practices (KAP) regarding dengue and its prevention in a community. We selected a community with 203 houses that had high baseline mosquito indices and community organizational level. KAP surveys were conducted in all houses before and after the intervention to determine if dengue knowledge and correct practices to reduce larval indices increased. The intervention consisted of house to house visits to eliminate breeding sites and distribution of targeted educational materials on dengue prevention. In 2005, 198 people were interviewed (69% females, median age=53 years). Response rate for 2006 was 88%. Few participants, in either pre or post-survey, correctly reported that dengue is a viral disease (27% in 2005 and 21% in 2006; p-value=0.2). Only one-third (30%) of participants knew that there is no dengue vaccine in 2005 but knowledge significantly increased by 2006 (51%; p-value<0.05). Only 36% reported that mosquitoes come from containers that accumulate water in 2005 but this proportion increased in 2006 to 58% (p-value<0.05). There was a significant increase in the percent of participants who reported checking their yards for containers that accumulate water (80% in 2005 and 93% in 2006; p-value<0.05) and indicated they do it one or more times per week (70% in 2005 and 80% in 2006; p<0.05). The majority of participants correctly indicated that dengue is transmitted by mosquitoes (80% in 2005 and 77% in 2006; p-value=0.5). In conclusion, KAP results for 2005 reflected that dengue knowledge was mixed with misconceptions. After the intervention, results showed significant improvements in knowledge but also some increases in misconceptions. Prevention activities continued in 2007 with the development of a video and a second post-intervention evaluation. Results of this project will be used to determine future community-based strategies that will effectively reduce disease transmission.

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ECOLOGICAL OBSERVATIONS ON THE WEST NILE VIRUS OUTBREAK IN DAVIS, CALIFORNIA, 2006

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During the summer of 2006, WNV transmission in Yolo County, California, reached epidemic levels with a human case incidence of 15.4 per 100,000. In Davis, a residential community in Yolo County with a population of over 64,400 residents, the human case incidence was 10.9 cases per 100,000 in July alone. Enzootic transmission was monitored by sampling mosquitoes weekly from April to October at 21 trap sites uniformly spaced 1.5 km apart over an area of approximately 26 km^2 and by collecting dead birds. All *Culex* and dead birds were tested for WNV RNA by RT-PCR. The onset of the outbreak was detected in dead birds, with episodic recovery of positive mosquitoes, dead birds and human cases subsequently following an extrinsic incubation degree-day model. Kriging interpolation delineated that *Cx. tarsalis* were most abundant at peripheral traps near surrounding agriculture, whereas *Cx. pipiens* were associated with residential and greenbelt systems within the older portion of the urban landscape. Cluster analyses were performed on WNV risk factors. Significant spatial-temporal clustering was detected among WNV-infected dead birds and WNV positive *Cx. tarsalis* and among WNV positive *Cx. pipiens* and high incidence of confirmed human cases. We conclude that WNV infected *Cx. pipiens* was the greatest risk factor for human WNV infection. Our results describe the 2006 WNV outbreak by investigating the epidemiologic factors which contributed to the high incidence of human infection.

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ADIPOCYTES ARE A POTENTIAL TARGET FOR WEST NILE VIRUS INFECTION

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Several epidemiological studies have suggested diabetes as one of the significant risk factors for West Nile encephalitis (WNE). Recent data suggests that increased adiposity is associated with insulin resistance and proinflammation in type II diabetes due to aberrant secretion of adipocytokines. We hypothesized that dysfunctional adipocytes in diabetic patients are more susceptible to WNV infection leading to perturbation in virus clearance, thereby increasing the probability of virus crossing the blood-brain barrier, resulting in WNE. Therefore, the aim of this study was to characterize WNV replication in preadipocytes and adipocytes derived from mouse 3T3-L1 cells. Adipogenesis was measured by triglyceride (TG) accumulation, while WNV replication was analyzed using immunofluorescence staining, plaque assay, and quantitative real-time RT-PCR (qRT-PCR). Relative mRNA expression of adipocytokines (adiponectin, resistin, leptin, tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) in infected-adipocytes, were measured by qRT-PCR. Adipocytes and preadipocytes consistently supported WNV replication without apparent cytopathic effect. The kinetics of WNV replication was similar in preadipocytes and adipocytes. Maximum viral titers in supernatants of preadipocytes and adipocytes were 7 and 6.5 $\log_{10}$ PFU/mL, respectively and viral titers were maintained at 5.5 $\log_{10}$ PFU/mL until day 15 post-inoculation (p.i.). WNV infection had no effect on TG content, and mRNA expression of adiponectin and resistin. Leptin transcripts were up-regulated by 2-fold at day 6 p.i., whereas TNF-α and IL-6 transcripts were up-regulated more than 10-fold till day 15 p.i. Overall our results suggest that preadipocytes and adipoctyes that are initially encountered by WNV in the subcutaneous tissue, may serve as one of the early targets for virus replication, and play an important role *in vivo* in viral persistence and subsequent dissemination. In addition, aberrant secretion of adipocyte-derived cytokines upon WNV infection may contribute to the pathogenesis of WNV-induced CNS disease.

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RAPID GIS-BASED PROFILING OF WEST NILE VIRUS TRANSMISSION: ENVIRONMENTAL FACTORS ASSOCIATED WITH AN URBAN-SUBURBAN OUTBREAK IN NORTHEAST OHIO, USA

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Human West Nile virus (WNV) infection was first detected in Cuyahoga County, Ohio in 2002. During that year's extensive epidemic/epizootic among non-immune human and bird populations, the county experienced 155 cases of severe WNV neuro-invasive human disease (WNND, incidence: 11.1 cases/100,000), with 11 fatalities. Structured serosurveys indicated that 1.9%, or ~ 26,000 of county residents (pop. = 1,372,303) were infected that year. To better focus monitoring and control efforts, in early 2003 we used a Geographic Information System (GIS)-based approach and spatial statistical analysis to identify the association of environmental factors plus human population structure with the observed local risk for WNV transmission. Within the varied range of component urban/suburban/rural habitats across the 458 mi^2 (1186 km^2) county setting, exploratory GIS-based spatial analysis indicated significant clustering of WNND risk in inner ring suburbs. Subsequent discriminant analysis based on inputs of census and land-use/ land cover data was used to effectively classify sub-areas of the county having low, medium, and high WNV risk. On a 4 mi^2 (1036 ha.), quadrat scale of resolution,

high risk of human infection was positively associated with low-income areas, increased fractionation of habitat, and older housing stock, while it was negatively associated with areas of agricultural land, wetland, or forest. The areal classification of WNV transmission risk has been subsequently validated in terms of increased local *Culex* spp. mosquito density (2002-2006), and increased frequency of WNV positive mosquito pools within the specified medium- and high-risk quadrats. This timely working identification of the WNV transmission scale effectively focused control interventions against this invasive viral species in a complex North American habitat.

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EVALUATION OF MOSQUITOES AS SYRINGES FOR ARBOVIRUS VIREMIA DETERMINATIONS IN SMALL VERTEBRATES

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Many vertebrate infection experiments require repeated blood sampling of the same individuals over specific time intervals. However, some animals cannot easily be bled repeatedly due to their small size and limited blood volume or lack of sufficient vein presentation. If mosquitoes can be used as tools to draw small quantities of blood from which virus titer can accurately be determined, then small vertebrates such as mice and nestling birds can be included in experimental infection studies. The specific aims of this study were to 1) test, and 2) validate the use of mosquitoes as syringes to assess virus titers in small animals. To test this technique, groups of chicks and hamsters were experimentally infected by needle inoculation (supplemented with mosquito saliva) with a flavivirus (West Nile virus), alphavirus (Highlands J virus) or bunyavirus (Snowshoe Hare virus - hamsters only). For each infected host, virus titers in blood samples collected by syringe, *Aedes albopictus* Skuse and *Culex p. quinquefasciatus* Say mosquitoes were determined by Vero cell plaque assay 2- and 3-days post infection and statistically compared to test the hypothesis that mean virus titers in syringe and mosquito samples were the same. Individual as well as pooled mosquitoes were tested. This technique was then validated by determining viremia profiles from syringe- and mosquito-drawn blood samples in groups of small birds (house sparrow) and mammals (laboratory mice) infected with each virus. Data generated from these studies is valuable for assessing the role of small vertebrates in relation to arbovirus ecologies of public health importance, and can be applied to a variety of disease systems.

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PREVALENCE OF HUMAN IGG ANTIBODIES AGAINST FLAVIVIRUSES IN CENTRAL AND NORTHERN AFGHANISTAN

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Data on the circulation of arboviruses in Afghanistan have not been available for more the 30 years. In 2006 we collected 139 human sera from Northern Afghanistan (Kunduz) and 134 sera from Central Afghanistan (Kabul). Sera were screened for IgG antibodies against Dengue viruses (DENV), West Nile virus (WNV), and tick-borne encephalitis virus (TBEV). For testing sera were screened by the respective ELISA assays (Euroimmun, Lübeck, Germany). Reacting sera were titrated by indirect immunofluorescence (Euroimmun, Lübeck, Germany). WNV positive sera were further tested by plaque reduction neutralisation test (PRNT) for differentiation of WNV and Japanese encephalitis virus (JEV).
15 of 139 (10.8%) sera from the Kunduz region reacted positive against WNV. In PRNT all positive sera were specific against WNV. 5 of 139 sera (3.6%) reacted specifically against DENV. No serum showed specific reactivity against TBEV. 20 of 134 sera (14.9%) from the Kabul region gave a specific positive reactivity against WNV. Also, none of the sera from the Kabul region reacted positive against TBEV. However, 12 of 134 sera (9.0%) from Kabul showed specific reactivity against DENV. Although only a rather small number of sera were tested, high IgG seroprevalence rates ranging from 10 to 15% against WNV were detected in both locations. In contrast, the IgG seroprevalence rate against DENV in the two studied areas was about 3 times higher in Central Afghanistan than in Northern Afghanistan. These results are in concordance with unpublished observations of dengue-like disease in the Kabul population in summer 2006. The low prevalence of DENV in the Kunduz region might be explained by the geographical barrier of the Hindukush Mountains between Kabul and Kunduz, which could form a barrier of distribution for DENV to the north. WNV, however, possibly can be distributed by birds and therefore can be found in Northern as well as in Central Afghanistan. The medical importance and impact of both, DENV and WNV infections, in humans remains to be determined.

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RELATIONSHIPS BETWEEN COMBINED SEWER OVERFLOWS AND WEST NILE VIRUS: SPATIAL PATTERNS OF MOSQUITO VECTORS, AVIAN HOSTS AND HUMAN CASES IN FULTON COUNTY, GA

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West Nile Virus (WNV) is transmitted in a bird-mosquito-bird cycle and is transmitted to humans through mosquito vectors that feed on both birds and humans. Since the introduction of WNV in Georgia there have been 149 human infections and 16 deaths, and in Fulton County there were at least 37 confirmed cases of WNV through 2006. One of the principal WNV vectors in Georgia, *Culex quinquefaciatus*, breeds in many different water sources including polluted water with high organic content. Combined Sewer Overflow (CSO)/CSO Regulator (CSOR) facilities, a common method of wastewater management in the US, release organically polluted waters into nearby streams on occasions when sewage and storm water exceeds the capacity of the treatment facility. Previous research has shown that the streams and creeks downstream of CSOs in Fulton County support mosquito vectors of WNV such as *Culex quinquefaciatus*. As part of an ongoing WNV surveillance effort the Fulton County Department of Health and Wellness collected data from 2001 to 2005 on mosquitoes, dead birds, and human cases. Mosquito traps were set up throughout Fulton County and 5875 pools of mosquitoes were tested for WNV and other arboviruses. In addition, 550 dead birds were tested for WNV. Geographic information was collected on the location of the mosquito traps, dead bird collections, human cases, and the 9 CSO and CSOR facilities. In all there were 160 mosquito pools, 339 dead birds, and 34 human cases found positive for WNV. The spatial distribution of WNV in mosquitoes, dead birds, and human cases in relation to the 9 CSO/CSOR facilities will increase our understanding of the role of CSO/CSOR in the epidemiology of WNV, and may provide insight for vector control planning with the goal of reducing human risk of WNV.

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CAPTIVE ANIMALS AS SENTINELS FOR WEST NILE VIRUS TRANSMISSION IN ZOOS FROM YUCATAN AND TABASCO STATES OF MÉXICO

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West Nile virus (WNV) is a virus, genus *Flavivirus* Its transmission cycle involves a variety of wild birds and *Culex* species mosquitoes. In July 1999, captive and free-flying birds in the Bronx Zoo in New York died of encephalitis caused by WNV. In March 2000, a program of avian surveillance was established in the Yucatán State of México in collaboration with Colorado State University. The first Mexican WNV positive bird was confirmed in December 2002. Between September 2003 and September 2004, 415 animals in El Centenario Zoo, Merida, Yucatan were tested for antibodies to WNV. 7 birds out of 257 (2.7%) and 2 mammals out of 52 (3.8%) tested positive to WNV by PRNT. The first WNV isolate in Mexico came from a captive American crow in the Yum-Ka Zoo, 10 Km outside Villahermosa, Tabasco. The aims of this study were to demonstrate active WNV transmission in "El Centenario" Zoo (downtown Merida), and to evaluate seroconversion in animals in the Tabasco zoos, Yum-Ka Zoo and La Venta Zoo (downtown Villahermosa). In March 2005, blood samples were collected in El Centenario Zoo. 11 out of 110 birds (10%) tested positive for WNV, and none of the 31 mammals and 10 reptiles sampled tested positive by either b-ELISA or RT-PCR. In March and October 2005, birds, mammals, and reptiles from the Tabasco zoos were tested by epitope b-ELISA to evaluate their seroconversion. 30 birds out of 111 (27%) tested positive for WNV in the first sampling and 39 out of 123 (32%) tested positive in the second sampling. 34% (10/29) of mammals tested positive in the first sampling, and 6 more tested positive in the second sampling, increasing the overall positive percentage to 55%. Six out of 7 (86%) crocodiles, *crocodylus moretii,* in La Venta Zoo tested WNV positive. The WNV positive all animals in "El Centenario" Zoo were: 10 of 151 with a 7.28% and the WNV positive of all animals in Tabasco zoos were: 91 out 270 with a 33.70%. In conclusion, the frequencies of infection between the 2 states were unlike and captive animals from zoos are useful sentinels to determine the establishment and transmission of WNV in urban areas.

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GENETIC VARIABILITY OF WEST NILE VIRUS IN SENEGAL

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West Nile virus (WNV) is an emerging mosquito-borne member of the flavivirus genus which is spread worldwide and has recently been introduced in the Western Hemisphere. Phylogenetic analyses have shown that WN viruses occur in two distinct major lineages ; lineage I viruses have a cosmopolite distribution whereas lineages II viruses have been isolated only from subsaharian Africa and Madagascar except once in Hungary in 2004. In Senegal, WNV has been repeatedly isolated from various hosts (mosquitoes, human and primate) since 1979. To better understand factors leading to WNV emergence in Senegal, we undertook phylogenetic analysis of 83 WNV isolates from various hosts, ecological contexts, in different regions of Senegal over 25 years. Isolates were sequenced in the envelope (E), polymerase (NS5) and NS5-3'UTR regions of the viral genome, and phylogenetic trees were generated with additional isolates from other african and european countries. The tree topology shows two distinct lineages. Lineage I isolates displayed important genetic variability, and a close relationship is found between senegalese, mediterranean, and european isolates. These data are compatible with exchanges of lineage I viruses between the latter regions and Senegal, probably through migratory birds. Conversely, lineage II isolates show high homogeneity -despite differences of hosts, year or site of isolation, or regions- compatible with an endemic mode of circulation. Interestingly, lineage I and II strains were co-circulating until 1998, whereas only lineage II strains were isolated in Senegal from that date. In addition, we identify a new lineage of West Nile virus isolated in south eastern Senegal at Kedougou in 1992. In conclusion, the data presented here provide new insight into the natural evolution, distribution, and dispersal of West Nile virus in Senegal, and should allow a better understanding of the factors leading to its emergence/maintenance in the other countries were it recently spread.

(ACMCIP Abstract)

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MULTIPLE FACTORS INFLUENCE WEST NILE VIRUS SEROPREVALENCE IN WILD MAMMALS

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Although mammals are frequently infected with West Nile virus (WNV), the factors that determine the intensity of exposure are poorly understood. We examined patterns of WNV seroprevalence in wild mammals along a forest to urban gradient in the eastern United States. We detected high WNV seroprevalence in four common peridomestic wild mammal species (opossums, raccoons, eastern gray squirrels and rats), and found that WNV antibody prevalence increased with age, urbanization, date of capture for juveniles, and varied between species. Our results offer important insights into the exposure of mammals to WNV and suggest that frequently exposed and abundant species such as gray squirrels are likely to be the best mammal sentinels of the spillover of WNV. However, our study also suggests that using squirrels as sentinels will require adequate samples of young of the year (juvenile) animals prior to dispersal, in order to accurately estimate current year transmission at the site of capture.

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INTRACELLULAR LOCALIZATION, MEMBRANE ASSOCIATION AND PROCESSING OF WNV NY99 STRAIN NS4B

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West Nile virus (WNV), replicates its RNA in association with newly rearranged host cell cytoplasmic membranes (CM), which are induced by viral proteins. Data suggest that WNV nonstructural protein 4B (NS4B) plays an important role in this process. The objectives of this study were to examine the role of the 2K signal peptide (SP) preceding WNV NY99 strain NS4B in intracellular localization, membrane association, CM formation, and proteolytic processing in mammalian cells. Various NS4B and Cycle 3 GFP or Histidine fusion proteins were constructed and tested in human embryonic epithelial kidney 293 (HEK293) cells using confocal immunofluorescence (IF) microscopy, phase separation and immunoblotting. NS4B was primarily localized to the endoplasmic reticulum (ER) and at a later time point translocated to the Golgi apparatus but not to the nucleus. Intense CM were evident in most cells at 40 hr post-transfection (p.t.) and the SP together with the NS4A played an important role in early initiation of these CM. Differential solubilization, and cellular fractionation in combination with triton X-114 phase

separation, indicated that the NY99 NS4B was first integrated into the ER membrane where it initiated the CM formation independent of the 2K SP. Evidence from three experimental approaches demonstrated that cleavage at the NY99 NS4A/4B signalase site occurs without the prerequisite cleavage at the putative NS4A/2K site. Furthermore, we established that post-cleavage at the signalase site, the NH2-terminal part of the WNV NS4B is localized in the cytoplasm, whereas the COOH-terminal part is in the lumen of the ER. Our data indicate that the SP preceding NS4B is not required for intracellular localization of NS4B, but it is essential for early induction of the CM in HEK293 cells. Further, specific amino acids in the NS4B allow the protein to assume a confirmation for the cleavage to occur at the signalase site, even in the absence of the 4A/2K cleavage.

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KNOWLEDGE, ATTITUDES, AND PRACTICES ABOUT WEST NILE VIRUS AMONG HISPANICS IN SAN DIEGO COUNTY - 2006

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West Nile virus (WNV), spread by infected mosquitoes, is a serious public health threat throughout the United States and can cause life-altering and even fatal disease. In San Diego County, one human WNV infection was reported in 2005 and 2006 combined. However, in neighboring counties of similar population, 121 and 11 human WNV infections were reported in 2005 and 2006, respectively. Personal protective behaviors (PPB) and education are the most important ways to prevent human WNV infection, because no cure or vaccine exists. We assessed the knowledge, attitudes, and practices (KAP) about WNV among Hispanics in San Diego County. We used a multistage cluster sampling scheme to administer an in-person KAP survey to 226 Hispanics over 18 years of age in three regions of San Diego County. Of the 420 homes approached, interviewers contacted a household member in 325 (77.4%) homes. Of these, 226 (69.5%) completed interviews. We found that demographics of Hispanics in the three regions sampled for this survey varied dramatically. Over 66% of the study population had heard of WNV, with older, U.S.-born respondents, and foreign-born respondents who had lived in the U.S. for more than 5 years reporting higher awareness than their counterparts. The news (newspaper, radio, television, etc.) was the most frequent source cited (93.2%) for WNV knowledge among the respondents, followed by doctor or health-care professionals (12.2%). Among the respondents who had heard of WNV, 41.6% had taken precautions to protect themselves or their family, with differences by age, gender, country of birth, and region. Knowledge of WNV and use of PPBs were lower among the study population than among predominantly non-Hispanic populations described in previous studies. Public health officials should implement educational and outreach efforts to the Hispanic community about WNV that are not only culturally competent but are also responsive to the heterogeneity within the Hispanic population.

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PHYLOGENETIC RELATIONSHIPS OF WEST NILE VIRUS ISOLATES COLLECTED IN FLORIDA DURING 2005

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During 2005, eight isolates of West Nile virus (WNV) were found in pools of Culex nigripalpus mosquitoes collected at three surveillance sites in Florida. Six isolates were collected from Duval County, one from Indian River County, and one from Manatee County. We sequenced a 1,500 base pair region of the envelope protein and a 1,600 base pair region of the NS3 protein to determine how these isolates related to each other, as well as other WNV sequences deposited into GenBank. All eight isolates were determined to be members of the North American clade of WNV. The envelope sequences also show the presence of a substitution at E159 (valine to alanine) that was previously described as a potential reversion to a less virulent strain. In addition to the field samples from 2005, we also sequenced the same envelope and NS3 regions for a 2003 isolate of WNV from Indian River County. The envelope amino acid sequence for this isolate does not have the alanine substitution at E159, thereby giving us a time frame for the introduction of the alanine-containing WNV strain into Florida. Additional evidence placing these Florida isolates within the North American clade as compared to NY99 is the presence of substitutions at nucleotide positions 6,138 and 6,426. With the exception of the Manatee County sample, the isolates we collected from Florida group together in our phylogenetic analysis. This indicates a spatio-temporal identity for this grouping, compared to the other reference samples from GenBank. At nucleotide position 6,238, we found a previously documented substitution in all field samples, except for the isolate from Manatee County. According to our phylogenetic analysis, the Manatee isolate is more similar to WNV isolates from Georgia and Texas than to our other Florida isolates. Manatee County is located on the Gulf Coast of Florida, while Duval and Indian River Counties are on the Atlantic Coast. Phylogenetic differences in WNV isolates could reflect adaptation occurring due to geographic host diversity, since flight patterns of migratory birds and overwintering sites may be dissimilar in bicoastal regions of Florida.

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OPTIMIZATION OF A CHIMERIC DEN-2/WEST NILE VACCINE

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West Nile virus (WNV) continues to impact public health worldwide, and there is an immediate need for a safe and protective WNV vaccine. The candidate dengue virus vaccine, DEN-2 PDK-53, was shown to be safe in early phase clinical trials. Chimeric vaccines expressing the prM and E genes of WNV in the attenuated DEN-2 PDK-53 background maintained the phenotypic attenuation markers of the DEN-2 PDK-53 virus and protected mice against lethal WNV challenge, as reported previously. We have engineered DEN-2/WN chimeras that replicate well in Vero cells, demonstrate reduced neurovirulence in mice, and fully protect mice from a lethal challenge with West Nile NY 99. Like other flaviviruses (e.g., the yellow fever vaccine, 17D), the parental DEN-2 PDK-53 virus and our lead DEN-2/WN vaccine candidate are very unstable in liquid formulations. Prior to human clinical testing of this vaccine, we sought to identify formulations that would significantly improve viral stability. The DEN-2 PDK-53 and DEN-2/WN vaccines were formulated with a variety of excipients including sugars, proteins, surfactants and polymers in phosphate-buffered saline and tested for stability by plaque assay. After 20 hours at 37C, the viruses were completely inactivated in most of the formulations. Some individual excipients provided a marginal increase in stability (1 - 8% titer remaining after 20 hours at 37C). Novel combinations of excipients provided enhanced thermal stability to the DEN-2-based vaccines. After incubation at 37C for 21 hours, lead vaccine formulations retained 30 - 70% of their starting infectious titers. In these formulations, the DEN-2 PDK-53 and DEN-2/WN viruses were stable for at least 7 days at room temperature, for at least 48 days at 4C, and after 2 cycles of freeze-thaw. We have identified candidate DEN-2/WN vaccine viruses and novel combinations of excipients that greatly enhance viral thermal stability. Enhanced thermal stability will facilitate distribution and administration of such vaccines worldwide.

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WNV-INDUCED MORBIDITY AND MORTALITY IN BALB/C MICE

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Recent data has shown that West Nile Virus (WNV) has spread to the Caribbean, including Puerto Rico (PR). Although WNV specific antibodies were detected in horses and resident birds, human cases have not yet been reported in PR. Since the Island is endemic to Dengue viruses (DENV), it is possible that prior immunity to DENV will protect the population against a WNV disease. To test this hypothesis, we are studying the effects of sequential DENV and WNV infections in Balb/c mice. For this purpose, we first determined the susceptibility of this mice strain to WNV infection at different ages. Groups of 20 mice at 9, 12 and 15 weeks of age were inoculated intraperitoneally with WNV doses ranging from 10^3 to 10^6 PFU. Morbidity and mortality were monitored for 28 days. We found that WNV-induced morbidity and mortality were dependent on mice age and virus dose. Signs of encephalitis were observed on day 6 post-infection in most inoculated animals. Mice began to die between day 7 and 8 after WNV infection. Viral loads in blood and brain were monitored by TaqMan assays. Our preliminary data showed that the viremia peak was observed around day 2 post-infection and was not associated with signs of disease. The viral load peak in brain was detected on day 6, which coincided with the appearance of sings of encephalitis. This data suggests that WNV mortality was associated to neuroinvasion. Antibody responses will be measured by indirect immunofluorescence and neutralization assays. Data from these tests will also be presented. Based in these results, 15-weeks-old mice will be used in future experiments to determine if DENV infection confers cross protection against WNV-induced morbidity and mortality.

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UPDATE ON THE STATUS OF THE NATIONAL WEST NILE VIRUS INFECTION AND PREGNANCY OUTCOMES STUDY

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Since 2005, the national registry tracking West Nile virus (WNV) infection of pregnant women and their pregnancy outcomes has been expanded to include enhanced follow-up of infant developmental outcomes. In addition, new information on maternal and infant health has been requested from women enrolled in the registry in 2003-04. Starting in 2005, newly infected pregnant women have been enrolled in a prospective case-control study to more thoroughly study the possiblity of adverse pregnancy outcomes associated with maternal WNV infection during pregnancy. Both the retrospective and prospective aspects of this study will result in a better understanding of the risks posed to children born to women infected with WNV during pregnancy. These studies are the first to address the lack of knowledge regarding WNV illness during pregnancy and adverse birth outcomes. Improved understanding of these risks will result in better counseling for these women and may lead to new options for treatment. Study design and results collected to date will be discussed.

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PREPARATION AND CHARACTERIZATION OF LIVE ATTENUATED STRAIN OF *LEISHMANIA MAJOR*

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Leishmaniasis is prevalent in 88 countries throughout the word, 21 in the new world and 66 in the old world; 16 of them are developed countries and the other 72 are developing countries. Leishmaniasis caused by different species of protozoan parasites belonging to the genus *Leishmania*. The combination of leishmaniasis and co-infection with HIV has been a major problem. Attempts to produce an effective vaccine have so far failed, and treatment of the disease in regions still primarily on the use of pentavalent antimonials, but the Resistance to drug is so high in some parts of the world and second - line drugs have not experienced wide spread use due to toxicity and cost. In this study a species-specific monoclonal antibody (mAb) recognize component on surface and culture supernatant of leishmania major (L.major). In ELISA and Western blotting this component is species-specific to *L. major* without cross-reactivity with other leishmania species, such as *L. donovani, L. infantum* and *L. tropica*. Since the mAb, can not reacted with component of medium, it is questionable that the parasite acquire unknown component from the growth medium and /or post translation modification antigens that parasite express on the surface or secrete to medium. We shown that the mAb can disrupt component(s) to parasite surface according to the presence of mAb in culture medium and un-coated parasite could not induce infection in susceptible BALB/c mice. Immunized mice compare with control groups could not induce infection after challenge with standard stain of *L. major*. In vitro results shown that vaccinated mice induce high level of IFN-γ, low Parasites load and resistant to L.major infection for at least 12 month.These observation explain a novel option that parasite acquire virulence factor as a post-translation modification of antigens.

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IDENTIFICATION OF GENETIC VARIATION AND POLYMORPHISMS PRODUCING DIFFERING MULTILOCUS ENZYME ELECTROPHORESIS PATTERNS IN NEW WORLD *LEISHMANIA* SPECIES

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Leishmaniasis in the New World is caused by *Leishmania* species that belong to the *Viannia* and *Leishmania* subgenera. All the species within this genus can cause characteristic cutaneous lesions, while only *Leishmania (Viannia) braziliensis* has been implicated in producing the metastatic mucocutaneous form. Multilocus enzyme electrophoresis (MLEE) is the gold standard for species identification, but its robustness and resolving potential is limited since it can only detect nonsynonymous substitutions that alter the net charge of the protein. We analyzed the coding regions of mannose phosphate isomerase (MPI), malate dehydrogenase (MDH), glucose phosphate isomerase (GPI), and glucose-6-phosphate dehydrogenase (G6PDH) from 63 strains belonging to 7 species of *Leishmania* previously typed by MLEE to identify the mutations responsible for the observed migration patterns for these enzymes. We confirmed the previously reported C1082G polymorphism in MPI that allows the differentiation of the highly related *L. (V.) braziliensis* and *L (V.) peruviana*, plus 2 additional species-specific polymorphisms in the MDH sequence. Several single nucleotide polymorphisms unique for every species were identified. Additionally, we identified a subpopulation of *L. braziliensis* isolates having atypical MLEE patterns for all the markers analyzed. When the corresponding genes were DNA sequenced, a

considerable level of genotypic divergence from the rest of the *L. braziliensis* strains was found, which was supported by PCR-RFLP data. DNA sequencing allowed the identification of polymorphisms not readily observable by MLEE and thus has a higher power of resolution for the identification of New World *Leishmania* species.

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PHASE III TRIAL OF PAFURAMIDINE MALEATE (DB289), A NOVEL ORAL DRUG, FOR TREATMENT OF FIRST STAGE SLEEPING SICKNESS

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Only a very limited number of drugs are available for treatment of sleeping sickness and none of them is applicable by the oral route. After successful preclinical testing, the oral prodrug pafuramidine was selected for clinical development for the treatment of first stage sleeping sickness in the year 2000 by the international consortium to discover new drugs for the treatment of parasitic diseases. The consortium is lead by the University of North Carolina, Chapel Hill, USA, and funded through the Bill and Melinda Gates Foundation. The compound successfully underwent extensive evaluation in several Phase I studies in healthy volunteers and Phase II (proof of concept in patients) clinical trials of trypanosomiasis caused by *Trypanosoma b. gambiense*. The clinical studies demonstrated the efficacy and good tolerability of pafuramidine, in particular as compared to standard treatment pentamidine. In the Phase II trials, the dosing regimen for pafuramidine was extended from 5 to 10 days in order to achieve sustained efficacy. A protocol for a pivotal Phase III confirmatory trial was developed in close collaboration with the US FDA. This open-label (sponsor-blinded), randomized, controlled clinical trial comparing pafuramidine with pentamidine was initiated in August 2005. Patients were enrolled in four centers in the Democratic Republic of Congo and in one center each in Angola and South Sudan. Enrollment of 274 patients, including adolescents and pregnant and lactating women, was completed in March 2007. Follow up of the patients will continue for two years. An interim analysis by the Data Safety Monitoring Board is planned for July 2007 after half of the recruited subjects have undergone the 12 month follow up examination. Should the outcome of this analysis be satisfactory, all subjects will undergo the 12 month follow up evaluation, the primary endpoint for the trial, and a registration dossier for submission to the US FDA will be prepared. Results will be presented on the safety and preliminary efficacy of pafuramidine.

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REPOSITIONING OF PDE TARGET CHEMISTRY TO PROMOTE DRUG DISCOVERY FOR SLEEPING SICKNESS

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Infection by African trypanosomes causes sleeping sickness, a lethal disease that threatens 60 million people. Existing treatments are inadequate and WHO has called for discovery of new drugs with better efficacy and safety. Such drugs must cross the blood brain barrier (BBB) to reach infection sites, eradicate the parasites, and be safe for humans. *De novo* discovery of such compounds is expensive and time-consuming. As a result there has been little progress in the delivery of drug candidates for sleeping sickness and other diseases that afflict poor populations. To facilitate new drug discovery we focus on *Trypanosoma brucei* proteins similar to known drug targets with existing chemistry expertise. Starting from 1200 targets in DrugBank we identified over 300 with *T. brucei* homologs. Two homologs, PDEB1 and PDEB2, were recently shown by RNAi to be essential for infection. These enzymes are similar to human phosphodiesterases (PDEs). Inhibitors of PDEs are selective and safe in humans, and several cross the BBB. We constructed homology models of the trypanosome PDEBs and compared these to crystal structures of human PDE-drug complexes. Binding site differences between human and parasite PDEs are consistent with the low potency of certain drugs against *T. brucei*. These differences suggest avenues for the design of compounds with better efficacy. Our results also suggest strategies for virtual screening of compound collections from companies that have worked on human PDE targets. Similar repositioning of existing target chemistry may be broadly useful to promote drug discovery for sleeping sickness and other neglected diseases.

(ACMCIP Abstract)

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AMERICAN TRYPANOSOMIASIS (CHAGAS DISEASE) AMONG LATIN AMERICAN IMMIGRANTS IN A CARDIOLOGY CLINIC IN LOS ANGELES, CALIFORNIA

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While the association between American trypanosomiasis (Chagas disease) and cardiomyopathy is well established, the prevalence of Chagas disease among Latin American immigrants with cardiomyopathy in the United States is unknown. We therefore conducted a cross-sectional study to estimate the prevalence of Chagas disease among Latin American immigrants with cardiomyopathy attending a cardiology clinic in a county medical center in Los Angeles, California. Charts of patients followed in the cardiology clinic were reviewed to identify patients with Hispanic surnames and a history of cardiomyopathy, defined as an ejection fraction (EF) < 55% demonstrated on transthoracic or transesophageal echocardiography. A questionnaire was completed by each study participant in either English or Spanish to obtain epidemiologic/risk data. Two cc of serum were obtained from all study participants. All samples were tested using a radioimmunoprecipitation assay (RIPA) that detects antibodies that bind specifically to the 72- and 90-kDa antigens of *Trypanosoma cruzi*. Of 85 Latin American immigrants with cardiomyopathy tested, 4 (4.7%) had Chagas disease, as evidenced by a positive RIPA. By contrast, all of the 54 non-Hispanic controls had negative RIPA results. These data demonstrate that a substantial portion of Latin American immigrants with cardiomyopathy attending our cardiology clinic in Los Angeles have Chagas disease. The diagnosis of cardiac Chagas disease has important management and prognostic implications. Our findings provide additional support for the view that Latin American immigrants with geographic risk for Chagas disease should be tested serologically.

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THE WIDE CLINICAL SPECTRUM OF *LEISHMANIA VIANNIA BRAZILIENSIS* INFECTION IN THE STATE OF CEARÁ, NORTHEASTERN BRAZIL

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Cutaneous leishmaniasis is an important endemic disease in Brazil with more than 25,000 new cases annually. *Leishmania Viannia braziliensis (LVb)* is the major etiologic agent in many states, including Ceará, which is located in northeastern Brazil and ranks fourth in the total number of cases. *LVb* is associated with a wide range of clinical manifestations. We assessed the clinical presentations of more than 1500 patients from 1986-2006. Ulcerated skin lesions with raised borders were the most common, but there many variations including patients with multiple lesions, different types of lesions and lesions in different stages of evolution. Patients who seek very early medical attention typically present with non-ulcerated nodular, papular or crusted skin lesions. Lymph node enlargement in the early stage of disease is an important clinical finding in *LVb* (> 60% of cases) and may be seen before, simultaneously with or after the appearance of skin lesion(s). Lymph nodes may be as large as 10 cm and are usually non-tender. Those in the axilla may be missed if a careful physical exam is not done. Skin lesions are usually < 10mm in diameter in persons with lymphadenopathy in the early stage of disease. Children may present with fever, adenopathy and small lesions. Disseminated cutaneous leishmaniasis occurs in a small percentage of cases, but can be dramatic. One patient had 749 skin lesions. Mucosal disease followed the pattern described elsewhere in Brazil, but accounted for less than 1% of patients. In one person the time between cutaneous disease and mucosal symptoms was 35 years. *LVb* disease in HIV-infected individuals has also been uncommon. In one patient with advanced AIDS, there was extensive skin involvement, with no ulceration. The skin was thickened and darkened. Buffy coat cultures in NNN were positive for *LVb* on multiple occasions. Despite the endemicity of *LVb* in Ceará, the diagnosis is still missed on occasion by local physicians. The challenge of making the diagnosis has been diminished by the development of a modified imprint test using skin biopsies (2mm punch); amastigotes have been identified in >90% of suspected persons using this method. Cutaneous leishmaniasis due to *LVb* is a major health problem in endemic areas of the state of Ceará in northeastern Brazil and has a wide spectrum of clinical findings.

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RAPID DIAGNOSIS OF HUMAN LEISHMANIASIS SPECIES USING A RAPID CELLULOSE ACETATE ELECTROPHORESIS (CAE)

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Human leishmaniasis caused by protozoan parasites of the genus *Leishmania* constitute a serious public health problem in several countries. Clinicians are confronted with steadily higher numbers of leishmaniasis patients not only in countries where the disease is endemic but also in countries where these parasites are not endemic. Unfortunately, there are no good established methods for the diagnosis of leishmaniasis at the species level. Current PCR techniques are plagued by a lack of adequate validation even if the correct primer / probe set are selected for speciation. The ability to provide a genus diagnosis is helpful, but can often be captured by the use of a slide and microscope alone. The challenge is to provide an accepted methodology that provides rapid turn-around to the species level. Multilocus Enzyme electrophoresis (MLEE) is an established species level technique but often takes several days to weeks to get a diagnosis. We have modified the MLEE technique so that it can be applied to diagnose a case with a small parasite sample or even a small amount of scraped tissue from the infected areas in just a few hours. In our laboratory, we have designed and standardized a simple (1-2 hrs) cellulose acetate electrophoresis (CAE) technique to characterize the infecting *Leishmania* species. In this technique, we take a small pellet of the *Leishmania* isolates or cultures and make a cellular lysates by freeze-thawing several times in liquid Nitrogen. Where a small tissue samples are available from the infected host, a tissue homogenate is made. A small amount of the clear supernatant (lysates/homogenate) from each sample is run in each well using cellulose acetate. Each membrane is stained for a single isozyme using the standard procedure and a final identification is made by comparing the standard. Within 1-2 hours, we can conceivably have a genus species diagnosis. Four isoenzymes were found to be very effective in the species identification of *Leishmania* sp. both the New World and Old World parasites viz., Glucose Phosphate Isomerase (E.C.5.3.1.9 GPI), Mannose Phosphate Isomerase (E.C.5.3.1.8 MPI), Phosphogluconate Dehydrogenase (E.C.1.1.1.44 GPGDH), Leucine Peptidase (E.C.3.4.11 or 13 LPI). Further studies are underway to find more isozymes of diagnostic importance.

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COST-EFFECTIVENESS OF ALGORITHMS FOR POPULATION SCREENING OF HUMAN AFRICAN TRYPANOSOMIASIS

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The control of *Trypanosoma brucei gambiense* Human African Trypanosomiasis (HAT) is compromised by the low sensitivity of the parasitological confirmation tests that are routinely used. More sensitive alternatives exist such as mini-Anion-Exchange-Centrifugation (mAECT) or Capillary Tube Centrifugation (CTC) but they are more expensive. We used formal decision analysis to assess the cost-effectiveness of alternative HAT confirmation algorithms in terms of cost per life saved. The effectiveness of the classical combination lymph node puncture (LNP), fresh blood examination (FBE) and thick blood film (TBF) was 36.80%, whilst the LNP-FBE-CTD-mAECT sequence reached almost 80%. The cost per person examined ranged from 1.56 € for LNP-FBE-TBF to 2.99 € for LNP-TBF-CTC-mAECT-CATT Titration. The algorithm LNP-TBF-CTC-mAECT was the most cost-effective in terms of cost per life saved. HAT confirmation algorithms that incorporate concentration techniques are more effective and efficient than the algorithms that are currently routinely used by several *T.b. gambiense* control programs.

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CANINE VISCERAL LEISHMANIASIS DIAGNOSIS IN BRASIL

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In Brazil, Canine Visceral Leishmaniasis (CVL) is caused by the parasite, *Leishmania (L.) chagasi*. The purpose of the present study was to evaluate the serological tests (RIFI and ELISA) in 34 *Leishmania*-infected dogs from Ilha Solteira, SP, Brazil. These tests were also compared with other methods used for diagnosis of this disease in dogs such as histological (H&E) and immunohistochemical (IMHC) in skin. When serological tests were done, 24 (70.6%) dogs were positives by ELISA or IFAT, but the number of dogs positives by ELISA was slightly higher than IFAT (56% for IFAT and 65% for ELISA), and in 7 (21%) dogs there was no agreement between ELISA and IFAT. There was also no agreement between serological tests and H&E or IMHC in 11 (32.4%), particularly in asymptomatic dogs. One asymptomatic dog was also positive in all tests (serological, H&E and IMHC), indicating that even dogs without any clinical signs can be infected. In symptomatic dogs, the agreement among tests was 100%, but 1/9 (11.1%) dog was negative and 8/9 (88.8%) were positives. The skin biopsies from asymptomatic dogs had negligible if any lesions, and parasite direct examination showed that the most of these dogs (62.5%) were negative or suspect, but 3 (37.5%) were positive in skins without any dermatological alterations. 100% of symptomatic dogs had several forms of dermatological alterations with strong parasite loud in their lesion skins. The results of the present study is in agreement with the observations that

only one test (serological or parasitological) may be not sufficient for CVL diagnosis. In addition, IMHC was considered a valuable method to support the diagnosis of the disease in addition to serological tests.

(ACMCIP Abstract)

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DEVELOPMENT OF A FIELD-USABLE ASSAY FOR DETECTION OF *LEISHMANIA* PARASITES IN SAND FLIES

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Specific antibody reagents that recognize promastigote surface components are being applied in the development and evaluation of prototype assays for the detection of various species of *Leishmania* parasites in sand flies. The assay is performed in a rapid, one-step immunochromatographic process for detection and identification of various *Leishmania sp* such as *L. major, L. tropica, L donovani,* and other epidemiologically relevant species in vectoring sand-flies. The procedure is initiated by grinding sand flies - individually or in pools and the sample used by the wicking assay that provides results within 15 to 30 minutes. Detection of infected sand flies and parasite numbers in the samples correlate with results obtained by the present dipstick assay format and the PCR assay. The developed dipstick assays will be stable at ambient storage conditions. A field-usable assay for the detection of Leishmania parasites in sand flies would allow for the accurate assessment of the leishmaniasis threat. Once threat assessment results are available necessary prevention and control efforts can be pursued in a focused and timely program.

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CHITINASE: ACTIVE RECOMBINANT PROTEIN FROM *PLASMODIUM VIVAX*

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To initiate invasion of the mosquito midgut, *Plasmodium* ookinetes secrete chitinases that are necessary to cross the chitin-containing peritrophic matix en route to invading the epithelial cell surface. Therefore the chitinases (EC 3.2.1.14) are potential targets of malaria parasite transmission-blocking interventions. *P. falciparum* chitinase PfCHT1 was successfully expressed already as an active recombinant enzyme in *E. coli*. PvCHT1 (GenBank#AB106896) is the sole chitinase from *P. vivax* identified to date, but it has yet to be characterized due to difficulty with recombinant protein synthesis. PvCHT1 differs from PfCHT1 in that *P. falciparum* gene lacks proenzyme and chitin-binding domains. Here we have tried to express recombinant PvCHT1, excluding N-terminal signal peptide and repeat/insert region, using wheat germ cell-free protein expression system. PfCHT1 was equally expressed cell-free for comparison. As is the case with PfCHT1, recombinant PvCHT1 hydrolyzes 4-methylumbelliferone (4MU) derivatives of chitin oligosaccharides. It shares substrate preference unique to *Plasmodium* endochitinases including PgCHT1 and PfCHT1: only 4MU-$GlcNAc_3$ of 4MU-$GlcNAc_{1\sim3}$ is hydrolyzed. Immunofluorescence assay with mouse antisera against purified recombinant demonstrated that PvCHT1 is expressed at as early as zygote and is concentrated at the apical end of ookinetes. One of the PvCHT1's uniqueness is lower sensitivity to allosamidin, the chitinase inhibitor: an IC(50) of 6 microM is >1,000 times higher than PfCHT1 (1 nanoM). The pH and temperature activity profile of PvCHT1 are also differently demonstrated from PfCHT1. The findings here on active recombinant chitinase from *P. vivax*, together with previous studies, should further contribute to developing strategies for interrupting the function of *Plasmodium* chitinases and eventually for parasite transmission-blocking interventions within the mosquito midgut.

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USE OF GLOBAL PROTEOMICS TO DEFINE PROTEIN PROFILES OF SEVERE DISEASE: AN INVESTIGATION ON SEVERE MALARIA

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Compounds directly involved in the mechanism of cerebral malaria (CM) continue to be a mystery due to lack of robust methods of identifying and quantifying proteins expressed in low abundance. Developments in proteomics have made it possible to identify low abundant proteins and may help identify new protein signatures for patients with various complications associated with severe malaria. We have used two-dimensional gel electrophoresis (2-DE) coupled to MALDI-TOF mass spectrometry and two-dimensional liquid chromatography (2D-LC) coupled to an ESI-ion trap mass spectrometer to identify differentially expressed proteins in archived CSF of children diagnosed with cerebral malaria (CM) compared with those diagnosed with acute bacterial meningitis (ABM) and slide negative encephalopathy (EN). Data were searched against the NCBInr and Sanger *falciparum* databases. Proteins identified from NCBInr were filtered to exclude viral and bacterial found. The results of these studies demonstrated that: *i)* An average of 150 spots were resolved on the CM and EN gels and 80 spots on ABM gels. Forty-five human proteins were found in both CM and EN gels. 20 human proteins were unique to ABM compared to CM. *ii) A* total of 202 human proteins were identified using the 2D-LC system. Of these 13 were unique to CM, 124 to ABM and 32 to EN. 6 proteins were found in both CM and ABM and 18 were found in EN and ABM. 9 proteins were common to all 3 disease groups. A total of 66 *falciparum* proteins were identified but of these 48 were hypothetical proteins. Of the non-hypothetical proteins, 2 were found in both CM and ABM and the rest were found only in ABM. In conclusion, proteomics can be used to create protein profiles of different disease groups. The use of 2D-LC enabled us to identify more low abundant proteins but some of the *falciparum* proteins identified by 2-DE were not seen in the 2D-LC method. Majority of the human proteins found were plasma proteins including common circulating proteins such as albumin and apolipoproteins, blood transporters and binding proteins, protease inhibitors, enzymes, cytokines and hormones, and channel and receptor -derived proteins. There seems to be a correlation between the number of proteins found in the CSF and the level of blood brain barrier break down. We are exploring the utility of this technique in discriminating between freshly collected plasma and cerebral spinal fluid from infected and healthy human subjects.

(ACMCIP Abstract)

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RECOVERY OF ENDOTHELIAL FUNCTION IN SEVERE FALCIPARUM MALARIA CORRELATES WITH RECOVERY OF PLASMA ARGININE CONCENTRATIONS AND FALL IN BLOOD LACTATE

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Severe malaria is associated with tissue ischemia, related to cytoadherence of parasitized erythrocytes to microvascular endothelium and reduced levels of nitric oxide (NO) and its precursor, L-arginine. As endothelial function can be improved by L-arginine in cardiovascular disease, we aimed to investigate the longitudinal relationship between endothelial function, tissue perfusion and plasma L-arginine concentrations during the clinical course of severe falciparum malaria (SM). Endothelial function, plasma L-arginine and blood lactate concentrations were measured longitudinally in 49 adult patients (18-56 years) with SM enrolled at Mitra Masyarakat Hospital in Timika, Papua, Indonesia, and in 48 healthy controls. Endothelial function was measured using peripheral arterial tonometry (PAT), and the reactive hyperemia PAT (RH-PAT) index calculated. Measurements were undertaken on admission and repeated daily following intravenous antimalarial therapy. RH-PAT index was impaired (<1.67) in 94% (46/49) of subjects with SM on admission. Following treatment, mean RH-PAT index rose from 1.41 [95%CI 1.33-1.47] to 1.72 [95%CI 1.62-1.82] at 48 hours. Mean plasma L-arginine concentration in SM was low on admission (49 µmol/L [95%CI: 43-55]) relative to controls (77 µmol/L [95%CI: 68-85]; p<0.0001), increasing to 76 umol/L [95%CI: 66-86] at 48 hours. In a longitudinal random effects model, there was a significant association between the improvement in RH-PAT index and the increase in L-arginine concentrations (p=0.01). RH-PAT values were inversely correlated with blood lactate concentrations in SM (r= -0.31; p=0.01). In the random effects model, improvement in RH-PAT was significantly associated with a fall in lactate levels (p=0.0001). In conclusion, patients with severe malaria have significant impairment of endothelial function and hypoargininemia. Recovery of endothelial function is related to the increase in plasma L-arginine concentrations and improved perfusion. L-arginine replacement may have a role as adjunctive therapy in severe malaria early in the course of illness by improving endothelial NO production and endothelial function.

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NITRIC OXIDE IN INTRAERYTHROCYTIC PLASMODIUM FALCIPARUM MAY BE PRODUCED IN THE FOOD VACUOLE BY AN ARGININE-INDEPENDENT MECHANISM

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Nitric oxide (NO) has diverse biological functions and its biosynthetic pathway has been documented in a wide variety of organisms. Within the family of protozoan parasites, NO production has been established in medically relevant organisms. Little is known, however, about NO production in Plasmodium falciparum, the agent responsible for the most lethal form of human malaria. In 1995, a nitric oxide synthase (NOS) activity was described in P. falciparum, although no other study since then confirmed that observation. NOS enzymes produce equimolecular amounts of NO and L-citrulline from the substrate L-arginine. Using diaminorhodamine-4M AM (DAR-4M AM), a nitric oxide fluorescent indicator, we obtained positive fluorescent signals suggesting nitric oxide production localized in the food vacuole of intraerythrocytic P. falciparum. Flow cytometry measurements showed that the median fluorescence intensity (MFI) of P. falciparum parasites cultured in arginine-free RPMI was at least as high as the MFI of parasites grown on standard RPMI containing L-arginine, suggesting that the parasite could utilize L-arginine derived from hemoglobin proteolysis for NO synthesis if no other source was available. However, using HPLC analysis, we found no evidence of L-citrulline production in P. falciparum cultures that could match the molar amounts of NO/nitrite produced by the parasite in vitro. Finally, western blot studies of P. falciparum protein extracts and database search of the P. falciparum genome did not reveal any sequence with significant homology to NOS molecules. In conclusion, DAR-4M AM images suggest that intraerythrocytic forms of P. falciparum parasites produced NO in or around the food vacuole, a critical parasitic compartment involved in hemoglobin degradation, heme detoxification and a target for antimalarial drug action. However, we did not obtain any conclusive evidence of the presence of an arginine-dependent P. falciparum NOS. NO production by alternative arginine-dependent or independent pathways should be investigated in P. falciparum.

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MOLECULAR BASIS OF *PLASMODIUM FALCIPARUM* RECEPTOR BAEBL FOR BINDING TO ERYTHROCYTE LIGAND GLYCOPHORIN C

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Plasmodium falciparum invades human erythrocytes by redundant pathways. Unlike *P. vivax* that has one Duffy Binding-Like (DBL) receptor, *P. falciparum* has four members of the DBL receptor family. Furthermore, one of these DBL genes, BAEBL, has polymorphisms at four amino acids in region 2, the receptor region of the protein; each polymorphism binds to a different red blood cell (RBC) ligand. One BAEBL variant (VSTK) binds specifically to erythrocyte glycophorin C and has no binding to neuraminidase-treated RBCs from which sialic acids were removed. When the amino acid threonine (T121) in BAEBL (VSTK) is changed to a lysine (K), it binds to a different RBC ligand that does not use sailic acid. We modeled the structure of region 2 of BAEBL (VSTK) on the crystal structure of a related DBL receptor, EBA-175. Four charged amino acids, Arg 52, Arg 114, Glu 54 and Asp 125, are predicted to surround T121 on the model of BAEBL (VSTK). They were individually mutated to alanine (R52, R114, E54, and D125) or lysine (R52, R114) and expressed on the surface of CHO cells. The wildtype binds poorly to Gerbich negative cells that have a deletion of exon 3 in glycophorin C, and poorly to neuraminidase-treated RBCs. Mutations in arginine 52 or 114 of BAEBL (VSTK) now could bind to Gerbich negative RBCs and neuraminidase-treated RBCs. Mutations in glutamic and aspartic acid had no effect on binding, that is, they had markedly reduced binding to Gerbich negative RBCs and neuraminidase-treated RBCs. These findings suggest that the two arginine residues surrounding T121 are critical for binding specificity of BAEBL (VSTK) to erythrocyte ligand glycophorin C and sialic acid.

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DIFFERENTIAL *IN VIVO* AND *IN VITRO* EXPRESSION OF MAESTRO PREDICTED MITOCHONDRIAL PROTEINS IN *PLASMODIUM FALCIPARUM*

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Bioinformatic tools for detecting signal sequences that direct proteins to the mitochondrion have limited precision in *Plasmodium falciparum* due to the high AT content of the genome. Predictions based on protein domains and expression data alone are also limited in sensitivity and specificity. Therefore, a Baysian probability based program, Maestro, was used to generate a list of core mitochondrial protein predictions by integrating data sets with complementary information. Using a 'gold-standard' set of proteins, the performances of current methods for predicting mitochondrial localization were evaluated. Valuable datasets were then combined in order to compile an inventory of high-confidence mitochondrial protein predictions. Microarray data from patient peripheral

blood samples collected at a field site in Velingara, Senegal in 2004 and 2005 was compared to expression data from cultured cell time courses for asexual and gametocyte stages. As expected, mitochondrial genes are more highly expressed in late asexual stages than in ring and merozoite stages. Hierarchical clustering of the predicted mitochondrial genes based on expression values revealed differential expression patterns between cultured parasites and field samples, with a subset of mitochondrial genes upregulated in both profiles. Within patient samples at least two distinctive expression profiles are evident. These expression profiles suggest the presence of an activated mitochondrial metabolic state *in vivo* and contrast with the expression profiles of 3D7 *in vitro* culture.

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THE *PLASMODIUM* SPOROZOITE AND ERYTHROCYTIC STAGE (SES) PROTEIN HAS A UNIQUE SURFACE LABELING PATTERN ON THE SPOROZOITE AND APPEARS TO PLAY A ROLE IN SPOROZOITE INVASION OF MOSQUITO SALIVARY GLANDS

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The *Plasmodium* sporozoite is infective for mosquito salivary glands and vertebrate host tissues. Although it is a key developmental stage of the malaria parasite, relatively few sporozoite surface or secreted proteins have been identified and characterized. A novel surface molecule, designated the Sporozoite and Erythrocytic Stage (SES) protein, is preferentially-expressed in salivary gland sporozoites versus oocyst and hemolymph sporozoites of *P. gallinaceum*. PgSES exhibits a spiral surface labeling pattern that overlays a known sporozoite surface antigen, the circumsporozoite protein, with only minor co-localization. It consists of 551 amino acids encoding a putative 63.2 kDa protein that has been shown to be expressed not only on particular sporozoite stages, but also during the erythrocytic stages. This novel protein has three conserved regions of unknown function that are present in eight *Plasmodium* spp. representing human, avian, non-human primate, and rodent malarias. Antibody blocking studies assessing the role of PgSES in sporozoite invasion of mosquito salivary glands show that anti-PgSES antibodies block invasion by 49-87%. The *P. falciparum* homolog, PfSES, also appears to have expression during the sporozoite and erythrocytic stages. Currently, studies assessing the function of both PgSES and PfSES in the sporozoite and erythrocytic stages are being conducted. Ultimately, if the SES protein is found to be critical to parasite development and/or invasion of host tissues, it could be a target for novel malaria intervention efforts.

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A CHEMICAL BIOLOGY APPROACH TO UNDERSTAND CYSTEINE PROTEASE FUNCTION IN *PLASMODIUM FALCIPARUM*

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The complete *Plasmodium falciparum* genome sequence is a rich resource in the search for targets of novel antimalarial therapies and allows the possibility of more global approaches for therapeutic target discovery. We have undertaken a chemical biology approach in *P. falciparum* to validate the therapeutic potential and functions of cysteine proteases expressed within the erythrocytic life cycle. Chemical genomic techniques were used to determine that the general cysteine protease inhibitor, E64d, elicits a cytostatic response during the intraerythrocytic life cycle and a cytotoxic response upon attempted parasite rupture form the host red blood cell. Chemical genomics and real-time PCR techniques were then performed to determine direct and indirect targets of the E64d. Coupling this transcriptional data to chemical proteomics studies, we determined that a novel family of cysteine proteases, the SERAs, play a role in the rupture of malarial parasites from host red blood cells and that this function is the key cytotoxic event for this enzyme family within the erythrocytic life cycle. Ultimately, this study demonstrates the utility of using general inhibitors to validate the therapeutic potential of enzyme families and the use of chemical genomics and proteomics techniques to identify the specific effects of these small molecules.

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IMPACT OF REPEATED ADMINISTRATION OF ACTS ON ANEMIA AND ANTIMALARIAL IMMUNITY IN MALI

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Most African countries have now changed their first line treatments from monotherapies to Artemisinin-based combination therapies (ACTs). ACT efficacy measured at Day 14 or 28 may not adequately reflect their true public health impact. Therefore, it is important to assess overall public health impact of the repetitive administration of these new combinations in the African context. In an ongoing randomized controlled Phase IV trial in Bougoula-Hameau, Mali we compare the impact of repeated administration of AS/AQ, AS/SP and AR-L for the treatment of consecutive episodes of uncomplicated malaria on anemia and antimalarial immunity. To date, we have screened 3850 subjects and included 780 patients (260 per arm) aged 6 months and above who have experienced a total of 1833 cases of uncomplicated malaria over 18 months.The geometric means of parasite density were compared during consecutive episodes of malaria. Hemoglobin concentration was measured on Days 0, 14 and 28. Incidence of anemia in each arm has been compared within the three treatment groups. All three arms significantly decreased the frequency of malaria attributable anemia during 28 days of follow up. Additional analysis on the incidence of anemia over time will be presented. To investigate whether repetitive treatment changes the number of parasites required for the appearance of malaria symptoms we compared the geometric means of parasite density during consecutive episodes of malaria and found that there were no significant differences between the three treatment regimens. Serum samples were collected during successive episodes of malaria. Antibody titers to various malarial antigens including *P. falciparum* 3D7 schizonte extract and blood stage antigens (MSP1-42, MSP3, AMA1, GLRUP, SERP) will be measured on these sera and presented to the meeting. The implications of these observations for large-scale ACT deployment in Africa will be discussed.

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ANALYSIS OF PFE0565W AND PF11_0394, TWO *PLASMODIUM FALCIPARUM* SPOROZOITE GENES

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Malaria is a resurging disease and thus, there is a need for better control methods. A key stage of the *Plasmodium* life cycle is the sporozoite because it exhibits dual infectivity for both the mosquito vector and vertebrate host. Therefore, sporozoites are a promising target for discovering effective ways of controlling malaria. Using data mining techniques, the *P. falciparum* genes, PFE0565w and PF11_0394, were chosen as candidates for study due to their potential role in the invasion of host tissues. These genes were selected based on data from PlasmoDB, indicating that these genes likely encode putative surface proteins and are expressed both at the transcriptional and protein levels in sporozoites. Additional sequence analysis shows that each gene has orthologs in other *Plasmodium* species. Both PFE0565w and PF11_0394 also express transcripts during the erythrocytic stages of the parasite life cycle as shown by RT-PCR. To analyze expression at the protein level, the production of polyclonal antibodies for PF11_0394 is in progress and antibodies for

PFE0565w have been generated and used to confirm its expression in asexual parasites via Western blot and to suggest its presence on the sporozoite surface using confocal microscopy. GFP trafficking constructs are being made to track the expression of both the PFE0565w and PF11_0394 proteins in various parasite stages, and gene disruption studies are in progress to ultimately assess the function of the genes in sporozoite biology and to determine if they play a role in host tissue invasion.

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COMPARISON OF CHLORPROGUANIL-DAPSONE WITH A COMBINATION OF CHLOROQUINE AND SULFADOXINE-PYRIMETHAMINE IN CHILDREN WITH MALARIA IN JOS, NIGERIA

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Effective, available and affordable treatment for uncomplicated malaria in children is critical in the face of increasing resistance of *Plasmodium falciparum* to CQ and SP. We compared the efficacy of chlorproguanil-dapsone(CD) with a combination of chloroquine and sulfadoxine-pyrimethamine(SP+CQ) in children under five years with malaria. Of 889 children presenting with fever, 264 met the inclusion criteria and were randomized to receive SP+CQ(n=136) and CD(n=128). Clinical and parasitological response was assessed on days 1,2,3,7 and 14. A total of 122 subjects in SP+ CQ group and 118 in CD group completed the study. By day 3, 96(78.7%) in SP+CQ and 94(79.7%) in CD had cleared their parasitaemia (p=0.79). By day 14, 111(91%) and 115(97.5%) respectively had cleared their parasitaemia (p=0.06). By day 3, 107(87.7%) in SP+CQ and 109(92.4%) in CD were symptom -free (p=0.32). Adequate clinical and parasitological response occurred in 111(94%; 95% CI 91.6-95.7%) in CD and 113(92.6%; CI 89.9-94.3%) in SP+CQ (p=0.85). In conclusion, SP+CQ and CD were not significantly different in their antimalarial efficacy and may provide an affordable choice in the treatment of uncomplicated malaria in children in northern Nigeria.

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COMPARATIVE EFFICACY OF AN ARTEMISININ COMBINATION THERAPY (ACT) AND A NON-ARTEMISININ COMBINATION THERAPY IN THE MANAGEMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN IBADAN, NIGERIA

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Artemisinin Combination Therapies (ACT) are now considered the gold standard for the treatment of malaria. However, they are often not affordable or available to those who need them most. We thus compared the relative safety and efficacy of an ACT with a non-ACT combination therapy for malaria. Method: In an open randomized, non-inferiority clinical trial, children aged less than 10 years with symptomatic acute uncomplicated malaria were allocated to 2 groups. Children in group 1 received artemether-lumefanthrine [AL] (6-doses), while those in group 2 received Amodiaquine (25mg/kg over 3days) plus sulfadoxine pyrimethamine (AMQ-SP) as a single dose, (25mg/kg of sulfadoxine to the next quarter tablet). Drugs were administered orally under supervision by medical staff. One hundred and twenty children (60 in each group) were evaluated. The mean fever clearance time was 1.19 ±0.44 and 1.52±0.78 for AL and AMQ-SP respectively, p = 0.0061. The mean parasite clearance time was 1.30 ± 0.49 and 2.22 ± 0.83 for AL and AMQ-SP respectively p = 0.0000001. Day 14 cure rate was 100% for both AL and AMQ-SP. Day 28 parasitological failure rates was 3.3% (2/60) for AL and 6.7% (4/60) for AMQ-SP (p = 0.079). Gametocyte carriage rate among AL treated patient was 0% after day 3 while the gametocyte persisted up to day 21 in those treated with AMQ-SP (p = 0.003). PCR analysis showed that all cases of parasite recurrence were re-infections. Both drug regimens were well tolerated and no patient was withdrawn as result of recurrent vomiting. In conclusion, AMQ-SP is as effective and safe as AL in the treatment of acute uncomplicated malaria and AMQ-SP will be a good alternative combination therapy in communities where AL is not affordable or not available.

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EFFICACY OF NON-CONTROLLED INTERMITTENT PREVENTIVE TREATMENT IN PREGNANT (IPTP) WOMEN IN CÔTE D'IVOIRE

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Placental malaria in Côte d'Ivoire is poorly documented. Intermittent Preventive Treatment of malaria during pregnancy (IPTp) is a key intervention in the national strategy for malaria control in Côte d'Ivoire. Sulfadoxine-pyrimethamineP, the current drug of choice is recommended to be administered in the second and third term of pregnancy during antenatal care (ANC) visits. This study aimed to assess the efficacy of non controlled Intermittent Preventive Treatment in Pregnant women in Côte d'Ivoire. The study was conducted from August to September 2006, in Anonkoua-kouté and Yopougon (suburban areas), Koumassi (urban area) and Bonoua (rural area). Socio-demographic data, clinical data and information about chemoprophylaxis were collected at delivery. One placental apposition was carried out and placental blood sample was collected for rapid diagnostic test HRP2 for each woman at delivery. The overall prevalence of placental malaria was 9, 56%. The four health care centers were significantly different in terms of placental malaria prevalence, which varied between 2,88% in Koumassi, 9,43%and 9,78% and was 15,89% in rural area (Bonoua).Prevalence of placental infection was significantly higher in primigravidae (13,79%) than in secundigravidae (9%) and multigravidae (8,26%). Among the 429 women at delivering, one dose of SP was received by 22 women (5, 13%), two or more doses were received by 112 women (25, 87%) and 296 (69%) women received others antimalarial for chemoprophylaxis. Placental parasitemia was identified in 29, 26% of delivering women who received two or more dose of SP. SP is not taken under supervision in the antenatal care visit (ANC). Intensified sensitization of pregnant women about the benefits of IPTp is suggested as an important approach for improving IPTp compliance. The successful implementation of the IPT strategy in Côte d'Ivoire depends on the proper planning of, and support to, the training of health staff and sustained sensitization of pregnant women at health facility and community levels about the benefits of IPT for the women and their unborn babies. It will be important to increase coverage of IPT.We also should propose DOT strategy for IPT in pregnancy

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USING SMOOTHED GROWTH CURVES FROM ANTHROPOMETRIC REFERENCE POPULATIONS IN MALARIA ENDEMIC COUNTRIES TO DESIGN AGE- AND HEIGHT-BASED ALTERNATIVES TO WEIGHT-BASED DOSING FOR ARTEMISININ-BASED COMBINATIONS IN THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA

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The regulatory development of drug regimens is based on dosing by weight. However, in practice, antimalarial treatment is often based on age resulting inevitably in some patients receiving a dose outside the therapeutic range. There are no standardized procedures to devise age-based proxies for weight-based recommendations. This has contributed to the considerable variability in existing age-based dose regimens for antimalarials, at times resulting in poor, but widely-used regimens. We developed a method to design practical age-based dose regimens that aim to maximise efficacy and minimise toxicity for the treatment of uncomplicated malaria based on pooled reference data. Using anthropometric data from individuals who took part in malaria treatment studies or population-based health surveys, we compiled a global weight-for-age reference database that is representative of the underlying populations at risk of malaria in sub-Saharan Africa, Asia and South America. Smoothed reference growth curves were generated for each region using the R GAMLSS package (R version 2.4.1). The modelled weight-for-age centiles have been used to design age-based dose regimens by comparing the proportions of patients predicted to receive doses within pre-defined therapeutic ranges for a range of age-dose categories. Inter- and intra-regional differences in modelled growth distributions and corresponding impact on optimal age-dose categories were assessed to guide decisions whether age- or height-based dosing regimens can be harmonized at global level or whether they should be restricted to regional or sub-regional levels because of inherent differences in growth potential. The application of this new method is illustrated using the regimen design for the new fixed-dose antimalarial combinations of artesunate/amodiaquine and artesunate/mefloquine. This proposed approach is applicable to a wide range of drugs used in the tropics.

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'MALARIA DISCIPLINE' AND NEUROPSYCHIATRIC CASES AMONG US TROOPS IN SE ASIA: 1960-1975

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Military campaigns have seldom escaped the toll that malaria exacted on troops in the field. During the Second World War, troops--Allied and Axis alike--served in malaria-endemic areas. Malaria-ridden units were unable to perform optimally in battle, thus jeopardizing the overall success of military operations. Addressing this problem, commanders such as General William Slim ordered a draconian policy of "malaria discipline" among his troops in Burma. Its success of enforced chemoprophylaxis helped to maximize troop strength. "Malaria discipline" also played a role in the Vietnam War. Shortly after the 1965 arrival of American troops in Vietnam, excessive malaria cases soon appeared in the ranks. Within a year, the medical command instituted a policy that resembled the Slim-model in Burma. The objectives were two-fold: Prevent malaria and maximize troop effectiveness. Directly Observed Therapy (DOT) provided the most efficacious administration of anti-malarial drugs; it also helped to ensure that troops, in fact, received regular dosing. US troops routinely received chloroquine, mefloquine, and dapsone in SE Asia. Following the implementation of "malaria discipline" in 1966-1967, malaria cases subsequently dropped, a trend that continued until the American withdrawal in April 1975. The apparent success of the program, however, may have come at an unexpected cost. As closely-monitored "malaria discipline" became commonplace among US troops in SE Asia, the rate of neuropsychiatric hospital admissions, suicides, and "self-destruction"--either "intentional" or "unintentional"--soon began a steady climb. The Southeast Asia Combat Area Casualty File (SEACACF) is the official mortality record of United States military personnel killed in the Vietnam Conflict from 1960 to 1975. Datasets such as the SEACACF include approximately 1,200 service personnel who died as a result of "suicide," "accidental self-destruction," or "intentional self-destruction," as well as tens of thousands admitted to hospital with neuropsychiatric diagnoses prior to the end of their 365-day tour of duty in Vietnam. This study will suggest that the chemoprophylaxis fundamental to "malaria discipline" may have exacerbated pre-existing psychiatric conditions, further aggravated by "Dear John Letters," homesickness, substance abuse and lagging morale.

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QUALITY OF ANTIMALARIAL DRUGS SOLD AT RETAIL OUTLETS IN TANZANIA, 2005. RESULTS OF A NATIONALLY REPRESENTATIVE SURVEY

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Retail pharmaceutical products are commonly used to treat malaria illness in subSaharan African countries. Ancecdotal reports have suggested that poor quality and counterfeit drugs are widespread throughout the region. Few of these are based on systematic sampling strategies that could lead to generalizable conclusions about the extent to which poor quality drugs are available in African communities. We conducted a nationally representative market survey at retail outlets in mainland Tanzania in 2005. A total of 1080 antimalarial drugs were collected including 668 antifol antimalarial samples, 274 amodiaquine samples, 77 quinine samples, and 51 artemisinin derivatives. A systematic sample of 301 products not already expired was assessed by laboratory analysis of dissolution profile and content using high-performance liquid chromatography. Of the antifol antimalarial drugs tested 38% were found to contain substandard content. Roughly one quarter (24%) of quinine tablets did not comply within the tolerance limits of the content analysis. Quality of amodiaquine drugs was good as only 7% did not comply within the tolerance limits of the analysis. All artemisinin compounds contained adequate active ingredient. In conclusion, substandard antimalarial drugs were widely available in Tanzania. Most of these represented genuine products that failed meet international standards for dissolution or contained inaccurate quantities of active ingredient. Complete fakes were not detected. Quinine and sulfadoxine/ pyrimethamine products were more likely not to reach quality standards than other malaria drugs. In addition, products obtained from drug stores and pharmacies were more likely to be of poor quality than those obtained at general shops. Substandard products were identified in all parts of the country and from both domestic and international manufacturers. As the retail pharmaceutical sector continues to expand, it will be necessary to monitor drug quality both at the factory and point of importation, but also at the level of consumer.

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HIV-1 INHIBITORS AND MALARIA

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Parasite resistance to antimalarial drugs is a serious threat to human health and novel drugs such as protease inhibitors, are attractive targets for drug development. Studies have indicated that antiretroviral protease inhibitors (PIs) may have an effect on malaria outcome. We have investigated the antimalarial activity of antiretroviral agents in clinical use, and showed that the HIV-1 PIs can directly inhibit the growth of *Plasmodium falciparum in vitro*. We have also shown that the oral administration of ritonavir, and ritonavir combined with saquinavir or lopinavir significantly attenuates parasitaemia in mice infected with *P. chaubaudi* AS. We hypothesize that the PIs kill malaria parasites by targeting an as yet uncharacterized non-digestive vacuole plasmepsin that once identified will lead to the development of a potent new class of antimalarial agent. We also believe that the use of these drugs for the treatment of HIV may be beneficial to those presenting with malaria and HIV. Studies designed to determine

the mechanism of action of the PIs against *Plasmodium* parasites and to examine the antimalarial activity of PIs in combination with known antimalarials and other antiretrovirals are now being performed. Our most recent findings and their implications for malaria and HIV treatment will be discussed. Our results are particularly important given the high rate of malaria and HIV co-infection in Africa and efforts to deploy antiretroviral therapy in these regions.

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MURINE MALARIA TREATMENT MODEL FOR ANTIMALARIAL COMBINATIONS

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Antimalarial drug combinations are often evaluated using *in vitro* techniques, with isobologram analysis to determine synergistic, additive or antagonist effects. Similarly, *in vivo* animal evaluations are commonly performed using the Peters 4-day Test to assess suppressive drug effects. We have investigated the use a murine malaria treatment model, using the principles of the Rane Test, in assessing combination treatments. Male Swiss mice were inoculated with 10^7 *P. berghei* parasites obtained from continuous passage through Balb/c mice. At 2-5% parasitaemia, mice received vehicle, individual drugs and combinations of: dihydroartemisinin (DHA, 30 mg/kg single dose) plus piperaquine (QC, 10 mg/kg single dose); DHA 30 mg/kg plus chloroquine (CQ, 30 mg/kg single dose); DHA 30 mg/kg plus doxycycline (DOX, 3 x 90 mg/kg doses 12 h apart). All treatments previously had been shown to produce subtherapeutic effects, with peripheral parasitaemia >0.002%, thus facilitating detailed monitoring of drug efficacy. DHA alone produced a prompt decline in parasitaemia, to a nadir that was 5 to 6-fold lower than the starting parasitaemia. PQ alone showed a slower decline in parasitaemia, to an 18-fold lower nadir. The combination of DHA + PQ resulted in a rapid decline in parasitaemia, consistent with DHA alone, to a 19-fold lower nadir, suggesting additive efficacy in this murine model. The nadir parasitaemia after CQ alone was 49-fold lower, while the nadir for DHA + CQ was 36-fold lower, with a similar rate of decline, demonstrating that the combination was no more effective than CQ alone. DOX alone did not cause a reduction in parasitaemia until 2 days after dosing, but the nadir (50-fold lower) was achieved 6 days after dosing. The combination of DHA + DOX resulted in a prompt decline in parasitaemia, with a 37-fold lower nadir after 6 days, indicating an additive effect from the combination. We conclude that a murine malaria treatment model could be a valuable component of pre-clinical efficacy studies, especially investigations of antimalarial drug combinations.

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MEFLOQUINE-INDUCED DISRUPTION OF CALCIUM HOMEOSTASIS IN MAMMALIAN CELLS MAY BE DUE TO AN IONOPHORIC EFFECT SIMILAR TO THAT OF IONOMYCIN

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In previous studies we have shown that mefloquine disrupts calcium homeostasis in neurons by depleting the endoplasmic reticulum calcium store, followed by external calcium influx across the plasma membrane. The associated upstream signaling events have not been characterized. In this study we explored two hypotheses. First, we investigated the possibility mefloquine activates non-NMDA receptors and the inositol triphosphate (IP3) signaling cascade leading to store discharge. Second, we compared the disruptive effects of mefloquine on calcium homeostasis to those of ionomycin in neuronal and non-neuronal cells. We chose ionomycin as a control ligand since it discharges the store (through an undefined mechanism) and subsequently induces store-mediated calcium entry (SMCE). In radioligand binding assays, mefloquine showed no affinity for the known bindings sites of several glutamate receptor subtypes. The pattern of neuroprotection induced by a panel of glutamate receptor antagonists was dissimilar to mefloquine. Both mefloquine and ionomycin exhibited a dose-related and qualitatively similar disruption of calcium homeostasis in both neurons and macrophages. The influx of external calcium was blocked by the inhibitors of SMCE in a dose-related fashion. Both mefloquine and ionomycin upregulated the IP3 pathway in a manner we interpret to be secondary to SMCE. Collectively these data suggest that mefloquine does not activate glutamate receptors and disrupts calcium homeostasis in mammalian cells in manner similar to that of ionomycin.

(ACMCIP Abstract)

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IN SILICO PHARMACOPHORE FOR ANTIMALARIAL ACTIVITY OF THE 4(1H)-QUINOLONES TO AID DISCOVERY OF NOVEL CAUSAL PROPHYLACTIC DRUG CANDIDATES

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4(1H)-quinolones and endochin (2-methyl-3N-heptyl-7methoxy-4(1H)-quinolone) were known causal prophylactic and potent erythrocytic stage agents in avain malaria but not against parasites in mammals. These observations led us to evaluate a series of endochin and ICI 56,780 analogues from WRAIR-CIS chemical inventory. Surprisingly, several 2-methyl-3-(1'-alkenyl) - or 3-alkyl-4(1H)-quinolones, such as WR193211 and its derivatives were found to have remarkable potency against erythrocytic stages of multidrug resistant isolates and clones of *Plasmodium falciparum in vitro*. WR193211 even demonstrated oral activity (SD90 = 97 mg/kg) in *P. berghei* infected mice. Structural similarities of the quinolones with naphthoquinones and ubiquinone led to further evaluate the efficacy of WR193211 analogues against atovaquone resistance in *P. falciparum*. The results not only indicated potent erythrocytic stage activity of these compounds but interestingly, the atovaquone cross resistance was not found to be complete across the chemical series. This clearly indicates that additional structure activity studies are necessary to design novel causal prophylactic drug candidates. In pursuit of these objectives, we report here a three-dimensional chemical feature based pharmacophore model for antimalarial activity of the 4(1H)-quinolones to provide a foundation for virtual screening of compound database searches and design of new prophylactic drug candidates. 3D QSAR-Catalyst methodology was adopted on a set of seventeen structurally diverse 4(1H)-quinolones together with chloroquine and mefloquine to develop the model. *In vitro* susceptibility of *P. falciparum* of the 4(1H)-quinolones was developed using the IC_{50} values of D6 (chloroquine and atovaquone susceptible) and TM90-C2B (chloroquine, mefloquine, and atovaquone resistant) strains. The pharmacophores for both the strains were found to contain two aliphatic hydrophobic functions and one aromatic hydrophobic (aromatic ring) function in specific geometric locations surrounding the molecular space. The pharmacophores were cross-validated and used for virtual screening of WRAIR-CIS and Maybridge databases to identify new compounds that are currently under evaluation.

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ASSESSMENT OF THE EFFECTIVENESS OF ARTEMETHER PLUS LUMEFANTRINE VERSUS ARTESUNATE PLUS AMODIAQUINE FOR THE TREATMENT OF CHILDREN WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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In the context of growing drug-resistance rapid replacement of failing drugs by artemisinine combination therapy (ACT) is strongly anticipated. The only two registered ACTs which are GMP-manufactured at an industrial scale contain either amodiaquine and lumefantrine as partner-drugs. High efficacy and good safety profiles of both regimen have been reported but data on their effectiveness and acceptability are scarce. The aim of this trial was to compare the effectiveness, safety and acceptability of artesunate-amodiaquine or artemether plus lumefantrine in children under five years of age with uncomplicated *Plasmodium falciparum* malaria in a holoendemic area. In a large open-labeled, randomized phase IV study childrenfrom 2003 to 2005 (ClinicalTrial.gov, NCT00206 who presented with fever at child welfare clinics of two rural district hospitals in Ghana were screened for eligibility. If microscopic assessment revealed *P. falciparum* monoinfection (parasitemia 2000-200000 asexuell parasites/µl) participants received a three-days treatment of either artesunate-amodiaquine (Arsucam®) or artemether plus lumefantrine (Coartem®) after informed consent was granted by their parents or legal guardians. The first weight-adjusted dose was applied under direct observation and caretakers were instructed on how to complete the full treatment course at home. Follow-up investiagtions were performed on day 3, 7, 14 and 28 to evaluate clinical, parasitological and hematological status. Re-infections were differentiated from recrudescent parasitemias by assessing length-polymorphisms of *msp-2* after PCR-amplification from DNA-filter papers. The primary endpoint was the clinical and PCR-controlled parasitological cure rate at day 28. Additional endpoints were drug safety. Protective efficacy was stratified for the six-month periods after each I (monitoring of adverse events by number, grade and relation to study drug) and acceptability (by standardized questionnaire) of both drug regimen. The protocol had been approved by the responsible Ghanaian and German Ethic Committees. This trial is registered at www.ClinicalTrials.gov (NCT00374205). The results that will be presented enable policy makers to make more evidence-based decisions in selecting the most effective ACT when implementing or changing first-line antimalarial treatment.

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EVIDENCE FOR THE NON-ESSENTIALITY OF THE *PLASMODIUM* CANDIDATE DRUG TARGET ENOYL ACP REDUCTASE

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The problem of *Plasmodium falciparum* resistance to existing drugs has intensified the need to identify novel targets and develop antimalarials with new modes of action. Of particular interest are the biochemical pathways specific to the parasite apicoplast, a relic plastid ancestrally related to cyanobacteria. These pathways include fatty acid synthesis (FAS), which in *Plasmodium* and bacteria is a dissociative type-II (FAS-II) process that is distinct from the large FAS-I multifunctional polypeptide present in mammals. Enoyl ACP reductase (ENR, also known as FabI) is a key FAS-II enzyme that converts *trans*-2-enoyl-ACP to acyl-ACP. ENR is a proven target of several important antimicrobials including triclosan, a topical microbicide, and the antitubercular drug isoniazid. Biochemical and structural studies reveal that triclosan can inhibit as well as bind to purified *P. falciparum* ENR (PfENR). To test the hypothesis that ENR is a valid drug target in *Plasmodium*, we implemented a double crossover strategy to delete the *pbenr* gene in the genetically facile rodent parasite *P. berghei*. Molecular studies have confirmed disruption of *pbenr*. HPLC analysis show that both the *pbenr* disrupted clone and the parental wild-type line generate similar fatty acid profiles with carbon chain lengths of C12 to C24. The IC50 value of triclosan was similar against both the Δ*pbenr* and parental lines, as measured *ex vivo* with parasites placed into culture for one day. Transfection studies in *P. falciparum* provide preliminary evidence that this gene can also be disrupted and we are currently attempting to obtain knockout clones for further phenotypic characterization. Our studies therefore suggest that ENR is non-essential for propagation of asexual blood stage growth and that this enzyme is unlikely to be the target of triclosan in *Plasmodium*.

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DISPOSITION OF ARTESUNATE AND MEFLOQUINE (ASMQ) AFTER ADMINISTRATION AS LOOSE (L) AND FIXED-DOSE COMBINATION (F) TO ADULT THAI HEALTHY VOLUNTEERS AND UNCOMPLICATED FALCIPARUM MALARIA PATIENTS

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Artesunate (AS) 4mg/kg/d for 3 days plus mefloquine (MQ) 15+10mg/kg on the 2nd and 3rd day of treatment (total dose AS12+MQ25mg/kg) is 1st-line therapy for uncomplicated falciparum malaria in multidrug resistant areas of SE Asia. The PKs of MQ 15 and 25mg/kg are known. AS4+MQ8mg/kg/d for three days would be easier to comply with and amenable to a fixed combination. Population PK data show better exposure with MQ than the 15+10mg split dose. We compared the disposition of AS and MQ given to volunteers and malaria patients as loose (L) or fixed (F) combination. The PKs of L vs. F were compared in a cross-over study (90d wash out) in 24 volunteers and a parallel-arm study in 50 uncomplicated falciparum malaria patients. Following Solid Phase Extraction AS and its metabolite DHA were measured by HPLC-EC (limit of quantification 10ng/0.5ml) and MQ by HPLC-UV (limit of quantification 25ng/0.5ml) to determine Tmax, Cmax, t1/2, AUC, Cl/F and Vd/F. In the volunteer study results were expressed as bioavailability ratios, ANOVA of PK parameters allowing for drug, period and carry-over effect, Geometric least squares means ratio with 90% confidence interval (Schuirmann TOST). In patients we used the one-way ANOVA of PK parameters and modelled MQ disposition. The ratios of the $AUC_{0\text{-inf}}$ geomeans of L vs. F were 88.2 (90%CIs 75.5-103) for DHA ($p_{L\text{-}TOST}$=0.14, $p_{U\text{-}TOST}$=0.002) and 87.5 (79.1-97.1) for MQ ($p_{L\text{-}TOST}$=0.92, $p_{U\text{-}TOST}$<0.001) in volunteers and were 78.7 (30.8-153.5) for DHA and 100.9 for MQ, respectively in patients. Disposition differed in volunteers vs. patients. For MQ, apparent clearance was 75-80% that of patients and volume of distribution 20-50% higher than patients (Cl/VD ratio in volunteers 50-60% of patients). In conclusion, in adult malaria patients ASMQ given daily as fixed combination produced similar exposure for MQ and lower for AS/DHA than the AS+MQ(15+10) regimen. The difference is probably biologically not relevant. Efficacy rates were similar. In healthy volunteers

exposures were similar. Both drugs produced higher exposure in patients than volunteers.

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ACUTE RESPIRATORY DISTRESS SYNDROME DUE TO VIVAX MALARIA SUCCESSFULLY TREATED WITH EXCHANGE TRANSFUSION

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Severe pulmonary involvement has been rarely reported in cases of *Plasmodium vivax*. There were only 12 previous cases of vivax malaria complicated with ARDS reported in the literature. We report the case of a 59 year-old male who presented to the ED at Northport Veterans Affairs Medical Center with a two-week history of intermittent fever, chills and profuse sweating. The patient took a five-week trip to Guyana and he returned to New York one day before presenting to the ED. His blood smear showed 5% parasitemia with many schizonts. He was treated with oral quinine and doxycycline but the patient developed respiratory distress and was subsequently intubated and admitted to ICU. CXR revealed bilateral infiltrates consistent with ARDS. Blood specimen was sent to NYDOH for *Plasmodium* PCR which showed the presence of *P. vivax*. The patient was started on IV quinidine gluconate but there were no clinical improvements after 2 days. RBC exchange transfusion was then performed via Quinton catheter with 11 units of PRBC. The patient responded well and was extubated 3 days after exchange transfusion. To our knowledge, this is the first case of vivax malaria complicated with ARDS that was successfully treated with RBC exchange transfusion and antimalarial therapy.

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LACK OF BENEFIT FROM ANTIMALARIAL TREATMENT TO CLEAR *PLASMODIUM FALCIPARUM* PARASITEMIA IN THE NORTH SAVANNA REGION OF MALI

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In the northern sudan-savannah zone of Mali, the prevalence of asymptomatic *Plasmodium falciparum* infection is ≥ 64% among children less than 10 years of age during the early rainy season (June to August) and peaks at 85% between September and October. We report a prospective study to evaluate the effect of treatment on the subsequent frequency of infection. Two groups of children were followed from July to October in 2006. The first group of 165 uninfected children received no treatment. The second group of 83 infected (smear-positive) children received chloroquine and/or coartem to clear their parasitemias. During the subsequent four months (July to October) the frequencies of infection were consistently higher among the children who had been treated: 26.5%, 30.1%, 57.8% and 84.9% vs. 17.6%, 24.9%, 57.6% and 62.9%. The risks of malaria re-infection were also higher among the treated children by 8.9%, 5.3%, 0.3%, and 21.9%, from July to October 2006. Differences in infectivity (Levene test) were higher in the treated group than the untreated group ($p = 0.002$) in October at the end of rainy season. During July and October, parasite densities were higher in the treated group ($p = 0.007$, $p = 0.022$). These results suggest that the risk of re-infection for children in malaria-endemic areas may be higher after antimalarial drug treatment to clear their parasitemias.

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STUDY DESIGN FOR ANTIMALARIAL DEVELOPMENT: INCREASING THE EFFICIENCY OF PHASE II EFFICACY STUDIES IN HUMAN SUBJECTS

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The development of new drugs proceeds through a series of steps from *in vitro* studies to studies in animals, and then to human safety (Phase I) and efficacy studies (Phase II and III). This abstract examines three strategies to increase the efficiency and thus reduce the cost of Phase II studies in human subjects. These strategies include: 1] using a gold standard (state-of-the-art) treatment as the control instead of a placebo, 2] testing several (2-3) doses of the candidate drug in the initial Phase II efficacy trial, and 3] using a Bayesian approach to data analysis. First, using the current gold standard, instead of a placebo, as the comparison group (performing a noninferiority study) addresses two important questions: 1] Is the new treatment at least as effective as the current state-of-the-art?, and 2] Does the new treatment have different pharmacokinetics or a different safety profile (either better or worse)? Second, testing more than one dose (performing a dose-finding study) in Phase II is more efficient than performing a separate trial for each dose and helps to clarify the maximal tolerable dose. This information is then extremely useful when extrapolating to potentially effective pediatric doses, and when considering doses for use in drug combinations. Third, the Bayesian approach permits serial examinations of the data as they accumulate during the course of a study. This in effect provides continuous updates on the performance of the investigational drug - i.e., which study arm has the best outcomes. Using this information to modify subsequent randomizations not only increases the efficiency of the study by increasing allocations to the most promising arms (thereby reducing the sample sizes required); it also has positive ethical implications because it uses continuously updated information to increase the probability of allocation to the most promising treatments/doses. These three strategies complement each other and potentially increase the efficiency of Phase II efficacy trials in human subjects.

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DIFFERENT APPROACHES TO THE EXOERYTHROCYTIC MODEL FOR HUMAN MALARIA IN MICE

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Research on the exoerythrocytic (EE) stages of human malaria parasites is a handicap for the therapeutic efficacy studies in the process of developing new antimalarial agents. The establishment of a suitable animal model, easier and more available than primates, will facilitate characterization of EE stage antigens and the assessment of stage-specific chemotherapeutic agents and candidate vaccines. We report here three different approaches to develop a chimerical mouse combining functional human hepatocytes with a severe combined immunodeficient mouse (scid/nod). The cells used to transplant were HepG2 selected by its availability, easy and fast growth. We have studied two kind of models based on the place of cell implantation: extrahepatic or hepatic. Extrahepatic localization includes two places: subcutaneous and renal. The engraftment areas respectively are subcutaneous between shoulder-blade and under renal capsule. Cells were suspended in Matrigel to increase the viability and to avoid dispersion of the engraftments and implanted directly in above mentioned places. For hepatic transplant we need to induce an acute hepatic failure, previous to the implantation of HepG2 cells. The hepatic failure consists in removing 42% of liver by mean surgery, then taking in advance the regeneratory stimulus to improve the engraftment the cells are introduced through the spleen. Every model has been evaluated

for 21 days after transplant, in order to establish the healthy condition of the animals and viability of implant. Physiological evaluation consists in biochemical and haematological analysis. Viability of implant was established by determination of human albumin (hA) and α-1-antitripsin (hAAT) levels in serum by ELISA and histology techniques. In any case were not detected signs of reject to human cells. Subcutaneous and renal approaches offer cell viability just 1 day after transplant as reveal hA and hAAT levels in serum. The implant still keeps on viable at the end of study. Hepatectomized group need at least one week to detect in liver human hepatic cells, and it need being done by mean not only hA or hAAT levels but histology preparations to confirm the hepatic location. Because that we consider the extrahepatic location a feasible and less aggressive approach to follow on the studies to develop the exoerythrociytic murine model for *Plasmodium falciparum.*

(ACMCIP Abstract)

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PRELIMINARY PHARMACOKINETIC/PHARMACODYNAMIC STUDY OF 4(1H)-PYRIDONE GW308678 IN A MURINE *PLASMODIUM YOELII* MODEL OF MALARIA

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4(1H)-Pyridones are a class of antimalarials acting as selective inhibitors of *Plasmodium* mitochondrial function with demonstrated activity both *in vitro* and in animal models of infection. The compound GW308678 was selected as lead compound for pre-clinical evaluation according to its good balance of efficacy, safety and pharmacologic profile. As part of the pre-clinical evaluation of GW308678, a preliminary pharmacokinetic/pharmacodynamic (PK/PD) study has been carried out to gain insight into the correlation of different PK parameters with the anti-plasmodial efficacy. A *Plasmodium yoelii* infection model in immunodeficient $CB17^{scid/beige}$ mice was used to perform the assays. Groups of 5 female mice were infected intravenously with 6.4×10^6 parasites. Treatment with GW308678 started 24 h after infection when parasitemia in peripheral blood was 0.3-1 % (Day 0). In this experiment, 30 different patterns of treatment were tested. Five doses (0.06, 0.25, 1, 4 and 16 mg/Kg) in three different dosage schedules (every 8, 12 or 24 hours) during 3 or 6 days were administered by oral route. Therapeutic efficacy was assessed measuring parasitemia in peripheral blood of mice at days 4 and 7 after infection. The ability of each treatment to radically cure the infected mice was assessed by following recrudescence up to day 60 after infection. In order to assess the pharmacokinetic parameters linked with the different treatment patterns, individual single dose oral PKs were performed in *Plasmodium yoelii* infected mice in the same conditions used for efficacy studies. Once blood concentration *versus* time profiles were obtained, best-fitted PK models were determined and PK profiles corresponding to the different 30 treatment patterns used were simulated. As for most anti-infective agents, exposure (AUC) over time seemed to be the main driver for efficacy of GW308678 against *Plasmodium* infection. Low doses (and related low exposures) were able of render parasitemias under the level of detection during or immediately after treatment. Interestingly, radical cure was also achieved in 75 % of mice treated three times a day with 16 mg/Kg for 6 days, corresponding to the highest drug exposure achieved in these experiments.

(ACMCIP Abstract)

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ANALYSIS OF *PLASMODIUM*-STAGE POPULATION DISTRIBUTION IN MURINE MODELS OF MALARIA BY FLOW CYTOMETRY USING AUTOFLUORESCENCE AND YOYO-1

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Flow cytometry is a powerful technique to measure parasitemias in peripheral blood of mice. We developed a new flow cytometry method based on the differences of autofluorescence and DNA content between infected and non-infected erythrocytes measured after staining with the nucleic acid dye YOYO-1 (Cytometry Part A 67A:27-36. 2005). The patterns of staining of infected erythrocytes using this technique were characteristic, allowing distinguishing infected reticulocytes from infected normocytes. In this work, we analyzed the potential of the flow cytometric technique for characterization of the population distribution of parasites in peripheral blood in murine models of malaria. We have analyzed the correlation between the distribution of parasite stages in peripheral blood measured by microscopy or by flow cytometry assessing the patterns of staining with YOYO-1 in bidimensional plots of fluorescence collected trough 530/30 and 585/42 bandpass filters. We have tested this correlation in murine models of infection by *P. yoelii, P. chabaudi, P. vinckei*, and *P. falciparum*. These species differ in selectivity to infect specific subpopulations of erythrocytes and in their degree of synchronicity. The results indicated that the pattern of staining obtained provided accurate information on the population distribution of parasites not only regarding DNA content but also the degree of maturity of each parasite stage. This might help to standardize analytical procedures that require control of purity of parasite stages or assess the *in vivo* susceptibility of the parasitic stages to new antimalarials.

(ACMCIP Abstract)

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CROSSOVER TRIAL TO TEST A 2100 MG DOSE OF AQ-13 AND THE EFFECT OF FOOD ON ITS BIOAVAILABILITY

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AQ-13 is a candidate antimalarial active against chloroquine (CQ)-resistant *Plasmodium falciparum.* AQ-13 doses up to 1750 mg have been tested in human subjects, found to be safe and to have pharmacokinetics (PK) similar to CQ, although AQ-13 is cleared more rapidly. We are currently performing a randomized crossover trial to examine the safety of the 2100 mg AQ-13 dose and the effect of a standard fatty meal on its bioavailability. This trial design increases the efficiency of the study by allowing each volunteer to serve as his/her own control with or without the fatty meal. Fourteen healthy volunteers are being randomized to receive the drug on an empty stomach or after a standard fatty meal while being monitored in the hospital. After a washout period of 8 weeks (four times the terminal elimination half-life of AQ-13), the volunteers will be re-admitted to the hospital to receive the same dose of AQ-13 after a fatty meal or on an empty stomach in reverse order. For the fasting arm, the 2100 mg AQ-13 dose is administered in three doses of 700 mg each day x 3 days after 10 hours fasting with 240 ml of water. For the fatty meal arm, the AQ-13 dose on day 1 is administered within 30 minutes of a standard fatty meal given after 10 hours fasting with 240 ml water. The fatty meal is eaten in no more than 30 minutes and the AQ-13 dose is administered within 30 minutes of beginning the meal. For both arms, water is not allowed for 1 hour before or after the dose; and food is not allowed for 4 hours after the dose. The results of this study will determine whether the initial dose-finding Phase II efficacy study of AQ-13 in persons with malaria

examines a 2100 mg AQ-13 dose, in addition to the 1750 mg dose which have already been shown to be safe.

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NOVEL *IN VITRO* CULTURE OF LIVER STAGE HUMAN MALARIA FOR SCREENING OF NEW ANTI-MALARIAL COMPOUNDS

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Establishment of a human hepatocyte cell line HC04 that supports exoerythrocytic (EE) development of *Plasmodium falciparum* and *P. vivax in vitro* has enable us to develop a new *in vitro* system for screening of new antimalarial compounds against both parasites. The HC04 cells maintain production of major proteins and CYP450 enzymes during *in vitro* cultivation which is an advantage of this cells comparing to HepG2. It is also more convenient than using primary cells for screening of the new compounds as results can be repeated with the same host cells. In this study we preliminary validate the use of this *in vitro* culture system to study antimalarial efficacy of known malarial activity drugs/compounds (primaquine, chloroquine and WR -compounds) against *P. falciparum* and *P. vivax* EE parasites. Toxic effect of drugs to HC04 was evaluated using cell proliferation, alamarBlue assay. Different drug concentrations were added to the HC04 cells cultured in 96 well plates. Culture medium containing drugs were replaced every 24 hrs and cell proliferation was evaluated 96 hrs later. Our results demonstrated that HC04 can tolerate up to 125 µM for QC, 62.5 µM for CQ and 62.5 - 15.6 µM for other compounds. Effect of drugs/compounds against *P. falciparum* and *P. vivax* liver stage parasites was evaluated. HC04 cells were cultured in 96 well plates 2 days prior to sporozoite inoculation. Sporozoites were harvested from mosquito salivary glands, inoculated into each well and incubated for 4 hrs in 5% CO_2, at 37°c. Then drugs/compounds dissolved in culture medium were added. The highest concentration of each drug used was the highest dose that was not toxic to the HC04 cells. The EE parasites were quantified by immunofluorescent assay with parasite-specific antibodies (anti HSP70, anti CSP) quantitative PCR (DNA and cDNA) and Giemsa staining. Production of major liver proteins and CYP450 enzymes under different culture conditions were measured by RT-PCR. Drug efficacy was evaluated on day 4 and 7 after sporozoite inoculation and interpreted as growth inhibition doses comparing to control well. Antimalarial efficacy using the *in vitro* culture of human malaria in HC04 has been compared to efficacy studies performed in animal models (mice/*P. berghei* and monkey/*P. cynolmolgi*). Further validation of this novel *in vitro* system for screening of new anti-malarial compounds and the potential to develop high throughput *in vitro* screening system will be discussed.

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PURINE TRANSPORT IN *PLASMODIUM FALCIPARUM*

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Since the malaria parasite is unable to synthesis purine *de novo* and has to salvage it from the host milieu through transporter(s), knowledge of the characteristics of these transporters should facilitate a rational, systematic purine-based chemotherapy of malaria. We investigated the mechanism of purine uptake in saponin-permeabilised *Plasmodium falciparum* trophozoite-infected erythrocytes. At room temperature up take of 0.25 µM [^{3}H]-hypoxanthine or [^{3}H]-adenosine was rapid and was appreciably inhibited by guanine and inosine but not adenine. [^{3}H]-adenine was also taken up rapidly. At 6°C, transport of 25µM [^{3}H]-adenosine was extremely rapid. When a gene encoding for the transporter *(ENT1)* was disrupted, uptake of 0.25µM [^{3}H]-hypoxanthine or [^{3}H]-adenosine was drastically reduced by over 80% compared with wild-type parasites. However, uptake of 25µM [^{3}H]-adenosine at 6°C was similar in both the knock out clone and wild-type parasites. Data obtained in this study suggest the existence of a single satuarable purine transporter with a high affinity for both hypoxanthine and adenosine, a second transporter with low affinity for adenosine only and the presence of a third transporter with high affinity for adenine only.

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SAFETY ASSESSMENT OF AZITHROMYCIN PLUS CHLOROQUINE FOR THE TREATMENT AND PREVENTION OF MALARIA

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We conducted a systematic assessment of the safety of combined use of azithromycin and chloroquine for the treatment and prevention of malaria in infants, children, and pregnant women. This study depended on the synthesis of pre-clinical, clinical and post-marketing data, the published literature, chloroquine and azithromycin product labels, and World Health Organization (WHO) and (CDC) guidance documents. Both products are approved and have been widely used for many years in both children and adults. Azithromycin pre-clinical, clinical and post-marketing safety data identified few adverse outcomes when used during pregnancy, lactation, or in children. Chloroquine non-clinical data suggest the potential for adverse effects during pregnancy at doses 16 to 40 fold greater than the treatment dose in humans. Published reports of chloroquine use in pregnancy identified no important adverse events when used according to WHO and CDC guidelines. In clinical trials using both products concomitantly, treatment was well tolerated with no additional adverse events beyond those listed in the individual product labels. The chloroquine label highlights the potential for toxicity when overdosed in infants and children, as well as the potential for QT prolongation at labeled doses. Mean increases in QTcF values were observed with chloroquine and chloroquine plus azithromycin combinations; however, no cardiac adverse effects were observed in clinical studies in adults. Non-clinical data on changes in electrical alternans suggest that the addition of azithromycin to chloroquine therapy does not increase the risk of arrhythmia liability at levels used in the treatment of malaria. In conclusion, the safety assessment supports the development of azithromycin plus chloroquine for the treatment and prevention of malaria in infants, children, and pregnant women. However, clinical trials are needed to confirm the safe use in these populations.

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DIFFERENTIAL EFFECTS OF CYSTEINE PROTEASE INHIBITORS ON *PLASMODIUM FALCIPARUM* SEXUAL STAGE PARASITES

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Many of the available anti-malarials do not inhibit development of sexual stage parasites and therefore the persistence of gametocytes after drug treatment allows continued transmission of the disease. Papain-like cysteine proteases, falcipains 2 and 3, have been reported to be involved in hemoglobin digestion and are the targets of current anti-malaria drug development efforts. Therefore, the malaria transmission blocking

potential of a number of different classes of cysteine protease inhibitors, epoxides, vinyl sulfones and fluoromethyl ketone derivatives, were tested. Only membrane-permeant cysteine protease inhibitor E64d significantly inhibited oocyst production (80-100 %). E64d reduced oocyst production more effectively than that reported previously for falcipain 1 knock-out parasites, suggesting that falcipains 2 and 3 may also be involved in malaria transmission. However, only falcipain 3, not falcipain 2, was found to be expressed in stage V gametocytes. Interestingly, during gametocytogenesis falcipain 3 is transported into the red blood cell and by stage V is localized in vesicles along the RBC surface, consistent with a role during gamete emergence. In contrast to the ability of E64d to inhibit oocyst production, it had no effect on the growth or differentiation of early stage gametocytes. The differential effects of distinct cysteine protease inhibitors during the *Plasmodium falciparum* life cycle emphasizes the importance of including evaluations of gametocytogenesis and sporogonic development in future drug design studies.

(ACMCIP Abstract)

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PREDICTORS OF OUTCOME IN THE PHASE II TRIAL OF DB289 AND ARTESUNATE FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Increasing drug resistance limits the choice of efficacious chemotherapy against *Plasmodium falciparum*. There is an urgent need to identify new treatment alternatives which are effective, safe and affordable. DB289 is a new drug that is being developed to treat malaria. We conducted a post hoc study to identify factors (socio-demographic and pharmacokinetic) that predicted treatment outcome in patients with uncomplicated *P. falciparum* malaria. We analyzed data from 90 adults with uncomplicated *P. falciparum* malaria who participated in a multi-center, open-label, randomized, phase II clinical trial. Patients were assigned to receive DB289 200 mg once daily or DB289 100 mg twice daily or a combination of artesunate and DB289 200 mg once daily. Patients were treated for 3 days and monitored for 28 days. Twelve individuals from each treatment group were selected for pharmacokinetic measurements. The main outcome was treatment failure. A logistic regression was used to identify significant predictors of treatment failure, using the intent-to-treat analysis principle. Treatment failure occurred in 44% (n=39/89) of the patients. In bivariate analyses significant predictors of treatment failure ($p<0.05$) were: gender; treatment allocation; DB289 Ctrough; and DB75 Cmax, Ctrough and AUC. In multivariate analysis, significant predictors remained for: gender, treatment allocation, DB75 Cmax and DB289 Ctrough. The odds of treatment failure was less in men (odds ratio (OR) 0.27, 95% confidence interval (CI) 0.07-1.05); was higher in patients treated with DB289 once daily (OR 6.37, 95% CI 2.02-20.04) or with DB289 twice daily (OR 1.32, 95% CI 0.42-4.16) compared with patients with a combination of DB289 and artesunate; was lower in patients who had DB75Cmax levels of at least 43.3 ng/mL (OR 0.05, 95% CI 0.003-0.81); and was lower in individuals who had DB289 Ctrough levels of at least 3.5 ng/mL (OR 0.13, 95% CI 0.01-1.42). In onclusion, we found that patients who had DB75 Cmax levels of at least 43.3 ng/mL and DB289 Ctrough levels of at least 3.5 ng/mL were much less likely to have treatment failure than individuals with lower levels. Men were less likely to have treatment failure than women. Thus, new DB-289 regimens which increase Cmax and Ctrough may have substantially improved efficacy.

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PHASE 1 INVESTIGATION TO ASSESS THE RENAL AND OPTHALMIC EFFECTS OF TAFENOQUINE, A NOVEL ANTIMALARIAL DRUG

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Tafenoquine (TQ) is an 8-aminoquinoline antimalarial drug under clinical development for radical cure of *Plasmodium vivax* malaria. Long term animal toxicity and findings in clinical studies led to a concern over potential renal and ophthalmic toxicity. We conducted a randomized, double blind, placebo (PLO) controlled trial in healthy volunteers over a 6 month, weekly dosing period to assess the renal and ophthalmic safety of TQ. Primary outcomes were mean change from baseline glomerular filtration rate (GFR) at 24 weeks and percentage of subjects in the TQ group with unimpaired night vision, as assessed by the forward light scatter test (FLST). Healthy volunteers, ages 18-55 were randomized in a 2:1 ratio (81 TQ, 39 PLO) to receive 200mg of TQ or matching PLO once daily for 3 days followed by once weekly for 23 weeks. Renal and ophthalmic assessments were carried out at baseline, 3, 6, 12, 18 and 24 weeks of therapy, with follow up at 12 and 24 weeks post-therapy. The adjusted mean difference in GFR ($ml/s/1.73m^2$) in the TQ group (+.023) compared to PLO (+.084) demonstrated non-inferiority [Treatment difference -0.061 (-0.168, 0.045); *a priori* non-inferiority margin of -$0.247ml/s/1.73m^2$]. There was no impairment in night vision observed by FLST in either the TQ or PLO group. The lower limit of the 95% CI for the TQ group was >90%, demonstrating no effect on night vision as assessed by FLST. Sensitivity analyses supported the negative findings of the primary endpoints. Secondary renal and ophthalmic endpoints, including analysis of urine, mean percentage change in GFR, serum creatinine, cornea and retinal abnormalities and macular function demonstrated no evidence of toxicity. New onset subclinical keratopathy occurred more frequently in the TQ (21%) group compared to PLO (11%), resolving completely by 24 weeks after therapy. The adverse event profile of TQ and PLO were very similar. TQ, given weekly for 6 months at a dose of 200mg is well tolerated and demonstrates no clinically significant renal or ophthalmic toxicity in healthy volunteers.

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DETECTION OF CHLOROQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM*: EVALUATION OF MOLECULAR MARKERS IN CLINICAL ISOLATES FROM NORTHEAST INDIA

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Chloroquine (CQ) is very effective and low cost anti-malarial drug widely used in India. In view of widespread CQ resistance, early detection of CQ resistance in clinical isolates of *Plasmodium falciparum* in endemic regions is important for effective therapy of potentially fatal falciparum malaria. Present study was aimed at evaluating genetic markers of CQ resistance in 105 CQ resistant and 23 CQ sensitive clinical isolates of *P. falciparum* collected from northeast (NE) India. In vitro sensitivity of isolates to CQ was determined by conventional method (W.H.O., mark III protocol). The N86Y mutation in *Pfmdr-1* gene and K76T mutation in *Pfcrt* were detected by PCR-RFLP while length polymorphism in kappa and omega repeats