TIONS, we examined the genetic composition of multiple clone infections, the relative importance of these processes for generating multiple infections, and the role of these infections in transmission. To determine whether the predominant clones were those isolated from naturally occurring multiple infections could be used for progeny from naturally occurring multiple infections could be used for linkage analysis, obviating the need for expensive and laborious genetic analysis. However, the data also reveals striking temporal trends in parasite population structure. First, we observed progressive decrease in the carriage of multiple infections over the course of the three year period. This suggests declining transmission in this area, most probably as a consequence of aggressive control using artemisinin-based combination therapy. Superimposed on the overall decline we see marked seasonal fluctuations. The highest levels of multiple infections and the lowest levels of inbreeding are from September to November, two months after the peak transmission season (April to July). The lowest carriage of multiple infections and maximal levels of inbreeding were from February to April. These data demonstrate that seasonality and control measures may generate a range of genetic population structures in a parasite population from a single location. However, time lags complicate the use of population genetic parameters as surrogate indicators of transmission intensity.

RELATEDNESS OF PARASITES FROM MULTIPLE CLONE MALARIA INFECTIONS

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In endemic areas Plasmodium falciparum infections containing multiple clones are frequently observed. These “multiple” infections are generally assumed to result from bites by two or more infected mosquitoes. However, in areas of low transmission, such infections can also result from the bite of a single mosquito infected with multiple parasite genotypes. To determine the relative importance of these processes for generating multiple infections, we examined the genetic composition of multiple clone P. falciparum infections collected from the border region of Thailand and Myanmar. We isolated 592 clones from 15 multiple clone infections by dilution cloning. Initially seven microsatellite markers were used to genotype these isolates, revealing 31 genetically unique clones, with between one and seven unique clones from each infection. We genotyped these unique clones using microsatellites distributed across the genome and measured relatedness by counting the proportion of shared alleles between pairs of parasites. These data demonstrate that the majority of multiple infections comprise closely related clones in Thailand, and rarely result from superinfection. These results have important implications for our understanding of malaria epidemiology, sex ratio, and virulence evolution and suggest that dissection of progeny from naturally occurring multiple infections could be used for linkage analysis, obviating the need for expensive and laborious genetic crosses.

G6PD DEFICIENCY PROTECTS HEMIZYGOUS MALES AGAINST SEVERE MALARIA IN BANDIAGARA, MALI

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy in humans and affects over 400 million people worldwide. Deficiency alleles for this X-linked disorder are geographically associated with historical patterns of malaria endemicity. The most common deficiency allele in Africa (G6PD A-) has been shown to confer resistance to severe malaria. The extent to which male hemizygotes and female heterozygotes are protected is unclear. Because of the physical location of the G6PD gene on the X-chromosome, we were interested in whether differences in severe malaria protection existed between male and female children. We conducted a community based case-control study in a population of 3500 children of Dogon ethnicity aged 6 months to 9 years in Bandiagara, Mali. DNA was extracted from individual blood samples and the presence of mutant G6PD A- alleles determined by RFLP analysis of PCR amplified DNA products. In Bandiagara, Mali, malaria is endemic and seasonal (June to November). Mild malaria attack rates per transmission season range from 25 to 75%.
1 to 2 episodes per child less than five years of age, and the annual incidence of severe malaria (cerebral malaria, severe anemia, hyperparasitemia, hypoglycemia, respiratory distress) is 4-5%. Case fatality rates from severe malaria range from 5 to 9%. In comparisons of children with uncomplicated \((N = 391)\) and severe \((N = 67)\) malaria, G6PD deficiency was associated with a significant reduction in risk of severe malaria \((P = 0.03)\). Stratification of the data by sex showed highly significant protection against severe malaria for male hemizygotes \((P = 0.01)\) but not female heterozygotes \((P = 0.56)\). Differences in mean parasite densities between male and female children were not statistically different \((P > 0.05)\). These data show that hemizygous G6PD deficiency protects male children against severe malaria in Bandagiara, Mali.

### DISTRIBUTION OF SEVERE MALARIA CLINICAL FORMS IN MALINKE CHILDREN IN MALI


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The regional intensity and seasonality of malaria transmission impacts the incidence and age distribution of severe forms of malaria. Kangaba, Mali resides in an area of the Southern Sudan Savanna, where six months of high malaria transmission are typical. In a community-based, case-control study spanning three transmission seasons \((2001\text{ to }2003)\), we enrolled 1942 cases of clinical malaria in children aged 3 months to 9 years. Two hundred and twenty five \((11.6\%)\) of these cases met World Health Organization criteria for severe disease. The peak incidence of severe malaria occurred in the months of September and October. Children under the age of 5 years represented 79.6% of the severe malaria cases. Repeated seizures, hyperpyrexia, and jaundice were frequent \((39.1\%, 29.8\%, \text{ and } 29.8\%, \text{ respectively})\). Hypoglycemia and severe anemia were less frequent \((3.1\% \text{ and } 16.7\%), \text{ respectively})\). The case fatality rate among all forms of severe malaria was 4%. Prognoses were found to be worse when severe malaria was associated with cerebral signs and/or respiratory distress.

### PRIMIPAROUS WOMEN ARE AT GREATER RISK OF GIVING BIRTH TO CHILDREN THAT ACQUIRE MALARIA INFECTION ANTENATALLY

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Malaria parasites have been identified in cord blood of newborns from malaria endemic areas. It is unclear whether these infections represent true congenital infection or admixture of maternal blood at delivery. This distinction is important since prenatal exposure to malaria may alter fetal immunity and affect subsequent susceptibility to infection and disease. To more precisely assess malaria infection in cord blood \((CB)\) and risk factors involved, we examined the quantity of malaria parasites by real-time quantitative PCR \((RTQ-PCR)\) and related these results to the presence and quantity of concurrent malaria infection in maternal and placental blood and parity from 216 paired Kenyan newborns and mothers.\(\text{Plasmodium falciparum}\) was detected in blood smears from 8.3, 1.6 and 3.4% of maternal peripheral and placental blood and \(CB\) respectively whereas the presence of malaria parasites by RTQ-PCR was 39, 12 and 9%. In the 19 \(CB\) samples positive by RTQ-PCR, there was no evidence for malaria infection by blood smear or PCR in paired maternal and placental blood in 3 newborns, and in 2 additional newborns the copy number of parasites was a log-fold higher than in maternal blood demonstrating contamination by maternal blood at delivery unlikely in these newborns. Because of the lack of strong correlation with concurrent maternal and \(CB\) infection at delivery in some newborns, we hypothesized that parity to be a risk factor for \(CB\) infection since primiparous women are at increased risk for malaria infection throughout pregnancy. This may result in infection of the fetus prior to delivery. We observed that 16.4% primiparous vs. 5.1% multiparous women had cord blood infection \((P=0.01)\) compared to 44% primiparous vs. 36% multiparous women with infection at delivery. Thus the fetuses of primiparous women are more likely acquire malaria infection transplacentally indicating true congenital infection that may affect newborns immunity to malaria during infancy. \((\text{AGCMCIP abstract})\)

### ASYMPTOMATIC AND SELF-LIMITED MALARIA INFECTIONS IN A RECENTLY EMERGED HYPOENDEMIC REGION OF PLASMODIUM FALCIPARUM AND P. VIVAX TRANSMISSION


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A \(\text{Plasmodium vivax}\) and \(\text{P. falciparum}\) epidemic emerged in the Iquitos region in 1991 and 1994, respectively. Now, there is a hypoenemic. By a passive-case detection the incidence is 421/person/year \((70\% \text{ P. vivax})\) in a community called Zungarococha \((N=1907)\). The malaria immunity paradigm predicts high morbidity and mortality and no acquisition immunity. In contradistinction, there is low morbidity and a cross-sectional study reported asymptomatic infections detectable by polymerase chain reaction (PCR). In Zungarococha, we instigated a prospective longitudinal cohort project. 957 individuals were sampled weekly for at least one month during February-July. There were 5558 visits. We used PCR to detect the \(\text{Plasmodium species-specific gene encoding} 18s\ RNA\). The force of infection \((\text{FOI})\) was 0.06 \(\text{P. falciparum}\) and 0.15 \(\text{P. vivax}\) infections/person/month during the higher transmission months: January-July. By microscopy we detected 62 \(\text{P. falciparum}\) and 161 \(\text{P. vivax}\) infections by active surveilance. The geometric mean densities were 627 \(95\% \text{CI: 369-1066}\) and 161 \(95\% \text{CI: 142-243}\). Only 24% and 12% of the \(\text{P. falciparum}\) and \(\text{P. vivax}\) infections were associated with fever, respectively. Parasite density and fever were correlated \((p=0.0002)\). We detected an additional 19 \(\text{P. falciparum}\) and 62 \(\text{P. vivax}\) infections by PCR-only. In total, including active and passive case detection and PCR, there were 118 \(\text{P. falciparum}\) and \(\text{P. vivax}\) infections detected during our study. We grouped parasitemias as “Symptomatic,” “Persistent,” or “Self-Limited.” The Symptomatic group had a reported/detected fever, a hemoglobin level \(<10g/dl\), or parasite density \(<5,000/ul\) blood and was treated within two days of detection. The later 2 groups were asymptomatic individuals and were visited 7 days later and were treated within 8 days. The Self-Limited group was individuals negative upon re-sampling at 7 and 8 days later or individuals whose parasitemia was detected by PCR. We classified 105 \(\text{P. falciparum}\) infections into groups: 57 Symptomatic (54%), 20 Persistent (19%), and 28 Self-limited (27%). The average age in each was 15 (median=15), 30 (median=23) and 30 (median=29) years, respectively \((P<0.0482)\). The high frequency of per-
sistent and self-limited asymptomatic makes a strong challenge to the existing malaria immunity paradigm. We discuss possible reasons for this and compare epidemiologic profiles seen in high and low transmission regions.

PYRIMETHAMINE AND CHLOROQUINE REDUCE THE TOTAL NUMBER OF B220 CELLS AND INCREASE THE EXPRESSION OF iNOS AND TNF-α IN MICE INFECTED WITH PLASMODIUM YOELII 17XL

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Therapy with antimalarials has been used successfully to control diseases where the immune system is disturbed, like the lymphoproliferative syndrome, in which pyrimethamine induces a decrease in the total number of lymphocytes, and such decrease is beneficial for the patient. However, it is not well known how these drugs modify the immune response when they are administered during an infection by Plasmodium. In this work, we studied how the treatment with chloroquine and pyrimethamine modifies the subpopulations of lymphocytes T and B. In addition, we studied how the treatment with these drugs modified the expression of the iNOS and TNF-α genes during the infection with P. yoelii 17XL.

Groups of BALB/c mice were infected with 5×10⁷ erythrocytes parasitized with P. yoelii 17XL, and on day 7th after infection they were treated with chloroquine or pyrimethamine or were left without treatment as controls. 24 hrs later mice were sacrificed and the spleen subpopulations of T lymphocytes and B220 cells were evaluated by fluorocytometry. In addition, expression of genes iNOS and TNF-α were measured by RT-PCR semiquantitatively. Results indicate that treatment with pyrimethamine did not modify the total number of CD3, CD4 or CD8 lymphocytes, but it did affect the total number of B220 cells, while chloroquine did not modify the total number of lymphocytes nor the CD8 subpopulation, but it did affect CD4 and B220 subpopulations. Pyrimethamine increased the expression of iNOS without modifying the expression of the TNF-α gene. In contrast, chloroquine increased both iNOS and TNF-α. These results suggest that both pyrimethamine and chloroquine not only eliminate the Plasmodium, affected B220 cells but also induce an expression increase of iNOS and TNF-α, molecules that are associated to a TH1 response and that have also been described as involved in parasitic elimination. This kind of studies provide more information of how antimalarials chloroquine and pyrimethamine modulate the immune response during an infection with Plasmodium. (ACMCP abstract)

ANTIGENIC DETERMINANTS EXPRESSED BY SOME PLACENTAL-BINDING PLASMODIUM FALCIPARUM-INFECTED ERYTHROCYTES ARE WIDELY DISTRIBUTED ACROSS DIVERSE POPULATIONS

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Infection with Plasmodium falciparum during pregnancy leads to the accumulation in the placenta of infected erythrocytes (IEs) with distinct antigenic phenotypes that adhere to chondroitin sulfate A (CSA) and other receptors. Antibodies to the surface of placental-binding IEs are acquired following exposure and may be involved in protective immunity or parasite clearance. The extent of antigenic diversity or conservation of placental IEs and the distribution of antigenic determinants in different populations is currently unclear. In separate studies, we have defined two genetically distinct clonal CSA-binding isolates that appear to express diverse and conserved antigenic determinants. Western blots suggest each isolate expresses a single dominant form of the parasite antigen P. falciparum erythrocyte membrane protein 1, the main target of acquired antibodies against the IE
ELEVATED NITRIC OXIDE PRODUCTION IN CHILDREN WITH MALARIAL ANEMIA: HEMOZOIN-INDUCED NOS2 TRANSCRIPTS AND NITRIC OXIDE IN BLOOD MONONUCLEAR CELLS

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The underlying mechanisms responsible for the pathogenesis of Plasmodium falciparum malaria remain largely undefined. One candidate effector molecule that remains controversial in the context of malaria pathogenesis is nitric oxide (NO). Although NO has potent antiplasmodial properties through inhibition of intraerythrocytic growth, the role of NO in regulating the severity of childhood malarial anemia (MA) has not been fully explored. To determine if NO production is altered during MA, we examined ex vivo peripheral blood mononuclear cell (PBMC) nitric oxide synthase (NOS) enzyme activity in healthy, malaria-exposed children and children with varying degrees of MA. NOS enzyme activity was also examined in cultured PBMC from healthy, malaria-exposed children and children with MA under baseline conditions and after treatment with LPS and IFN-γ. In addition, NO and NOS2 (inducible NOS, iNOS) were determined in PBMC from healthy, non-malaria-exposed donors stimulated with malarial pigment (hemozoin). PBMC NOS enzyme activity was significantly elevated in children with mild and severe malaria, and inversely associated with hemoglobin levels. In addition, NOS enzyme activity in cultured PBMC from children with malaria was elevated under baseline conditions and following stimulation with LPS and IFN-γ. Stimulation of cultured PBMC from healthy donors with physiologically relevant amounts of hemozoin (10, 1.0, and 0.1 μg/mL) dose-dependently augmented NO and NOS2 transcripts. Our results demonstrate that high production of NO from PBMC is associated with MA, and that hemozoin promotes NOS2-derived NO production. (ACMCIP abstract)
p-value = 0.03. In commune 5 (urban area), we did not find any difference on malaria prevalence between primigravidae and multigravidae. On the set of *Plasmodium falciparum* isolates responsible for gestational malaria, we had observed a mean prevalence of CSA phenotype of 53.2% ± 13 (n = 6 placental sections), determined by indirect immunofluorescence with monoclonal antibody anti-DBL3 γ. We also had observed an important individual variability in the percentage of HP-Cytoadherence inhibition on endothelial cells. Although this percentage of inhibition was higher in serum of infected women with third and fourth gestation, it was not significantly different from infected women with first and second gestation. 

[(13/51), 56.8% ± 14 versus 42.1 ± 9.3, p = 0.1]. These findings are important in the area of malaria anemia treatment. The identification of other parasites populations on the placenta could contribute to open the way of vaccine therapy in pregnant women living in malaria endemic areas. *(ACMCIP abstract)*

**REGULATION OF CYTOKINES AND EFFECTOR MOLECULES IN RHESUS MACAQUES INFECTED WITH *PLASMODIUM COATNEYI***

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Non-human primates such as rhesus macaques (*Macaca mulatta*) infected with *Plasmodium coatneyi* are a useful model system for studying cerebral malaria. Since the animals do not develop neurologic signs until the parasitemia reaches high levels and becomes life-threatening, we have been investigating the model for studies on malarial anemia (MA) at lower levels of parasitemia. To determine if the *P. coatneyi* infection in rhesus macaques elicits a similar immunologic profile to that seen in human MA, we measured cytokines and effector molecules previously established as immune correlates in childhood MA. Immune mediators (* interleukin (IL)-10, IL-12p40, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and nitric oxide (NO)*) were examined in malaria-naïve animals (n=4) at 8 different time points during infection. Elevated IL-10 and TNF-α transcripts (determined by real time RT-PCR in circulating blood mononuclear cells) and plasma levels (determined by ELISA) were associated with enhanced disease severity (peak parasitemia, fever, and anemia). IFN-γ transcripts peaked at day 2, while plasma levels increased with parasitemia, and remained elevated even after recovery. Transcripts for IL-12p40 increased up to day 5, but were suppressed below baseline levels at peak parasitemia (day 8) where they remained for the duration of the infection. IL-12p40 plasma levels were highest at peak parasitemia in 3 of 4 monkeys. Nitric oxide (NO*) and NO* were detected. Three other donors failed to show IFN-γ production in culture supernatants but short term T cell lines (STCLs) from these donors showed lytic responses. Five donors showed IFN-γ responses but no CTL activity. In summary, residents in holoendemic regions of malaria have low levels of CTL responses and the detection of IFN-γ response do not reliably predict the CTL response. We also investigated the relationship between the presence of blood stage parasites at the time of blood draw or recent infection (positive blood film in peripheral blood within 3 months of blood draw) and T cell responses. T cell responses were not associated with parasitemia at the time of sampling (p<0.25 p=0.2). However, the odds of having a positive CTL response or IFN-γ production was lower among participants who had a malaria infection 3 months prior to blood draw (OR 0.28 p=0.07). *(ACMCIP abstract)*

**EFFECT OF MALARIA PIGMENT ON THE KINETICS OF CYTOKINE EXPRESSION DURING DIFFERENT STAGES OF SIV/AIDS-INFECTION***

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Based on the geographic overlap between malaria and human immunodeficiency virus (HIV), HIV/malaria co-infection presents a significant public health problem for tropical regions. One common feature of both diseases is dysregulation of cytokines, which appears to enhance pathogenesis. Since immune dysregulation during co-infection is largely unexplored, peripheral blood mononuclear cells (PBMC) were isolated from rhesus macaques (*Macaca mulatta*) chronically infected with a simian immunodeficiency virus (SIVdeltaB670). PBMC were stimulated with physiologic concentrations of hemozoin (malarial pigment) at distinct phases of disease, non-infected (CON n=4), SIV, non-AIDS (SIV n=3), and AIDS (n=3). Transcriptional regulation of interferon (IFN)-γ, interleukin (IL)-12, IL-10, and tumor necrosis factor (TNF)-α was determined by real time RT-PCR. Hemozoin-treatment resulted in a rapid and transient increase in IFN-γ transcripts in the CON group, modest and sustained elevation by 120hrs in the SIV group, and significant suppression of message by 120hrs in the AIDS group. Stimulation with hemozoin caused a slight decrease in IL-12 message over 120hrs in the CON group, and a rapid and transient increase in SIV-infected animals, with the effects being more pronounced in the AIDS group. Levels of IL-10 in hemozoin-treated cells increased slightly in the CON and SIV groups, and rose to high levels by 48hrs in the AIDS group before declining to near baseline levels by 120hrs. Treatment with hemozoin elicited peak levels of TNF-α message at 48hrs in all groups, with the AIDS group having significantly higher transcript levels. Taken together, these

**CYTOTOXIC T CELL AND INTERFERON-γ RESPONSES TO *PLASMODIUM FALCIPARUM* HLA-CLASS I RESTRICTED EPITOPES IN RESIDENTS OF A HOLOENDEMIC MALARIA REGION***


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Cytotoxic T cell (CTL) responses and interferon-gamma (IFNγ) responses are believed to play a critical role in arresting the development of pre-erythrocytic stage of malaria parasites. CTL assays can be laborious in field settings and the frequency of CTL response is often low in endemic areas. Therefore, investigators are increasingly attempting to use IFNγ production as a surrogate for CTL response. In this study we investigated, whether there is a correlation between CTL responses and IFNγ responses in individuals residing in a holoendemic region for malaria in Kenya. Peripheral blood mononuclear cells from 57 individuals were tested against previously defined HLA-class I restricted peptides from Liver Stage antigen-1, circumsporozoite protein and thrombospondin related adhesion protein. Eighteen of 57 individuals responded to peptide stimulation by either IFNγ production or target cell lysis. The donors were from various HLA backgrounds although a majority (63%) of them were positive for HLA-A2. We were able to test 11 of the responding individuals for both IFNγ release and target cell lysis. In 4 of these individuals both CTL and IFNγ production were detected. Three other donors failed to show IFNγ production in culture supernatants but short term T cell lines (STCLs) from these donors showed lytic responses. Five donors showed IFNγ responses but no CTL activity. Stimulation with hemozoin caused a slight decrease in IL-12 message over 48hrs in the AIDS group, and rose to high levels by 48hrs in the AIDS group before declining to near baseline levels by 120hrs. Treatment with hemozoin elicited peak levels of TNF-α message at 48hrs in all groups, with the AIDS group having significantly higher transcript levels. Taken together, these
results illustrate that malarial products elicit differential effects on pro- and anti-inflammatory cytokines during different stages of SIV. Overproduction of TNF-α and suppression IFN-γ during AIDS may account for the increased pathogenesis observed in co-infected individuals during end-stage disease.

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DYNAMICS OF ACQUISITION OF ANTIBODY RESPONSES AND PROTECTIVE IMMUNITY TO PLASMODIUM FALCIPARUM AMONG CHILDREN OF DIELMO, SENEGAL

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A long-term prospective study of naturally acquired immunity to Plasmodium falciparum has been conducted for ten years in Dieumo, a village of Senegal where malaria is holoendemic. Longitudinal clinical, biological, parasitological and entomological data were collected to assess the dynamics of acquisition of protective immunity. In this study, we investigated the development and maintenance of antibody responses of 50 children towards different P. falciparum vaccine candidate antigens in relation to epidemiological data. Antibody responses against MSP3, LSA3, GLURP (R0) and R23 peptides were studied longitudinally every year over a period of at least five years up to ten years. Our data showed different dynamics of IgG, IgM and isotype responses depending upon the antigens. Globally, antibody responses were acquired early and tended to increase with age. Logistic regression models taking into account the effect of age and transmission indicated that some isotype responses against the antigens tested were significantly associated with a decreased risk of malaria attacks among children. Isotypes associated with protection were mainly cytophilic IgG1 and IgG3 subisotypes (MSP3, GLURP-R0, R23) but some non-cytophilic isotypes (IgG2/LSA3, IgG4/GLURP R0) were also found associated with a decrease risk of malaria attack. Results showed that some responses are significantly associated with an increased risk of malaria attack stressing the necessity in a vaccine strategy to select epitopes that induce the protective isotype. Analysis of individuals responses suggested that there is no switch from non protective isotypes to protective isotypes. Instead regular increased of defined isotypes depending of the subject was observed. These results confirmed that different mechanisms are involved in malaria protection probably associated to the genetic polymorphism. (ACMCIP abstract)

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HEALTHY CHILDREN WITH A HISTORY OF PRIOR MILD PLASMODIUM FALCIPARUM MALARIA PRODUCED SIGNIFICANTLY HIGHER LEVELS OF MACROPHAGE MIGRATION INHIBITORY FACTOR THAN CHILDREN WITH A HISTORY OF PRIOR SEVERE MALARIA: IMPLICATIONS FOR MIF IN PROVIDING IMUNOPROTECTION

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Plasmodium falciparum malaria remains one of the most lethal diseases affecting children in sub-Saharan Africa. Clinical presentations range from mild symptoms in some children to severe life-threatening complications such as, hyperparasitaemia and severe anaemia in others. The molecular mediators that determine disease severity remain largely undefined. Recent studies in murine models have identified macrophage migration inhibitory factor (MIF) as an important regulator of innate and inflammatory responses required for clearance of both bacterial and parasitic infections. To investigate the role of MIF in human malaria, circulating levels of MIF were measured in healthy Gabonese children (ages 2-8 years) with a history of prior mild malaria (PMM) or prior severe malaria (PSM). In addition, since MIF has reciprocal immunologic interactions with tumour necrosis factor α (TNF-α), this soluble mediator was also examined. Children with PMM had significantly higher plasma MIF levels than the PSM group (P < 0.001). Consistent with its role in the pathology of severe malaria, TNF-α plasma levels were higher in the PSM group relative to the PMM group (P < 0.05). Measurement of MIF transcripts by real time RT-PCR showed that MIF gene expression did not significantly differ in circulating blood mononuclear cells from the two groups. Findings presented here suggest that increased levels of MIF may be necessary for an effective immune response and protection against severe disease. Since all subjects were aparasitaemic at least two months prior to sampling, we postulate that differential production of MIF in the groups is due to host genetic factors. (ACMCIP abstract)

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DEVELOPMENT AND STANDARDIZATION OF AN IN VITRO PLASMODIUM FALCIPARUM GROWTH INHIBITION ASSAY UTILIZING MEASUREMENT OF LACTATE DEHYDROGENASE (LDH) ACTIVITY

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The process of vaccine development for the erythrocytic stages of malaria parasites would be greatly facilitated by an in vitro assay which would correlate with in vivo protective immunity. One frequently used assay is the malaria parasite growth inhibition assay (GIA) which usually involves incubation of synchronized late-stage parasites with antisera followed by enumeration of newly invaded erythrocytes to measure the capacity of a specific antisera to inhibit parasite invasion or growth. Although many investigators perform GIA, the methods widely used to count the newly invaded erythrocytes have disadvantages (e.g. time-consuming, need radioisotopes or expensive equipment, etc.), and there is no standardization of GIA protocols. We have developed and standardized a method for performing GIA by measuring parasite lactate dehydrogenase (LDH) activity. We have examined the variables involved in the assay and evaluated the reproducibility of results using anti-AMA1 antisera and the IgG fractions of such sera from multiple species. After incubation with parasites and antisera, it takes < 2 hours to obtain the % inhibition of 72 samples tested in triplicate by one operator, excluding a freezing step to release parasite LDH from the erythrocytes, with a % coefficient of variation in triplicate wells < 5 %. When anti-AMA1 antisera were tested with various concentrations 6 times independently, the SD of % inhibition was < 9 in each tested concentration of antisera. Similar data have also been obtained with IgG fractions of antisera and specificity has been demonstrated by reversal of GIA with antigen. This method is inexpensive and can be performed by many laboratories which can reliably culture P. falciparum parasites and operate a spectrophotometer. Given the numbers of pre-clinical and clinical studies in progress with various blood-stage vaccine candidates, this method provides an important tool to evaluate the in vitro biological activity of both animal and human
We have expressed and purified an iron-regulatory-like protein of *Plasmodium falciparum* (PIRPa) in a bacterial expression system. The obtained protein was labile and sensitive to oxidation, therefore its kinetic properties were determined under anaerobic conditions. Aconitase activity was detected using a colorimetric NADPH-MTT assay. The results show that PIRPa displays aconitase activity under conditions favoring the formation of an iron-sulfur cluster. Kinetic properties of PIRPa were further assessed spectrophotometrically by monitoring the hydration of cis-aconitate at 240 nm, using a molar absorption coefficient of 3600 M⁻¹ cm⁻¹. The initial rates of cis-aconitate hydration were plotted against different concentrations of cis-aconitate, from which the values for *Kₘ*, *Vₘₐₓ*, and *Kₖₐₜ* were calculated. Recombinant PIRPa showed high affinity for cis-aconitate (*Kₘ* = 3.5 µM), but a low turnover number (*Kₖₐₜ* = 3.3 sec⁻¹). The overall catalytic efficiency of rPIRPa was of similar magnitude to human cytosolic IRP1/aconitase and human mitochondrial aconitase. Immuno precipitation of PIRPa from these lysate preparations using two anti-PIRPa rabbit sera further confirmed the capacity of PIRPa to demonstrate aconitase activity.

Immunofluorescence analysis showed that the protein was localized in both the cytosol and mitochondrion of the parasite. The results of differential digitonin permeabilization of isolated trophozoites with subsequent Western blotting are consistent with the localization of PIRPa in the membranous compartments of the parasites such as the mitochondria. Our results provide evidence for the potential of PIRPa to switch between RNA binding and aconitase activity. In addition, the presence of an iron-sulfur cluster in the enzyme conformation of PIRPa suggests that this protein can also serve as an iron-storage protein in this parasite. Knowledge of the role of PIRPa/aconitase in the regulation of iron homeostasis in the asexual stages of *P. falciparum* may contribute towards the development of novel antimalarial strategies against plasmodial species.

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**PREFERENTIAL TRANSCRIPTION OF A SUBSET OF var GENES DURING SHORT-TERM CULTURE OF ex vivo SAMPLES**

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Antigenic variation in *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) has been detected both in vivo and in vitro, however the switching process of the genes responsible for this phenomenon remains undetermined. Our previous work described the presence of a dominant transcript in two ex vivo samples prior to any in vitro cultivation. In this study, we examined the transcription of var genes in these ex vivo human samples during limited cultivation. During 28 days of *in vitro* culture, the prevalence of this initially dominant transcript decreased at a constant rate. Twelve different transcripts were detected in these samples at frequencies >10%. There was considerable overlap in the transcripts detected at these frequencies even though the samples originated from two different individuals, suggesting preferential transcription of some genes during the acute phase of an infection and subsequent short term cultivation. Alignment of the observed var transcripts against the genome database indicated that these transcripts are products of a group of var genes with similarities on various levels including domain structure, 5' and 3' UTRs and chromosome location. RT-PCR, real-time PCR and mathematical modelling are used to estimate the switch away rate of the initial transcript and several other transcripts during this culture period. The results suggest that once a parasite commits to switching, it continues to do so at a constant rate irrespective of the environment.

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**EVOLUTION OF THE GENES ENCODING THE TRANSMISSION-BLOCKING VACCINE CANDIDATES Pv25 AND Pv28 OF PLASMODIUM VIVAX**

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We have investigated the genetic diversity and evolution of the genes encoding the transmission-blocking vaccine antigens Pv25 and Pv28 of *Plasmodium vivax*. We studied the genetic polymorphism by sequencing alleles from 12 isolates that are representative of the worldwide distribution of *P. vivax* (Asia and the Americas). Overall, we found that these antigens are less variable than others expressed in the sporozoite and merozoite stages with *π* values of 0.00185 and 0.00245 respectively (the hypervariable region of AMA-1 has a *π* > 0.037 in a comparable sample). No evidence of positive natural selection was found. Specifically, we could not reject the null hypothesis that the synonymous vs. non-synonymous substitution ratio was > 1. No evidence of positive selection was found by comparing with *P. cynomolgi*, a close related species that is parasitic in macaques. We have preliminary data on the homologous genes in non-human primate malaria that are closely related to *P. vivax*. We have not found evidence of selection affecting the divergence of these genes during the radiation of primate malaria parasites including *P. vivax*. Our intra-specific and inter-specific data demonstrate that genes encoding transmission-blocking antigens have not been under selective pressure for accumulating mutation; thus, our data is consistent with the assumption that these genes are conserved because of lack of selective pressure by the host immune system.
The owl monkey, Aotus nancymai, is widely used in malaria vaccine research, but only some Plasmodium falciparum parasites are virulent in this model. To identify molecular determinants of parasite virulence to A. nancymai, we have developed a P. falciparum genetic cross through anopheline mosquitoes and a chimpanzee. A virulent P. falciparum clone was mated with a clone unable to establish an infection in A. nancymai. Thirty-three independent recombinant progeny have been isolated and typed with microsatellite maps at 5 cm resolution. The phenotypes of these progeny fall into two distinct parental groups based on their infectivity or non-infectivity to A. nancymai red blood cells. A major gene for infectivity maps to a 36 kb region, containing 7 predicted genes. We are currently screening candidate genes within this locus for their role in virulence.

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TARGETING OF RIBOSOMAL PROTEINS IN MALARIA PARASITES

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A total of 131 genes can be identified as encoding ribosomal proteins in malaria parasites through bioinformatic approaches. Expression profiles of these genes in the DeRisi database show detectable expression for most of these proteins at different stages of intraerythrocytic developmental cycle. As reported by the DeRisi group, putative cytoplasmic and organellar ribosomal protein genes are expressed at distinguishable times during the life cycle. We have combined these expression data with extensive sequence analyses to derive a list of putatively mitochondrial ribosomal protein genes to distinguish them from those encoding cytoplasmic and apicoplast ribosomal proteins. Thus, 82 of the proteins can be proposed as cytoplasmic, 29 proteins as plastid and 20 proteins as mitochondrial ribosomal proteins. All mitochondrial ribosomal proteins are nuclearly encoded requiring import into the organelle for subunit assembly, whereas the majority of the plastid ribosomal proteins are encoded in the plastid genome and thus do not require translocation. We are using the large subunit ribosomal protein L2 as a prototype to investigate the transport machinery needed for mitochondrial ribosome assembly. Malaria parasites encode three L2-like proteins with distinct expression profiles and different sizes. Two of those proteins are nuclearly encoded; therefore, one is likely to be targeted to the cytoplasm while the other to the mitochondria. The third L2 protein is encoded in the plastid genome. We have constructed plasmids encoding full length L2 proteins as well as the putative mitochondrial targeting leader sequence tagged with GFP. These constructs have been transfected into Plasmodium falciparum with a view to investigating targeting of the proteins as well as the possible assembly of these proteins into mitochondrial ribosome like structures.

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ANTIMALARIAL DRUG SCREEN TARGETING KASIII (β-KETOACYL-ACP SYNTHASE III) IDENTIFIES COMPOUNDS THAT INHIBIT PLASMODIUM FALCIPARUM IN VITRO.

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In Plasmodium falciparum, the fatty acid biosynthetic pathway requires the successive action of five enzymes (MCAT, KASIII, BKR, HAD, and ENR) along with the use of ACP as a substrate. The enzymes of fatty acid biosynthesis in malaria are attractive targets for antimalarial drug discovery because malarial enzymes are homologous to the Type II enzymes found in plants and microorganisms, but not to the Type I enzymes found in humans. The inhibitor screen uses radioimabeled (14C)-acetyl-Coenzyme A to monitor the transfer of the (14C)-acyl group to ACP generating (14C)-acyl-ACP. An iteratively refined three-dimensional pharmacophore model was developed to screen several chemical databases. We have been able to screen 453 compounds in the KASIII inhibitor screen. At 10µM, 40 drugs have shown greater than 60% inhibition against KASIII. All 40 compounds were sent to the in vitro parasite screen and tested against the D6 (chloroquine-sensitive) and W2 (multi-drug resistant) strains of P. falciparum. Of the 40 compounds, 23 had IC50 values in the low micro-molar range against P. falciparum in the in vitro parasite screen. The IC50 values for 17 of 23 of these compounds are in the low micro-molar range against both the D6 and W2 strain (8 of 17 show activity less than 10µM).

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ELISPOT ASSAY FOR IFN-γ PRODUCTION IN RHESUS MACAQUES IMMUNIZED WITH PLASMODIUM FALCIPARUM-BASED ADENOVIRUS CONSTRUCTS

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The circumsporozoite protein (CS) of Plasmodium falciparum has been proven partially effective in humans and is a viable candidate antigen for pre-erythrocytic vaccine development. CS-expressing, recombinant, non-replicative Adenovirus 35 and 5 vectors (rAd35-CS and rAd5-CS, Crucell, Netherlands) were given to rhesus macaques in order to evaluate their subsequent immune response to CS. We undertook an extensive analysis of methods and quality assurance techniques to optimize an antigen-specific ELISPOT assay. Cryopreserved peripheral blood mononuclear cells (PBMC) from the immunized monkeys were stimulated with pooled peptides and whole proteins. The assay included simultaneous stimulation of cells with media (negative control), Concanavalin A (ConA, positive control), and various vaccine-related peptide pools and proteins. This sensitive and reproducible assay has been useful at evaluating both acute and chronic responses. The non-challenge rhesus macaque model is useful for careful analysis of immunogenicity and can help to focus attention on the most promising vaccine candidates and combinations prior to design of human clinical trials. (ACMCIP abstract)

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ENHANCED IMMUNOGENICITY OF PLASMODIUM KNOWLESI VACCINES IN RHESUS MONKEYS BY LONGER INTERVAL BETWEEN DNA PRIME AND VIRAL BOOST


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In this study we compared the effect of 1 versus 6-month intervals between last DNA priming and recombinant vaccine virus boosting on a malaria vaccine in rhesus monkeys. We measured in vivo protection against sporozoite
challenge and in vitro antibody and T cell responses against Plasmodium knowlesi antigens. The rhesus monkeys were primed 3 times 4 weeks apart with the Pk4 DNA vaccine, a cocktail of four Pk antigens: Pk CSP, Pk AMA1, Pk MSP2, and Pk SSP2. After priming with DNA, monkeys were boosted at either one-month or six-months by the Pk4 COPAK that is a mixture of four attenuated recombinant poxvirus encoding the same four antigens. All animals were challenged iv with 100 P knowlesi sporozoites 2 weeks after boosting. Compared to the 1-month interval, the 6-month interval induced stronger serum antibody responses against the Pk 4 antigens as assayed by ELISA. The longer interval also induced stronger IFN-γ ELISPOT responses with both Pk CSP and Pk AMA1 antigens (other 2 antigens not tested). Five of 5 animals in this group produced 750-2000 spot-forming cells/million PBMC that was significantly higher than that induced by the shorter interval (P< 0.005), particularly for the Pk AMA1 antigen. However, despite these improved immune responses, there were no significant differences in parasitemias between the groups having 1 and 6-month intervals before boost, and only slight protection as compared to control infected animals. Using IL-2 Ig DNA as adjuvant for DNA priming did not alter vaccine induced immunogenicity and protection. These results suggest that a longer period between DNA prime and viral boost improves immunogenicity from vaccination. Optimization of interval between priming and boosting could be critical to application of vaccine candidates from animal to human clinical trials.*

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**PLASMODIUM FALCIPARUM APICAL MEROZOITE ANTIGEN-1 ELISA DEVELOPMENT AND VALIDATION**

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The apical merozoite antigen-1 of *Plasmodium falciparum* (PfAMA-1) is a blood-stage malaria antigen thought to be involved in red blood cell invasion, and thus is a recognized vaccine candidate antigen. A Phase I clinical trial was recently conducted to assess the safety and immunogenicity of an AMA-1 recombinant vaccine candidate. ELISA was used to test the IgG γ specific antibody responses after vaccination. In order to ensure that the assay would generate accurate and precise results, the antigen and secondary antibody concentrations were first optimized by finding the concentrations yielding the maximum signal-to-noise ratios of a known positive sample to a known negative sample. Assay and technician accuracy and reproducibility were then validated by serial dilution of nine samples in triplicate plus a negative and a positive control on nine separate days. Mean, standard deviation and coefficient of variation (CV) were determined for sample and control titer values, for curve slope, for the plate blank and for optical density (OD) values at each dilution for all samples over this period. None of the samples had titer value CVs higher than 12.2%. The CV for the curve slope of the positive control was 6.1% and the plate blank CV was 6.4%. The average CV of OD values per dilution was found to be 2.6%. Acceptable limits for future quality control assays and for plates containing clinical trials samples were then determined by using the mean ± two times the standard deviation. This assay has been proven robust and repeatable in detecting human responses to AMA-1, and can be validated to meet regulatory requirements. (ACMCIP abstract)

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**FEVER PREVALENCE AND THE PYROGENIC THRESHOLD FOR DEFINING MALARIA DISEASE AT THE MALARIA VACCINE TESTING SITE OF DONEGUEBOUGOU, MALI**


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The goal of blood stage malaria vaccines is to reduce malaria-related morbidity and mortality. Doneguebougou, Mali, has been prepared for malaria vaccine testing and a Phase 1 trial was started in May 2004. Defining precise, convenient, and meaningful endpoints will be critical for Phase 2 and 3 blood stage malaria vaccine trials. However, in endemic areas, malaria disease is difficult to define because parasitemia is common and symptoms are not specific. Modelling malaria parasitemia as a function of fever to determine a pyrogenic threshold has been proposed as a useful alternative to defining malaria disease in endemic areas. To establish such a threshold in Doneguebougou and 4 surrounding villages located within 15 km of Doneguebougou, we conducted five cross-sectional surveys in children aged 0 to 9 years from March 2002 to December 2003. Among children between the ages of 2 and 5 years, 11.2% and 5.2% had fever during the rainy and dry seasons, respectively. The overall proportion of fever attributable to falciparum malaria in this age group was 30.7% during the rainy season and 13.4% during the dry season. The optimal threshold values - with the highest sensitivities and specificities - were approximately 6400 pl/mcL (corresponding to 85% sensitivity and 85% specificity) during the rainy season and 1600 pl/mcL (corresponding to 86% sensitivity and 86% specificity) during the dry season. Malaria remains a major cause of fever in Doneguebougou and its surrounding villages in Mali. Malaria disease is more frequent during the rainy season and the parasite density threshold for defining disease varies according to the season. This seasonal variability in the pyrogenic threshold must be taken into account when planning endpoints for future Phase 2 and 3 malaria vaccine trials in this area.

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**DEVELOPMENT OF COMBINATION MALARIA VACCINE AGAINST PLASMODIUM FALCIPARUM BASED ON 19 kDA C-TERMINAL DOMAIN OF MSP-1 AND FUNCTIONAL BINDING DOMAIN OF EBA-175**

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A vaccine for *Plasmodium falciparum* malaria is urgently needed. It may be necessary to combine multiple merozoite antigens in order to develop an effective blood stage vaccine for *P. falciparum* malaria. We propose to develop a combination vaccine based on the 19 kDa C-terminal domain of MSP1 (PfMSP1-19) and the functional binding domain of EBA-175 (PfEBA-175). We have designed a synthetic gene coding for PfMSP1-19 based on codon bias
Humans who receive a candidate malaria vaccine containing Merozoite Surface Protein-142 (MSP-142) can exhibit antibodies to MSP-142 that are measured by Enzyme-Linked Immunosorbent Assay (ELISA). To make a standard human reference pool for MSP-142 antibody measured in absolute quantity units, we pooled plasma samples known to contain high titers of MSP-142 antibody based on previous ELISA results. Immobilized metal affinity chromatography (IMAC) was employed to determine the amount of MSP-142 antibody in this reference pool. Hexahistidine-tagged MSP-142 antigen adsorbed to nickel-chelating resin was used to capture MSP-142 antibody in the reference pool. The intact MSP-142 antibody-antigen complexes were eluted and total immunoglobulin measured by ELISA standardized to purified human IgG. In this way, the reference pool was determined to contain 48.3 µg/ml of MSP-142 antibody. This serum pool may be useful as a quantitative working standard for MSP-142 antibody responses in future vaccine trials involving MSP-1. (ACMCIIP abstract)

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EVALUATION OF THE IMMUNE RESPONSE TO A RECOMBINANT LSA-1 LIVER STAGE VACCINE FOR MALARIA IN SMALL ANIMAL MODELS

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Liver Stage Antigen-1, LSA-1, expressed on Plasmodium falciparum parasites during their liver stage development, has been related to humoral and cellular immune protective responses in persons residing in malaria endemic areas and persons exposed to multiple bites from radiation-attenuated P. falciparum-infected mosquitoes. For the first time, we have expressed the LSA-1 protein from the P. falciparum 3D7 strain in E. coli using harmonized codons. Our construct, LSA-NRC, contains the N-terminal and the C-terminal regions, linked by two 17-amino acid sequences from its repeat region. To accomplish the initial stages of evaluation of this protein as a vaccine against P. falciparum malaria we evaluated the antigenicity of LSA-NRC in mice and rabbits. After intraperitoneal immunization with LSA-NRC, NZW rabbits were immunized subcutaneously with 100 µg of LSA-NRC in Montanide ISA 720. NZW rabbits were immunized subcutaneously with 100 µg of LSA-NRC in Montanide ISA 720. Rabbits, A/J mice and BALB/c mice produced high titers of antibodies that recognized liver stage parasites in infected hepatocytes. As a measure of the magnitude of the specific Th1 and Th2 responses to LSA-NRC, we evaluated INF-γ and IL-5 production by the Elispot assays. Interferon-γ producing cells, which have been associated with LSA-NRC mediated protection, were found in high numbers in spleens from immunized A/J and Balb/c mice when stimulated with LSA-NRC or with four of ten peptide pools overlapping the complete protein. No IFN-γ producing cells were found in C57BL/6 mice, but in contrast a significant number of IL-5 producing cells were detected. Dissimilar patterns of IFN-γ responses to peptides were found between A/J and Balb/c mice. Differences in antibody and cytokine production suggests a genetic restriction for LSA-NRC immune responses as observed with other LSA-1 constructs. More detailed information about immune responses induced by LSA-NRC in mice and rabbits will be presented. (ACMCIIP abstract)

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CHARACTERIZATION OF A STANDARD HUMAN REFERENCE POOL FOR MEROZOITE SURFACE PROTEIN-142 ANTIBODY

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234 table of E. coli and expressed this antigen as a soluble intracellular protein with N-terminal histidine-tag. High cell density fermentation was developed to express recombinant PfMSP1-19 at high levels. A simple two step purification strategy was developed to purify recombinant PfMSP1-19 using Streamline chelating chromatography followed by ion exchange chromatography. The purified PfMSP1-19 is pure, monomeric and is recognized by conformation sensitive monoclonal antibodies. PfF2, the binding domain of EBA-175, was expressed in E. coli, purified from inclusion bodies under denaturing conditions by metal affinity chromatography, renatured by oxidative refolding and purified to homogeneity by ion-exchange chromatography. Recombinant PfF2 was characterized and shown to be pure, homogeneous and functionally active in that it binds to human erythrocytes with specificity. We have studied the humoral immune responses of the combination vaccine composed of recombinant PfMSP1-19 and PfF2 formulated with four different human compatible adjuvants namely Montanide ISA 720, Montanide ISA 51, AS02A, and Alum in mice. Characterization of immune responses including ability of sera to block erythrocyte invasion by P. falciparum in vitro will be presented.

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DEVELOPMENT OF VACCINE AGAINST PLASMODIUM VIVAX MALARIA BASED ON FUNCTIONAL BINDING DOMAIN OF P. VIVAX DUFFY BINDING PROTEIN

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Molecular interaction of P. vivax Duffy binding protein (PvDBP) with its Duffy receptor on erythrocyte surface is a necessary event for erythrocyte invasion by P. vivax merozoites. We propose to develop vaccine against P. vivax malaria based on functional binding domain, region II, of PvDBP (PvRII). A synthetic gene encoding PvRII was designed and expressed in E. coli along with C-terminal 6-histidine tag. Expression level of synthetic gene was enhanced two fold and low molecular weight fragments appeared during expression of native gene were absent in case of synthetic gene expression. High cell density fermentation was developed to express high level of PvRII. PvRII was purified from inclusion bodies by Streamline chelating chromatography in denaturing conditions and refolded to its native conformation by rapid dilution method. PvRII was further purified to homogeneity by ion exchange chromatography. The purified PvRII is monomeric, homogeneous and functional in terms of its binding to human erythrocytes with specificity. Immunogenicity studies of PvRII formulated with various human compatible adjuvants in mice and rhesus monkeys suggested that formulation with AS02A, Montanide ISA 51, and Alum in mice. Characterization of humoral immune responses including ability of sera to block erythrocyte invasion by P. falciparum in vitro will be presented.
A NOVEL AND SCALABLE VACCINE APPROACH AGAINST MALARIA: Ad35 VECTORS PRODUCED ON PER.C6® CELLS

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Protection against malaria, both in mice and human challenge models, is conferred by the circumsporozoite protein antigen (CS). This protection is limited when insufficient T cell immunity to CS is induced. Replication-incompetent adeno-virus 5 vectors (Ad5) expressing the CS of Plasmodium yoelii have been shown to provide protective immunity against malaria infection, a protection conveyed predominantly by CS-specific T cells, in particularly, CD8+ CTLs. The clinical utility of common Ad5 vectors is seriously impaired by the fact that the majority of humans has pre-existing infection, a protection conveyed predominantly by CS-specific T cells, in particularly, CD8+ CTLs. The clinical utility of common Ad5 vectors is seriously impaired by the fact that the majority of humans has pre-existing immunity against Ad5, as a result of natural exposure. We designed vectors derived from rare Ad human serotypes, like Ad35, that show a very low seroprevalence worldwide, including malaria endemic areas, and are efficiently produced on PER.C6® or derivatives thereof, as reported previously. We showed the ability of Ad35 vectors expressing P. yoelii CS to protect mice against the murine malaria challenge and, subsequently, that pre-existing Ad5 serum titers do not hamper Ad35-mediated T cell responses against foreign antigens, as reported previously. We present the generation on PER.C6®-derived cells of Ad35 vectors expressing the CS of P. falciparum; their stability and production to high titers as well as their immunogenicity in mouse models. In collaboration with the WRAIR, immunogenicity of these vectors in non-human primates is evaluated. (ACMCIP abstract)

CONSTRUCTION OF A NOVEL MALARIA ANTIGEN EXPRESSION VECTOR.

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Development of an effective malaria vaccine is an area of active interest to the Army Medical Department. Currently, there are a number of malaria antigens under investigation as potential vaccine candidates. These include sporozoite antigens (circumsporozoite protein), merozoite surface proteins (e.g. MSP-1), erythrocyte-binding antigen (EBA) 175, rhesus-associated protein (RAP) 1, apical merozoite antigen (AMA) 1, and gametocyte antigens (e.g. Pfs25, Pfs230). Regardless of the antigen studied, the conventional approach to recombinant vaccine construction entails cloning the gene of interest into an expression vector, which is then used to transform a non-pathogenic bacterium. The transformant then elaborates the encoded protein, eliciting an immune response without causing disease. There are however a number of limitations with current cloning vectors. These include plasmid segregation in the absence of antibiotic selective pressure, and poor yields of soluble protein. Here we describe the construction of a novel malaria antigen expression vector pGENΔzap by modification of a plasmid constructed by Galen’s group, as reported previously. Features of the construct include the hob-soh post-segregation killing system (obviating the need for antibiotic selective pressure), par loci (to ensure plasmid partitioning to bacterial progeny), a PompC1 promoter (enabling optimum transcription or translation, depending on the salinity of the culture medium), and a customized poly linker into which genes of interest may be cloned. The first of the cloned sequences, Plasmodium vivax merozoite surface protein, encodes an antigen expressed by the blood-stage parasite prior to its entry into the liver. The resulting construct, pGENΔzap-Pvmsp1, contains an in-frame Pvmpl locus under the regulation of the upstream PompC1 promoter, and is stably maintained within Escherichia coli in the absence of antibiotic selective pressure. (ACMCIP abstract)

CELLULAR AND HUMORAL RESPONSE OF PLASMODIUM VIVAX IRRADIATED SPOROZOITES IN AOTUS MONKEYS

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Exposure to radiation attenuated Plasmodium sp. sporozoites has proven to be the best way to elicit immune responses that protect mice and humans against malaria. The model was developed in the late 1960s and early 1970s. The Plasmodium falciparum model has been extensively studied during the past 15 years but, the P. vivax model, has not been reproduced since 1975, and even then was only studied in a few volunteers. Because of the potential importance of this model for characterizing protective immune responses against pre-erythrocytic stage antigens and identifying novel vaccine candidates for vaccines against P. vivax, we are currently evaluating immune responses in pre-clinical studies and setting up the conditions for clinical trials. In order to evaluate humoral and cellular immune responses, we have developed a protocol using 30 naïve Aotus monkeys, separated into three groups. Group I (n=18) was immunized with P. vivax 15 Krad irradiated sporozoites (irr-spz) and was subdivided into three groups of 6 animals each (Ia, Ib, Ic), which received 10, 5 or 2 immunizations respectively of approximately 100,000 irr-spz per immunization. Group II (n=6) was immunized with extracts of salivary glands from non-infected mosquitoes. Immunizations were done every two weeks by i.v. injection in the femoral vein. Group III (n=6) was used as a source of normal blood samples for the immunological assays. Antibodies in sera from immunized monkeys recognized long synthetic peptides of the PvCSP as well as the native protein on sporozoites. IFN-γ secretion was detectable in PBMC of all immunized animals in response to PvCSP peptides and sporozoites. These results indicate that vaccination of Aotus monkeys with radiation attenuated P. vivax sporozoites induced an immune response that is similar to immune response previously reported in humans, and suggest that the Aotus monkey model may prove to be important for the pre-clinical development of preerythrocytic-stage P. vivax vaccines.
The *Plasmodium vivax* Pvs25 is a zygote/ookinete surface protein able to elicit malaria transmission-blocking (TB) antibodies in animals immunized experimentally and is therefore considered an important vaccine candidate for human use. Here we have addressed several critical issues relevant for further development of Pvs25 as a vaccine for human use. First, we assessed, in Aotus monkeys, the immunogenicity and the duration of the antibody response of a Pvs25 recombinant protein produced in Saccharomyces cerevisiae. Animals immunized three times with 100 µg of the protein formulated in Montanide ISA-720 developed specific anti-Pvs25 antibodies that appeared by day 30 after the first immunization to levels of 1:103-1:104 as determined by ELISA. Titers started decreasing by day 180 and were still detectable on day 300, (180 days after the last boost). Second, we assessed if these antibodies would display TB activity when tested in laboratory reared Anopheles albimanus mosquitoes fed with *P. vivax* human isolates, using an artificial membrane feeding assay. Plasma from all immunized Aotus, but not from the control animals, was able to completely block the development of the sporogonic parasite cycle in fed mosquitoes. Third, we addressed the boosting effect of blood infection on the anti-Pvs25 antibody titers. No boosting was observed during infection despite the presence of infective gametocytes, indicating that this protein might not be expressed in sufficient quantities in erythrocytic stages of the parasite. In conclusion, Pvs25 protein formulated in Montanide ISA-720 induces efficient and long lasting TB antibodies that can not be boosted by parasite infection, indicating that revaccination would be required to maintain the immunity and produce significant epidemiological impact in endemic areas.

**VACCINATION WITH A PLASMODIUM CHABAUDI ADAMI MULTIVALENT GENOMIC LIBRARY CROSS PROTECTS MICE AGAINST *P. C. CHABAUDI* INFECTIONS AND INDUCES CROSS-REACTIVE RESPONSES TO MOUSE AND HUMAN MALARIA PARASITES**

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The development of an effective anti-malarial vaccine has been considerably delayed due to the strain-specificity of acquired immune responses to the candidate antigens under study. In order to achieve a level of immunity comparable to the state of premonition reported in humans, a malaria vaccine will have to be multivalent and include conserved target epitopes that will assure its success independently of the parasite strains present in the different areas of transmission. We have previously reported the protective efficacy of multivalent malaria DNA vaccines comprising from 30,000 (30K vaccine) down to 56 plasmids containing genomic sequences from *Plasmodium chabaudi adami* parasites. The immunity induced by our vaccines, characterized both by the induction of opsonizing antibodies as well as IFN-γ secretion by splenic T cells, protected BALB/c mice both against homologous and heterologous challenge with two different *P. c. adami* strains. In the present study we have further assessed the protective capacity of the 30K vaccine against heterologous infection with *P. chabaudi chabaudi* AS parasites in susceptible A/J mice. Immunized A/J mice effectively controlled patent infection which resulted both in a significant reduction in cumulative parasitemia (50%) as well as enhanced survival. In addition, the 30K vaccine induced cellular and humoral immune responses cross-reacting with *P. chabaudi chabaudi* and *P. yoelii* parasites. Moreover, mice immunized with the 30K vaccine developed antibodies capable of specifically opsonizing human red blood cells infected with *P. falciparum* parasites. Considering that DNA sequencing of plasmids in the 30K vaccine has identified epitopes which are conserved in sequence among malaria species, we propose that further studies will allow us to identify new multi-epitope or poly protein-based vaccines capable of inducing strain-transcending immunity against asexual blood stage malaria.

**PRELIMINARY EVALUATION OF PLASMODIUM VIVAX MSP1 (r200L) SUBUNIT VACCINE: ANTIGENICITY, IMMUNOGENICITY AND PROTECTIVE EFFICACY IN AOTUS MONKEYS**

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Merozoite surface protein-1 (MSP-1) is a family of proteins expressed in *Plasmodium* species studied to date. With *P. falciparum* this protein has been considered a major vaccine candidate and several subunits are being tested in clinical trials. The PfMSP1 protein is located near the N-terminal fragment of PfMSP-1 and contains a binding domain to the erythrocyte cytoskeleton protein spectrin. Immunization of *Aotus* monkeys and humans with this subunit is known to induce protective immune responses. After...
sequence analysis we found that the *P. vivax* MSP-1 protein contains a region that shares >60% homology with *Pf*190L. It was termed *Pv*-200L and selected for study as a potential subunit vaccine. *Pv*-200L was expressed as a His-tagged recombinant protein in *E. coli* (*r*200L), including part of a high-binding region to reticulocytes (HBR-I). The protein was purified using standard Immobilized Metal Ion Affinity Chromatography (IMAC) to obtain a bench-quality material more than 90% free of *E. coli* contaminants with an endotoxin level below 1000 EU/50 µg of protein. We found that immune IgG recognized r200L specifically by immunoblot; seroepidemiological studies were then carried out to determine the antigenicity of *Pv*-200L. Sera from 150 individuals of a malaria-endemic region of Colombia were assessed to determine the immune recognition of the protein using an ELISA technique. More than 70% of *Pv*-infected patients and more than 50% of exposed but non-infected individuals had IgG antibodies that reacted with r200L. To determine the immunoreactivity of the protein, BALB/c mice were injected IP 3 times with 50 µg using Freund’s adjuvant. Immunization induced high levels of IgG antibodies (titer >10⁶) recognized the native MSP-1 protein in IFAT assays. Protective immunity is currently being determined. Detailed results from these studies will be discussed.

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**ANTIBODIES TO RAMA PREDICT RESISTANCE TO PLASMODIUM FALCIPARUM RE-INFECTION IN WESTERN KENYA**

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Residents of Plasmodium falciparum endemic areas develop protective immunity that limits parasitemia and disease. In previous studies, we collected sera from an extensively characterized cohort of 143 male volunteers aged 12–35 years residing in a holoendemic area of Western Kenya. Volunteers were drug-cured of current malaria infection and blood was collected from each individual two weeks post treatment but prior to reinfection. Participants were followed for 16 weeks with weekly blood smears to quantify reinfection. Using reinfection data, we identified and pooled sera from the 10 most resistant individuals (RS) and the 7 most susceptible individuals (SS), matching for age, sickle trait, and mosquito exposure. Pooled RS and SS sera were utilized in a differential screen of a *P. falciparum* cDNA expression library. We screened 550,000 clones and identified 7 clones which were uniquely recognized by RS but not by SS.

One of these clones had a high degree of homology to the C-terminal region of RAMA, a recently described rhesus-associated membrane antigen, and rabbit antibodies raised against this clone recognized a 50 kDa antigen in *P. falciparum* infected RBCs. RAMA has been implicated in both rhoptry bio-logical studies were then carried out to determine the antigenicity of *Pv*-200L. Sera from 150 individuals of a malaria-endemic region of Colombia were assessed to determine the immune recognition of the protein using an ELISA technique. More than 70% of *Pv*-infected patients and more than 50% of exposed but non-infected individuals had IgG antibodies that reacted with r200L. To determine the immunoreactivity of the protein, BALB/c mice were injected IP 3 times with 50 µg using Freund’s adjuvant. Immunization induced high levels of IgG antibodies (titer >10⁶) recognized the native MSP-1 protein in IFAT assays. Protective immunity is currently being determined. Detailed results from these studies will be discussed.

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One of these clones had a high degree of homology to the C-terminal region of RAMA, a recently described rhesus-associated membrane antigen, and rabbit antibodies raised against this clone recognized a 50 kDa antigen in *P. falciparum* infected RBCs. RAMA has been implicated in both rhoptry biogenesis and erythrocyte invasion. We sub-cloned, expressed and purified this C-terminal region of RAMA (RAMA-pr) and used it as the target antigen in isotype-specific antibody assays. We assayed sera from 139 of the 143 volunteers and evaluated the relationship between isotype-specific antibody levels and re-infection indices in multivariate models. Individuals with high levels of IgG1 anti-RAMA-pr (n=24) had 8% lower frequencies of reinfection on the 16 post treatment blood films than individuals with medium or low (n=115) levels after accounting for age, sickle trait and mosquito exp-

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**GENETICALLY ATTENUATED PLASMODIUM SPOROZOITES PROTECT AGAINST INFECTION**

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Malaria is transmitted to the mammalian host by infectious mosquito bite, which inoculates sporozoites. Sporozoites invade hepatocytes and transform into liver stages. Liver stages reside within a parasitophorous vacuole (PV) where they grow and produce merozoites, which initiate red blood cell infection. Although parasite residency in the liver is short, immune responses against liver stages can be sterilizing thus completely preventing infection. Here we report a sporozoite-expressed protein, UIS4 that is also expressed in the PV membrane of liver stages as shown for the rodent malaria species *Plasmodium berghei* and *P. yoelii*. Using gene targeting we demonstrate that UIS4 has no apparent role for sporozoite invasion of hepatocytes. However, UIS4 is necessary for successful intrahepatic development and the generation of infective merozoites. Immunization of mice with usi4(-) sporozoites protects against subsequent challenge with wild type sporozoites. These results show the feasibility to create genetically attenuated parasites by manipulation of distinct genes. Such parasites may be used as life-attenuated malaria vaccines. (ACMCIP abstract)

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**PREVALENCE OF TRANSMISSION-BLOCKING IMMUNITY (TBI) IN NATURAL GAMETOCYTE-CARRIERS FROM A MALARIA ENDEMIC VILLAGE OF MALI**

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Malaria Research and Training Center, Bamako, Mali

The main objective in this study is to determine transmission reduction by specific antibodies to *Plasmodium falciparum* detected in sera of natural gametocyte-carriers. In previous studies undertaken in 2002 and 2003, we determined the prevalence of transmission blocking activity (TBA) in natural gametocyte carriers of different age groups in relation to seasonal changes and parasite genotypes. In this study, sera from the same carriers were studied by ELISA to identify antibodies to Pf6230 and Pf648/45 and their role in transmission reduction from mosquitoes to man. We will discuss the relevance of such study for TBV field-testing sites. (ACMCIP abstract)
Differential effects of rice growth on anopheline sp larva densities in two types of water management systems in Niono, an irrigated rice cultivation area of Mali

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We performed this study in 18 villages located in an irrigated rice cultivation zone of Niono, Mali, to assess 1) the impact of a poor water management scheme on mosquito larval density; 2) the spatial and temporal relationship between mosquitoes larval density and environmental factors. Four rice fields were randomly selected within a 2-km radius buffer around each study village. A total of 72 rice fields were randomly selected from two types of water management systems (a high-quality drainage system and a poor water drainage system) to assess the size of each type and follow-up from March 1999 to February 2001. Seasonal variation of larval density was more prominent at the end of the rainy season in field with a poor-quality water management system: geometric mean = 1.43, 95%CI [1.27-1.58] vs. 0.71, 95%CI [0.66-0.76] in field with high-quality water management systems. In both types of fields setting, larval density varied with the rice development stage. However, a negative association between the rice growth and larval density was found in fields with a high-quality drainage system. Larval geometric means for each rice development stage were 1.23, 1.45, 1.26, 0.80 at tillering, elongation, gaining and maturation stages respectively. In contrast, a positive association was observed in rice fields with a poor-quality drainage system, with larval geometric means for each rice development stage = 1.13, 1.74, 1.81, 1.67 at tillering, elongation, gaining and maturation stage respectively. To assess for spatial and temporal variations of larval density in relation with environmental variables (type of water management system, season, rice growth stage, vegetation, water state, soil type, agricultural activity and rice crop ), we fitted a Bayesian spatio-temporal Poisson model in WinBugs®. Results of the study revealed marked inter-villages and temporal variations in the larval geometric means. High larval density ratio was observed at the fertilizing stage. A spatial-temporal analysis showed strong spatial correlations, which reduced to less than 5% at distance larger than 25 km. This study was able to assess the effect of environmental parameters on mosquito larval density in rice fields, taking into account the amount of spatial and temporal correlation in mosquito larval density.

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Bicarbonate production and transport inhibitors interrupt development of Plasmodium and Leishmania parasites.

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Malaria and leishmaniasis are transmitted by Plasmodium and Leishmania parasites respectively. These parasites require a mosquito (in the case of Plasmodium) or a sand fly vector (in the case of Leishmania) to develop and complete their life cycle. Both parasites propagate naturally in the alimentary tract of the female mosquito and the sand fly, primarily in the midgut in which the environmental pH is slightly alkaline. Furthermore, Plasmodium gametocytes and Leishmania promastigotes require bicarbonate to develop inside the midgut of these insects. Since the parasitic intracellular pH is maintained close to neutrality in both cases, pH maintenance must involve molecular switches and transport molecules for modulating pH regulation. At present, the physiological and biochemical basis of these mechanisms are poorly understood. We are using molecular techniques as well as pharmacology to study these mechanisms and interrupt parasite development. We have treated Plasmodium and Leishmania infected blood with bicarbonate production inhibitors (such as methazolamide) and transport inhibitors (such as DIDS) and we have observed inhibition of development of Plasmodium parasites after treatment. We have used An. stephensi, An. freeborni and An. albimanus as well as P. falciparum and P. vivax as models. In the case of An. albimanus, the positive control for infection (blood infected with P. vivax) resulted in an average of 233 oocysts per midgut. Decarbonated plasma mixed with blood infected with P. vivax resulted in 38 oocysts per midgut in average. Treatment with 25 mM bicarbonate resulted in a higher number of oocysts as predicted. Treatment of P. vivax infected blood with methazolamide resulted in 100% reduction in the number of oocysts at 10^-3 M. We have obtained similar results for An. stephensi and An. freeborni using methazolamide and DIDS. Studies are currently underway to investigate the effect of these compounds on L. (V) panamensis y L. (L) infantum development as well. We postulate that these compounds inhibit the development of the parasites by inhibiting either production and/or transport of bicarbonate in the midgut of the insect vector.

A molecular marker for mosquito aging

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We have shown that long-term viability of mosquito cells is accompanied by high expression of EF-1α mRNA and protein. When maintained in serum-free medium, Aedes albopictus C7-10 cells continued synthesizing protein, albeit at a slow rate. When radiolabeled proteins were examined on SDS gels, we noted a prominent band measuring ~ 50 kDa, as well as 4 additional co-expressed proteins. We used tandem mass spectrometry to identify the 50 kDa protein as EF-1α. Commercially-available antibody was used to verify the increase in EF-1α expression in serum-deprived cells. EF-1α expression has been correlated with longevity in a wide range of organisms, including Drosophila melanogaster, and longevity of mosquitoes is an important component of vector competence. Most organisms contain two
functional EF-1α genes. We have cloned an EF-1α cDNA, and are using unique sequence in the 3'UTR to obtain the other gene. Gene-specific probes will be used to determine which of these genes correlates with viability in cultured cells. Experiments will be designed to test whether one of the genes affects longevity in adult female Aedes aegypti. These investigations extend a provocative observation with cultured cells to adult female mosquitoes, provide new information on expression of a mosquito protein known to be involved in viral replication cycles, contribute to an understanding of the role of protein synthetic machinery in mosquito aging and longevity, and provide a new avenue to reduce mosquito-borne disease by transgenic manipulations.

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DETECTION OF PEROXIDASE ACTIVITY OF HEME ASSOCIATED TO THE PERITROPHIC MATRIX OF MOSQUITOES FED ON MICE IMMUNIZED WITH A GENE ENCODING A PERITROPHIC MATRIX PROTEIN
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Iron is both an essential nutrient and a potent toxin for organisms. Many proteins are known to interact with iron and its reactivity with oxygen, including heme, iron-sulphur proteins, and iron-binding proteins. Heme is a toxic product of hemoglobin degradation and the female mosquito must have mechanisms for heme detoxication after a blood meal. The involvement of the peritrophic matrix on the iron detoxication process has been demonstrated. As part of an anti-mosquito approach using DNA vaccination with mosquito genes, we recently immunized mice with Ag-Aper1, a gene encoding a peritrophic matrix (PM) protein from Anopheles gambiae. The gene was inserted in a mammalian expression vector and the control group consisted of immunization with vector alone. After feeding mosquitoes on immunized animals, we assessed the mortality by constructing life-tables and analyzing survival curves using Kaplan-Meier log rank analyses. The mortality of mosquitoes fed on Ag-Aper1 group was higher than mosquitoes fed on Vector group at a significant level (p=0.03). After 7 days the proportion of mosquitoes surviving in the Ag-Aper1 group was 68% compared to 77% in the Control group. To detect any differences in the amount of heme bound to the PM, we checked for heme peroxidase activity by sectioning guts of mosquitoes and assaying them with tetramethylbenzidine (TMB), a peroxidase substrate. Interestingly, guts from Ag-Aper1 group had stronger heme peroxidase activity when compared to control groups when analyzed by light microscopy. A possible explanation for this higher heme peroxidase activity is that digestion was occurring faster in the Ag-Aper1 group. Immunohistochemistry with gut sections is being done to demonstrate in situ antibody binding and to test for possible differences in PM thickness. Our results so far reinforce the interaction of heme and the PM.

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COMPARATIVE ANALYSIS OF Aedes aegypti AND HUMAN KYNURENINE TRANSAMINASE
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Kynurenine transaminase and 3-hydroxylkynurenine transaminase are two key enzymes involved in mosquito tryptophan metabolism and play a vital role in the normal development of mosquitoes. These proteins are therefore potential targets in the development of insecticides for mosquito control. Our data show that the biochemical characteristics of mosquito kynurenine and 3-hydroxylkynurenine transaminases are quite different from their counterpart enzymes in other species. This study focuses on the extensive biochemical characterization and comparison of Aedes aegypti kynurenine transaminase with its counterpart human enzyme. Our data showed that a number of keto acids function as the amino group acceptor for both mosquito and human kynurenine transaminase and that the overall level of keto acid has a major impact on kynurenate synthesis. Comparative analysis of the mosquito and human kynurenine transaminase revealed some unique biochemical characteristics of the two enzymes. For example, cysteine at physiological concentrations greatly stimulates kynurenine transamination activity of the mosquito enzyme, whereas at identical conditions, this amino acid significantly inhibits the human counterpart enzyme. Our comparative analysis also revealed several efficient inhibitors of the human enzyme. The biochemical characteristics of proteins are dictated by their 3-dimensional structures; therefore, it is likely that mosquito enzymes and counterpart enzymes from other species exhibit apparent differences in their respective 3D structures, which also makes it possible to develop insecticide or inhibitor specific for the mosquito enzyme with minimal impact on similar enzyme from other species. Both Aedes aegypti and human kynurenine transaminase have been crystallized and their 3D structures provide some explanation regarding their catalytic mechanisms. In-depth analysis of the overall structure/function relationship of the mosquito and human enzymes is currently underway, which should provide insight toward possible mosquito controls through the targeting of mosquito kynurenine transaminase.

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POST-TRANSLATIONAL MODIFICATION OF CHORION PEROXIDASE IN RELATION TO ITS BIOCHEMICAL AND BIOPHYSICAL PROPERTIES
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Our previous studies dealing with Aedes aegypti chorion hardening determined that peroxidase is present in the egg chorion and mediates chorion protein crosslinking during chorion hardening. Subsequent purification and biochemical characterization of the mosquito chorion peroxidase revealed that the enzyme is highly resistant to various denaturing conditions. For example, the enzyme remained active being stored in 1-5% SDS for several months. The chorion peroxidase band on the SDS-PAGE gel was positively stained in peroxidase substrates following a staining/destaining process in 40% methanol and 7% acetic acid. To understand its structural mechanisms against denaturation, we further analyzed the structural characteristics of A. aegypti chorion peroxidase with emphasis on its post-translational modifications. Our data suggest that after translation, chorion peroxidase undergoes extensive post-translation modifications, which includes proteolytic processing, disulfide bond formation and glycosylation. Through N-terminal labeling of the intact chorion peroxidase, chemical and enzymatic deglycosylation of the native enzyme and its reduced and heat-denatured form, trypsin digestion of its intact and deglycosylated forms, protease K digestion of its reduced and unreduced form, and subsequent analysis and comparison of peptides, glycopeptides, and glycoconjugates using various biochemical techniques, including HPLC with UV and fluorescent detection, MALDI/TOF/MS and HPLC/MS/MS, we were able to establish the starting N-terminal amino acid residue of the mature enzyme, the composition of glycoconjugates, the site of glycosylation, and residues of cysteines involved in disulfide bond formation. Our data suggest that post-translation modification of the chorion peroxidase substantially con-
MOLECULAR IDENTIFICATION OF SELECT CONTAINER BREEDING Aedes/Ochlerotatus MOSQUITOES.

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Mosquito surveillance requires accurate, and often rapid, identification of the medically important (disease) vectors. Species identification of these mosquitoes is especially important in urban surveillance programs where there is endemic disease or epidemic potential. Likewise, introduction of exotic mosquito species is an increasing problem. Because container-breeding Aedes/Ochlerotatus mosquitoes are poorly represented in CO2-baited CDC light trap collections, their eggs are typically collected with oviposition traps. Identification of these eggs in situ by morphological characters is impractical at best, especially in areas where multiple species oviposit concomitantly. Typically species identification is determined by adult emergence or rearing through several larval instars. These processes may take several days to more than a week, assuming the eggs hatch. In an effort to develop a rapid (i.e. one day) method to aid in the identification of medically important mosquito species from their eggs, we developed a species-specific PCR assay for the following mosquitoes: A. aegypti, Ae. albopictus, Oc. bahamensis, Oc. triseriatus, Oc. mediovittatus, Oc. japonicus, and Oc. atropalpus. Briefly, this assay is designed to amplify interspecies differences in the noncoding regions of the ribosomal DNA (rDNA) gene group based on ITS2 size polymorphism. We used a single oligonucleotide pair designed to anneal at the 5.8s and 28s regions flanking the ITS2 to produce amplicons for all of the above species. Sequence data were used to design specific internal oligonucleotides to differentiate the species with similar ITS2 amplicon sizes. (ACMCIP abstract)

EVALUATION OF CANDIDATE GENES CYP9-J AND SDR AS DETERMINANTS OF Aedes aegypti SUSCEPTIBILITY TO Plasmodium gallinaceum

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Susceptibility of Aedes aegypti to Plasmodium gallinaceum on the molecular genetic as well as a physiological basis is poorly understood. However, in previous investigations we have identified several candidate genes associated with susceptibility. Real time PCR techniques showed that these genes are upregulated in the susceptible Red-eye A. aegypti strain after a Plasmodium infected blood meal, whereas expression is greatly reduced in the refractory Moyo-R strain. Two of these candidate genes are SDR and CYP9-J. Expression of both genes is increased with uninfected as well as infected bloodmeals, although levels are higher with the infected bloodmeal. A definitive connection between the expression of these candidate genes and the degree of susceptibility to Plasmodium has not been determined. One possibility is that these genes are involved in the detoxification of oxidative active radicals. The hypothesis is that the gene products are targeted against radicals and, therefore, an upregulation results in decreased concentrations of radicals and thus in lower negative affects on parasites in the gut. In an effort to investigate how expression of CYP9-J and SDR genes impact susceptibility of A. aegypti, we have exposed six A. aegypti strains (Rockefeller, Rubio, Moyo-S, Moyo-R, Liverpool-SB and Red-Eye) to P. gallinaceum. The quantitative expression of CYP9-J and SDR was analyzed and compared to the susceptibility of the six strains. Additionally, the potency for removing oxidative active radicals (H2O2) in highly susceptible Red-eye versus refractory Moyo-R was assayed. We have found different degrees of susceptibility to P. gallinaceum among the six A. aegypti strains and are evaluating variability in quantitative expression of the candidate genes. We determined that peroxidase activity was not different in Red-eye and Moyo-R larvae, but was significantly higher in carcasses and the hemolymph of adult Red-eye versus Moyo-R females. Our results suggest that susceptibility is linked to a higher peroxidase activity in susceptible strains, although the role of SDR and CYP9-J in this process has yet to be validated.

ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS IN GENES LOCATED THROUGHOUT THE Anopheles gambiae GENOME

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Single Nucleotide Polymorphisms (SNPs) were identified in genes throughout the Anopheles gambiae genome. Most correspond to enzyme loci, but some are candidates for Plasmodium falciparum vector competence. Mosquitoes were collected as larvae from different sites throughout Mali to generate a core collection. DNA was extracted and whole genome amplification was performed on DNA from 95 individuals representing different chromosomal forms. PCR and SSCP analyses were performed and novel genotypes were sequenced. We modified the original Oligonucleotide Ligation Assay (OLA) to work at high temperatures with PCR products and a thermal stable ligase. Following 50-60°C hybridization to complementary template, an oligonucleotide with a 5’ biotin and an allele specific 3’ nucleotide was ligated to a second oligonucleotide with a 5’ phosphate and a 3’fluorescein reporter. This ternary complex was melted at 90°C and then returned to the hybridization temperature. Melting/hybridization was repeated for 25 cycles to produce multiple ligated products that were then captured on streptavidin coated plates, washed, exposed to peroxidase labeled anti-fluorescein antibodies, washed again, and ligation was visualized by peroxidase staining. Oligonucleotides for detecting SNPs have been developed for Glycerol-3-phosphate dehydrogenase (2L), Superoxide dismutase (CuZn)(3L), Alkaline phosphatase(2L), Adenylate kinase(3R) and developed for Glycerol-3-phosphate dehydrogenase (2L), Superoxide dismutase (CuZn)(3L), Alkaline phosphatase(2L), Adenylate kinase(3R) and Acetylcholine esterase(Ace-X). Abundant polymorphisms were detected in all genes except Ace-X.

CHARACTERIZATION OF AN ACETYLCHOLINESTERASE GENE IN Aedes aegypti ORTHOLOGOUS TO THE GENE RESPONSIBLE FOR INSECTICIDE INSENSITIVITY IN OTHER MOSQUITOES.

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Acetylcholinesterase (ACHE) hydrolyzes the neurotransmitter acetylcholine (ACh) to terminate neuronal excitation at the postsynaptic membrane and is a target for organophosphorus (OP) and carbamate insecticides. In several dipterans, including Drosophila melanogaster, Musca domestica and Lucilia cuprina, the amino acid polymorphisms in AChe associated with decreasing sensitivity to OPs and carbamates have been identified. However, the...
orthologous acetylcholinesterase gene (Ace) in mosquitoes and some other insects was determined to not be involved with resistance. Instead, it was determined that AChE-mediated insecticide-insensitivity was localized to different linkage groups than the Ace gene in *Culex pipiens* and *Culex tritaeniorhynchus*. Recently, a paralogous gene (Ace1) was identified and characterized in the greenbug, *Schizaphis graminum*, that is responsible for resistance. The Ace1 ortholog was identified following genome sequence determination in the mosquito *Anopheles gambiae*, and has been associated with AChE-mediated insecticide-insensitivity in *C. pipiens*, *C. tritaeniorhynchus* and *Anopheles gambiae*. We have initiated efforts to isolate and characterize the Ace1 gene in *Aedes aegypti*. A partial Ace1 gene sequence was recently made available in GenBank. We isolated a BAC genomic clone containing Ace1 using specific primers for a PCR library screen and confirmed that the initial PCR product has high amino acid identity to other mosquito Ace1 genes. We also isolated a partial cDNA sequence using RT-PCR with a specific primer based on the BAC clones sequence analysis. We have determined the complete genomic sequence for the *Ae. aegypti* Ace1 gene and determination of the complete cDNA sequence is ongoing. The genome positions of the Ace and Ace1 genes were mapped genetically on chromosomes 1 and 3, respectively (ACMGIP abstract).

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IDENTIFICATION OF A FEMALE-SPECIFIC ACTIN GENE IN *Aedes aegypti*

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Population reduction of mosquitoes is an effective method for controlling dengue fever and malaria transmission. Recent developments in control techniques include proposals to construct transgenic strains of mosquitoes carrying dominant, conditional-lethal genes under the control of a sex- and stage-specific promoters. In order to identify such promoters, subtractive cDNA libraries derived from male and female pupal mRNA of the yellow fever mosquito, *Aedes aegypti*, were constructed and screened. A cDNA clone, F-49, corresponds to a gene expressed specifically in female pupae. Sequence analyses revealed that this gene belongs to the actin gene family, and therefore was designated *Aedes Actin-4* (*AeAct-4*). Transcription analyses demonstrated that this gene is expressed in the indirect flight muscles of developing female mosquitoes. The promoter of this gene may be a powerful tool for developing conditional lethal strains of mosquitoes.

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CHARACTERIZATION OF THE ANOPHELES GAMBIAE *opsin* GENE FAMILY: MOSQUITO PHOTORECEPTION STUDIES

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Behaviors that significantly impact mosquito survival and vector capacity such as host location, mating and oviposition represent potential targets for mosquito control. In the malaria vector mosquito, *Anopheles gambiae* and other mosquitoes that are active during crepuscular (dusk/dawn) time periods, vision is a fundamental component of these behaviors. Opsins are cellular receptors that have a critical function in vertebrate and invertebrate vision, their interaction with photons of a specific wavelength initiates the photo-transduction signaling cascade that ultimately induces a visual response. We have identified a family of *opsins* (GPRrep-1-12) in the genome of *A. gambiae*. Our studies indicate a specific expansion in the *A. gambiae* long wavelength sensitive opsins relative to *Drosophila*, suggesting a potentially unique functional role for these proteins in visually-dictated mosquito behaviors. At present, the significance of this expansion and the roles of the *A. gambiae* opsins in visual processes and vision related behaviors are poorly understood. Our preliminary quantitative expression analysis indicates that the *opsins* may have differential roles in male and female *A. gambiae* vision during diurnal light/dark cycles and suggests possible specialized roles of the *opsin* genes that may enable *A. gambiae* to function at low light intensities. We have initiated ultra-structural and immuno-localization studies to determine the organization of photoreceptors in the retina of male and female *A. gambiae*. The overall objective of this research is to elucidate the molecular basis of *opsin* mediated vision in *A. gambiae* and to identify unique mosquito visual behaviors that may facilitate the development of novel mosquito control strategies.

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KNOWLEDGE DATABASE PIPELINE FOR MOSQUITO IMMUNE EST ANNOTATION

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We analyzed a library of *Anopheles gambiae* immune-regulated (AGIR) ESTs, made by subtractive cloning from the transcriptome of *A. gambiae*. It is a challenge to assign the correct gene identity and function based a collection of ESTs. Here we present an *A. gambiae* knowledge database pipeline for automated annotation of ESTs, which can easily be adapted for other species. Our AGIR ESTs were annotated through this pipeline in minutes. Results indicated that 68% of ESTs were successfully assigned to annotated (known or predicted) genes, and 32% of ESTs matched unannotated (unpredicted) genes, or unfinished regions of the genome. For the ESTs that did not match annotated genes, at least 71% had independent support by dBEST, mass spectrometry peptide sequence, and/or computational gene re-prediction, and thus represent new mosquito immune-response genes. Most of the ESTs were assigned to innate immune response functions by this knowledge pipeline.

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POSITIONAL CLONING LINKED A SERINE PROTEASE GENE FROM THE AFRICAN MALARIA MOSQUITO, *ANOPHELES GAMBIAE*, TO THE *PLASMODIUM* ENCAPSULATION TRAIT

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A laboratory *Anopheles gambiae* mosquito strain (L35) exhibits the ability to encapsulate multiple *Plasmodium* species in a melanin/protein matrix, killing the parasites as they pass through the mosquito midgut epithelium. This phenotype is genetically determined by at least three quantitative trait loci, named *Plasmodium* encapsulation 1 to 3 (*Pen1* to *Pen3*). In an attempt to molecularly identify the *Pen1* locus at 8C-8D division on the polynucle chromosome of *A. gambiae*, we have sequenced 528 kb in our laboratory, and in combination with the published genome sequence, have assembled and annotated the *Pen1* region of 1.2 Mb for candidate gene(s). Our annotation revealed a serine protease with sequence similarity to a number of insect prophenoloxidase-activating enzymes. These enzymes are a component of the melanotic encapsulation pathway. Furthermore, adult mosquitoes injected with double-stranded RNA from this serine protease showed sig-
significant reduction in their ability to encapsulate *P. berghei*. Co-localization at the *Pen1* region and its effect on encapsulation phenotype suggest that this serine protease is the *Pen1* genetic locus.

### 815

**THE DISTRIBUTION OF CHROMOSOMAL FORMS OF *ANOPEHES GAMBIAE S.L.* (DIPTERA: CULICIDAE) IN URBAN, PERI-URBAN AND RURAL LOCALITIES IN GREATER ACCRA.**

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*Anopheles gambiae s.s.* is the most anthropophilic malaria vector worldwide. Polyteny chromosome studies of *An.gambiae s.s.* have revealed five inversion chromosomal forms associated with ecotypic differences. Point maps have shown the correlation of inversion frequency variations to changes in climatic or ecological conditions. Accurate identification, distribution and knowledge of population structure of disease vectors are fundamental to the design of effective vector control. The main objective of the present study was to karyotype *An. gambiae sensu stricto*, determine their distribution and relate inversion frequencies to different ecological changes. The study was carried out at sites along a specified transect from rural (Dodowa), peri-urban (Madina) to urban (Achimota) communities in the greater Accra, region of Ghana. Larvae and pupae were collected from various breeding sites ranging from heavily polluted to clean water, reared to adults and blooded on immobilized chicken and on human volunteers to obtain half gravid. A total of 202 chromosome preparations were made, of which 60 preparations were readable. In urban Accra (n=23), a mixed population of SAVANNA (65%) and MOPTI (22%) chromosomal forms and SAVANNA × MOPTI heterozygotes (13%) were observed, whereas in peri-urban Accra (n=20), SAVANNA chromosomal forms (70%) were observed, with low proportions of both FOREST (10%) and MOPTI (20%) chromosomal forms, and no hybrids were recorded. FOREST (59%) and SAVANNA (41%) chromosomal forms occurred in sympathy in the rural community (n=17) and no hybrid was recorded. Significant heterozygote deficiency was observed in all the three communities suggesting partial reproductive isolation between the three major chromosomal forms. Interestingly, a substantial number of MOPTI/SAVANNA hybrids and inversion heterozygote frequencies (+/+a) were found in the urban area and might represent adaptation to the urban environment.

### 816

**ANOPEHES GAMBIAE S.L. OVIPOSITION AS INFLUENCED BY TIME IN THE DIEL CYCLE**

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A battery-powered and horizontally positioned clock was modified to automatically present a unique section of dark and wet substrate upon which the malaria mosquito, *Anopheles gambiae s.l.*, could oviposit at hourly intervals over 24 h. The clock face was filled with wet, black agricultural soil covered with removable brown paper towelling. A circular plate of thin plastic was attached to the hour-hand drive of the clock so that all oviposition substrate was covered except for the equivalent of one hour. The clock turned this panel one revolution per 12 h in the presence of 30 - 60 house-caught gravid females deployed in a 60 x 60 x 60 cm screened cage held under the natural light cycle for equatorial Kenya. Two discrete periods of oviposition were documented by summing eggs laid per hourly period over seven experimental runs. Of the ca. 12,000 total eggs recovered, 61% were laid during Period 1, beginning at dusk, peaking at 19-20:00 hrs, and ceasing at 1-2:00 am. Thereafter, no eggs were laid until dawn when a second ovipositional period commenced that accounted for 39% of the total eggs. Period 2 peaked at 8-9:00 am and continued until noon when mosquitoes were held in the shade. PCR analysis is underway to document whether these ovipositional peaks are shared by or unique to *Anopheles gambiae vs. arabiensis*.

### 817

**CLIMATIC DETERMINANTS OF THE DISTRIBUTION AND DENSITY OF *ANOPEHES QUADRIMACULATUS* IN FLORIDA: A SPATIO-TEMPORAL MODEL**

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The presence and abundance of anopheline vectors that have the potential for transmitting malaria in areas where it has been eradicated, and the possible increase in transmission risk with changing climatic conditions has attracted some research interest in recent years. Malaria was endemic in the USA until its eradication in the 1950s. Since then a number of outbreaks have occurred in different states as a result of autochtonous transmission, most recently in Florida in 2003. *Anopheles quadrinaculatus* was the principal malaria vector in the eastern half of the USA, where the disease was most prevalent. With the purpose of gaining a better understanding of the association between vector density and historical malaria transmission in the USA, this study examines temporal and spatial relationships between climatic factors and anopheline densities in the state of Florida, using monthly data on mosquito catches conducted in over 200 locations during the period 1950-1972 to develop a predictive model based primarily on variations in temperature and rainfall. Validation is done by comparing model predictions with vector density data that has been excluded from the data series on which the model is based. Although this model will be used to predict mosquito densities in the past, the methodology used can also be applied to predicting densities of nuisance mosquitoes and vectors of diseases such as Eastern Equine Encephalitis or West Nile Virus, as data for these is also available. Potential effects of global warming on mosquito densities can also be quantified, and results can aid in a more strategic planning of mosquito control activities in Florida and the rest of the USA.

### 818

**OVIPOSITION OF AFRICAN MALARIA MOSQUITOES, *ANOPEHES GAMBIAE* (S.L.) AS INFLUENCED BY SUBSTRATE TEXTURE, MOISTURE, AND DARKNESS**

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Larvae of *Anopheles gambiace* (s. l.) typically develop in temporary puddles over bare soil disturbed by humans. Longevity of puddles has been established as the most critical determinant of pupal productivity in research sites near Kisian, Kenya. Thus, we postulated that *An. gambiae* should oviposit on substrates of fine (clay) rather than course particle sizes (sand and gravel) as well as those fully rather than partially hydrated. Effects of these substrate variables were measured by choice-tests (> 6 replicates per experiment) in 60 x 60 x 60 cm laboratory cages populated with 40 - 100 gravid *An. gambiae* from laboratory strains or house-collected individuals from Kisian. In contradiction to our hypothesis, *An. gambiae* oviposited equally on all fully hydrated silica substrates segregated into eight different
particle size-classes varying from over 850 µm to less than 38 µm. However, moisture content of both fine sand and Kenyan soil strongly influenced oviposition by this mosquito. Cups of soil shallowly covered with standing water received the most eggs in choice tests. However, soils approaching saturation were also excellent releasers of oviposition, indicating that mud as well as water is likely to receive many eggs in the field. Interestingly, females in choice tests deposited some eggs on Kenyan soils with moisture contents ranging down to 18% water, even when well-hydrated (30%) or saturated (33%) soils were nearby. Thus, An. gambiae females will distribute some eggs on seemingly poor ovipositional resources despite proximity to excellent ones. Egg output of An. gambiae on soils of differing moisture levels correlated nearly perfectly with soil conductivity readings obtained using a modified leaf-wetness sensor (Spectrum, Inc.). Thus, a new tool is now available for easy assessment of the likelihood that particular soils in nature will be conducive to An. gambiae oviposition. We also confirmed that black substrates stimulate much more oviposition (> 10 fold) than light-gray or white substrates. Lastly, ovipositional cups presenting fully hydrated and coarsely ground black Kisii stone received as many eggs as hydrated black Kenyan soil from typical An. gambiae larval habitats. Collectively, these data support a conclusion that wetness and darkness are the two key properties most responsible for oviposition by this malaria mosquito.

819
VECTOR COMPETENCE OF OCHLEROTATUS TRISERIATUS (DIPTERA: CULICIDAE) FOR WEST NILE VIRUS

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The ability of Ochlerotatus triseriatus to become orally infected with West Nile virus (WNV) and to transmit the virus by bite was investigated in the laboratory. This mosquito, the primary vector of LaCrosse Encephalitis, fed readily on WNV infected 2-4 day old chickens with various titers. Infection rates varied from 16% to 67% of Ochlerotatus triseriatus that fed on chickens with viremas of 4.6 log_{10} PFU/ml and 7.0 log_{10} PFU/ml respectively. Ochlerotatus triseriatus orally infected by viremic chickens subsequently transmitted West Nile virus to uninfected chickens.

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QUANTITATIVE ANALYSIS OF PLASMODIUM YOELII SPOROZITE PRODUCTION AND MOVEMENT INTO MOSQUITO SALIVARY GLANDS

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Malaria is a disease caused by blood infection with Plasmodium parasites. It is initiated when an infected mosquito bites a host and injects saliva containing sporozoites into the skin. This study focuses on sporozoite development of rodent malaria parasites (Plasmodium yoelii) within the mosquito vector. The main objectives of the study are, 1) to estimate the number of sporozoites produced per oocyst, and 2) to estimate the efficiency of sporozoite movement into the mosquito salivary glands. Infected mice were fed upon by two species of mosquitoes (Anopheles stephensi and A. freeborni). From day 9 up to day 20, individual mosquitoes were sampled for sporozoites in the hemolymph, sporozoites in the salivary gland, and oocysts on the midgut. First, the number of sporozoites in the body cavity was determined by extracting hemolymph with a perfusion technique and placing the extracted fluid on a hemocytometer. Next, the number of sporozoites in the salivary glands was estimated by excising the glands, grinding them in fluid and examining the fluid on a hemocytometer. Lastly, the number of oocysts on the midgut were determined by dissecting mosquito midguts and examining them under a microscope. This procedure was performed every 1 to 2 days (4-10 mosquitoes per date) during the time of sporozoite release and invasion into the salivary glands. Oocyst densities were consistently greater in A. freeborni than in A. stephensi, but the average number of sporozoites produced per oocyst was greater in A. stephensi (610 sporozoites/oocyst) than A. freeborni (440 sporozoites/oocyst). In both mosquito species, many oocysts failed to mature and produce sporozoites by day 20. Nevertheless, many thousands of sporozoites were present in the hemolymph by day 14. But the estimated number of hemolymph sporozoites that successfully invaded the salivary glands was low, with an average of 19% for A. stephensi and 5% for A. freeborni. We concluded that P. yoelii sporozoites produced in A. stephensi and A. freeborni were relatively inefficient in invading the salivary glands of these mosquito species compared to what has been reported for P. falciparum sporozoites developing in A. gambiae and P. vivax sporozoites developing in A. dirus and A. minisinos mosquitoes.

821
GENE EXPRESSION OF ANOPHELES GAMBIAE IN RESPONSE TO O’NYONG-NYONG VIRUS INFECTION

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Anopheles gambiae transmits not only malaria but also O’nyong-nyong alphavirus (ONNV). Culicine mosquitoes are normally natural vectors for most arbovirus transmission. Interestingly, however, ONNV is transmitted by anopheline mosquitoes such as An. gambiae and An. funestus. In order to study gene expression resulting from An. gambiae/ONNV interactions at the genome level, we utilized cDNA microarrays including about 20,000 normalized cDNAs. For the study, female An. gambiae (G3 strain) were fed on ONNV-infected bloodmeals and harvested at fourteen days post-infection. Gene expression was then compared to that of the uninfected control females. The fourteen days post-infection time point was chosen because it is the most competent stage for ONNV transmission to humans and also known as the highest titer of ONNV in An. gambiae. As a result, fifty-nine genes were identified as being at least two-fold up-regulated in the ONNV infected mosquitoes compared to the uninfected control females. In addition, thirty-eight genes displayed at least two-fold down-regulation in the ONNV infected mosquitoes compared to the uninfected controls. Among the two fold up-regulated genes, twenty genes that are homologous to known genes in the sequence databases were further analyzed by qRT-PCR to confirm their respective microarray data. Subsequently, five of these genes were validated to be up-regulated by qRT-PCR.

822
INSECTICIDE RESISTANCE SURVEILLANCE IN ANOPHELES DARLINGI, AN. ALBIMANUS, AND Aedes aegypti IN THE COASTAL DESERT AND AMAZON BASIN OF PERU

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The use of insecticides for the suppression of mosquito vector species remains the primary control measure for both endemic and epidemic
malaria and dengue in Peru. Long-term use of insecticides in both an agricul-
tural and public health context may potentially reduce the suscep-
tibility of key vector species to these products. To assess the current level of
insecticide resistance and establish a mechanism to monitor changes in
resistance levels over time, personnel from the Naval Medical Research
Center, Detachment and Peruvian Ministry of Health implemented a
national insecticide resistance surveillance program. Utilizing the CDC bot-
tle bioassay, over 2,200 assays were performed on over 47,000 Anopheles
daringi, An. albimanus from the coastal desert town of
Tumbes and the Amazonian city of Iquitos. From these assays, diagnostic
dosages were established for the pyrethroida alpha-cypermethrin,
cyfluthrin, deltamethrin, lambda-cyhalothrin, and permethrin, the
organophosphates fenitrothion and malathion; and the organochlorine
DDT. These baseline concentrations along with organized training pro-
grams to transfer the bottle assay technology to local public health and vec-
tor control organizations will ultimately assist in monitoring the efficacy of
current control strategies and guide the selection of the most efficacious
insecticides for the control of malaria and dengue.

824
UTILIZATION OF A NOVEL PORTABLE HUT DESIGN TO DEFINE THE FLIGHT BEHAVIOR OF MALARIA VECTORS IN BELIZE, CENTRAL AMERICA

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Knowledge of the flight behavior of local anopheline vectors is of para-
mount importance in malaria control programs. The following study defined the recapture rates of wild-caught, marked Anopheles darlingi, An. vestitipennis and An. albimanus females at 0 M, 400 M and 800 M from a
fixed release point using a novel portable experimental hut design. Three
trials, each consisting of two consecutive 12-hr human-baited landing col-
lections, were performed at each distance at two separate field sites. One
location was typical for An. darlingi breeding sites and the other location
was typical for both An. vestitipennis and An. albimanus larval habitats. For
each species, recapture rates of marked females were highest at the 0 M site
and on the first collection day at each distance. Overall, the recapture rates
of An. darlingi were significantly greater than both An. vestitipennis and An.
albimanus at the 0 M (29.0%, 8.3% and 1.1%, respectively); 400 M (11.6%, 3.1% and 0.0%, respectively) and 800 M (5.8%, 0.2% and 0.0%, respectively) distance locations. The peak recapture time for An. darlingi at both the 0 M and 400 M sites occurred within two hours post-sunset, while the
peak recapture time at the 800 M site occurred during the seventh hour
post-sunset. The peak recapture time for An. vestitipennis at both the 0 M
and 400 M distances occurred during hours 4–10 post-sunset, coinciding with biting activity patterns of unmarked populations. Results from the
present study can be used to conduct malaria vector density risk assess-
ments based on distances from species-specific anopheline breeding sites.

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POST-OVIPOSITION EFFECT OF DIFFERENT CONSTANT TEMPERATURES ON EGG HATCHING OF ANOPHELES GAMBAE

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Anopheles gambiae Giles sensu stricto (Diptera: Culicidae) egg development and its relation to environmental parameters is a critical yet understudied
aspect of vector biology. In this study we examined the effect of tempera-
ture on egg hatching of this malaria vector. Mosquito eggs were incubated under moist conditions at temperatures of 12, 22, 27, 33 and 42°C for
intervals of 1, 3, 7 and 10 days after which they were flooded with distilled
water and hatchability observed for up to 7 days. Mosquito eggs held at 22
and 27°C after 10 days had the highest hatching rates of 38.7% ± 4.0 and
47.3% ± 4.0 respectively. Temperature of 42°C drastically reduced the mos-
quitos egg viability since few to no eggs hatched in this temperature regime.
Though egg hatching rates of 33°C tended to be high at 1 and 3-day incu-
bation periods (79.3% ± 3.3 and 66.7% ± 3.8 respectively), this decreased
dramatically during 7 and 10-day incubation periods (14.0% ± 2.8 and 8.7%
± 2.3 respectively). Mosquitoes eggs held at 12°C also showed a rapid decline in hatchability at 7 and 10-day incubation periods. Additional stud-
ies also show that temperature during early embryonic development appears to have a major effect on egg development while temperatures later
in embryonic development appear to have less of an effect. This study demonstrates that temperature is an important ecological parameter for
early embryonic development of malaria vectors and an important regula-
tor of vector populations.

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EFFECTS OF PYRIPROXYFEN ON EGGS, PUPAE AND FECUNDITY IN Aedes aegypti (L) (Diptera: Culicidae) FROM PERU

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Pyriproxyfen is a juvenile hormone analogue that has been used against a
range of arthropods since its introduction to the agrochemical market in the
early 1990s. The World Health Organization has recently recommended
that pyriproxyfen be used for the control of some mosquito species.
Pyriproxyfen is not an adulticide, but is toxic to the larvae and / or eggs of
a number of insect species and is frequently reported as being effective
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HIGH INCIDENCE OF BACTERIA IN ANOPHELES GAMBAE S.S. MOSQUITOES

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A symbiont that has the potential of being useful to express anti-Plasmodium proteins in mosquitoes will require one present in both immature and adult stages. The aim of the present study therefore was to isolate, identify and characterize bacterial symbionts in Anopheles s.s. mosquitoes, and ultimately to select one which is universal in all the life forms. Larvae, pupae and wild adult An. gambiae mosquitoes from Ghana, wild adult An. gambiae mosquitoes from Kenya, and laboratory colonies of adult An. gambiae mosquitoes from Tanzania, Liberia and Kenya were used. Each specimen was surface sterilized before DNA extraction was carried out. The PCR method of Scott et al. (1993) was used to identify single species of the An. gambiae complex. PCR using universal eubacteria 16S and 23S rDNA primers was first used to detect the presence of the microorganism’s DNA sequences in the specimens, then PCR using WOLB16S/F1/WOLB16SR1 and fssZ/fssZ2 primers carried out to determine whether they were Wolbachia sp. In silico analyses were performed with DIGEST software and the results were compared to those provided by the experimental restriction patterns of the amplified 16S and 23S rDNAs. Of the 395 PCR positive specimens, 373 (94.4%) were identified as An. gambiae s.s. These consisted of 274 (73.5%) adults, 28 (7.5%) pupae and 71 (19.0%) larvae. DNA fragments of the predicted sizes were successfully amplified in 81.8% (305/373) and 75.9% (283/373) of the specimens using the 16S rDNA and 23S rDNA primers, respectively. Bacterial DNA sequences were amplified from all life form stages, from both wild and laboratory reared adults irrespective of the geographical location from which they were obtained. Out of 281 specimens, PCR positive for both 16S and 23S rDNA primers, selected and screened for Wolbachia, 94 (33.5%) were positive for the WOL16S rDNA primers but for none fssZ2 primers. Comparison of the results of the experimental restriction patterns of 16S and 23S rDNA amplicons and those obtained from the in silico analyses revealed that none of the products could be that of either Escherichia coli or Pantoea agglomerans. Since a high number of bacteria occur naturally in wild mosquitoes, it may be possible to modify anophe- line vector competence by introducing or indigenous bacteria.

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IDENTIFYING MOSQUITO GUT BACTERIAL CANDIDATES FOR PARATRANSGENESIS

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Progress in understanding the mechanisms of disease transmission and vector-pathogen interactions has led to interest in developing alternative, more immediate, methods of transmission blocking. One of these strategies involves the use of paratransgenesis, the genetic modification of obligate vector gut symbionts or commensals with transmission blocking genes. Understanding the natural vector gut microbial community is a prerequisite for paratransgenesis. Use of genetically engineered microbes that are transient or not associated with structures involved in disease transmission will not be effective in reducing transmission. Very little is currently known about gut communities in mosquito larvae and adults that transmit arboviruses. During the last 15 years, application of comparative 16S ribosomal RNA analysis to microbes in natural ecosystems has shown that microbes do not exist as isolated species, but rather as members of structured communities. Insects have been shown to contain complex and diverse communities of microbes in their guts, yet relatively little is known about how these resident microbes influence the physiology of their hosts. We used comparative 16S ribosomal RNA analysis to characterize bacterial species in the gut of the West Nile virus vector, Culex pipiens; and Aedes sp. that vector encephalitis viruses from multiple locations in Larimer County, Colorado, and to determine if these species form stable communities and are directly associated with structures in the midgut. Both culturable and non-culturable species were examined. Species diversity in larval and adult midguts was estimated. Abundance of 120 species in adults and larvae from different locations within and between mosquito species was compared using Reverse Southern Analysis. Several bacterial species were identified that merit further investigation.

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EVALUATION OF VOLATILE COMPOUNDS FROM BLOOD AS ATTRACTANTS OF ANTHROPOPHILIC AND ORNITHOPHILIC MOSQUITOES

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Research on mosquito attractants has primarily focused on responses of anthropophilic mosquitoes such as Aedes aegypti. With the introduction and spread of West Nile virus across North America, ornithophilic mosquitoes such as Culex spp. have become the focus for surveillance efforts, however, these species do not respond as readily to attractants effective for anthropophilic species. In this study, we focus on volatile compounds identified from bovine and avian blood and compare responses of anthropophilic (Ae. aegypti) and ornithophilic (Cx. quinquefasciatus and Cx. nigripalpus) mosquitoes. Volatiles from blood samples were collected using various SPME (solid phase microextraction) fibers, purge and trap and solvent extractions with analysis by GC/MS. Dual choice olfactometer assays confirmed significant attraction of Ae. aegypti and Cx. quinquefasciatus but not Cx. nigripalpus to bovine blood. All three species responded significantly to avian (chicken) blood. In olfactometer assays, Ae. aegypti responded to carbon disulfide, dimethyl disulfide and 2-pentanone, Cx. quinquefasciatus responded only to dimethyl disulfide and Cx. nigripalpus did not respond to any compounds tested. A bioassay was developed to evaluate landing responses of all three
species to blood and components from the blood. All three species responded significantly to both bovine and avian blood in this assay. Lancing responses of Ae. aegypti were strong to a wide range of carboxylic acids, sulfide compounds and cholesterol. In contrast, Culex responded poorly to sulfide compounds, cholesterol and all carboxylic acids except for stearic acid. Clear differences exist in responses of these mosquito species to host-derived odors and a discussion on these results relating to surveillance are discussed.

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QUANTITATIVE ANALYSIS OF VECTOR COMPETENCE FOR DENGUE 2 VIRUS IN INDIVIDUAL Aedes aegypti Mosquitoes

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Understanding the relative vector competence of mosquitoes at the species, population, and individual level is critical to the study of vector biology and the success of future vector-borne disease control programs. At the level of individual mosquitoes, vector competence is typically characterized qualitatively by the presence or absence of pathogen-specific antigens. Vector competence for a species or population is estimated by the proportion of tested individuals that develop a disseminated infection. We have developed a quantitative assay using real-time RT-PCR to measure dengue 2 virus (JAM1409) RNA copy number in individual Aedes aegypti mosquitoes. We also employ this technique to quantitate antisense viral RNA in order to assess the kinetics of viral replication in specific tissues of laboratory infected Ae. aegypti. The assay shows a high intra and interassay reproducibility with CV values ranging from 2.6 to 5.6% and 1.1 to 5.6%, respectively over a 6 log10 dynamic range. The lower detection limit was approximately 500 copies per reaction. However, due to the non-specific nature of SYBR® Green I binding, quantitation became unreliable in reactions with less than 3 log10 of the target sequence. Our results show that disseminated viral RNA copy number varies significantly between mosquitoes (range at 14 days post infection = 6.11-6.75 log10/mosquito, p < 0.0001). The observed variation in viral load between individual mosquitoes highlights the importance of incorporating quantitative analyses in future studies aimed at exposing determinants of vector competence.

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DNA MICROARRAYS REVEAL INDUCTION OF SYNAPTIC VESICLE PROTEIN-2 SUGGESTING A NOVEL VIRAL RELEASE MECHANISM BY SINDBIVUS IN THE MIDGUT OF THE DISEASE VECTOR, Aedes aegypti

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The midgut of hematophagous insects is the primary site of infection by viral pathogens, therefore playing a crucial role in disease transmission. To further understand processes that occur in the midgut in response to viral infection, we employed DNA microarrays to analyze gene expression changes upon infection by the alphavirus, Sindbis (MRE16 Malaysian strain). Midgut transcription profiles from infected mosquitoes were compared with those from mosquitoes receiving non-infectious blood meals at 1, 4, and 8 days post feeding. Among the many changes that occurred, the most dramatic involved three genes demonstrating nearly identical transcript patterns with approximately forty-fold increases at 4 days post infection. These genes were synaptic vesicle protein-2 (SV2), potassium-dependent sodium/calcium exchanger (K-NCX), and a homologue of C. elegans Unc-93, a two-pore potassium channel regulatory protein. Because SV2 and K-NCX are clearly involved in calcium-mediated exocytosis and Unc-93 has a plausible role in this process, we believe that these changes represent viral induction of vesicle fusion machinery that enhances release of virus filled vesicles from infected cells.

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ANOPHELES GAMBIAE HOMOLOGUES OF PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN (PfCSP) RESPONSIVE GENES IN DROSOPHILA MELANOGASTER

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Malaria continues to be a deadly disease that kills approximately 3000 people everyday in Africa alone, which is caused by the incessant transmission of Plasmodium sporozoites by the Anopheles vector. Our long-term goal is to exploit the mechanism of sporozoite survival in the mosquito to inhibit malaria transmission. To understand how insects respond to sporozoite release into insect hemolymph, using a high throughput screen earlier we identified P. falciparum circumsporozoite protein (PfCSP) responsive Drosophila melanogaster genes. To examine if homologues of these genes also respond to malaria sporozoite infection in mosquitoes, we first selected 100 most highly responsive Drosophila genes. Fifty-four of these showed high homology with identified Anopheles gambienses genes. Between 500 to 800 base-pair fragments of 23 of these have been cloned and sequenced. Gene Ontology (GO) definition of these genes showed they are involved in a diverse array of functions, including metabolism, DNA and RNA binding, biosynthesis, and organelle and cytoskeletal organization. This suggested that malaria sporozoite survival in vector mosquitoes is a complex biological process. Probes are being prepared with the cloned gene fragments to determine their expression profile in different parasitic developmental stages and after infection with bacteria, fungus and Plasmodium parasites. We are also analyzing the expression of these mosquito genes after recombinant PfCSP injection using Real Time Quantitative PCR. We expect this pilot project will lead us to pursue a more comprehensive high-throughput screening of the Anopheles gambienses genes responsive to Plasmodium sporozoites and allow us to elucidate the molecular mechanism of sporozoite survival in vector mosquitoes. (ACMCI9P abstract)

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SEASONAL DYNAMICS OF Culex pipiens MOSQUITOES AND THE TRANSMISSION OF WEST NILE VIRUS

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We sought to determine the seasonal dynamics of Culex pipiens and the contribution of such trends to the force of transmission of West Nile virus
Kdr RESISTANCE GENE AND ANOPHELES GAMBAE S.S. CHROMOSOMAL FORMS IN DONEGUEBOUGOU (A MALARIA ENDEMIC AREA OF MALI)

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Molecular studies have shown the presence of Kdr genes in both molecular M and S forms of Anopheles gambiae s.s. However, it is not clear which of the 2 components (Savana et Bamako) of the S molecular form carried the Kdr genes. The aim of this study was to assess for Kdr gene distribution in relation with the chromosomal forms (Savana, Mopti, and Bamako) of An. gambiae s.s. The study was carried out from June to December 2002 in the village of Doneguebougou, a Sudan-savanna area where malaria transmission is endemic and dominated by An. gambiae s.s (98.63% of An. gambiae s.s and 1.17% of An. funestus). An. gambiae complex was composed of 73.7% (n=824) An. gambiae s.s. and 26.3% (n=294) of An. arabiensis. The presence of Kdr was screened using Martinez Torres & al, 1998 method and the mosquitoes were identified by cytogenetic (chromosomal reading of polytene chromosomes of the half gravid mosquitoes). The study has revealed the presence of Kdr gene in both Bamako and Savanna of the S molecular form. The frequency of the kdr gene was 1.46 % (12/824) in An. gambiae s.s. All observed cases of resistance were heterozygote (RS). The frequency of the kdr gene among the Bamako form was 2.73 % (3/1110) relatively higher than that observed among Mopti 1.24 % (6/483) and Savanna (1.52 %, 3/198). These observations confirm a progressive diffusion of the kdr resistant gene into the An. gambiae s.s populations. (ACMCIP abstract)
American robins and common grackles were the most common avian hosts identified. These birds are locally abundant in the peri-domestic environment throughout the Northeast. Only two bloodmeals were identified as American crow and 6 as Fish crow. A subset of *Aedes vexans*, *Ochlerotatus japonicus*, *Ochlerotatus triseriatus* and *Ochlerotatus trivittatus* examined fed exclusively on mammals. Host species identifications are pending.

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**PREDICTING WEST NILE VIRUS RISK IN CONNECTICUT USING LIGHT TRAP DATA AND ENVIRONMENTAL CORRELATES**

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West Nile virus (WNV) seems now firmly established in the United States, which calls for better surveillance systems to minimize risk of transmission to humans. The best surveillance data presently available is derived from mosquito light traps, which sample only a very small area. In order to estimate entomological risk (ER) - or density of infected vectors, in the area between traps, we need to develop predictive models of the presence and abundance of the mosquito vectors. The risk model would then integrate ER with measures of virus activity and human exposure. We developed a regression-based spatial model to predict the presence and abundance of *Culex* spp. caught bi-weekly in 32 light traps in Fairfield County, Connecticut, between 2001 and 2003. In our study area, *Cx. pipiens* and *Cx. restuans* play an important role as enzootic and epizootic vectors, while *Cx. salinarius*, a more catholic feeder, may be acting as a bridge vector to humans and other mammals. We used both 'static' variables (derived from remote sensing and geographic information systems) with 'dynamic' ones (temperature, humidity and rain) to model the abundance and phenology of these species in areas with different dominant land uses. *Cx. pipiens* and *Cx. restuans* numbers where higher in areas of low values of normalized difference vegetation index (NDVI), high percentage of impervious surfaces, high population density and low altitude, all indicators of high levels of urbanization. Both the numbers caught in traps and their seasonal patterns changed in different landscapes, which emphasized the importance of a spatio-temporal approach. We expect development of this model will assist health agencies in estimating risk at unsampled locations, improve trap placement to minimize gaps and predict the distribution of WNV earlier in the season.

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**ALBENDAZOLE/IVERMECTIN TREATMENT EFFECT ON WUCHERERIA BANCROFTI TRANSMISSION IN SIKASSO DISTRICT, MALI**

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To provide baseline entomological data to the National Filariasis Control Program we assessed in May 2003 the impact of alben-dazole/ivermectin on transmission intensity in 6 endemic villages of Sikasso District, southern part of the country. Entomological baseline data was collected in 2001 before an intervention consisting of a mass drug administration with alben-dazole/ivermectin combination. The intervention was followed by two different surveys 2002 and 2003 to assess the effect of mass treatment on vector transmission parameters. This study found that lymphatic filariasis transmission was mainly assured by *Anopheles gambiae sensu lato* (~80% of vector population) and *Anopheles funestus* (~20%). Over the three years of study, the annual infection rate for the two vectors combined decreased significantly by 71% from 2001 to 2003 (*P*<10⁻⁷). The annual infectivity rate for the two vectors combined decreased by 91.3% with *P*<10⁻⁶. The annual entomological inoculation rate and the means number of L3 per infective mosquito decreased respectively by 92.1% and 9.9%. In 2003 no infective mosquito was carried more than one infective larvae. The annual transmission potential in 2001 (84 infective bites per man per year) was 2.9 fold higher than that of 2002 (28.1 infective bites per man per year) and 12 fold higher than that of 2003 (6.9 infective bites per man per year). The reduction rate from 2001 to 2003 was 91.8%. These findings suggest that mass drug administration with albendazole/ivermectin may decrease considerably the entomological parameters within 3 year period, however the potential for *W. bancrofti* transmission may still exists.

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**COMPARING POISSON AND LOGISTIC REGRESSION FOR MODELING ANOPHELES (DIPTERA: CULICIDAE) AQUATIC LARVAL HABITATS IN KISUMU AND MALINDI, KENYA**

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Cross-sectional studies with binary outcomes analyzed by logistic regression are frequent in the entomological literature. However, the odds ratio can importantly overestimate the prevalence ratio, the measure of choice in these studies. In this research effort, Poisson regression and logistic regression modeling techniques for determining environmental variables for urban aquatic larval habitats were compared. Data from field and satellite surveys of 185 mosquito aquatic larval habitats in the urban area for Kisumu and 145 for Malindi, Kenya collected from March, 2001 to August, 2003 were collected. A Poisson regression model of the count of *Anopheles* mosquitoes per habitat sampled and logistic regression model of the presence or absence of *An* were estimated for each urban area and the pooled data for Kisumu and Malindi. All multivariable analyses were performed in GENMOD procedure of SAS 8.01. (Carey, North Carolina). Results indicate the $R^2$ measure of the Poisson regression quantified the predictability of environmental variables for aquatic larval habitat development and *Anopheles* species better than the binary logistic regression analysis. While logistic regression correlated predictor variables with the presence or absence of *An* larvae and provided information about the variance of the binary proportion of the data to obtain efficient estimates, the Poisson regression with the actual counts estimated the frequencies and their variation for all multivariable models in Kisumu, Malindi and the dataset of both cities, and for all *An* species-specific counts with greater explanatory power.

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**EXPERIMENTAL TRANSMISSION OF WEST NILE VIRUS BY CULEX NIGRIPALPUS CAPTURED IN HONDURAS**

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Due to concerns regarding the geographic spread of West Nile (WN) virus to Central America, we evaluated the potential for Honduran *Culex nigripalpus* Theobald to transmit this virus. We tested individual mosquitoes captured in Olancho province, Honduras, in September 2003. Mosquitoes were fed upon 2- to 3-year-old chickens previously inoculated with a New York strain (*Crow 397-99*) of WN virus. Infection rates in *Cx. nigripalpus*
Materials may be as effective as WHO standard bednets for use as ITNs. These results suggest that deltamethrin-treated bednets from recycled installations (25.0 min). After washing, the mean median knockdown times for the recycled nets (80.0, 72.0, and 57.8 min) were not significantly different from that for the standard net (76.2 min). Thus the recycled nets behaved similarly to the WHO standard net (40.0 min, p=0.6, ANOVA). As expected, knockdown times were longer for the deltamethrin resistant population, but the post-wash times for the recycled nets (21.2, 20.8, and 21.4 min) were not significantly different from that for the standard net (21.2 min). Washing all the nets thrice prolonged the mean median knockdown times, but the post-wash times for the recycled nets (25.0 min). After washing, the mean median knockdown times for the recycled nets (80.0, 72.0, and 57.8 min) were not significantly different from that for the standard net (76.2 min). Thus the recycled nets behaved similarly to the standard WHO impregnated net when tested against either deltamethrin sensitive or resistant mosquitoes either before or after washing. These results suggest that deltamethrin-treated bednets from recycled materials may be as effective as WHO standard bednets for use as ITNs.

**Efficacy of Deltamethrin-Treated Recycled Net-Curtain Materials Against *Anopheles Gambiae* Sensu Lato Giles (Diptera: Culicidae)

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The use of insecticide-treated bednets (ITNs) has been widely adopted as an important method of malaria control. The cost of ITNs remains a major obstacle. In Ghana, the use of untreated locally tailored bednets made from imported recycled net-curtain materials is gaining popularity. These bednets are cheaper, more durable, more attractive, and more widely available than standard nets. To assess the possibility of impregnation, we compared the insecticidal efficacy of three common recycled materials and one standard polyester bednet treated to a concentration of 55 mg/m2 deltamethrin. We performed standard wire frame knockdown assays with two to five-day old non-blood fed adult female *Anopheles gambiae* of both a deltamethrin-susceptible laboratory strain (Kisumu) and a wild, deltamethrin-resistant population (Dodowa). A standard WHO deltamethrin susceptibility test showed 90-100% and 50-73% corrected mortalities for the Kisumu strain and Dodowa population respectively after 24 hours holding period. For the susceptible strain, the mean median knockdown times for the three recycled nets (21.2, 20.8, and 21.4 min) were not significantly different from that for the standard net (21.2 min). Washing all the nets thrice prolonged the mean median knockdown times, but the post-wash times for the recycled nets (55.0, 56.8, and 50.8 min) were only marginally longer than the time for the standard net (40.0 min, p=0.6, ANOVA). As expected, knockdown times were longer for the deltamethrin resistant population, but the mean median knockdown times for the three recycled nets (22.7, 24.3, and 21.6 min) were not significantly different from that for the standard net (25.0 min). After washing, the mean median knockdown times for the recycled nets (80.0, 72.0, and 57.8 min) were not significantly different from that for the standard net (76.2 min). Thus the recycled nets behaved similarly to the standard WHO impregnated net when tested against either deltamethrin sensitive or resistant mosquitoes either before or after washing. These results suggest that deltamethrin-treated bednets from recycled materials may be as effective as WHO standard bednets for use as ITNs.

**Penetration of *Aedes aegypti* Midgut by Ingested Microfilariae of *Mansonella ozzardi*

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Mosquito transmission of arboviruses may be enhanced when mosquitoes ingest blood containing both virus and microfilarial worms (i.e., dually-infected host). If ingested microfilariae (mf) penetrate the mosquito midgut, then virus may be introduced directly into the mosquito hemocoel. This accelerates viral development, making mosquitoes become infectious much sooner than normal. Thus, human filariasis may enhance the transmission of dengue viruses by *Aedes aegypti* mosquitoes. There are several species of filarial nematodes that parasitize humans: *Mansonella ozzardi* is a benign human filarial parasite of the Neotropics transmitted by black flies and biting midges. We wanted to know if *M. ozzardi* mf could also penetrate the midgut of *Ae. aegypti* and thus play a role in mf enhancement of dengue transmission. To test this, we fed local *Ae. aegypti* (F1) mosquitoes on the forearms of 6 microfilaremic volunteers in an endemic area in northern Trinidad. Mosquitoes were dissected at various times after feeding and the midguts and thoraces were examined for mf. The percentages of mosquitoes that ingested mf ranged from 0 to 94%, depending on the microfilaraemia of the volunteer fed upon. Numbers ingested were uneven. Even on the most heavily microfilaremic volunteer, some mosquitoes ingested many mf (up to 62 per mosquito), whereas other mosquitoes feeding nearby ingested few or none. Some *M. ozzardi* mf penetrated the midguts of *Ae. aegypti*. Peak penetration occurred at 3 to 5 hours after mosquito feeding. Overall penetration success ranged from 0 to 33%, with greatest mf penetration occurring in mosquitoes that fed on the volunteers with the highest microfilaraemias. This study demonstrates that *M. ozzardi*, like species causing lymphatic filariasis (*Wuchereria bancrofti* and *Brugia malayi*), can penetrate the midgut of *Ae. aegypti* mosquitoes and thus, may contribute to mf enhancement of dengue transmission.

**Inter-Epidemic Yellow Fever Ecology in Kenya, East Africa**

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In East Africa, Yellow fever outbreaks are characterized by a focal periodicity spaced by lengthy inter-epidemic periods. The two most recent outbreaks in the region were detected in Sudan (2003) and in Kenya (1992/1993). In an effort to better understand the ecology of the disease in the region ongoing research (expected completion Oct. 2004) has involved a number of investigations including a retrospective serological survey of close to 1,000 monkeys from Kenya, temporal and spatial field collections of vector mosquito species from two areas in Kenya (the area of the previous outbreak and an area of recognized potential activity), and vector competence and trans-ovarial transmission studies of domestic, peridomestic, and forest vector species. At the time of this writing the primate serological survey has been completed, half of the field collection work has been completed (5 out of 10 months), and vector competence and transovarial transmissions experiments are currently in progress and have targeted *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) bromeliae* species from multiple locations in Kenya. Additional studies involving two other species *Aedes*
(Stegomyia) africana and Aedes (Stegomyia) kenensis are being pursued but because of their sylvatic nature are proving difficult to study under laboratory conditions. It is our hope that the multi-disciplinary and comparative approach of this study will be useful in better describing the ecology of the disease in the region and provide data essential to identifying areas of potential risk. An overview of the most relevant findings are presented here.

**SUSCEPTIBILITY OF ANOPHELES MACULATUS AND AN. ACONITUS TO COINDIGENOUS PLASMODIUM VIVAX IN CENTRAL JAVA, INDONESIA**

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Anopheles aconitus and An. maculatus are considered important malaria vectors in Indonesia. However, only limited information is available on susceptibility to Plasmodium infections. To better understand vector competence, co-indigenous Plasmodium vivax gametocytes were provided to laboratory-reared mosquitoes using a direct feeding method on infectious human volunteers. All P. vivax gametocyte positive volunteers (N = 172) with uncomplicated illness were recruited from medical clinics in Purworejo District, Central Java, Indonesia. Nulliparous female An. maculatus and/or An. aconitus were allowed to feed on P. vivax positive volunteers for a maximum of 15 - 20 minutes or until replete. Fully fed mosquitoes were maintained under controlled laboratory conditions during incubation. Portions of the fed cohorts were examined periodically to determine state of oocyst and sporozoite development. Among An. aconitus cohorts, 55% (33/60 volunteers) developed oocysts on the midgut, of which 21 (64%) produced salivary gland sporozoites. Among An. maculatus cohorts, 33% (55/168 volunteers) developed oocysts, of which 22 (40%) produced sporozoites. For both species combined, nearly 49% of oocyst positive cohorts produced detectable salivary gland sporozoites. From 57 matched feedings, An. aconitus and An. maculatus had nearly identical oocyst rates (53 and 51%, respectively). However, An. aconitus produced significantly more sporozoite-infected salivary glands (37% vs. 21%) than An. maculatus. These results combined with vector bionomic findings, indicate malaria transmission in the inland hill regions of central Java is maintained by a combination of different vector species during the year. The susceptibility rates obtained in this study demonstrate both An. maculatus and An. aconitus can serve as competent malaria vectors of P. vivax under favorable conditions.

**CORRELATION BETWEEN ENТОMΟLOGIC AN CLIMATIC VARIABLES WITH THE INCIDENCE OF DENGUE IN COLIMA, MEXICO**

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The association between dengue transmission and entomologic parameters related to Aedes mosquito populations have been established from the beginning of the XXth century; however, formal reports regarding this association are very scarce, and controversial, limited to larval indices. In the present work we review the entomologic parameters, regarding larval (Breteau index) and adult populations of Aedes aegypti, including man landing rate, resting adult female per house and gonotrophic discordance rate (checking the Christopher's stage of follicular development according the blood meal digestion), recorded from 20 selected sampling places in the urban zone of Colima City; at the Mexican Pacific coast, during dry and rainy seasons, in a period of 7 years (1996-2003), checking the annual environmental temperature and pluvial precipitation and explored their correlation with the reported number of cases of classic and hemorrhagic dengue in the zone. The results show that during years 1997 and 2002 two epidemic outbreaks of dengue and dengue hemorrhagic fever occurred, in frank coincidence with elevated Aedes aegypti man-landing rate, female mosquito resting population, and gonotrophic discordance; meanwhile, Breteau index showed a very weak positive correlation. Environmental temperature and pluvial precipitation had no correlation with these entomologic variables, neither with dengue fever incidence. These findings compel the necessity of maintaining continuous entomologic survey systems in endemic zones for dengue transmission including adult mosquito parameters in those surveys as a rationale prediction of epidemic outbreaks.

**CHARACTERIZATION OF ENTAMOEBA HISTOLYTIKA ISOLATED IN GEORGIA**

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Entamoeba histolytica, the major cause of colitis and liver abscess in humans, infects about 500 million people annually, but only 10% experience symptomatic disease. People infected with E histolytica without clinical manifestation of the disease spontaneously spread the infection. The most common way of transmission of this infection is through contaminated food and water. There was a significant number of cases of intestinal amebiasis (1334 in 1998 and 660 in 1999) registered in capital of Georgia - Tbilisi, with 404 cases of amebic liver abscesses. The municipal drinking water was suspected as a major source of the outbreak. The real range of amebiasis in Georgia was unknown; the number of registered patients does not show the complete picture. Therefore, the improvement of monitoring and surveillance systems of E histolytica and other pathogenic protozoan infections in Georgia is of high priority. To reveal the current range of infection, stool samples of former patients and risk group members have been tested on the presence of Entamoeba histolytica and/or E dispar using several methods such as microscopy, culture, antigen detection and PCR-based identification test. Our investigation has shown that although the range of newly developed symptomatic amebiasis in Georgia has significantly decreased, there is still number of asymptomatic cases, infected with E histolytica as detected by enzyme immunoassay analysis for the rapid identification of E histolytica-specific adhesin. High level of amebic liver abscess in Tbilisi has suggested the possible existence of unique genotypes granting the Georgian strains with propensity to cause liver abscesses. Our study is also focused on elucidation and understanding of genomic diversities contributing to invasiveness and hepatotropicity of the parasite. Identified differentially expressed genes will form the basis for development of new fast and specific diagnostic test systems. (ACMICP abstract)
IMPORTANCE OF ANTIGEN DETECTION IN STOOL SAMPLES FOR DIAGNOSIS OF ENTAMOEBA HISTOLYTICA INFECTION

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Entamoeba histolytica is the main parasite which causes amoebiosis, a substantial and common disease throughout the world. The diagnosis of amoebiosis by microscopic identification of the parasite in stool is insensitive and incapable of differentiating pathogenic *Entamoeba histolytica* from the commensal parasite *E. dispar*. In this study, we have tested a monoclonal antibody ELISA technique for the detection of the adhesin of *E. histolytica* and compared it with by microscopy. These assays were applied to stool specimens taken from 279 patients with diarrhea in Adana, Turkey, including 43 (15.41%) stools with microscopy confirmed *E. histolytica* infection, only 7 (2.51%) with adhesin monoclonal ELISA technique confirmed *E. histolytica* infection, other 36 (12.90%) samples confirmed as *E. dispar*, and 236 (84.59%) specimens that were negative by microscopy and monoclonal ELISA technique for both *Entamoeba* species. In conclusion, antigen detection test was both simple and rapid for specific identification of *E. histolytica* in stool.

ISOSPORA BELLI IN AIDS PATIENTS: EOSINOPHILS AND STEATORRHEA IN STOOLS

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A rapid and easy technique has already been described to detect eosinophils in stool specimens. It consists of emulsifying a small particle of faeces with a drop of Gomori Trichrome. Patients with *Isospora belli* infection may present with marked blood eosinophilia, but also with eosinophils in their stools. Seven AIDS patients originating from Africa were found positive for oocysts of *I. belli* after stool examination and nine AIDS patients also originating from Africa, negative for oocysts of *I. belli*, were used as controls. Among the seven found positive for *I. belli*, stool eosinophils were present in six, with Charcot Leyden crystals only in three of them. Amongst the nine controls, no eosinophils or Charcot Leyden crystals was found in their stools. Steatorrhea is frequently observed in patients with *Isospora belli* infection, may present with marked blood eosinophilia, but also with eosinophils in their stools. Steatorrhea was seen in none of the nine controls. We, therefore, suggest that some of the steatorrhea cases correlate with the presence of eosinophils in stools as we have already noticed, particularly in patients with irritable bowel disease.

DETECTION OF TOXOPLASMA GONDII BRADYZOITES IN RAW MEAT AND MEAT BYPRODUCTS BY REAL TIME PCR ASSAY

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Humans acquire toxoplasmosis mainly through ingestion of oocysts from undercooked cyst-contaminated meat or from fecal contaminated hands or foods, and through trans-placental transmission. Newly infected fetuses during early pregnancy are at risk for developing severe birth defects. A reliable method to identify *Toxoplasma gondii* contaminated meat, therefore, is important. A real-time PCR method for *T. gondii* was used in a small-scale survey to detect contaminated meat. Various types of tissues were obtained from nine small vendors or farmers markets, and from two nationwide grocery chain stores in Atlanta, GA. These tissues included muscle, brain, kidney, heart, liver, tongue, intestine, and stomach from swine. In addition, we also obtained pork sausage, and bovine and lamb muscle samples. Only 3 samples out of a total of 186 (1.61%) tested positive for *T. gondii* bradyzoite DNA. These positive samples were from porcine muscle, brain, and sausage. The positive muscle and pork sausage samples were obtained from one vendor at the same time. These data suggest a large-scale survey study may be needed to obtain the true prevalence of *T. gondii* contamination in meat and meat products.

VARIATION IN THE BREEDING STRUCTURE OF TOXOPLASMA GONDII AND THE ROLES OF SELFING, DRIFT, AND EPISTATIC SELECTION IN MAINTAINING LINKAGE DISEQUILIBRIA


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Previous studies of *Toxoplasma gondii*, based on samples dominated by clinical isolates, have concluded that its population structure is clonal, despite the sexual reproduction that occurs in cats. To determine whether this applies to non-clinical isolates, we compared patterns of linkage disequilibrium (LD) among seven loci in samples of *T. gondii* from Brazil and the US. LD was detected in both locations, but it was substantially lower in Brazil probably because a higher rate of sexual reproduction between different genotypes (outcrossing) due to higher rate of transmission. The extent of LD between pairs of physically unlinked loci varied significantly in each location. Moreover, the magnitude of LD between corresponding locus pairs in Brazil and the US was correlated, despite minimal gene exchange between the continents (mean FST=0.19). The heterogeneity among locus pairs and the correlation in LD between physically unlinked locus pairs from different continents suggests that locus-specific factors, such as epistatic selection are involved in maintaining LD in *T. gondii*. The usefulness of typing isolates based on physically unlinked loci is questioned by the low overall predictability of the genotype of one locus based on the genotype in another (unlinked) locus. This predictability ranged between 23% and 45%, but was close to nil for a considerable fraction of locus pairs.
DIAGNOSIS OF HUMAN FASCIOlOSIS:
EVALUATION OF EXCRETORY-SECRETORY AND A
RECOMBINANT CATHEPSIN L5 AS ANTIGENS FOR
ELISA
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Fasciolosis, caused by the parasites Fasciola gigantica (tropical liver fluke) and Fasciola hepatica (temperate liver fluke), is now recognized by the World Health Organization as a serious public health problem. An estimated 17 million people have fasciolosis worldwide. Previously developed ELISA methods have used either crude somatic extracts from parasites or a preparation of molecules secreted by the parasite in vitro (excretory/secretory or ES antigen). The use of a complex antigen preparation can result in reduced specificity since parasites often share immunogens. Recombinant cathepsin L5, cloned from adult F. hepatica (FhCatL5) RNA, was assessed for its potential in the serological diagnosis of human fasciolosis. Using microscopy as the gold standard, we compared the performance of ELISAs based on ES antigens and FhCatL5. A panel of 312 sera were used, 107 samples from Cuban subjects positive for F. hepatica by microscopy; 80 samples from healthy Canadians and, 125 samples from subjects with other parasitic diseases. Both assays achieved 100% sensitivity. Specificity was quite different however: 96% in the FhCatL5 assay and only 83% in the ES antigen-based assay. These data demonstrate that recombinant FhCatL5 is an excellent antigen for the serological diagnosis of human fasciolosis.

TREMATODE INFECTIONS IN PUERTO RICAN
BIOMPHALARIA GLABRATA
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Numerous species of trematode parasite are found in the populations of Biomphalaria glabrata that now exist in Puerto Rico. While Nasir in the 1960s described perhaps 9 species of cercariae from B. glabrata collected in Venezuela, other workers have reported about 9 other species from Brazil and/or other endemic areas. It is assumed that some species have been described more than once and given different names. Therefore, it is not clear just how many trematodes can use B. glabrata as their first intermediate host. We have studied B. glabrata collected in various sites around Puerto Rico and have determined that to date at least 8 different species of trematode are represented by the cercariae shed on the island. Ribeiroia marini and Echinostoma barbovae are present in most sites with up to 40% of the adult snails infected. These two life cycles have been completed. The other species appear to be strigeids, or echinostomes and there is at least one xiphidiocercaria. Each type of cercariae is being studied by light and scanning electron microscopy and compared to published work. Efforts to complete the life cycle of each type of cercariae are in progress. The metacercarial stages of many of these cercariae encyst in fish are being used as sentinels for this snail host in a survey of Biomphalaria populations across the island. It is noted that while infected snails perish during the dry season, the aestivating survivors or their adult offspring are readily susceptible during the next wet season. Also, those adult snails collected that are not already infected are highly susceptible to a local human strain of S. mansoni. In conclusion, whereas in competitions between species of trematode within a snail, the schistosomes usually loose, in a population of snails in nature there are enough snails to go around so that all species survive.

BG-BB, A BAC LIBRARY FOR BIOMPHALARIA
GLABRATA, INTERMEDIATE HOST OF
SCHISTOSOMA MANSONI
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Biomphalaria glabrata, the intermediate snail host of Schistosoma mansoni remains poorly understood genetically. Less than 0.1 percent of its 931 megabase (Mb) genome has been characterized. The Biomphalaria glabrata Genome Initiative, a consortium of international investigators, was formed to encourage and pursue molecular study of this species, with an emphasis on gene discovery. Support was obtained from the National Human Genome Research Institute (NHGRI) to produce a bacterial artificial chromosome (BAC) library. BACs stably maintain large DNA inserts of up to 2 megabases (Mb). Genomic BAC libraries can be used for the cloning of genes, characterization of regulatory sequences, constructing of physical maps and ultimately genome sequencing. The B. glabrata BAC library (BG-BB) was constructed using genomic DNA (partially digested with HindIII) from the BB02 strain, a new field isolate from Brazil that is susceptible for S. mansoni. The library consists of 61,440 clones, with an average insert size of 144 Kb, representing 9.5x coverage of the genome. Probing with single/copy number genes from B. glabrata and B. asper end sequencing of 96 clones indicated that the BAC library sufficiently represents the gene complement and is of good quality. The Biomphalaria glabrata Genome Initiative encourages use of this BAC library for characterization of individual genes and sequencing of random BAC ends toward construction of a physical map of the genome of B. glabrata. Ultimately, the BG-BB BAC library may be used toward full genome sequencing, thus complementing genome information of S. mansoni and Homo sapiens, the other partners in the schistosome life cycle. This library is available at cost from the Arizona Genomics Institute to the research community.

EFFECT OF INFECTION WITH SCHISTOSOME
PARASITE ON ALLERGIC RESPONSES TO
OVALBUMIN IN MURINE MODELS
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Helminth infection and allergic diseases, such as asthma, often induce similar inflammatory responses characterized by the generation of a significant Th2 immune response, peripheral blood eosinophilia, and elevated serum IgE levels. The nature of the relationship between the two diseases remains uncertain and controversial. The aim of this study was to determine if a pre-existing schistosome parasite infection would affect subsequent inflammatory responses in mice that were exposed to a non-parasite allergen. C57BL/6 mice received ovalbumin (OVA) immunization at different lengths of infection, and were challenged with OVA by either peritoneal cavity injection or intratracheal inhalation. Inflammatory cell recruitment was examined by peritoneal cavity lavage (PCL) and bronchoalveolar lavage (BAL). Cytokines and IgE levels in the BAL fluid and mouse sera were measured. As compared, CCR5 KO mice (CCR5-/-) with their wild type counterparts were used to check their responses to S. mansoni infection and OVA challenge. We found in C57BL/6 mice, early infection with S. mansoni (<21 days) did not significantly influence inflammatory cell population and
eosinophil counts recruited by OVA challenge. Total cell and eosinophil counts started to significantly increase around 42 days post S. mansoni exposure, which was consistent with occurrence of egg granuloma and a predominant Th2 immune response. Peak cell infiltration occurred at 9-10 weeks post exposure. Th2 cytokines and IgE levels were significantly higher in the sera and lavage fluid from mice with infections and OVA immunization/challenge, compared to those either with S. mansoni infection alone or those receiving OVA alone. When mice infected with S. mansoni were exposed to aerosolized methylcholine following OVA challenge, however, they did not show a prolonged asthmatic response or increase in mucus production. In CCR5 KO mice, OVA immunization/challenge without S. mansoni infection showed a decreased eosinophil recruitment to both BAL fluid and BAl fluid, but their total cell count was significantly increased. The lower levels of IL-5, IL-4, and IgE indicated an impaired Th2 response to S. mansoni infection, but they did not present an obvious change in the immune response to OVA challenge. Our results suggest that pre-existing S. mansoni infection would promote inflammatory responses to inhaled allergens. (ACMCIF abstract)

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ASSESSING THE IMPACT OF CONNECTED ENVIRONMENTS ON SCHISTOSOMIASIS CONTROL

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An often overlooked risk factor in the transmission of parasitic diseases is the fact that people and villages are connected to one another socially and via the environment. Such connections can have important ramifications on the spatial patterns of disease occurrence, spread, and reemergence after control. In this study, the transmission of Schistosoma japonicum is modeled for a 12 x 12km region in Sichuan province, China, consisting of 200 villages that are connected to one another via surface hydrology. A subset of the villages in this region is part of an ongoing research project that involves the collection and analysis of epidemiological and environmental data, and most recently, the design and implementation of intervention strategies. For this reason, the focus here is on describing how the model can be used to identify suitable interventions. The model consists of a remote sensing-based snail density prediction for each village, the construction of a spatial interaction matrix from a digital elevation model that describes how cercaria and miracidia flow between adjacent villages, and a set of deterministic differential equations that predict worm burden within each village. Simulations of the model demonstrate the ability to identify areas that serve as sinks in the network of connected villages and have the potential for high worm burdens. Combinations of chemotherapy, snail control, and region-wide control scenarios are used to explore the impact of connectivity on reinfection and overall reduction of worm burden in the region. The reintroduction of parasites from adjacent villages renders haphazard administration of chemotherapy unsustainable, while environmental modification and region-wide control scenarios that take advantage of connectivity are more sustainable.

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SCHISTOSOMA JAPONICUM AND OCCULT BLOOD LOSS IN ENDEMIC VILLAGES IN LEYTE, THE PHILIPPINES

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Schistosoma japonicum has been related to anemia, but the mechanisms mediating this relationship remain unresolved. This study was undertaken to 1) To assess the role of occult blood loss in mediating S. japonicum-associated anemia after adjusting for confounders; and 2) To identify intensity categories of risk for occult blood loss for trichuris and hookworm after adjustment for the presence of other helminth infections. We enrolled 729 individuals, aged 7-30 years, living in three rural, rice-farming villages in Leyte, The Philippines. Three stool specimens were examined in duplicate for helminth eggs using Kato-Katz method. A socio-economic status (SES) questionnaire was administered. Hemoglobin and presence of fecal occult blood were assessed and cross-sectionally related to intensity of helminth infection. Multivariate models were made to adjust for confounding by other helminths and SES. Least square means (LSM) analyses were conducted to assess adjusted mean hemoglobin across infection intensity categories. Adjusted mean hemoglobin significantly decreased with increasing infection intensity of S. japonicum, N. americanus, and T. trichuria (P<0.0031, <0.0001, <0.0001, respectively). The relationship between S. japonicum infection and hemoglobin remained even when presence of fecal occult blood was retained in the model (Heavy S. japonicum LSM hemoglobin =10.95, Uninfected, Low, and Moderate S. japonicum LSM hemoglobin =11.63, P<0.01). In multivariate models, individuals with higher intensity S. japonicum and T. trichuria were significantly more likely to be fecal occult positive (OR=3.54, P = 0.008, OR = 2.68, P = 0.013, respectively). In conclusion, it is likely that occult blood loss plays a role only at higher intensity S. japonicum infections and some other mechanism, such as anemia of inflammation, may be contributing to anemia. No previous studies have examined the relationship between fecal occult blood loss and S. japonicum after controlling for important confounding covariates. In addition, no large scale, community studies have addressed the risk for fecal blood loss due to Trichuris across a range of infection intensities.

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MODEL BASED CONTROL STRATEGIES OF SCHISTOSOMIASIS FOR HETEROGENEOUS POPULATIONS IN DISTRIBUTED ENVIRONMENT

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We developed mathematical models of schistosomiasis transmission for distributed human-snail populations that account for age-behavioral heterogeneities, environment (geographic proximity of human and snail sites), and its long term effects (chronic morbidity and immune protection). Our models, based on differential equations exploited Macdonald - type (worm burden + snail prevalence) formulation, and a suitable representation of “behavior, age, environment”. These mathematical models enable investigators to quantify the concept of infection potential to assess the associated (environmental) risk factors, to examine long term effects of chemotherapy and snail control, and to design optimal (in terms of cost and long term effect) control strategies.

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ASSOCIATION BETWEEN WATER CONTACT FREQUENCY AND AGE, SEX AND IRRIGATION METHODS IN SAMAR AND SORSOGON PROVINCES, THE PHILIPPINES

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Schistosomiasis japonica is a chronic helminthic disease contracted through water contact that has significant public health impact in the Philippines and South East Asia. The objective of this study was to compare the frequency of water contact between irrigated (2) and rain-fed (2) villages, by sex and age. 286 individuals were followed over a four or six month period in 4 villages of Western Samar and Sorsogon Provinces. Each individual filled in daily map-based interviews on frequency and reason (farming, household, other) for water contacts. The number of water contacts was adjusted for the total number of days each individual was followed. Summary statistics were completed on adjusted average frequency of water contacts stratified by age group, sex, and water management type. Males age ≥ 19 yrs had higher average number of water contacts than any other sex and age group. Large temporal variations in the number of water contacts were noted, particularly in individuals age ≥ 19 yrs. There was a larger adjusted number of daily water contacts in irrigated villages compared to that in rain-fed villages. However, the adjusted number of water contacts associated with farming activities was higher in the rain-fed villages. Irrigation at the village level affects the relative frequency of water contacts associated with different types of activities. It is possible that the presence of irrigation canals promotes more frequent water contacts associated with household activities. Alternatively, rain-fed areas will flood the farms and increase the number of contacts.

THE ANTIBODY RESPONSES TO ADULT-WORM ANTIGENS OF SCHISTOSOMA HAEMATOBIUM, AMONG INFECTED AND RESISTANT INDIVIDUALS FROM AN ENDEMIC COMMUNITY IN SOUTHERN GHAN

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Antibody responses to antigens from adult Schistosoma haematobium were investigated in an endemic community in Ghana, using microplate-ELISA. The results of a survey of egg output in urine and of a questionnaire-based investigation of water-contact activities were used to select ‘endemic normal’ (EN) and patent infected (PI) individuals as subjects. The plasma levels of antibodies reacting with the adult-worm antigens were determined and compared and the correlations between these levels and the age, water-contact index and egg output of each subject were evaluated. Compared with the EN subjects, the PI generally had higher levels of anti-worm IgG and IgE but lower levels of anti-worm IgA. When the data for the EN and PI groups were combined, the levels of anti-worm IgG and IgE were found to be positively correlated with egg output and with each other. Whichever the antibody class considered, levels of anti-worm antibodies were not negatively correlated with egg output. These results indicate that anti-worm IgE and IgG could be used as markers to reflect current infection intensity, and that anti-worm antibodies may not act as protective antibodies in the natural course of urinary schistosomiasis. (ACMCIP abstract)

ADAPTATION AND EVOLUTION OF SCHISTOSOMA SPP. IN RESPONSE TO CHEMOTHERAPEUTIC PRESSURE

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Knowledge on how the phenotype and genotype of Schistosoma populations may change in response to Praziquantel (PZQ) pressure may raise important theoretical and applied implications, such as that for the success of control programmes where mass human chemotherapy is implemented. This study concerns the development and application of genetic and phenotypic markers for the monitoring and surveillance of the human paratypes, Schistosoma mansoni, S. haematobium and S. japonicum under differing chemotherapeutic and environmental pressures, both within the laboratory and the field. Focusing initially on S. mansoni, data are presented characterising life-history traits and trade-offs across a range of PZQ-tolerant and susceptible schistosome strains under differing environmental conditions. Preliminary data on the population genetic structure of S. mansoni before, during and following mass PZQ chemotherapy will also be presented.

UNDERSTANDING UNCERTAINTY OF LOCAL SCHISTOSOMIASIS TRANSMISSION IN MOUNTAINOUS REGIONS OF SICHUAN, CHINA

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Previously we developed a quantitative framework for study of site-specific schistosomiasis transmission in endemic mountainous regions of China. The model is parameterized to encompass both biologic and site-specific information. Uncertainty in model outputs (i.e. human infection intensity and prevalence, infectious snail density) can be attributed to uncertainty and variability associated with these model parameters that are taken into account in the model. In this work, we calibrated the model via a Bayesian approach to three endemic villages (Shan 5, Xinlong 7, and Xinning 3) in Xichang County with the objective of reducing the uncertainty associated with the biologic parameters that are assumed to be regionally invariant. A univariate Kolmogorov-Smirnov statistical analysis of prior (before calibration) and posterior (after calibration) parameter spaces at the individual village level indicated that, among the 17 parameters (10 biologic and 7 measurable), four biologic and two measurable parameters were highly important in all three villages. The biologic parameters were eggs excreted per worm pair per gram stool (h), probability of human infection per cercariae per m² contact (α), probability of snail infection per miracidium per m² surface water contact (ρ), and threshold temperature for sporocyst development (T). The measurable parameters were the worm aggregation parameter (κ) and spatial interaction between cercariae and human water contact (γ). Because some biologic parameters occur only as products in the model, notably ρh and ασ, the parameter dimension was reduced for multivariate analysis. Preliminary results showed that uncertainty associated with these biologic parameter sets is reduced substantially in the posterior distributions but that there are biologic parameter sets that meet calibration criteria for all three villages supporting the original assumption of regional invariance. However, there are both similarities and differences in the importance of site-specific parameters by village that suggests parameter interaction and sensitivity will also differ at the village level. The application of statistical approaches to our transmission model demonstrates that...
the combination of sites-specific information to inform priors on model outputs as well as information on model inputs can be used to reduce parametric uncertainty for further studies.

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INTESTINAL SCHISTOSOMIASIS PROFILE IN A RURAL IRRIGATE AREA OF KOU VALLEY, NORTHERN BOBO-DIOULASSO, BURKINA FASO (WEST AFRICA)


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Schistosomiasis remain one of the major public health concerns in the world, particularly in Africa where 85% of the persons infected or at risk to be infected are located. For assessing these importance and propose a diagnostic tools, we conducted a survey in Burkina Faso in October, 2002. A sample random method was used to enroll the 694 subjects for the study. A questionnaire was administrated to record their behaviour and knowledge about disease due to water, in general, and intestinal schistosomiasis, in particular. Each subject also provided a stool sample. Stool specimen were transferred to the parasitological laboratory of Muraz Center and processed according to the Katz method. The mean age of the population was 18 with a range between 7 and 46 years. Among the subjects, intestinal schistosomiasis prevalence was 48%; 52, 8% for male and 42, 2% for female. Most of infections were moderate, and geometric mean decreased with age. During the survey, 80, 1% works in rice fields. Most of our study population (70%) didn't use water closer. Only 5% of the subjects were aware of intestinal schistosomiasis. Therapeutic coverage with praziquentel reached 93, 6% in our sample where the most infection were moderate. This rural area is an endemic zone for intestinal schistosomiasis, 48% as prevalence and only 5% know it, the concern is serious and required a closeness follow-up and an adapted control program.

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EXPERIMENTAL IMMUNIZATION AND IDENTIFICATION OF ANTIGENS USING ADULT SCHISTOSOMA MANSONI AND S. INTERCALATUM

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We have run a study on the possible role of adult Schistosoma mansoni and S. intercalatum in host-parasite relationship in schistosomiasis through the capability of whole worm extract to induce protective immune response in mice versus naive mice as control and a screening of the antigenic composition after polyacrylamide gel electrophoresis. Infestation of naive mice (control groups) with cercariae of either specie led to 100% susceptibility rate. Cercarial penetration rates were high and sex-ratio of adult worms was desequilibrated in favor of males. Immunization of mice by injection of either worm extract led to a significant reduction of parasitic loads and maturation rates to challenge infection when compared to control groups. Reduction rates were significantly high towards challenge infection with homologous specie than to heterologous. In either groups, sex-ratio was desequilibrated in favor of males. The immunization process did not affect cercarial penetration rates nor susceptibility rates. Immunization of mice by injection of either whole worm extract induced a significant but partial resistance to challenge infection by homologous or heterologous species. These results give hope for a vaccine using local strains of schistosomes against intestinal schistosomiasis due to of S. mansoni or S. intercalatum.

These parasitic infections are well established in Cameroon and neighbouring countries. Analysis of antigenic profiles of the extracts was done through analytic separation by SDS-PAGE in 10% polyacrylamide gel followed by western blot and immunodetection with pools of human sera. Sera tested consisted of of pool control sera, a pool of human sera harboring monospecific infection with S. mansoni, S. intercalatum or S. haematobium as shown by parasitologic analysis. These processes showed that no antigen reacted with the pool of control sera in the two extracts. In S. mansoni whole worm extract, 10 antigens reacted with homologous infection serum, 3 antigens reacted with S. intercalatum infected serum and 3 antigens reacted with S. haematobium infected serum. 9 antigens were specific to S. mansoni whereas 1 antigen reacted with all three sera. In Schistosoma intercalatum whole worm extract, immunoprecipitation revealed that 8 antigens reacted with S. intercalatum infected sera, 9 antigens reacted with S. haematobium infected sera and 7 antigens reacted with S. mansoni infected sera. 5 antigens reacted with three sera, 1 antigen appears to be specific to S. intercalatum. Some crossreactive antigens reacted only one serum. Two antigens reacted both with pools of sera from S. intercalatum and S. haematobium infected patients. One antigen reacted both with S. intercalatum and S. mansoni infection sera. This study shows that S. mansoni and S. intercalatum adult worm extracts are mixtures of antigens of different molecular weights among which there are species specific antigens and antigens shared between two or three schistosomes species.

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SIMPLE AND COST EFFECTIVE QUANTIFICATION OF INFECTIOUS PATHOGENS USING REAL-TIME NESTED PCR AND SYBR GREEN CHEMISTRY

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Quantitative PCR plays an important role in the prevention and management of infectious diseases. It is recommended to monitor pathogen load, response to treatment and to differentiate between latent and active infection. Detection of pathogens at low concentration is aided by nested PCR. Sensitivity and specificity are enhanced because the target of the inner primers is “nested” within the outer primers. Real-time PCR measures the amount of product generated by each PCR cycle using fluorescence chemistries. The fluorescent detection chemistry is either SGI dye or sequence-specific labeled probes (e.g. Taqman probes, molecular beacons and Light Cycler probes). The latter formats are expensive and require optimization for each target. The main advantages of SGI are versatility (sequence independent), ease of use and low cost. The objective of this study was to develop a simple, rapid, and cheaper alternative to sequence-specific fluorescence-based quantification assay, and to apply this method in the detection and quantification of infectious pathogens. Nested PCR protocols were adapted for real-time quantification. The exponential phase of the outer amplification was determined first followed by real-time nested SGI fluorescence detection. Pathogen standards of defined concentration and varied clinical specimens with known diagnosis were utilized for quantitative analysis of Mycobacterium tuberculosis (MTB), cytomegalovirus (CMV), herpes simplex virus (HSV) and parvovirus B-19. Demonstration of a single, specific product with optimized nested protocols was verified by gel electrophoresis. Accurate quantification of all pathogens target DNA was achieved by: 1. Determining the exponential phase amplification of outer PCR. 2. Generation of linear relationship between pathogen DNA concentration and cycle threshold. 3. Measurement of fluorescence at the optimum temperature for each target DNA and use of HotStart Taq™ to improve the reaction specificity. In conclusion, a novel real-time nested PCR with SGI fluorescence is a simple, economic, rapid and effective method to quantify
infectious pathogens DNA present over a wide dynamic range. The assay is inexpensive, easy to handle, has high sample throughput and is completed in less than 3 hours.

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A C-TERMINAL BASIC AMINO ACID MOTIF OF ZAIRE EBOVIRUS VP35 IS ESSENTIAL FOR TYPE I INTERFERON ANTAGONISM AND DISPLAYS HIGH IDENTI FI CATION WITH THE RNA-BINDING DOMAIN OF ANOTHER INTERFERON ANTAGONIST, THE NS1 PROTEIN OF INFLUENZA A VIRUS

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Previous work has shown that the Zaire ebolavirus VP35 protein antagonizes the cellular type I interferon response by blocking phosphorylation of IRF-3, a transcription factor that turns on the expression of a large number of antiviral genes. To identify the domain of VP35 responsible for interferon antagonism, we generated mutations within the VP35 gene and found that a C-terminal basic amino acid motif is required for inhibition of ISRE reporter gene expression as well as IFN-β production. Remarkably, this basic amino acid motif displays high sequence identity with part of the N-terminal RNA-binding domain of another interferon-antagonizing protein, NS1 of influenza A virus.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES SPECIFIC FOR THE RNA BINDING DOMAIN OF THE HANTAAN VIRUS NUCLEOCAPSID PROTEIN

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We generated a panel of monoclonal antibodies (mAbs) that recognize the RNA-binding domain (RBD, amino acids 175-300) of the Hantaan virus (HTNV) nucleocapsid (N) protein. Mice were vaccinated with purified full-length HTNV N protein cross-linked to S-segment vRNA and digested with RNase V1; purified full-length HTNV N protein cross-linked to S-segment vRNA; HTNV truncated N protein, amino acids 175-300; or HTNV truncated N protein, amino acids 175-300, cross-linked to S-segment vRNA. Animal sera were analyzed by ELISA for the production of HTNV N-specific mAbs using purified full-length N protein and repeated with truncated N, amino acids 175-300. From these data, one animal from each group was chosen for hybridoma production. Over 130 pre-clones secreting HTNV-specific antibodies were identified by ELISA using either purified full-length N protein or truncated N protein, amino acids 175-300, and a subset of these was selected for cloning expansion based on their ELISA-reactive titers to truncated N protein, amino acids 175-300; amino acids 137-214; or amino acids 208-262. Additional peptide mapping and competition studies identified 6 hybridomas that secreted mAbs specific for distinct epitopes that span the region encompassing the RBD of the HTNV N protein. Antigen specificities of the mAbs were confirmed by radio-immunoprecipitation and immunofluorescence assays. We are currently conducting studies to evaluate the ability of these mAbs to recognize the N protein-vRNA complex and to, thereby, potentially inhibit viral assembly. These data will provide a basis for future studies aimed at the production of immunotherapeutic reagents for the treatment and/or prophylaxis of hantavirus infections.

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AEDES AEGYPTI SALIVARY GLAND EXTRACTS MODULATE MURINE ANTI-VIRAL AND TH1/TH2 CYTOKINE RESPONSE TO SINDBIS VIRUS INFECTION

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Intradermal inoculations of Aedes aegypti salivary gland extract (SGE) and Sindbis virus (SINV) were used to investigate the effect of mosquito feeding on the vertebrate immune response to infection with an arthropod-borne virus. Murine cytokine expression in the skin at 24 and 72 hours post inoculation (pi) was quantified by means of real-time RT-PCR and primer-probe sets specific for anti-viral, Tβ1, and Tβ2 cytokine mRNAs. In response to co-inoculation of SGE with SINV, interferon (IFN)-β expression at 24 and 72 hours pi was significantly reduced by 2.2- and 2.3-fold, respectively, when compared to injection of virus alone. Similarly, IFN-γ expression in response to SINV infection was significantly decreased by 1.6-fold at 24 hours pi when SGE was co-inoculated. In contrast, interleukin (IL)-4 expression was significantly up regulated when SGE was co-inoculated at 24 hours pi becoming a 3.3-fold increase by 72 h pi. Furthermore, compared to expression with SINV alone, IL-10 expression showed a 7.6-fold increase by 72 hours pi in mice receiving SGE concurrently with virus. The injection of SGE alone as compared to inoculation of PBS led to an enhancement of IL-4 and IL-10 expression of approximately 4.0-fold at 24 h that persisted to 72 h pi. This study demonstrates that the response to virus is significantly different when an infection is initiated in the presence of mosquito salivary factors, and suggests a possible mechanism for potentiation of viral infections initiated by the natural mosquito vector or in the presence of mosquito saliva. To our knowledge this is the first report of an in vivo effect of mosquito SGE on the cutaneous immune response to a viral infection.

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PHYLOGENETIC RELATIONSHIPS AMONG MEMBERS OF THE GENUS PHLEBOVIRUS (BUNYAVIRIDAE) BASED ON S SEGMENT SEQUENCE ANALYSIS

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The genus Phlebovirus (family Bunyaviridae) currently consists of 68 antigenically distinct virus serotypes, which are distributed in two groups: the Phlebotomus fever (PFL) group and the Uukuniemi (UUK) group. Human infections with phleboviruses usually result in acute non-specific febrile illness; but some (i.e. Toscana, Rift Valley fever) can also cause meningitis, encephalitis, hemorrhagic symptoms, retinitis or death. Phleboviruses contain a negative-sense, single-stranded RNA genome, consisting of three segments, designated large (L), medium (M) and small (S). The S segment exhibits an ambisense coding strategy and encodes the N and NSs proteins from mRNAs of opposite directions. In this study, RT-PCR with “cocktail” primers were performed to amplify the first 600 bp region of the S segment from 33 phleboviruses included in the sandfly fever Naples, sandfly fever Sicilian and Punta Toro serocomplexes. Phylogenetic analysis was performed on the sequences. The three resultant genotype lineages were consistent with serological data and with analysis of the M segment performed previously. This suggests that the M and S segments co-evolved; no evi-
dence of reassortment between these two segments was identified in the viruses studied. In addition, the sequence identities were: 76.8% (nucleotide) and 30.3% (amino acid) within the Sicilian serocomplex; 81.7 (nucleotide) and 69.2 (amino acid) within the Naples serocomplex; and 77.0 (nucleotide) and 55.2 (amino acid) within the Punta Toro serocomplex. The higher nucleotide sequence identity than amino acid sequence is rather unusual. Further studies, including the analysis of the full-length sequence, should provide a better explanation.

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INVESTIGATING THE VIRAL MOLECULAR DETERMINANTS OF PATHOGENESIS OF PUNTA TORO VIRUS IN THE SYRIAN HAMSTER

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The phleboviruses Toscana, Rift Valley fever (RVF) and Punta Toro (PT) are important causes of morbidity, and in the case of RVF, mortality in human populations around the Mediterranean, Africa, and Panama respectively. PT infection with strains that produce lethal (Adames (PT-A)) and non-lethal (Balliet (PT-B)) infections in the Syrian hamster (Mesocricetus auratus) is a model for Phlebovirus pathogenesis. Previous studies showed that the LD50 for PT-A in hamsters was a million-fold lower than that for PT-B and that PT-A titers rise rapidly early in infection, suggesting that this strain has an advantage over the early innate immune system as compared to PT-B. The inhibition of the early innate immune response has been implicated in the pathogenesis of some members of the Bunyaviridae family. The NSs gene on the S segment determines aβ IFN inducibility and sensitivity in RVF infection. The overall objective of this study is to investigate the respective roles of the 3 viral RNA segments in the differential pathogenesis of the PT-A and PT-B in hamsters using genetic reassortants generated between the two strains. Reassortants were produced through dual infection of PT strains in Vero E6 cells and genotyped by RT-PCR. A pilot mortality study was conducted using an ABA (LMS convention) reassortant virus genotype. Comparison of geometric mean viral titers in liver and serum revealed a trend showing highest titers in the AAA group followed by the ABA and SBB groups. Hamsters infected with the ABA genotype also displayed intermediate histopathology. In conclusion, the M segment is at least one factor affecting PT virulence. In addition, genomic analysis between PT-A and PT-B revealed the following amino acid changes within proteins coded by the M and S segments: 12 (NSs), 28 (NSm), 18 (Gp1), and 11 (Gp2). Residue changes within the NSs and NSm proteins resulted in changes in predicted secondary structure between the two strains. These residues and structural changes may be pathogenic determinants in PT infection and are targets for mutagenesis studies.

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POTENTIAL ANTIVIRAL DRUGS FOR TREATMENT OF HANTAVIRUS DISEASE

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Hantaviruses first came to the attention of western medicine in the early 1950s when more than 3000 US troops stationed in Korea became ill with a disease that later came to be known as hemorrhagic fever with renal syndrome (HFRS). The prototypic virus for HFRS, Hantaan virus (HTNV), was identified and cultivated from the tissues of the field mouse Apodemus agrarius. Since this initial discovery, numerous hantaviruses have been discovered throughout the world, most recently in the Americas in 1993. These newly identified hantaviruses were discovered to be the agents of a second illness, hantavirus pulmonary syndrome (HPS). Effective vaccines and antivirals for the treatment or prophylaxis of hantaviral infections for both syndromes are currently unavailable. Except for ribavirin, no other antiviral drugs for treating hantaviral diseases have been identified. There are no published papers that have analyzed the mechanism of action of ribavirin for hantaviruses or other members of the Bunyaviridae. Its mechanism of action has been difficult to elucidate, primarily because of its pleiotropic effects. We have previously shown that viral RNAs isolated from HTNV-infected cells treated with ribavirin have a high mutation frequency (Severson et al. 2003). To further explore the hypothesis that this drug may cause error catastrophe, we are exploring the mechanism of action of the drug through additional approaches that include an examination of the metabolism of this drug. This study now includes both HFRS and HPS viruses. Further, we are exploring the mechanism of additional synthetic analogs, and structurally related C-nucleoside drugs tiazofurin and selena-zofurin. Progress in defining the mechanism of action and potential for error catastrophe of ribavirin and related nucleosides will be presented.

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GENETIC CHARACTERIZATION OF M AND S GENOMIC SEGMENTS FROM CANADIAN SNOWSHOE HARE AND JAMESTOWN CANYON VIRUS STRAINS

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Snowshoe Hare (SHH) and Jamestown Canyon (JC) viruses are mosquito-transmitted bunyaviruses belonging to the California serogroup. SHH virus has been documented across Canada and has been associated with cases of meningitis and encephalitis in Quebec, Ontario, New Brunswick, and Nova Scotia. JC virus has been isolated from Aedes mosquitoes collected from several western provinces and associated with neurological disease in Ontario. As part of a study to determine the genetic diversity of SHH virus in Canada, viral isolates collected from five provinces and two territories during the 1960s, 70s, and 80s were characterized by PCR amplification and amplicon sequencing and compared to each other, JC strains, and other California serogroup viruses. Sequencing of amplified portions and full length M and S genomic segments indicated that the isolates could be grouped into several distinct genotypes. G2 glycoprotein encoding regions exhibited nucleotide sequence divergence as high as 23%; however, all but one isolate displayed 100% amino acid sequence similarity within the G2 portion analysed. All isolates from Ontario showed high RNA sequence homology (> 98%) despite being collected over a 15 year time period. In contrast, phylogenetic characterization of SHH strains from the Yukon revealed the existence of at least two distinct co-circulating genotypes. A SHH “variant” isolated from a larval mosquito in Saskatchewan displayed significant divergence at both the nucleotide and amino acid levels when compared to both Canadian and American isolates. This genetic variant provides further evidence for the extensive evolution that these RNA viruses may undergo during their transmission / amplification cycles and the high degree of genetic diversity that may exist among strains within the same serotype.
PRELIMINARY STUDIES ON THE TRANSMISSION DYNAMICS OF SIN NOMBRE VIRUS IN NATURALLY-INFECTED DEER MICE (PEROMYSCUS MANICULATUS) COLLECTED IN MANITOBA, CANADA

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Sin Nombre virus (SNV) is a rodent-borne virus, which in Canada is the only etiological agent of hantavirus pulmonary syndrome, an often-fatal disease in humans. In August 2003, a pilot study was conducted to quantify the prevalence of SNV in naturally-infected deer mice (Peromyscus maniculatus) from sites in southern Manitoba, Canada. Positive and equivocal SNV specific IgG titres were detected in 17.2% and 5.7% of the captured deer mice (n=122), respectively. Using real-time (Taqman®) RT-PCR, SNV S segment RNA was detected in 80 to 82% of a variety of samples from different tissues (i.e., heart, lung, kidney, spleen, salivary gland, muscle and fat) from 15 of the mice with anti-SNV antibodies. In contrast, SNV RNA could be detected in only 40% of bladder and 67% of liver samples. Sin Nombre virus cDNA was also amplified from 6 of 122 (4.9%) saliva and 2 of 11 (18%) urine samples from these mice. Based on these results, in May 2004 an intensive mark-recapture study was initiated to better define the seasonal transmission dynamics of SNV within deer mouse populations. Results from the 2004 field season will be discussed in detail; however, as of this writing, SNV RNA was detected in the excreta and blood samples of relatively few mice despite high prevalence of antibodies in adult animals collected during May and June.

SOCIODEMOGRAPHIC AND BEHAVIORAL FACTORS ASSOCIATED WITH NON-ADHERENCE TO HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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Although behavioral factors are known to affect adherence to HIV treatment regimens, it is unclear whether sociodemographic characteristics affect adherence. The purpose of this survey was to identify factors associated with non-adherence to highly active antiretroviral therapy (HAART) and to identify means of reducing secondary HIV transmission by decreasing non-adherence. This cross-sectional study surveyed 101 patient volunteers from an inner city clinic, aged 18 to 64, who had been on HAART for at least six months. Face-to-face interviews using a structured questionnaire addressed factors previously identified as potentially associated with non-adherence and additional factors that had not been measured before. The survey population of seventy males and thirty-one females were 53.5% African American, 23.8% Caucasian, 13.9% Latino, and 8.8% other races, with an average age of 43.8 years. More than 80% had missed at least one dose of medication, while 40.6% described taking a longer, non-prescribed “drug holiday” at some point. Of those with an appointment scheduled in the previous month, 18.8% reported missing the appointment. Nearly half were sexually active, with 19.8% reporting that they did not use protection. Several factors were found to be associated with non-adherence, including drug use, side effects of medications, employment, fear of being seen taking the medication, lack of a support system, being away from home, and simply forgetting to take their medications. Potential interventions should address interruptions of drug therapy, such as support groups focused on adherence, and patient-centered programming to increase awareness and education about medication resistance and secondary transmission issues.

MODELING EEE TRANSMISSION IN ALABAMA


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Recent studies at a site in Alabama have demonstrated that avian host choice by vectors of Eastern Equine Encephalomyelitis (EEE) at this site is not random. Chosen host species are selected more often than predicted based upon the density of the species at the site. The temporal feeding pattern on the preferred hosts appears to be restricted, and suggests the hypothesis that vector mosquitoes are specifically targeting nestlings and/or young of the year. To explore this hypothesis further, the data collected during the 2001 EEE transmission season were used as the basis for the development of a computer model. The model uses a second-order Runge-Kutta algorithm to simultaneously iterate parameters on a daily time-step. The model is based upon three interacting modules, each with several states. These modules are the adult bird population, the young of the year bird population, and the vector mosquito population. A key feature of the model is that it allows for temporal changes in the feeding preference of the vector mosquitoes. This permits us to model the effect of preferential feeding on young of the year. Initial values for the model's parameters were determined from a variety of published sources and from data collected at our Alabama study site in 2001. The performance of the model was validated with 2002 and 2003 observations, which were independent of the data used to develop the model. The model was extremely successful in matching the observed data for both 2002 and 2003. Sensitivity analyses were then conducted on the 27 variables in the model. The input variables were then categorized as drivers, those having a large effect on the output and non-drivers, those that had a small impact. Of the 11 variables found to be drivers, four related to the avian young of the year. The model therefore predicts that the degree that young of the year are successfully fed upon by EEE vectors may play a significant role in determining whether or not an EEE enzootic will develop.

OPTIMIZATION OF EXPRESSION OF FULL LENGTH NATIVE SARS SPIKE GLYCOPROTEIN IN HUMAN CELLS

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Expression of recombinant eukaryotic proteins in transfected mammalian cell lines has become an important approach for the characterization of the structure and function of these proteins. The spike protein, a membrane component of severe acute respiratory syndrome coronavirus (SARS-CoV) is anticipated to be an important component of candidate vaccines. We utilized a transient expression system to determine the optimal design for the recombinant expression of the extracellular domain (without transmembrane and cytoplasmic regions) of the Spike protein (S-Ex) with a C-termi-
nal his-tag in human cell lines. Levels of expression were assessed by SDS-PAGE and immunoblot using polyclonal antibodies specific for S-Ex. The parameters studied included secretion signals, culture conditions, transfection methods, and cell lines. We found that the native SARS and human tissue plasminogen signal sequences were equally efficient at expressing recombinant S-Ex as a fully folded and soluble protein in culture media of transfected cells. Human HEK293T and U449 cells were employed for the comparison of protein expression. Studies on optimization of transfection methods including DNA cell ratio, chemical transfection and media types showed that Opti-MEM serum-reduced media generated material that allowed most efficient purification. Recombinant S-Ex was purified by standard nickel column. Biochemical characterization showed that recombinant S-Ex contained the intact N-terminus of the full length glycoprotein. These results indicate that this optimized transient system can produce quantities of characterized SARS-CoV Spike glycoprotein in a reproducible, robust and efficient system. This material is being used to support the analysis of clinical materials for lot release.

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HANTAVIRUS DISEASE IN PANAMA - HIGH PREVALENCE OF MILD OR ASYMPTOMATIC CHOCLO VIRUS INFECTION

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Hantaviruses causing cardiopulmonary syndrome in the Americas are rare infections associated with high mortality of 20-45% yet low seroprevalence rates. Crude estimated ratios of hospitalized to mild/asymptomatic infection appear to be greater than 10:1 for Andes and Sin Nombre viruses. An exception to this mortality-seroprevalence dichotomy is found in the Gran Chaco of western Paraguay among two populations residing in the same region. Ferrer and colleagues documented that Amerindians experienced few symptomatic infections in the context of a seroprevalence rate of 40%. It is unknown whether all seropositivity in this region is due to Laguna Negra virus infection. Another exception to the mortality-seroprevalence dichotomy is found in Panama where all hospitalized hantavirus infections are due to Choclo virus, based on blood-borne nucleotide sequences, and no infections with Calabazo virus have been found. The mortality rate of the 44 patients described since year 2000 is 20%. Community-wide seroprevalence rates in the Azuero peninsula range from 16% to 45%, with linear increases in seroprevalence between 4 and 40 years of age in all 5 communities tested. Choclo virus has been cultured in Vero E6 and A549 cells and complete nucleotide sequence determined. In focus neutralization assays using both cells lines, sera from hospitalized cases neutralize Choclo virus in dilutions of 1:400 to 1:3200, while seropositive sera from individuals denying any history of respiratory illness neutralize Choclo virus in dilutions of 1:200 to 1:1600. The calculated ratio for hospitalized to mild/asymptomatic infection with Choclo virus is 1.20, strikingly lower than other American hantaviruses. While cross-reactions with Calabazo virus can not be ruled out in focus assays, and host genetics will modulate disease phenotype, Choclo virus should be investigated for reduced virulence characteristics.
A COMPARISON OF FLUORESCENCE-BASED METHODS FOR IN VITRO ANTIMALARIAL DRUG TESTING

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Fluorescence-based alternatives to traditional radioisotopic methods have recently been described for in vitro antimalarial drug testing. As these appealing methods gain popularity, it is essential that their strengths, weaknesses and characteristics be carefully explored. We have performed well over 2000 fluorescence-based EC₅₀ assays with P. falciparum and report our experience here, including analysis of the effects of varying assay growth duration, parasite strain, parasite developmental stage, parasitemia, hemocrit, lysis buffer, and microplate well volume. Results using several fluorophores, including SybrGreen I, PicoGreen and several other nucleic acid stains have been compared, as have the differences between several microplate styles and three varieties of plate-reading fluorometers. In addition, drugs with intrinsic fluorescence have been tested to assess whether unacceptable interference results. Finally, a comparison of EC₅₀ determinations involving fluorophore-stained parasites and parasites expressing Green Fluorescent Protein will be presented. The results unequivocally confirm the utility of fluorescence-based in vitro antimalarial drug testing, and demonstrate that any number of methods can be used. With an understanding of how the above variables impact results, robust methods can be selected to fit individual needs. In order to develop method standardization for most drug testing uses, recommendations based on simplicity, cost and breadth of applicability will be presented.

EVALUATION OF THE EMBRYONIC TOXICITY OF ARTESUNATE IN RATS

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Artesunate (AS) has been used as treatment for different forms of malaria in Asia and Africa for years and has been generally recognized as being effective and safe. However, concerns about its safety in pregnancy have been raised following detection of embryotoxicity in animals exposed to AS during pregnancy. With the increasing amount of interest in this drug, there is a need for careful evaluation of adverse effective dose(s) of AS on embryonic development and its use in different trimesters of pregnancy. This study, therefore, investigated the adverse effects of AS on pregnant outcome of rats. Mature SD female rats were mated with males and selected by sphen in vaginal smears. AS (GMP product) was administered by IM injection. At the first stage of study, AS at a series doses of 0 (vehicle control), 0.5, 0.55, 0.6, 0.65, 0.75, 1.0, 1.25, 1.5, and 4.5 mg/kg/d was injected for 13 days from gestation day (GD) 6 to GD 18. Dams were euthanized on GD 20 and fetuses were examined clinically and microscopically. The defined non-adverse-effective (NAED), moderate-effective (MED) and maximal-effective (MXED) embryotoxic doses of AS were then tested in a three-days dose regime from GD6 to GD8. The moderate-effective toxic dose of AS was subsequently tested in the first (GD 3-5), the second (GD 8-10) and the third (GD 15-17) periods of pregnancy. There were no clinical or microscopic abnormalities of dams. All pregnant rats had fetuses or resorbed placentas in uteri. All fetuses except 1 in vehicle group were alive with normal placentas when released from uteri. In contrast, all fetuses in groups of AS 0.75mg/kg/d and up were completely resorbed. In range of 0.5, 0.55, 0.60 and 0.65 mg/kg/d, the rates of survived fetuses from 100% to 7% were inversely correlated to AS doses. The total NAED, the MED and the MXED of AS were 6.5, 8.45 and 9.75 mg/kg, respectively. AS effects on different periods of pregnancy are under test. In conclusion, AS applied during pregnancy exhibited a clear embryotoxicity in rats at relatively low doses. This effect of AS seems not related to maternal toxicity.

ASSESSMENT OF THE EFFECT OF A CANDIDATE ANTIMALARIAL DRUG ON CARDIAC QT INTERVAL

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The QT interval is an electrocardiographic index which measures the time in milliseconds (msec) from the beginning of the QRS complex to the end of the T wave. It represents the time between the onset of ventricular depolarization (beginning of the QRS) and the end of ventricular repolarization. However, the QT interval is inversely correlated with heart rate. Therefore, it must be adjusted for heart rate (heart rate-corrected QT interval = QTc) in order to obtain a heart rate-independent measurement to compare different individuals or to compare the same individuals at different heart rates. QTc prolongation is of interest because many drugs (including the aminoquinolines) prolong the QTc interval, and because extreme QTc prolongation is associated with a rare, potentially fatal ventricular arrhythmia: Torsades de pointes. Normal QTc values are <450 msec for males and <470 msec for females. We examined the effect of a new candidate antimalarial (AQ-13), which is effective against chloroquine- (CQ) and multi-resistant P. falciparum, on the QTc interval and compared its effects with those of CQ. Twenty four healthy volunteers received 600 mg of CQ and 12 received an equivalent dose of AQ-13. QTc intervals were determined from 12-lead electrocardiograms (ECGs) taken 4 hours after dosing, i.e., at the time of peak blood concentrations. The QTc was prolonged similarly in both groups (from 406 to 421 msec with CQ (p<0.01), from 403 to 418 msec with AQ-13 (p<0.01). However, there were no arrhythmias or clinically significant cardiac events in either group. In addition, the QTc interval returned to baseline within 2 weeks in both groups. Holter monitoring is now being used to obtain a more rigorous assessment of the effects of AQ-13 and CQ at the 1500 mg dose. The advantage of Holter monitoring is that it permits continuous monitoring of the QTc and the ECG. In summary, these studies have found no evidence for cardiotoxicity with AQ-13 or CQ doses up to 600 mg.

GENETIC AND WHOLE CELL IN VITRO INVESTIGATIONS INTO PLASMODIUM FALCIPARUM ENOLY ACP REDUCTASE INHIBITORS

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The emergence and spread of antimalarial drug resistance in Plasmodium falciparum and P. vivax makes it imperative to discover and develop new chemical entities that are not compromised by existing mechanisms of resistance. Previous studies have shown that malaria parasites employ a type II fatty acid biosynthesis (FAS-II) pathway that is distinct from the type I pathway utilized in humans. FAS-II in P. falciparum has been localized to the apicoplast, an organelle with cyanobacterial and plastid origins. The
FAS-II enzyme enoyl ACP reductase (ENR, also known as FabI or InhA) has been validated as an effective target for antimicrobials, including the antituberculosis agents isoniazid and ethionamide.

Previously we and others identified the \textit{P. falciparum} ENR (PFENR) as the target of triclosan, a commonly-used topical antimicrobial. This work has included elucidation of the crystal structure of the PFENR-triclosan-NAD complex in order to gain insight into the structural basis of triclosan binding and develop a structure-based method to improve inhibitor affinity. A medical chemistry approach is also being taken to develop triclosan analogs and test these for efficacy in the \textit{P. berghei} rodent malaria model.

Here, we will present our \textit{in vitro} data on triclosan analogs, showing our progress in identifying compounds with good inhibitory activity against \textit{P. falciparum} growth, determined using a 72-hour \textsuperscript{[3H]}-hypoxanthine assay. Several compounds have now been identified with similar potency to triclosan. We will also present our transfection work aimed at engineering \textit{P. berghei} strains that express the \textit{P. falciparum} or \textit{P. vivax} \textit{enr} in the place of the rodent ortholog, as a means to generate \textit{in vivo} models to evaluate compound efficacy against the human malaria parasite target enzymes.

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\textbf{FIXED DOSE COMBINATION THERAPY FOR CHLOROQUINE-RESISTANT MALARIA}

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The availability of user-adapted, fixed-dose oral forms of drugs containing artemisinine derivative is central to the introduction of combination therapy for malaria. The FACT (fixed dose artemesia combination therapy) project aims at a fast track development and testing of two such combinations (artesunate-amodiaquine, AS/AQ; artesunate-mefloquine, AS/MQ) which, as freely associated individual components, have been proven effective and well tolerated. The targeted indication for these new products, for which two strengths are developed, is the first line treatment of paediatric and adult uncomplicated falciparum malaria for use (AS/AQ case) in Africa-where artemisinine resistance is low- and in endemic regions including multidrug resistance areas (AS/MQ case) with priority to South America and Asia. The organisation of development activities for the FACT project supported by the European Community (INCO-Dev Program), the ongoing studies, and first results of mainly preclinical investigations will be presented. The steps to Registration will be outlined.

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\textbf{RELATIONSHIPS BETWEEN THE ENТОMOLOGICAL FORCE OF MALARIA PARASITE TRANSMISSION BY MOSQUITOES AND THE PUBLIC HEALTH BURDEN OF MALARIA IN AFRICAN COMMUNITIES}

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Every malaria endemic African community is faced with exposure to \textit{Anopheles} mosquitoes carrying infective-stage sporozoites that are transmitted by mosquito bites. The force of transmission can be measured by the entomological inoculation rate (EIR), the product of the man-biting rate and the proportion of mosquitoes carrying sporozoites in their salivary glands. One infective bite is enough to cause a malaria infection and associated life-threatening illness. In malaria endemic areas of Africa, EIRs can range from less than one infective bite per year to over 1,000. The relationships between the EIR and epidemiological measures of malaria in humans are central to developing sustainable malaria control programs. This review describes standard entomological procedures for estimating EIRs, and provides four case studies from research in Kenya that illustrate basic relationships between EIRs and measures of malaria incidence, malaria parasitemia (proportion of infected red blood cells), the incidence of severe disease, and malaria prevalence. From this comparative evaluation, there are two important points. First, any decreases in EIRs attained through vector control operations can correspondingly decrease malaria incidence and also can decrease parasitemias during peak transmission periods in highly endemic areas. Second, because transmission is very efficient even at low levels, decreases in EIRs through vector control may not affect the incidence of severe disease or malaria prevalence unless EIRs are reduced to levels below one infective bite per person per year. In terms of malaria control operations, it is important to understand the likely thresholds of transmission necessary to protect children from life-threatening malaria infections and to protect communities from extremely high prevalence of malaria associated with very low levels of transmission.

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\textbf{GENETIC STRUCTURE OF ANOPHELES FUNESTUS POPULATIONS IN BURKINA FASO, WEST AFRICA}

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Although \textit{Anopheles funestus} is a primary vector of malaria, its population genetic structure has been largely neglected, especially in comparison to other equally important vector mosquitoes. Analyses across large distances using a variety of molecular markers revealed very high and significant differentiation values. This finding emphasized the need to investigate \textit{A. funestus} populations at smaller geographical scales ranging from 0 to 750 km. However, this need has been somewhat complicated by results from recent population genetic studies in Burkina Faso using paracentric chromosomal inversions and molecular markers. In Burkina Faso, these studies suggested population structure between two forms designated Folonzo and Kiribina, initially based on the observation of heterokaryotype deficits and linkage disequilibrium among inversions located on different arms from single locales. These forms were independently corroborated with 16 microsatellite loci and 834-bp of the \textit{mtDNA ND5} gene. Although significant molecular differentiation was found between forms, no differentiation was found within forms between small geographical scales (0.5-2 km). To fully understand the population genetic structure of \textit{A. funestus} in Burkina Faso, a mesogeographic study was carried out between villages on an East-West transect with distances measuring from 2 to about 300 km. Preliminary data using 10 microsatellite loci between 4 villages showed a slight, but positive, correlation. However, some of these loci were unmapped and village sample sets included both forms, potentially biasing differentiation estimates. Collections for a more robust study were made in December 2002, and, after karyotyping of half-gravid females, villages will be chosen based on an adequate sample size of each form. After species identification by PCR, samples will be genotyped at 16 physically mapped microsatellite loci, chosen from an \textit{A. funestus} reference set, including 3 within inversions known to be important in form designations, and 834-bp of the \textit{mtDNA} \textit{ND5}. Pair-wise population differentiation values within and between villages for each form will be presented, as well as differentiation values between forms collected in the same village.
USE OF THE GAL4-UAS TRANSACTIVATION SYSTEM TO CHARACTERIZE PROMOTERS AND EXPRESS ANTI-PATHOGEN MOLECULES IN TRANSGENIC Aedes aegypti

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An effective genetic control strategy for mosquito-borne pathogens will require more than simple proof-of-principle pathogen-resistant transgenic mosquitoes. Once candidate effector genes and promoter elements are identified, they must be rigorously examined to ascertain whether they may be further improved, and to determine the precise conditions under which they may fail. This requires not only the generation of many different transgens, but also depends upon a direct and meaningful comparison between them. Such a comparison is made difficult by the fact that the transposable elements used to generate transgens integrate randomly throughout the genome, leading to variation in expression due to position effects. One solution to this problem is the use of the yeast GAL4-UAS transactivation system. A single transgenic line, expressing the GAL4 transactivator under the control of a specific promoter, could be crossed to several effector gene lines under the control of the UAS element. This would allow direct side-by-side comparison of several effector genes, or the optimization of a single effector gene. Conversely, a single UAS-effector gene transgenic line could be crossed to several different promoter-GAL4 lines to compare the effectiveness of each promoter, or to study the effects of variations on a single promoter. In this study, 2 promoter-GAL4, 2 UAS-reporter, and 3 UAS-effector constructs were used to transform Aedes aegypti. The promoters chosen were derived from the salivary gland specific genes D7 and Apyrase. The two marker genes used under UAS control were Luciferase and EGFP, while the three effector genes were inverted repeat constructs used to generate a RNAi response against DEN-2 virus. Preliminary crosses between a D7-GAL4 line (#6) and a UAS-Luc line (#71) indicate at least a 10-100 fold induction of Luciferase activity in the female salivary glands compared with line #71 alone.

MICRO- AND MESO-GEOGRAPHIC ANALYSIS OF CHROMOSOMAL INVERSION POLYMORPHISM OF ANOPHELES FUNESTUS FROM BURKINA FASO

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To investigate the effect of distance and karyotype on the genetic structure and the level of population differentiation in Anopheles funestus from Burkina Faso, we examined the pattern of chromosomal polymorphism at two spatial scales: village-level (micro-geographic), comparing compounds dispersed over a 6 km² area in the village of Koubri, at 35 km southwards of the capital Ouagadougou, and regional (meso-geographic), comparing 15 villages aligned along a transect approximately centred in Ouagadougou ranging 350 km on a west-east axis. Indoor-resting half-gravid females were collected by knock-down spray-sheet catches from 39 compounds, representing 40% of the total number of compounds in Koubri. Collections were performed during the course of one week to minimize the effect of temporal variation in inversion frequencies, as observed occurring in this village. For the meso-geographic analysis, villages were surveyed during two months yearly for two successive years. In both studies, inversion frequencies were found significantly different either among villages or among compounds. At the micro-geographic scale, higher inversion frequencies were found in compounds near the border of a large swamp, while they markedly decreased in compounds near an artificial lake devoid of emergent vegetation only 2 km from the swamp. Both water reservoirs are potentially good breeding sites for An. funestus. At the meso-geographic scale, most villages presented contrasting levels of chromosomal polymorphism, with fewer showing intermediate inversion frequencies; however, villages with a higher or lower degree of polymorphism were spatially arranged in patches rather than in a pattern suggestive of genetic introgression. There is increasingly independent chromosomal and molecular evidence supporting the existence in Burkina Faso of two reproductive (semi)isolated chromosomal forms characterised by contrasting degrees of inversion polymorphism. Under this scenario, our results suggest that their distribution is mainly governed by micro-spatial environmental factors presumably associated to the presence of alternative larval habitats.

EVALUATION AND OPTIMIZATION OF MEMBRANE FEEDING AS AN ALTERNATIVE TO DIRECT FEEDING AS AN ASSAY FOR INFECTIVITY

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Between August 1996 and December 1998, we used direct feeding of mosquitoes to evaluate malaria-transmission dynamics and compared infectivity between direct feeding and membrane feeding among children ages 4—18 years in Bancoumana, Mali. Results from 372 Plasmodium falciparum gametocyte carriers showed that children ages 4—9 years were more infectious than older children (p=0.039), especially during the rainy season. Data from 35 carriers showed that mosquitoes who were fed by direct feeding were about 1.5 times more likely to feed (p<0.001) and two times more likely to become infected if they fed (p=0.001) than were those who used membrane feeding. Overall, infectivity rate was about three times higher for direct feeding than for membrane feeding (p<0.001). Although intensity of infectivity was lower for membrane feeding, it could be an useful alternative to direct feeding for evaluating transmission-blocking activity of candidate malaria vaccines. Optimization of the method for future trials would involve using about three times more mosquitoes than would be used for direct feeding.

FIELD AND LABORATORY ADVANCES TOWARD STERILE INSECT TECHNIQUE (SIT) FOR CONTROL OF ANOPHELES ARABIENSIS

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One of the few available methods of genetic control of insects including mosquitoes is SIT. For mosquitoes, this method relies on the release of large numbers of genetically sterile males that mate with wild virgin females leading to reduced adult population fecundity. When successful, population suppression or elimination results. Previous SIT programs against anopheline species ended 30 years ago and consisted only of feasibility studies. In view of
the availability of new technologies and the increasing burden of malaria, the International Atomic Energy Agency (IAEA) is conducting a program for SIT against Anopheles arabiensis. The program primarily consists of a laboratory component for development of methods for factory production, sterilization, distribution, and sex separation of this African malaria vector. This requires the development of novel technology and knowledge that consider the limited - yet specific - goals of SIT: male vigor, dispersal, and competitive mating behavior. In this presentation, we detail these critical laboratory and field research questions that must be answered to ensure success of SIT. Because the factory production methods determine the performance of the males in the field site, concerns include ecological, physiological, behavioral, and genetic knowledge. The Agency is developing such SIT laboratory technology in coordination with studies of select field sites at which releases are being considered. We summarize the current activities and scientific progress in these areas and describe how these field sites meet the criteria for SIT studies. The two sites in Sudan and the French island of La Réunion identified thus far are very different economically, geographically, and epidemiologically, yet robustly satisfy the clear criteria for successful genetic control.

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**HUMAN BLOOD FEEDING PATTERNS OF THE DENGUE VECTOR, Aedes aegypti**


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Feeding patterns of the dengue vector, Aedes aegypti for individual human hosts were examined from Thai villages during high and low dengue transmission seasons from February 2000 to January 2003. We used PCR-based profiling of human DNA in mosquito blood meals. Aspiration collections were conducted inside homes in four Thai villages near Mae Sot, Thailand. Human microsatellite markers were amplified at six polymorphic loci and one region of the human x and y-chromosomes. A computer-match program was employed to match human DNA fingerprints in mosquito blood meals to profiles of community resident volunteers and field crew members. The person(s) who were fed on and the frequency of feeding from different people were determined. Feeding frequency across human host age classes was analyzed after correcting for population age structure. Controlled time-series experiments with one and multiple hosts were conducted to identify the limits of DNA detection using this approach. The frequency of alleles in the human host population was also determined to identify rare alleles that would be useful in matching partial DNA profiles. Results over multiple years, dengue transmission seasons and villages will be presented.

**COMPREHENSIVE MONITORING OF THE IMPACT OF A PILOT MASS DRUG ADMINISTRATION PROJECT FOR FILARIAISIS IN PAPUA NEW GUINEA**

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This study was designed to assess the impact of mass drug administration (MDA) on bancroftian filariasis in Papua New Guinea and to gain experience with different monitoring methods. Single dose DEC with albendazole was administered in 12 villages in the Usino district, near Madang. Sentinel villages were studied before and one year after MDA. Treatment coverage in the target population over 2 years of age was 72.7%. Microfilaria (MF) prevalence (1 ml night blood by filter) fell from 18.9 to 8.3% (56% reduction). Notably, 75% of MF-positive subjects treated in year 1 were MF-negative one year later. Median MF/ml in positives decreased from 166 to 89, and MF/ml/person decreased by 81%. Filarial antigen prevalence (a marker for adult worm infection, assessed by card test) decreased from 49 to 34.2% (30% reduction). Antibody to Bm14 (a marker for filarial infection or heavy exposure) decreased from 56.3 to 41.7% (26% reduction). Antigen and antibody prevalence rates decreased more in children under 11 years of age (by 40% and 70%, respectively), perhaps reflecting their lighter infections and shorter durations of exposure/infestation. The prevalence of parasite DNA in household samples of fed or gravid mosquitoes (assessed by PCR) decreased from 41.4 to 21.5% (48% reduction). This study has provided useful information on the value of comprehensive monitoring for assessing effects of MDA. Each test provides different, useful information. Modest coverage with a single dose of MDA had a dramatic effect on filariasis in the study area. However, our results suggest that additional cycles of MDA (preferably with improved coverage) will be necessary to eliminate filariasis infection and transmission in this area.

**MOLECULAR XENOMONITORING AS A MEANS OF ASSESSING PROGRESS TOWARD ELIMINATION OF FILARIAISIS IN EGYPT**

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The government of Egypt initiated a National Filariasis Elimination Program in September, 2000. This program calls for repeated, annual mass drug administration (MDA) of single-dose diethylcarbamazine with albendazole in all known endemic areas. Molecular xenomonitoring (MX, detection of parasite DNA in vectors) has been proposed, but never before tested, as a tool for monitoring the impact of MDA on filariasis prevalence and transmission. We now present results of early studies performed to validate the approach of MX in Egypt. All samples were tested in Egypt for the presence of the Wuchereria bancrofti Spt repeat DNA sequence by PCR. Preliminary studies led to the approach of testing pools (range 5-25 per pool) of resting mosquitoes (mainly Cx. pipiens, the principal filariasis vector in Egypt) collected late at night in randomly-selected households in study villages. This provides estimates of household and (with Poolscreen2 software) mosquito infection rates. Four sentinel villages (2 high prevalence
villages and 2 with low prevalence rates more typical for Egypt) were studied in early 2000, prior to the first round of MDA. Annual follow-up data are available through 2003, reflecting effects of 3 rounds of MDA. Household and mosquito infection rates fell by 62-78% and 75-80% in the high prevalence villages, respectively; low-level transmission continues in these villages. No infected mosquitoes were detected after 3 rounds of MDA in the low prevalence villages (suggesting interruption of transmission in these locations). These results are consistent with changes in other parameters that reflect MDA impact (prevalence rates for microfilariaemia, parasite antigenemia, and anti-filarial antibodies). Data for the fourth round of MDA will also be presented at the meeting. We conclude that MX is a powerful tool for monitoring effects of MDA and progress in filariasis elimination programs. It also holds great promise as a means of demonstrating interruption of transmission.

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**COMPARISON OF A NOVEL REALTIME-PCR PROTOCOL WITH CONVENTIONAL-PCR FOR DETECTION OF WUCHERERIA BANCROFTI DNA IN MOSQUITOES COLLECTED IN EGYPT AND PAPUA NEW GUINEA**


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Molecular xenomonitoring (MX) has been proposed as a potentially useful tool for monitoring progress in filariasis elimination programs. High-throughput methods are needed for the practical application of MX in large-scale programs. We have developed a realtime-PCR method for detecting *Wuchereria bancrofti* DNA with primers specific for the LDR1 repeat DNA through (C-PCR, amplification of DNA with primers specific for the LDR1 repeat DNA with *SspI* repeat DNA with NV1/NV2 primers and detection of product by agarose gel electrophoresis). The two methods had comparable sensitivity for detection of genomic DNA from microfilariae. We performed blinded testing of DNA extracts from mosquito pools collected in houses. Realtime-PCR detected parasite DNA in 42 of 222 *Culex pipiens* pools from Egypt (a mix of samples collected prior to and after MDA). Realtime-PCR results agreed with C-PCR in 95% of these samples. Twelve mosquito pools were initially positive only by realtime-PCR. Six of these pools were weakly positive when they were retested by C-PCR. Realtime-PCR detected parasite DNA in 60 of 162 *Anopheles punctulatus* pools collected in a filariasis-endemic area in Papua New Guinea. Some pools contained recently fed mosquitoes; others were host-seeking. Concordance between the two methods was 97% for PNG samples. Ongoing studies are using realtime-PCR to compare mosquito pool infection rates before and after mass drug administration. Realtime-PCR appears to be slightly more sensitive than C-PCR for detection of *W. bancrofti* DNA in pooled mosquitoes. Other advantages of realtime-PCR include its high-throughput capacity (amplification and readout for 96 reactions in 2 hours) and elimination of the need for a separate procedure for detecting PCR products. This reduces the risk of cross-contamination in the lab. Apart from the cost of the instrument, the cost per test for realtime-PCR is similar to C-PCR. The advantages of realtime-PCR make it an attractive option for regional laboratories responsible for testing large numbers of mosquito samples to monitor progress in filariasis elimination programs.

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**WUCHERERIA BANCROFTI INFECTION RATES IN HUMANS AND MOSQUITOES REMAIN SUPPRESSED AT NEAR ZERO LEVELS FOUR YEARS AFTER CESSATION OF MASS TREATMENT IN PAPUA NEW GUINEA**

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The Global Program to Elimination Lymphatic Filarialis currently relies on mass treatment with four to six annual doses of antifilarial drugs. The goal is to reduce the reservoir of microfilariae in the blood to a level that is insufficient to maintain transmission by the mosquito vector. Between 1994 and 1998 four annual mass drug administrations using single dose of DEC plus ivermectin or DEC alone were successfully carried out in 8 villages in Papua New Guinea at a coverage rate of over 80%. Impact assessment performed soon after the fourth treatment in 1998 revealed over 90% reduction in infection rates in both humans and mosquitoes. Pre-treatment microfilaria (mf) rates ranging from 32% to 68% were reduced to less than 4% in all villages. Annual infectivity rate of the *Anopheles punctulatus* vector decreased from 2% before treatment to 0.1% after treatment. Further treatment did not occur in any of these villages until July 2003 when surveys were conducted in three of them to determine new rates of microfilaraemia. Monthly entomological investigations were also carried out from August 2003 to January 2004 in the village with the highest mf rate (48%). An assessment of mf rates in the three villages showed that they have continued to decline despite the cessation of mass treatment with village specific mf rates ranging between zero to 1%. Only three of the 598 people examined were mf positive and none of them had been previously treated. Also none of the 750 mosquitoes dissected had L3 larvae and only one was infected with a single L1 larvae. Our results suggest that fewer than six rounds of mass treatment may be necessary to interrupt *Anopheles*-transmitted *W. bancrofti* in areas with pre-treatment mf rates of less than 50%.

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**IVERMECTIN TREATMENT MAY SELECT FOR A β-TUBULIN GENOTYPE IN ONCHOCERCA VOLVULUS: IMPLICATIONS FOR WORMS FERTILITY, CMFL AND NODULE DENSITY**

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A reduction in response to ivermectin (IVM) in *Onchocerca volvulus*, could adversely affect the onchocerciasis control programs. *O. volvulus* was obtained from patients in Cameroon, sampled before (1994), and after 3 years of IVM treatment (1997). There were 4 treatment groups: 150µg/kg (1x or 4x/pa) and 800µg/kg (1x or 4x/pa). The nodules were digested to release microfilariae. DNA was extracted (230 female worms from 1994, 165 female worms in 1997) and genotyped, blinded, for β-tubulin. IVM treatment selects for β-tubulin heterozygote genotype “ab”. In 1994, ab made up 18.7% of the worms. Following 3 years of IVM, the proportion of ab increased to 50.5% in the *O. volvulus* from the 1x/pa, and to 63.9% in
worms from the 4×pa treatment. Furthermore, following 3 years of IVM, the frequency of ab was 90% in worms collected in highly endemic villages (community microfilarial load > 70 microfilariae (mf)/mm²), and only 50.7% in those with lower endemicity. The reproductive status of female worms was determined, by blinded microscopic exam for the presence of mf and embryo. Prior to treatment, heterozygotes were less fertile than β-tubulin homozygous worms (respectively, 37.2% vs. 67.9% producing mf). However, this difference decreased with frequent treatment. The proportion of infertile worms, respectively for untreated, yearly treated (1 year after last IVM) and quarterly treated patients (3 months after last IVM), increased from 3.7% to 14.6% to 40.9% for the homozygotes. For the β-tubulin heterozygotes, the proportion infertile was 16.2%, increasing to 46.9% and remaining at 46.1%, for the same respective treatment histories. These results indicate that, (1) the frequency of heterozygous female worms increased markedly with treatment, suggesting that heterozygotes may be better able to survive repeated IVM, and (2) following heavy, or recent, IVM treatment (4×/p.a.), the reproductive disadvantage of the heterozygotes largely disappeared. The ability to survive better with a comparable fertility after recent IVM treatment, could allow the heterozygous worms to have an advantage under IVM treatment, compared with the wild-type “aa” worms. This study indicates how IVM resistance may develop in O. volvulus. Further work is urgently required to (i) confirm that there is a sub-population of O. volvulus better able to tolerate IVM, and (ii) to fully characterize the relationship between responses to IVM and genotypic changes. (ACMCIP abstract)

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**BRUGIA PAHANGI LARVAE DISPLAY DIFFERENCES IN GENE EXPRESSION FOLLOWING INTRADERMAL AND INTRAPERITONEAL INJECTION IN THE MONGOLIAN GERBIL**

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Under natural conditions, *Brugia* larvae are deposited on the skin of a human by an infected mosquito, and must then migrate through the various skin layers to establish infection within the lymphatics. This migration has been mimicked in our laboratory with the development of an intradermal (ID) inoculation model using the gerbil and *B. pahangi*. Experiments investigating this migration model have shown that at 3 days post infection (DPI) a majority of the larvae inoculated ID are present in the popliteal lymph node (PLN). Following 3 DPI, most of the larvae have left the PLN. We have classified larvae isolated from the PLN at 3 DPI as migrating larvae (IDL3). In contrast, larvae that are injected intraperitoneally (IP) do not migrate away from the peritoneal cavity and have been classified as non-migrating larvae (IPL3). Total RNA was extracted from 3 day IDL3 and IPL3, and suppressive subtractive hybridization was conducted. Analysis of the resulting subtracted cDNA showed the presence of 4 strongly expressed bands in IDL3. These bands have been identified as encoding tropoion, hemolysin, abundant larval transcript-2 and cytochrome oxidase 1. Preliminary data using semi-quantitative and quantitative RT-PCR suggests these genes are up-regulated in IDL3, compared to IPL3. These data suggest that larvae inoculated into different sites within a host show altered gene expression profiles. Whether this is due to differences in larval activity, such as migration versus no migration, or is a result of varying host responses directed at larvae within each site is currently unknown. (ACMCIP abstract)

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**EFFECT OF FUNCTIONAL ABOLITION OF MACROPHAGES IN BRUGIA PAHANGI INFECTIONS OF MICE**

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Previous studies have used carrageenan as a macrophage toxic agent to demonstrate that they are involved in the immune resistance to experimental filarial infections. Intraperitoneal injection of carrageenan abolishes resistance of mice to brugian infections, making them more permissive. This has been assumed to be due to the macrophage toxic effect of carrageenan. In order to better understand this phenomenon we injected wild type B6 mice with 250µg of type II carrageenan in 500µl PBS i.p. one day prior to intraperitoneal infection with *Brugia pahangi*. Consistent with previous observations, we observed a significant increase in the number of larvae in treated mice compared to controls. This was associated with a decrease in the numbers of peritoneal exudate cells (hereafter PEC). The decrease involved all cell types. Increased numbers of worms were present as late as seven weeks post infection. The global deficit in cell recruitment is reminiscent of a T-cell defect, as seen in TCRβδ−/− mice. We hypothesized that carrageenan was phagocytosed by antigen presenting cells and blocked their ability to prime T-cells. To test this hypothesis, we primed mice with *B. pahangi* with or without carrageenan pretreatment and challenged them with *B. pahangi* after 6 to 8 weeks. 9 days post secondary infection, mice that had been treated with carrageenan before the priming infection had significantly greater worm burdens from both the primary and secondary infections as compared to PBS treated mice. However macrophage and total PEC numbers were comparable in the two groups. Treatment of mice 6 to 8 weeks prior to infection eliminated the larvae with kinetics similar to wild type mice suggesting that the defect was dependent on the presence of carrageenan at the time of priming. These data suggest that carrageenan has at least two modes of action. One appears to be a delay in T cell priming. The other may be a direct action at the effector phase of macrophage function. (ACMCIP abstract)

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**CIRCULATING IgM IS ESSENTIAL FOR THE CLEARANCE OF BRUGIA PAHANGI INFECTION IN MICE**

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Our laboratory has previously demonstrated that B6.129S2-Igh-6tm1Cgn/µMT mice, which lack mature B lymphocytes, are severely impaired in clearing intraperitoneal infections with *Brugia pahangi*. Similarly C57BL/6 mice, which are deficient in B1 cells, clearing filarial infections more slowly than CBA/CaJ/J mice. These results suggest that B lymphocytes, specifically the B1 subset and/or their products, play critical role(s) in establishing anti-parasite immunity. One of the predominant functions of B1 cell subset is to secrete IgM isotype antibodies with broad specificity and low affinity. To investigate the role of natural IgM in our parasite model, we utilized mice that specifically lack secreted form of IgM (slgM−/−). Membrane IgM and other immunoglobulin isotypes are normal. Peritoneal infections with *B. pahangi* reveal striking differences in inflammatory responses at the site of infection between slgM deficient mice and wild type cohorts. The mean peritoneal cells numbers at two weeks post infection were around ~40x10⁶ in slgM−/− mice, and 25x10⁶ in C57BL/6 mice. Wt mice harbor approximately 5% worms at two weeks post-infection; slgM−/− mice have 25-30%. These results suggest that IgM immunoglobulin is not essential for leukocyte recruitment to the inflammatory site, but is important for worm elimi-
infection. We then examined the role of circulating IgM in secondary immune responses against the parasite. Normal wt mice exhibit accelerated clearance of the challenge infection while slgM-/- mice bore high parasite burdens from both primary as well as challenge infections. In this study demonstrates a crucial role for secreted IgM in eliminating both primary as well as challenge infections of the helminth parasite Brugia pahangi.

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ANTIGENIC PROFILE OF NYMPHAL DERMACENTOR VARIABILIS SALIVARY GLAND PROTEINS FED ON A SYLVTIC HOST, PEROMYSCUS LEUCOPUS: CONSTRUCTION AND ANALYSIS OF A D. VARIABILIS SPECIFIC SALIVARY GLAND LIBRARY

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The American dog tick, Dermacentor variabilis, is the most common vector of Rocky Mountain Spotted Fever in the eastern United States. Larval and nymphal D. variabilis readily feed on white footed mice, Peromyscus leucopus, without rejection. When a tick feeds many immunogenic molecules found in the saliva are injected into the hosts which, in turn, cause host responses such as antibody production and cellular response. These molecules may also be involved in down regulation of host responses to tick salivary gland proteins, thus allowing the tick to feed to repletion. Such a response was evaluated in P. leucopus by salivary gland proteins from D. variabilis. An immunogenic 25 kD protein was identified by western blot analysis of whole gland homogenates from nymphal ticks blotted with sera from infested mice. Edman degradation of the immunogenic protein was performed and the resulting peptide sequence was compared to a full-length cDNA sequenced library of Ixodes scapularis with no matches. To further confirm the presence and evaluate the role of this protein a full length cDNA library was constructed using #5 four day fed D. variabilis nymphs. 2000 randomly selected clones were sequenced resulting in 704 clusters of which 130 were secreted salivary gland proteins. Salivary glands of D. variabilis contain many proteins of unknown function and several proteins that are homologous to other blood feeding arthropods. The 25 kD immunogenic protein of interest was found among the sequences and was cloned into a PCR NT7-TOPO expression vector and expressed using a cell free rapid translation system. Sera from a subset of 802 field caught P. leucopus were tested by western blot against the purified expressed protein. Exposure to D. variabilis was confirmed in wild caught rodents, thus suggesting immunogenic response to salivary gland proteins could be used to assay for tick exposure.

900

CO-INFECTION OF FRANCISELLA TULARENSIS AND A FRANCISELLA SYMBIONT IN DOG TICKS ON MARTHA’S VINEYARD, MASSACHUSETTS

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Dog ticks, Dermacentor spp., are known vectors of Franciscella tularensis, the agent of tularemia. Despite their widespread distribution across North America and their anthropophily, tularemic cases due to dog tick bite are relatively uncommon. Field studies suggest that the prevalence of F. tularensis in dog ticks is usually very low. The factors that limit the prevalence and distribution of F. tularensis in dog ticks remain undescribed. Similar questions concerning Rickettsia rickettsii, the agent of Rocky Mountain Spotted Fever, have been explained by the presence of a non-pathogenic rickettsia, Rickettsia peacockii, in its vector, Dermacentor andersoni. Infection of germline tissues with one rickettsia species tends to prevent superinfection and subsequent transovarial transmission of a second rickettsia. In this manner, infection by a preexisting related rickettsial species prevents perpetuation of another within a site. With the reclassification of the Dermacentor variabilis symbiont (DVS), formerly known as Wolbachia persica, as a Franciscella, we sought to evaluate whether the analogous situation may exist between DVS and F. tularensis. Accordingly, we collected dog ticks from Martha’s Vineyard, Massachusetts, the site of a recent outbreak of tularemia, and tested them for evidence of co-infection with Franciscella spp. As many as 87% of 243 dog ticks (95% CI [82, 91]) on Martha’s Vineyard are infected with the Franciscella symbiont. Furthermore, ticks harboring the symbiont were equally likely to be infected with F. tularensis as those without (OR=1.06, 95% CI [0.1, 8.9]). Therefore, we conclude that DVS is a common infection of dog ticks and that its presence does not necessarily prevent an enzootic cycle of F. tularensis on Martha’s Vineyard.

901

THE IXODES SCAPULARIS GENOME PROJECT

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Ticks transmit the greatest variety of human and animal pathogens of any arthropod vector and are second only to mosquitoes as vectors of human disease. Diseases transmitted by blood feeding ixodid ticks are global medical and veterinary health problems and include bacterial, rickettsial, viral and protozoan diseases. In addition, a number of tick-borne pathogens are considered to be emerging infectious diseases or potential agents of bio-terrorism. In the United States, Ixodes scapularis is the most important tick species from a human health perspective. Ixodes scapularis transmits Lyme disease, babesiosis, human granulocytic anaplasmosis and possibly the flaviviral agent of Powassan encephalitis. An international consortium of scientists has been established to sequence the I. scapularis genome. The I. scapularis Genome Project (IGP) was recently approved through the NIAID Microbial Sequencing Centers Program. The overall goal of the IGP is to generate draft I. scapularis genomic sequence as a resource for the scientific community. The IGP represents an important scientific advance and will provide opportunities to identify new strategies for control of ticks and tick-borne diseases. The IGP will also provide the first genomic analysis of the taxonomically diverse subphylum Chelicerata and will significantly expand the scope of comparative and evolutionary eukaryotic analysis. We are currently generating I. scapularis cDNA, Bacterial Artificial Chromosome (BAC) and genomic libraries. In the initial phase of the IGP we aim to undertake extensive sequencing of pooled, normalized cDNA libraries representing a variety of I. scapularis tissues, extensive BAC end sequencing and complete sequencing of four BAC clones. This sequence data will provide a preliminary analysis of the Ixodes genome and will be used to guide subsequent whole genome random shotgun sequencing efforts. Our progress towards these and future goals will be discussed.

902

CIRCULATION OF MULTIPLE BORRELIA BURGDORFERI CLONES IN PEROMYSCUS LEUCOPUS

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Lyme disease is caused by the spirochete *Borrelia burgdorferi* that is maintained in a rodent reservoir, primarily *Peromyscus leucopus*. Once infected, *P. leucopus* remains infected for life. However, new infections may be introduced through subsequent bites by the vector *Ixodes scapularis*. Over a period of two years, *P. leucopus* were sampled multiple times. Thirty animals were captured and sampled at least twice. Tissue from these animals was analyzed for *B. burgdorferi* infection by PCR for outer surface protein C (ospC). OspC is highly variable among clonal populations of the bacteria. Samples from ospC-positive individuals were analyzed for genetic variability using single-strand conformation polymorphism (SSCP) analysis and sequencing. The SSCP banding pattern shows that, of the thirty individuals, 11 appear to be infected with different ospC genotypes at different sampling times. Polymorphism analysis based on PCR from a potentially mixed clone source could present bias in the genotypic representation of the sample. Therefore, cloning and re-analysis by SSCP were used to confirm the observed clonal changes in ospC. Preliminary results from SSCP of cloned samples confirm that multiple genotypes of ospC can be found in an individual at different time points. However, some individuals remain infected with only one genotype throughout the sampling period. Understanding how frequently reservoirs are re-exposed to locally circulating populations of *B. burgdorferi* will further our understanding of the maintenance and transmission of this pathogen.

### 903

**IDENTIFYING AREAS OF INCREASED RISK FOR INFECTION WITH BABESIA MICROTI IN CT USING GIS AND CLUSTER ANALYSIS**

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*Babesia microti* is an intraerythrocytic protozoan parasite that can be transmitted by the deer tick, *Ixodes scapularis*, as well as by blood transfusion. There have been over 40 documented cases of transmission through blood transfusion in the US. This is a concern for the blood supply because some individuals do not exhibit symptoms of the infection; however, the infection can be severe or even fatal in immunocompromised, elderly or asplenic individuals. Geographic Information Systems (GIS) are computer-based mapping programs that can be used to assess and monitor such infectious diseases. Thus, this tool can be utilized in blood centers and other facilities to map individuals' risk-factors in space and/or time. This information can then be utilized to analyze the endemicity of the agent and consider the feasibility of geographic exclusion criteria for blood donation. An ongoing study is being conducted to determine the seroprevalence of *B. microti* in CT blood donors. Blood collections from southeastern CT were screened for *B. microti* antibodies using IFA (positive > 1:64) during the months of May through December in 2000 through 2003. Areas of increased risk were identified using GIS and cluster analysis based on donor residence. GIS ArcView 8 software was used for mapping and the Poisson model of SaTScan 3.1 software was used to identify purely spatial and retrospective space-time clusters of increased risk. Statistically significant areas of increased risk (clusters) were identified within southeastern CT using these methods. One prominent cluster overlapped an eleven town area. With space time analysis there appeared to be westward movement of seroprevalence within this cluster during the study period. Additional analyses can be conducted by adding environmental risk factors such as vegetation and soil type, which are associated with tick-borne disease spread. In conclusion this technology can be used to identify geographic areas of increased risk for vector-borne diseases and thereby enhance blood safety.

### 904

**IS THE U.S. BLOOD SUPPLY SAFE FROM HUMAN GRANULOCYTIC EHRLICHIOSIS (HGE)? SEROPREVALENCE AND PERSISTENCE OF ANAPLASMA PHAGOCYTOPHILUM IN CONNECTICUT BLOOD DONORS**

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HGE is a recently identified tick-borne disease caused by *Anaplasma phagocytophilum*. Although this agent produces mild disease in healthy individuals, it can be severe or fatal in the immunocompromised, including many blood transfusion recipients. While *A. phagocytophilum* has been reportedly transmitted through blood transfusion, its prevalence among US blood donors is not known. Therefore, we evaluated the seroprevalence and persistence of *A. phagocytophilum* in Connecticut blood donors. Donors were invited to participate in an ongoing *Babesia microti* seroprevalence study during the 2001-2003 transmission seasons. Donor consent permitted further research of tick-borne diseases, including HGE. Samples were tested by IFA (cutoff of 1:64). Several donors were repeat donors; all donations were analyzed. In 2001, 2,007 samples were tested, 83 positive (4.1%). In 2002, 2,105 samples were tested, 68 positive (3.2%). In 2003, 1,952 samples were tested, 70 positive (3.6%). Of the seropositive donors, 52 were repeat donors, donating at least three times in the 3-year period. Fourteen donors were seronegative and seroconverted subsequently, 3 of these donors cleared the infection. Seventeen initially seropositive donors later cleared the infection. Twenty-one donors identified as seropositive remained as such at each subsequent donation. Examination of repeat donations demonstrates several new infections, antibody persistence over time, and clearance of antibodies and presumably the agent. It is unclear if antibody persistence or apparent acute infections pose a risk for transfusion transmission. While only one transfusion case has been reported, the agent has the ability to survive in stored blood at 4°C. It is possible that *A. phagocytophilum* is removed from blood prior to transfusion through leukoreduction, the reported case of transfusion-transmitted HGE involved 2 units of non-leukoreduced RBCs. Further research is needed to define the blood safety risks associated with this agent.

**ACMCIP abstract**

### 905

**A SUSTAINED-RELEASE FORMULATION OF DOXYCYCLINE HYCLATE (ATRIDOX™) PREVENTS SIMULTANEOUS TRANSMISSION OF ANAPLASMA PHAGOCYTOPHILUM AND BORRELIA BURGDORFERI BY IXODES SCAPULARIS TICKS**

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Given the lack of a vaccine, prophylaxis for infected tick bite consists only of personal protective measures directed against ticks. The need for effective prophylaxis is underscored by an increase in Lyme disease cases and the seriousness of untreatable sequelae in a subset of individuals. This study compares the efficacy of a single oral dose of doxycycline hyclate to that of an injection of sustained-release doxycycline in a mouse model of Lyme borreliosis and *Anaplasma phagocytophilum*. Pharmacokinetic studies indicated peak plasma values of 2mg oral doxycycline hyclate (2.43+/-0.9
A PROSPECTIVE STUDY OF DAILY COTRIMOXAZOLE PROPHYLAXIS IN KENYAN HIV-INFECTED ADULTS AND THE DEVELOPMENT OF ANTIMICROBIAL RESISTANCE

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Cotrimoxazole (CTX) prophylaxis reduces morbidity and mortality in HIV-infected African adults. In 2000, UNAIDS/WHO recommended adults with symptomatic HIV receive daily CTX. In Africa, the effect of daily CTX on antimicrobial resistance in common pathogens is unknown. In a prospective study, we measured prevalence and resistance among colonizing S. pneumoniae (Spn) and E. coli and prevalence and incidence rate of Plasmodium falciparum (Pf) parasitemia. We assigned 690 HIV infected adults with CD4 counts >350 cells/µl daily CTX (arm1) and 338 with CD4 counts ≥350 cells/µl daily multivitamins (arm2). We measured changes in resistance over 6 months. Incidence rate of Pf parasitemia was 18 and 140/100 person-years in arms1 and 2 respectively (Rate Ratio = 0.13 [95% Confidence Interval 0.09-0.18], p <0.001). Sulfadoxine-pyrimethamine (SP) treatment failure of clinical malaria while receiving study drugs was low in both arm1 (5% [4/84]) and arm2 (2% [3/186]) at day 7 or 14 (p=0.21). Among isolated E. coli, baseline CTX-resistance increased from 79% (413/522) to 97% (161/166) at 2 weeks and to 94% (260/275) at 6 months in arm1. Among isolated Spn, baseline CTX resistance increased from 91% (93/102) to 100% (58/58) at 2 weeks in arm1. Bacterial resistance did not change from baseline in arm2. Overall carriage of resistant Spn slightly declined from 21% to 18%. Pf parasitemia incidence rate was much lower among clients taking daily CTX. SP remained effective for breakthrough Pf parasitemia. High baseline resistance in Spn increased with CTX, but overall, the percent of adults carrying resistant Spn decreased. CTX use increased already elevated resistance in commensal E. coli, which might reflect increased CTX-resistance in enteric pathogens, such as Shigella. Daily CTX prophylaxis is unlikely to lead to widespread increases in antifolate drug resistant Pf or Spn in Kenya, but could increase CTX resistance among enteric pathogens. If daily CTX prophylaxis is adopted into national policy, use of alternative antibiotics to treat enteric diseases may be necessary.

RISK FACTORS FOR MOTHER-TO-CHILD TRANSMISSION OF HIV IN MALAWI

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This study was undertaken to examine the clinical features and evolution of patients with multidrug-resistant tuberculosis (MDRTB) and HIV receiving individualized MDRTB treatment in Perú. A retrospective review was conducted of infected patients treated with individualized MDRTB regimens between August 1996 and December 2003. All patients with MDRTB enrolled in our cohort were screened for HIV. Among the 1687 patients enrolled through 12/03, 22 (1.3%) were coinfected with HIV. The median age was 27 years and 36% of the patients were female. The median CD4 count was 154 and the median viral load during MDR treatment was 5.03 log (n=90). Eight of 22 patients in this cohort received combination antiretroviral therapy (ARTs). Of those with viral load (VL) monitoring on therapy (n=5), the median VL on ARVs was 2.94 log. The median number of prior TB treatments was 2, with resistance to a median of 5 TB drugs. Extrapolmonary disease was present in 9 of 22 patients (41%) with the most common sites of involvement being lymphatic (55%) followed by TB-meningitis (33%). The most common opportunistic infections included oral candidiasis in 82%, esophageal candidiasis in 18%, recurrent bacterial pneumonia in 14% and cryptococcosis in 9%. Important complications of MDRTB therapy included reversible renal dysfunction requiring adjustment or temporary discontinuation of injectables (aminoglycosides and capreomycin) in 40% and peripheral neuropathy in 20%. The TB-related outcomes of this cohort include: 1 patient has completed MDRTB treatment and is cured, 2 patients have defaulted and 10 patients (45%) have died (median time 5.6 months) with only 2/10 (20%) deaths ascribed to TB. Among alive patients treated for >4 months, the median time on treatment is 15 months. Smear and culture conversion among all patients in treatment for >4 months occurred in 13 of 18 patients (72%), yet despite smear/culture conversion, 6 out of 13 patients (46%) developed new AIDS-defining illnesses during MDRTB treatment. Two patients among the 8 patients receiving ARVs have died (25%), compared to 8 out of 14 not on ARVs (57%). In conclusion, coinfected patients can have significant HIV-related morbidity and mortality despite effective individualized regimens for MDRTB therapy. Increased access to antiretroviral therapy in resource-poor settings is urgently needed to improve clinical outcomes and slow progression to AIDS and death in coinfected patients treated for MDRTB.
We designed a case-cohort study nested within an on-going study of pregnant women at Queen Elizabeth Central Hospital in Blantyre, Malawi to describe maternal risk factors associated with mother-to-child HIV transmission. Cases of vertical transmission were categorized as either intrapartnerine (IU, n = 39) or intrapartum (IP, n = 49) and both were compared individually to a randomly chosen subcohort of HIV positive pregnant women (n=144). The main factors investigated included the following: maternal HIV RNA viral load, maternal malaria, maternal HIV proviral load, placental microtransfuson, and standard obstetric and gynecological features. HIV proviral load was assessed by a quantitative Real-Time PCR assay and it was strongly correlated with HIV viral load (regression coefficient: 2.44, 95% CI 1.97-2.90). In a univariate analysis, proviral load was significantly associated with IU transmission (P=0.023), while HIV RNA load was not significantly associated with either type of transmission. Placental microtransfuson was assessed by quantification of placental alkaline phosphatase activity in umbilical cord serum, and in a univariate analysis, it was associated with IP transmission. No association between malaria and transmission was evident. These data suggest that HIV transmission could occur in part as a result of a breakdown in the placental barrier before and during parturition.

**909**

**VIRAL, HLA AND T CELL ELEMENTS IN CROSS REACTIVE IMMUNE RESPONSES TO HIV-1 SUBTYPES A, CRF01_AE AND A CRF02_AG VACCINE SEQUENCE IN IVORIAN BLOOD DONORS**

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The most desirable HIV vaccine will induce immune responses against multiple HIV subtypes and recombinants. Our objective was to study the role of HLA, virus and T cell degeneracy, in the establishment and maintenance of cross reactive immune responses. The ELISPOT assay was used to study T-cell cross reactivity in 15 HIV-1 infected blood donors from Cote d’Ivoire to overlapping gag and env peptides from a CRF02_AG vaccine sequence, a western Kenya subtype A virus and a consensus CRF01_AE sequence from Thailand. Peptides were tested in subtype-specific pools or peptide matrices. HLA typing was done by PCR. Infecting viruses were classified by sequencing viral DNA from PBMC. All donors had at least one positive response. For gag, epitope mapping showed that 7 of 8 (87.5%) individuals responded to all three subtypes. The number of gag epitopes recognized by an individual ranged from 1-7. All donors were heterozygous for the 3 HLA class I loci. There was no association between the level of cross reactivity or the number of epitopes an individual recognized and HLA heterozygosity, measured as supertypes. Six of 8 (75%) individuals recognized at least 1 epitope that was conserved among the subtypes. For env, all the 3 individuals, for whom epitopes were mapped, recognized 2 subtypes, however, cross reactive responses were to alternative and not corresponding epitopes. In general, within env, abolition of a response in a subtype was characterized by at least 3 amino acid changes in the peptide. In some individuals, the peptide recognized was a variant of the infecting viral sequence, varying by up to 3 amino acids. The presence of broad cross subtype responses suggests that a single viral vaccine may be used in regions with different circulating viruses.

**910**

**COMPREHENSIVE HIV/AIDS TRAINING FOR NURSES IN EL SALVADOR**

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We describe planning and implementation of an HIV/AIDS nurse-training program in El Salvador. Aims were to provide focused, comprehensive, interdisciplinary training in HIV/AIDS for registered nurses serving adult and pediatric patients and to establish a local capacity for prevention of HIV infection and care of individuals with HIV/AIDS. The curriculum uses adult learning principles, emphasizes voluntary counseling and testing, decreasing stigma and discrimination, and had a computer literacy component. Each month long course had 20 students. The best students from the first two courses were trained to serve as instructors for subsequent courses. Instruction was provided in the classroom and in institutions caring for people with HIV/AIDS. The first course began in February, 2004; 100 nurses had been trained by the end of July, 2004. Planning involved several key activities: development of the structure of the program and curriculum, identification of leaders and their responsibilities and of local resources (e.g., educators and educational materials); and recruitment and selection of students. Key participants included representatives of the Salvadorian Ministry of Health, a local nursing society and its school, and local experts in HIV/AIDS and nursing education. The early phase of implementation was characterized by close monitoring of the course’s administration and participants; we modified the structure and content of the course as a direct result of these evaluations. The middle and late phases of implementation concentrated on retaining the best teachers and organizing student projects. Communication among team members through e-mail, workshops, and internet conferencing was key to accomplishing objectives. Course evaluations by students were uniformly positive. The course was well suited to the Salvadorian students. Success was achieved through careful organization, consideration of local needs, creative use of available resources, and flexibility to meet students’ needs.

**911**

**CHANGE IN HEALTH-RELATED QUALITY OF LIFE (HRQOL) INDICATORS AMONG HIV-INFECTED AND UNINFECTED KENYAN ADULTS ENROLLED IN A RESEARCH STUDY TO MEASURE ANTIMICROBIAL RESISTANCE DEVELOPMENT ASSOCIATED WITH DAILY COTRIMOXAZOLE (CTX)**

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Daily cotrimoxazole (CTX) prophylaxis reduces morbidity in HIV-infected African adults. HRQOL indicators provide subjective measures to evaluate CTX prophylaxis programs. As part of an antimicrobial resistance study, we provided HIV-infected adults with CD4 cell counts <350 cells/µL daily CTX (arm 2). Adults with CD4 cell counts ≥350 cells/µL (arm 3) and HIV-negative clients (arm 1) received daily multivitamins. All were followed monthly in clinic and received medical care during routine and sick visits for 6
months. Clients were interviewed at enrollment, when the study drug was provided, and at month 6 visit. We measured change in HRQOL in 8 dimensions of health. Effect size to analyze clinical significance of HRQOL changes was calculated. Findings were controlled for age, gender and marital status. We enrolled 205 clients: 50 (24.4%) in arm 1, 100 (48.8%) in arm 2, and 55 (26.8%) in arm 3. In total, 147 (71.7%) completed month 6 visit. All arms showed significant improvements in HRQOL in Overall Health and General Health Perceptions. In addition, arm 2 showed significant improvements in Social Functioning. (Mean change (MC) +20.3; p<0.002), Emotional Well-Being (MC +18.1; p<0.001), Energy Levels (MC +17.7; p<0.001), and Pain (MC +26.3; p<0.001). Worsening HRQOL was observed in Physical Functioning for HIV-infected clients (arms 2 and 3) and in Role Functioning for all arms. Arm 2 showed the largest effect size in 5 dimensions. Older age, female sex and unmarried status were risks for worsening HRQOL in specific dimensions. In conclusion, clients in arm 2 reported greater overall improvement in HRQOL, and were more likely to report greater improvements than those in arms 1 and 3, suggesting that CTX prophylaxis provided in a clinical care program is associated with improved HRQOL. Improvements were also observed in 2 dimensions among those in arms not receiving CTX, suggesting that improved HRQOL occurs without CTX prophylaxis, and may be related to general health care provision, the development of support networks or the effect of the multivitamins.

**HIV-1, HTLV-1 AND HEPATITIS CO-INFECTIONS AMONG MULTITRANSFUSED PATIENTS IN PERU**

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In addition to human immunodeficiency virus type 1 (HIV-1), other retroviruses such as human T-cell lymphotropic virus type II (HTLV-I/II) and hepatitis viruses B and C have been recognized worldwide in individuals exposed to transfusions. Limited data are available on the infection rates with these pathogens in this setting. A PAHO sponsored soroepidemiologic study was conducted among adult multitransfused patients in Peru as part of a strategy to improve blood safety in Latin America. Inclusion criteria, 1) reception of a total of at least ten units of allogenic blood or blood products (i.e. whole blood, plasma, red blood cells or platelets), on at least two different occasions and/or 2) hemodialysis in at least 3 occasions previously. During February 2003 and March 2004, volunteer blood samples were collected in 7 hospitals in Peru and tested for evidence of infection with hepatitis C virus (HCV), HIV-1, HTLV-I/II, hepatitis B virus (HBV) surface antigen (HBsAg) and anti-core antibody (anti-HBc). Samples were screened by enzyme-linked immunosorbent assay (ELISA); neutralization was used to confirm HBV infection; Western blot (Genelabs) was used as a confirmatory assay for HTLV-I/II ELISA-positive samples. A total of 322 volunteers were enrolled in the study, of which 125 (39%) were found to have been infected with HBV,19(6%) of the volunteers were found to be HBsAg/neutralization-positive. HTLV-I/II infection was confirmed in 11 (3.4%), 5(1.6%) of which were co-infected with HCV and 2(0.6%) with HBV. In addition, HBV/HCV and HIV-I/HCV co-infections were documented in 7(2.2%) and 2(0.6%) of patients, respectively. One patient showed unusual HCV/HBV and HTLV co-infection. These findings document an increased risk for transfusion-transmitted HTLV-I/II infection in Peru. The relatively high rate of HTLV-I/II and HCV co-infections may reflect the high prevalence for infection with these viruses in Peru.

**MULTIPLE ERYTHROCYTE POLYMORPHISMS INCLUDING A GLYCOPHORIN B VARIANT IN A MALARIA ENDEMIC AREA OF PAPUA NEW GUINEA**

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Red blood cell (RBC) polymorphisms have been described in malaria endemic areas of Papua New Guinea and are thought to play a role in decreased malaria susceptibility. Common polymorphisms include deletions within genes for glycoporin C (GYPC∆ex3) and band 3 (SLCA4A1∆27), as well as α-globin (α-globinA∆2). Both GYPC∆ex3 and SLCA4A1∆27 are integral RBC membrane protein polymorphisms which are associated with ovalocytosis and influence malaria susceptibility in vitro. Through our previous studies, we identified another integral RBC membrane protein polymorphism, a glycoporin B (GYPB) variant, with altered mobility by electrophoresis on Western blot. In this study, we investigated the molecular basis of the GYPB variant and its relationship to other RBC polymorphisms. DNA sequence analysis revealed that a recombination event between glycoporin A (GYPA) and GYPB introduces a 682 bp portion of GYPA spanning the exon 3/intron 3 junction and replaces the homologous region of the GYPB pseudogene 3. A functional 5’ splice site from the GYPA gene becomes a part of the GYPB gene through the recombination event to produce a GYP-A-B recombinant allele (Morobe variant) and results in a higher molecular weight protein observed on Western blots. The Morobe variant was observed in 89 of 150 (59%) individuals in our study population; the allele frequency was estimated to be 0.363. In addition, there were 67 (45%) GYPC∆ex3 homozygotes, 30 (20%) SLCA4A1∆27 heterozygotes and 134 (89%) α-globinA∆2 homozygotes. Preliminary data analyses revealed that the 3 integral RBC membrane protein polymorphisms are independently distributed (χ2, p=0.695). Furthermore, all 4 polymorphic genotypes studied were simultaneously present in 20 (13%) individuals. Multiple RBC protein polymorphisms co-exist with high frequency in populations residing in malaria endemic areas of Papua New Guinea. These polymorphisms may have arisen to protect the individual from malaria morbidity and are likely to alter the ability of merozoites to invade erythrocytes. (ACMCIIP abstract)

**PREMATURITY SENECEENCE AND OXIDATIVE DAMAGE OF HEMOGLOBIN C ERYTHROCYTES: A PROTECTIVE MECHANISM AGAINST SEVERE FALCIPARUM MALARIA**

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Epidemiological studies indicate that homozygous hemoglobin C (CC) individuals are protected against severe Plasmodium falciparum malaria when compared to their normal hemoglobin A (AA) counterparts. According to our recent findings, one of the mechanisms of CC protection against malaria involves the formation of abnormal knobs, electron dense protrusions on the surface of infected erythrocytes.
These abnormal knobs are unable to mediate cytoadherence, rosetting or agglutination in vitro, which may reduce the pathological effects of microvasculature sequestration of the parasites in vivo. Although the mechanism by which abnormal knobs are formed is unknown, their morphology suggests major structural perturbations in the CC erythrocyte membrane. In addition to morphological changes in the knob structure, we observed signs of premature senescence and increased oxidative activity in uninfected CC erythrocytes, as evidenced by the three-fold increase in hemichrome levels we found in CC membranes. We therefore determined whether erythrocytic membrane proteins known to participate in knob structures (e.g., spectrin, protein 4.1) showed signs of such oxidative damage in uninfected CC cells. Immunoblotting of AA and CC erythrocyte membrane protein extracts with anti-nitrotyrosine polyclonal antisera revealed that several erythrocyte proteins (45, 65, 90 and 120 kD) were nitrosylated exclusively in CC cells. One of these bands was confirmed to contain protein 4.1 by mass spectroscopy. Oxidative injury of protein 4.1 may explain the characteristic rigidity of uninfected CC erythrocytes as well as abnormal knob morphology of infected CC erythrocytes. Further characterization of oxidative damage in other structural membrane proteins will help to better understand the pathophysiology of CC erythrocytes and to elucidate the molecular mechanism of abnormal knob formation and protection against falciparum malaria.

**915**

**UNEXPECTED INCIDENCE OF CONCURRENT INTUSSUSCEPTIONS IN CHILDREN DYING OF CEREBRAL MALARIA**

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The annual incidence of intussusceptions (involutions of the intestinal tract) in infants and children in the US is 1 in 500 while the incidence in African children has not been reliably established. The most common ‘lead point’ finding in histological preparations of intussusceptions is either lymphoid hyperplasia or the presence of lymph nodes, but 71-100% of intussusceptions do not have an identifiable cause in African children or in the US. A clinical history of an antecedent upper respiratory infection is common, however. Intussusceptions have been observed commonly in our ongoing autopsy-based study. Half of the autopsies (22/43) have yielded gross evident of intussusceptions. In patients with cerebral malaria (on clinical grounds, confirmed by autopsy), intussusceptions were more common than in patients dying with non-malarial coma (16/26 or 62% vs 6/27 or 22%, p=0.005). Histological examination of stomach, small bowel, and colon from a subset of these patients failed to reveal a correlation with lymphoid hyperplasia. Immunohistochemical findings for inflammatory mediators (iNOS, serum hsCRP) will be described, and the association between cerebral malaria, concurrent intussusceptions, and putative pathogenic mechanisms will be discussed.

**916**

**CRANIAL ABNORMALITIES ASSOCIATED WITH MALARIA**

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Malaria during pregnancy causes increased perinatal morbidity and mortality. Associated pathologies include abortion, premature delivery, IUGR, LBW, and failure to thrive but not misshapen heads. We have shown that malaria (Plasmodium coatneyi) in pregnant rhesus monkeys (Macaca mulatta) accurately models human malaria. In the present study, 73 infants were measured in vivo by ultrasound (US) and at delivery with electronic calipers. Both malaria naïve and semi-immune malaria-infected (MI) thescus monkeys delivered infants with microcephaly, dolichocephaly, and/or IUGR. Based on gravidity ranging from 1-4 and P. coatneyi infections 0-3, the monkeys comprised 11 groups including un inoculated controls (gravid 1 & 2) (C). Weekly US measurements included biparietal diameter (BPD) and head circumference (HC). Delivery day measurements included BPD and occipital front diameter (OFD). US HC measurements, although apparently accurate for gestational age, failed to reveal significant (sig) differences in skull shape due to elongation of the OFD and, less often, a narrow BPD. The ratio of the OFD:BPD between infants from MI and C (student T-test) at birth was sig different with MI having larger ratios (p = 0.00424). Infant head comparisons showed a sig longer OFD in MI (p = 0.00172) indicating that the ratio differences were driven by a narrow, more elliptical shaped head (dolichocephaly). A one-way ANOVA comparing the means of 11 groups was highly sig for OFD (0.000) and OFD:BPD ratio (0.008) differences. Post hoc analysis of OFD by (Tukey) and (LSD) revealed sig differences between 5/11 and 10/11 groups respectively. Some infants from naïve MI had small for gestational age HC with smaller BPD and OFD (microcephaly). IL-12 and TNFR 1 and 2 levels in cord blood revealed sig differences between 5/11 and 10/11 groups respectively. Some infants from naïve MI had small for gestational age HC with smaller BPD and OFD (microcephaly). IL-12 and TNFR 1 and 2 levels in cord blood were elevated in dolichocephalic groups while IFNγ levels were lower when compared to unaffected groups. Associated hormone data will also be reported. Our studies indicate that fetal osteogenesis may be dysregulated in MI pregnancies. Cytokines and hormones, important for normal osteogenesis, may be involved.

**917**

**MAXIMAL CEREBELLAR EXPRESSION OF CYTOKINES AND ADHESION MOLECULES IN FATAL HUMAN CEREBRAL MALARIA**

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Although the role of systemic proinflammatory cytokines, IL-1β and TNF-α, and their up-regulation of adhesion molecules, ICAM-1, VCAM-1 and E-Selectin, in the pathogenesis of cerebral malaria (CM) is well established, the role of local cytokine release remain unclear. Immunohistochemistry (IHC) was used to compare the expression of ICAM-1, VCAM-1, E-Selectin, IL-1β, TNF-α and TGF-β at light microscopic level in cerebral, cerebellar and brainstem postmortem cryostat sections from 10 CM, 5 severe malarial anaemia (SMA), 1 purulent bacterial meningitis (PBM), 2 non-central
nervous system infections (NCNSI) and 3 non-infections (NI) deaths in Ghanaian children. Fatal malaria and Salmonella sepsis showed significantly higher vascular expression of all 3 adhesion molecules, though their expression in the sepsis case was not as intense as that in the fatal malaria sections. There was highly significant co-localization of receptor expression with sequestration in the malaria cases, though there was negligible difference in their expression between the CM and SMA sections. TGF-β showed intravascular and perivascular distribution in all cases, but expression was most intense in the PBM case and CM group. TNF-α and IL-1β showed prominent brain parenchymal staining, in addition to intravascular and perivascular staining, in only the PBM case and CM group. The increased expression of the adhesion molecules was associated with increased local proinflammatory cytokine release in the CM sections, but not in the SMA group. The maximal expression of all 6 antigens studied was in the cerebellar sections of the malaria cases. Endothelial activation is a feature of fatal malaria and Salmonella sepsis, with adhesion molecule expression being highly co-localized with sequestration in fatal malaria. IL-1β and TNF-α are expressed in only cases with neurodegenerative lesions, whilst TGF-β is present in all cases. Both cytokines and adhesion molecules were maximally expressed in the cerebellar sections of the malaria cases. (ACMCIP abstract)

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ANALYSIS OF β-CHEMOKINE EXPRESSION IN PERIPHERAL BLOOD OF CHILDREN WITH VARYING DEGREES OF MALARIA DISEASE SEVERITY

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Induction of proinflammatory cytokines and chemokines is believed to be critical in the immunopathogenesis of Plasmodium falciparum infection. Since the role of malaria-induced chemokines in P. falciparum-infected individuals is largely undefined, we investigated the profile of β-chemokines (CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES)) in Gabonese children (n=46) with varying degrees of malaria disease severity. Circulating levels of chemokines were determined by ELISA, and chemokine gene expression profiles were determined in peripheral blood mononuclear cells (PBMC) by real time RT-PCR. Plasma levels of CCL3 and CCL4 were significantly elevated in children with mild malaria (MM) and severe malaria (SM), relative to healthy controls (HC, *P < 0.05). Circulating levels of CCL3 were also significantly higher in children with SM versus those with MM. In contrast, plasma CCL5 levels significantly decreased with increasing disease severity. Additional analyses revealed that healthy, malaria-exposed children with prior severe malaria (PSM) had significantly lower levels of CCL5 than those with prior mild malaria (PMM), while no significant differences were observed between levels of CCL3 and CCL4. Gene expression profiles for the chemokines closely resembled results obtained in plasma, suggesting that circulating blood mononuclear cells primarily account for the observed pattern of chemokine expression in different disease states. Taken together, results here demonstrate a distinct profile of β-chemokine expression in which severe malaria is characterized by increased production of CCL3 and CCL4, and decreased production of CCL5. (ACMCIP abstract)

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DIFFERENTIAL GENE EXPRESSION ANALYSIS OF PATIENTS WITH ASYMPTOMATIC AND SEVERE PLASMODIUM FALCIPARUM MALARIA: A COMPARISON OF CASES FROM BAMAKO AND THE VILLAGE OF MISSIRA, MALI

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Microarray studies can provide a comprehensive picture of gene expression in both diseased and healthy individuals. However, the interpretation of human microarray data is frequently compromised by the variability of the results, by limitations in study design, and by inadequate baseline and control samples. To address these problems in a study of gene expression in malaria, we have taken 3 serial blood samples for RNA isolation and microarray analysis from individuals with symptomatic Plasmodium falciparum infection. The first sample is taken at the time of the patient’s initial presentation (positive thick smear, positive Optimal antigen test, fever or other symptoms or signs). The second sample is taken 3 days later (after 3 days of antimalarial treatment). The third is taken after finishing 3 days of oral treatment (10 days after presentation) when the patient has recovered and is again asymptomatic. Confounding by interindividual differences is controlled by using three samples from the same individual. Controls for assay variability include include the use of duplicate chips at each time point, and the elimination of data for oligonucleotides that are discordant between the two chips. Confounding by changes in baseline gene expression is addressed by using the expression of cytoskeletal and cytoskeleton genes (Gene Ontology classification) as internal controls. Using this strategy, we examined changes in gene expression in children with malaria. Malaria produced significant increases in the expression of genes in the GO classifications for defense response, immune response, response to external stimuli, response to pathogen or parasite, innate immune response, and response to stress (*p<10^-6 to 3 x 10^-7). In contrast, there were no significant changes in the expression of genes in the cytoskeleton or cytoskeleton organization/biogenesis GO classifications (*p>0.38 and 0.62). (ACMCIP abstract)

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A DOUBLE BLIND, RANDOMIZED, CONTROLLED, DOSE ESCALATION PHASE I FIELD TRIAL IN 12 TO 47 MONTH OLD CHILDREN IN WESTERN KENYA TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF FMP1/AS02A MALARIA VACCINE VERSUS RABIES VACCINE

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The falciparum merozoite protein 1 (FMP1) is a lyophilized recombinant antigen expressed in Escherichia coli at the Walter Reed Army Institute of
Research. FMP1 is derived from the 3D7 clone of Plasmodium falciparum and consists of the 42-kDa C-terminal portion (392 amino acids) of the Merozoite Surface Protein-1 (MSP-1\textsubscript{19}). In this, as in four previous clinical trials, it was reconstituted in GlaxoSmithKline Biologicals\textsuperscript{2} proprietary AS02A adjuvant system prior to injection. The FMP1/AS02A candidate malaria vaccine has previously been shown to be safe and immunogenic in adult volunteers in the US and Kenya. We carried out a phase I, double-blind, randomised, controlled, dose-escalation field trial in 135 children aged 12-47 months in Kombewa Division, western Kenya. We evaluated the safety, reactogenicity and immunogenicity of 10, 25 and 50 µg of the vaccine formulated in 100, 250 and 500 microliters in preparation for a phase Ib proof-of-concept study in the same population. Children were recruited into one of the three dosage cohorts and were randomized in a 2:1 fashion to receive either the test product or a comparator (Imovax® rabies vaccine). Vaccinations of the dose cohorts were staggered by 2 weeks. A Data and Safety Monitoring Board reviewed safety reports after each dose and approved the initial dose-escalations. Monitoring of safety and reactogenicity included detailed clinical and laboratory analyses and assessment of adverse events. Safety and immunogenicity results will be presented.

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**DOUBLE BLIND RANDOMIZED CONTROLLED PHASE I Trial TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF WRAIR'S MSP1 CANDIDATE MALARIA ANTIGEN (FMP1) ADJUVANTED IN GLAXOSMITHKLINE BIOLOGICALS' AS02A VS. RABIES VACCINE IN SEMI-IMMUNE ADULTS IN BANDIAGARA, MALI**

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Merozoite surface protein 1 (MSP1), a 195 kDa antigen found on the surface of Plasmodium falciparum merozoites, undergoes processing to a 19 kDa fragment that has been implicated in the invasion of erythrocytes by the merozoite. This antigen has been identified as a promising blood stage malaria vaccine candidate. The FMP1/AS02A malaria vaccine consists of a lyophilized recombinant 42 kDa carboxy-terminal end of MSP1 produced in and purified from E. coli bacteria at the Walter Reed Army Institute of Research, adjuvanted with AS02A, a proprietary formulation developed and produced by GlaxoSmithKline Biologicals, Rixensart, Belgium. In clinical studies in malaria-naïve adults in the U.S. the FMP1/AS02A vaccine was safe and highly immunogenic, and a study to evaluate its safety and immunogenicity in malaria-experienced individuals was recently conducted in Kenya, an area where malaria transmission is intense and perennial.

Here we report the second study in an endemic area, designed to evaluate the safety and reactogenicity of the FMP1/AS02A in a population exposed to more limited and seasonal malaria transmission pressure. Beginning in July 2003, in Bandiagara, Mali, forty healthy adults aged 18-55 years were randomized to receive either the FMP1/AS02A vaccine or rabies vaccine at 0, 1 and 2 months, and are being followed for one year. The mean age of study participants is 44.1 years, and 82.3% are male. All 40 participants received all three vaccinations. The only grade three reactions related to vaccination were swelling in 9, 11 and 6 of the volunteers in the week following the first, second and third immunizations, respectively. All other adverse events were mild or moderate and all resolved within seven days following immunization. As of May 2004, no severe adverse events related to vaccination had been reported. Titters of anti-MSP1\textsubscript{19} antibodies will be measured by ELISA on sera collected at pre- and post-vaccination time points. The study will remain blinded until July 2004. Full unblinded safety and immunogenicity data will be reported.

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**PHASE I Trial of MalariaVax, a Recombinant Hepatitis B Virus Core Particle Containing Repeat and T Cell Epitopes of the Plasmodium Falciparum Circumsporozoite Protein**

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The first Phase I, dose-escalating trial of MalariaVax (ICC-1132) in the United States is nearly complete. ICC-1132 is a recombinant protein vaccine comprised of Plasmodium falciparum circumsporozoite (CS) protein repeat epitopes (B and T1) and a universal T cell epitope, fused to a modified hepatitis B virus core protein, which when expressed in E. coli, forms multimeric, virus-like particles. This vaccine, when adjuvanted with Seppic ISA 720, has been shown to be immunogenic in humans. The primary objectives of this trial were to assess the safety, reactogenicity and immunogenicity of ICC-1132 formulated with alhydrogel. Three dose levels, 10, 20 and 50 µg were compared, each injected intramuscularly on study days 0, 2 and 6 months. Preliminary results show the vaccine to be safe, well tolerated and immunogenic. Antibody and cellular responses were measured from blood samples collected on the day of immunization and at days 14, 28, 56 and 84 post-immunizations. Anti-CS antibody was detected in all volunteers receiving 10 µg + alhydrogel. High levels of antibody to hepatitis B core and ICC-1132 were also detected in these volunteers. Responses were adjuvant dependent, as only three of seven volunteers receiving ICC-1132 without adjuvant converted to anti-CS positive, all with significantly lower antibody titers to ICC-1132 and hepatitis B core. PBMCs from volunteers immunized with 10 µg ICC-1132 + alhydrogel proliferated and produced IL-2 in response to stimulation with recombinant P. falciparum CS and ICC-1132. Cellular and serological responses of volunteers immunized with 20 or 50 µg of ICC-1132 + alhydrogel are pending. These encouraging results have led to plans for a Phase IIa trial to assess the protective efficacy against P. falciparum sporozoite challenge.
IMMUNITY INDUCED BY RECOMBINANT MEROZOITE SURFACE PROTEIN 1 (MSP1) AGAINST PLASMODIUM FALCIPARUM CHALLENGE IN AOTUS MONKEYS STRONGLY CORRELATES WITH ELISA AND IN-VITRO PARASITE INHIBITORY ACTIVITY

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A number of malarial blood stage candidate vaccines are poised for testing in human clinical trials, but our understanding of the relation between protective immunity in-vivo and data obtained from in-vitro assays of host responses remains inadequate. An in-vitro assay which can reliably predict protective immunity in-vivo would greatly facilitate vaccine development. Merozoite Surface Protein1 (MSP1) is a leading blood stage malaria vaccine candidate, and anti-MSP1 antibodies from individuals that are clinically immune to malaria inhibit the invasion of Plasmodium merozoites into erythrocytes in-vitro. We have produced two allelic forms of MSP1(42) (FVO and 3D7) expressed in E. coli and refolded. Aotus nancymai monkeys were immunized with FVO, 3D7 or a combination of FVO and 3D7 allelic forms of MSP1 and subsequently challenged with P. falciparum-FVO parasites. The immune sera taken prior to challenge and purified IgG from the same sera were tested by a standardized ELISA and by an in-vitro growth inhibitory assay (GIA). Regardless of immunogen used, all monkeys that had >200,000 ELISA units of antibody to MSP1(42) (FVO) at the time of challenge self-cured their infections. Monkeys with lower GIA activity (60%) to P. falciparum (FVO) required treatment for high parasitemia after challenge. There is a strong correlation between ELISA units (Spearman Rank Correlation, rs is ~ 0.75) or GIA (rs is ~ 0.7) and protective immunity as judged by various parameters (e.g., day of patency, peak parasitemia). These data indicate that, in this monkey model, the ELISA and GIA can reasonably predict protective immunity induced by a blood stage vaccine. (ACMCIP abstract)

PHASE I DOSE ESCALATION STUDY OF MALARIA BLOOD STAGE VACCINE BASED UPON RECOMBINANT APICAL MEROZOITE ANTIGEN-1 (AMA-1) FORMULATED WITH GSK BIOLOGICAL’S ADJUVANT AS02A

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Prior to field testing, we conducted an open label Phase I dose escalation study to evaluate the safety, reactogenicity, and immunogenicity of candidate Plasmodium falciparum asexual stage malaria antigen (FMP2.1) administered intramuscularly with GSK Biologicals’ adjuvant AS02A. FMP2.1 is a lyophilized recombinant protein produced in E. coli that represents the ectodomain of apical merozoite antigen-1 (AMA-1) from the 3D7 clone of P. falciparum. Twenty-three healthy malaria-naive adults were enrolled and randomized to one of three groups. Vaccinees received approximately 10, 25, or 50 micrograms of FMP2.1 in a fixed volume of 0.5 ml of AS02A on a 0, 1, and 2 months schedule. Follow up continued for 6 months after the last dose. The FMP2.1/AS02A was well tolerated and there were no vaccine-related severe adverse events. The vaccine proved to be immunogenic and induced growth inhibitory antibodies active against homologous P. falciparum merozoites in a standardized growth inhibition assay. Encouraged by the safety and immunogenicity results, we plan a Phase I trial of FMP2.1/AS02A in 2004 in Mali.

INDUCTION OF PROTECTIVE ANTIBODIES AGAINST PLASMODIUM FALCIPARUM BY IMMUNIZATION OF AOTUS MONKEYS WITH PFEMP-CIDR1A PROTEINS

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PIEPI is both a target of naturally acquired immunity and is involved in the development of severe malaria. CIDR1 is the PIEPI domain present on the surface of infected erythrocytes (IE) that is responsible for cytoadherence via CD36. To determine the protective efficacy of recombinant CIDR1 antigens, Aotus monkeys were immunized with either FVO-CIDR1 or a combination of three proteins (FVO-, A4tres- and MC-CIDR1) in Freund’s adjuvant. The monkeys were challenged with FVO parasites (FVO-1) and monitored for parasitemia. To compare the effect of vaccination and infection, versus infection alone on protection against a different antigenic variant of FVO, the monkeys were re-challenged with parasites collected from one of the delayed infections (FVO-2). Serum samples were collected appropriately and ELISA titers determined. Antibody binding to CIDR1-PIEPI1 on the surface of FVO-infected RBC was also assessed by flow cytometry. In the first challenge, compared to control monkeys immunized with Pf625, CIDR1-immunized animals showed a 5-day delay in patent and a 5-day delay in peak parasitemia. Despite the delay, the rise in parasitemia was similar in all groups, once patent, and all animals were treated for high parasitemia (>5%). 4 of 7 control monkeys required treatment on re-challenge whereas none of the vaccinated animals were treated. Two of the vaccinated monkeys had no patent parasitemia; 7 of 9 had recrudescence compared to 1 of 3 control monkeys. Pooled sera from the immunized animals (FVO alone and FVO, A4tres, and MC) before the first challenge showed antibodies against the surface of FVO-1 IE; none had antibodies to FVO-2. All had antibodies to FVO-1 after the first infection including the control Pf625 immunized monkeys. Low-level antibodies to FVO-2 before the second challenge were observed in the immunized but not in the controls. In conclusion, in contrast to the protection previously seen with CIDR1 from the MC clone, CIDR1-immunization followed by challenge with FVO parasites gave more limited protection i.e. delay in patenty and peak parasitemia. (ACMCIP abstract)

THE COST OF DEVELOPING, LICENSING, MANUFACTURING, AND DELIVERING A MALARIA VACCINE FOR AFRICAN INFANTS: WHO WILL PAY AND FOR WHAT TYPE OF VACCINE?

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The primary burden of malaria occurs in infants, young children, and pregnant women in sub-Saharan Africa who are infected with Plasmodium falci-
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B. nasutus shed for a period of 5 weeks while B. globosus continued shedding for 9 weeks. The longest individual continuous shedding by B. nasutus was 58 days. On average, B. globosus emitted significantly higher numbers of cercariae than B. nasutus. These figures suggest that under laboratory conditions is significantly a better host for transmission than B. nasutus. The implication of these findings and their roles in transmissions of the local strain of the parasite is discussed.

Schistosoma haemotobium is prevalent in various parts of Kenya especially Coast Province. The Vector snails Bulinus globosus and B. nasutus both exist but their transmission of the parasite is not clearly understood. In an effort to establish their role in transmission of S. haemotobium, laboratory bred B. nasutus and B. globosus were exposed to infections with the local parasites under the same conditions. Confirmation of the identity of the two Bulinus was carried out using PCR techniques in Israel. All exposed snails were examined for cercariae-shedding daily from day 21 from the date of exposure. The significant observation was that both snail species started shedding on day 21, with maximum number of snails shedding on days 24 and 25 (week 4) for B. globosus and B. nasutus respectively, after exposure.

Transmission of Schistosoma mansoni infection is intense along the Ugandan shore of Lake Albert, Uganda. Pre-treatment surveys of cohorts aged 7-60 years in 4 neighbouring villages situated along a 20km stretch of coastline revealed 100% prevalence with mean egg counts exceeding 700egp in each cohort. However, follow-up surveys conducted 1 and 2 years after praziquantel treatment in each village indicated that re-infection levels varied considerably between villages. An investigation into factors affecting re-infection levels in these villages revealed that tribal differences in behaviour, particularly those of women belonging to the Bagungu tribe, explained most of the variation in the different infection levels. Changes in the level of the lake between the times of the surveys in these villages may have been influential due to associated changes in the population ecology of the two main intermediate hosts - Biomphalaria stanleyi and B. sudanica. The results indicate that the apparent success of control programs in terms of re-infection may depend on demographic and ecological factors not routinely recorded in parasitological surveys.

Schistosoma mansoni infection can lead to hepatosplenic morbidity, with a hardfimd organomegaly and the sequelae of portal-hypertension. Classic autopsy studies established that this severe hepatoplenic form of schistosomiasis is associated with gross hepatic portal fibrosis, usually in adults who have been exposed to infection for many years. However, recent studies, combining hepatic ultrasonography (US) with detailed clinical examinations, have reported that children from a moderate transmission area of Kenya were suffering similar hepatoplenomegaly with signs of elevated portal pressure, in the absence of US-detectable portal fibrosis. We have also observed, in a fishing community in Uganda, that the major risk factor for development of portal fibrosis is duration of exposure to S mansoni for more than 15 years. However, in the neighbouring communities we have examined adult and child cases of hepatoplenomegaly in the absence of hepatic fibrosis, particularly in children or adults who have residen for less than 10 years. Here we present clinical and hepatic ultrasound
data that demonstrates that hard/firm hepatosplenomegaly, sometimes accompanied by portal hypertension and evidence of hepatic and splenic varices, occurs in the present of little or no US-detectable portal fibrosis. To characterise the epidemiological features of this form of hepatosplenic morbidity in children and adults, we also carried out a clinical survey of representative cohorts, aged 6-60 years, from two of these Ugandan communities, Bugoigo and Walakuba, where the majority of individuals had been resident for less than 10 years. Age-infection profiles were typically convex in both villages, with the peak level of infection in adolescent children. Spleen enlargement, recorded as extension below the rib cage along the mid ancillary line declined with age, but was greater in cases of peri-portal fibrosis (grade C or higher on the Naamey scale). Liver enlargement, recorded as extension below the rib cage along the mid sternal line was high in all ages, but was also exacerbated by the presence of fibrosis. The results indicate that intense exposure to S. mansoni infection of less than 10 years is associated with hepatosplenic morbidity in the absence of fibrosis across all age groups.

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THE ROLE OF LIVESTOCK AS RESERVOIR HOSTS OF SCHISTOSOMA JAPONICUM IN EASTERN SAMAR, PHILIPPINES
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Livestock animals such as cows, water buffaloes (carabao) and pigs have long been considered important reservoir hosts of Schistosoma japonicum in endemic rural farming areas of China and the Philippines. In China treatment of cows and water buffaloes is an important component of the national schistosomiasis control program. In addition, schistosomiasis japonica vaccine development has been based on a strategy of having a livestock vaccine which would block transmission to humans. This study aims at a baseline examination of the prevalence of S. japonicum infection in humans, carabao and pigs at the community level in Eastern Samar, the Philippines. Fecal samples were taken rectally from 35 carabao and pigs when possible from each of 50 eligible East Samar villages. Each animal was sampled for 3 consecutive days when possible. The samples were processed and examined for S. japonicum eggs using the modified Danish Bilharziasis Laboratory filtration-sedimentation method. In each village 35 eligible households were selected at random and 4-6 persons in each were asked to provide stool samples for 3 days. The human fecal samples were processed and examined using the duplicate slides Kato-Katz method. Results: Preliminary estimates from half of the study villages indicate relatively low prevalences of S. japonicum infection in both carabao and pigs while moderately to high human prevalences have been observed in the same villages. In villages with the highest human prevalences sampled pigs and carabao were found to be free of infection. Preliminary results suggest that carabao and pigs are exposed to lower pressures of infection than humans and that these livestock animals may play a minimal role as reservoir hosts perhaps as a result of local agricultural practices, ecological factors or other reasons. The results may suggest some association between water management and infection in carabao but not pigs. More data from the remaining villages will contribute to verifying these initial results.

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INTERMEDIATE HOST DYNAMICS REVISITED: AN APPLICATION OF MECHANISTIC AND STATISTICAL POPULATION MODELING AND LONGITUDINAL FIELD METHODS TO ONCOMELANIA HUPENSIS CONTROL IN SICHUAN, CHINA
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A synthesis of mechanistic and statistical modeling approaches was undertaken to develop a mathematical population dynamics model for Oncomelania hupensis, the intermediate host for Schistosoma japonicum, with the objective of informing control decisions. The model was parameterized with data from a novel, longitudinal mark-recapture technique incorporating enclosures so as to distinguish birth from immigration, and death from emigration. Snail density, birth and death rates were thus estimated monthly, and environmental variables recorded continuously, at three sites in a mountainous region of southwestern China. Competing mechanistic functional forms were assessed using nonlinear least squares fitting techniques. Moreover, a repeated measures, mixed effects modeling approach was used to relate birth and death parameters to lagged and unlagged environmental variables, clustered by site. V-fold cross-validation and time-series probes were used to estimate the prediction error of competing models against observed population dynamics. O. hupensis seasonal cycling was largely governed by temperature, and less so by precipitation, with a characteristic late summer population peak and weak density-dependent effects. An application of the model optimizing the timing of molluscicidal snail control is demonstrated, and the implications for reducing schistosomiasis transmission are discussed.

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SPATIAL ANALYSIS OF SCHISTOSOMIASIS IN TANZANIA AND ZAMBIA
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The Schistosomiasis Control Initiative (SCI) has been established with funding from the Bill and Melinda Gates Foundation to initiate large-scale, sustainable national schistosomiasis control programmes in six Sub-Saharan African countries (Burkina Faso, Mali, Niger, Tanzania, Uganda and Zambia). Spatial analytical methods are being employed for a number of reasons, including: 1) to provide an objective framework for spatial targeting of praziquantel drug treatment, 2) to assist planning of longitudinal monitoring and evaluation strategies and 3) to gain an improved understanding of the spatial processes involved in schistosomiasis transmission. In Tanzania and Zambia, prevalence surveys of school-age children have been undertaken in over 300 locations using standardised protocols for both countries, with a wide geographical coverage. The data were analysed in three stages: 1) visual analysis, including overlays of prevalence data and biologically important ecological variables derived from remote-sensing, 2) exploratory analysis to quantify spatial heterogeneity and 3) Bayesian multi-variable logistic regression and subsequent predictive risk-mapping.
Preliminary results demonstrate significant spatial heterogeneity in *Schistosoma haematobium* and *S. mansoni* infections. We discuss the results in terms of their applications to SCI and similar large-scale parasitic control programmes.

### ASSOCIATION BETWEEN PRO-INFLAMMATORY MEDIATORS AND MALNUTRITION IN SCHISTOSOMIASIS JAPONICA


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Pro-inflammatory responses induced by eggs trapped in host tissues, may mediate in part the nutritional deficiencies of schistosome infection. In the present study, we examined the relationship between inflammatory mediators and nutritional status in an *Schistosoma japonicum* endemic region of the Philippines. We enrolled 624 *S. japonicum* infected individuals, aged 8-30 yrs, living in a rural, rice farming village in Leyte, the Philippines. At baseline, serum was obtained and analyzed for: C-reactive protein (CRP), IL-1, IL-6, TNF-α, TNF receptor 1 (TNFR-I). Anthropometric measurements were obtained to calculate indices of nutritional status. These measures included body mass index z-score (BMIZ), weight for age z-score (WAZ), weight for height z-score (WHZ) and mid-upper arm circumference z-score (MUACZ). Individuals were treated with Praziquantel, and 4 weeks post treatment, PBMCs were obtained (n=601) and stimulated with soluble worm (SWAP) and egg antigens (SEA). Culture supernatants analyzed for IL-1, IL-6 and TNF-α. Two months after treatment, anthropometric measurements were repeated (n=561). Data were analyzed with Spearman’s correlation. At baseline, serum CRP was associated with significantly decreased WAZ (Rho = -0.11, P = 0.01), MUACZ (Rho = -0.14, P = 0.009) and BMIZ (Rho = -0.155, P = 0.007). TNFR-I was associated with decreased WHZ (Rho = -0.28, P = 0.008).

SWAP and SEA stimulated TNF-α measured 1 month post treatment was associated with significantly decreased HAZ measured at 3 months post treatment (Rho = -0.09, P = 0.05, Rho = -0.11, P = 0.02). Similarly, SEA stimulated TNF-α was associated with significantly decreased WAZ (Rho = -0.1235, P = 0.01). When analyzed dichotomously, individuals with high SEA stimulated TNF-α (n=194) had mean WAZ scores that were 0.3 SD lower than individuals with low TNF-α levels (n=223). We demonstrate that circulating mediators of inflammation are associated with several measures of under nutrition in individuals infected with *S. japonicum*. In addition, we show that egg induced TNF-α production predicts decreased nutritional status as measured by WHZ. These data suggest that pro-inflammatory responses to egg antigens mediate malnutrition in schistosomiasis japonica.

### ENHANCED WEST NILE VIRUS INFECTION IN YOUNG CHICKENS INFECTED BY MOSQUITO BITE

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Arboviruses delivered to vertebrate hosts by mosquito vectors have been shown to cause higher viremia than if delivered by needle inoculation. Immunomodulatory effects of mosquito saliva have been implicated as a potential cause of this enhanced infection, however other differences exist between needle inoculation and mosquito bite that may account for altered host infection. We determined the effect of viral delivery methods on host infection response by comparing viremia and viral shedding in 1-day and 5-day old chickens infected with West Nile virus (WNV) by subcutaneous inoculation or by the bite of WNV infected *Culex pipiens*. Infected chickens exhibited high viremia (~10⁸ PFU/ml) and shed significant amounts of virus in oral (~10⁷ PFU/ml) and cloacal (~10⁶ PFU/ml) secretions. Mode of infection had a significant effect. Viremia of chickens infected by mosquitoes was 1,000x higher at 12h and 100x higher at 24h PI than viremia of chickens infected by needle inoculation. Mosquito-infected chickens also had significantly higher titers in oral and cloacal swabs at these two time points and cleared virus faster than needle inoculated chickens. Enhanced infection due to mosquito bite was seen in both one-day old and five-day old chickens and was not species specific. Chickens fed upon by WNV infected *Cx. tarsalis* had increased viremia and oral shedding at 12 and 24 hours compared to needle inoculated chickens. Differences in infection response between needle and mosquito inoculated chickens were not solely due to different doses of virus inoculated by mosquitoes vs. needle. Chickens needle inoculated with increasing doses of WNV (up to 10⁸ PFU/ml) exhibited higher viremia and oral swab titers but not to the levels seen in chickens infected by mosquito bite. Future studies will determine whether mosquito saliva, location of virus inoculum, and virus source affect chicken infection response. Our results underscore the importance of studying natural modes of WNV infection in vertebrate hosts.

### EVALUATING HERD IMMUNITY FOR WEST NILE VIRUS IN AVIAN AMPLIFYING HOSTS

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Herd Immunity is a phenomenon that prevents transmission of pathogens by reducing the risk of infection in susceptible hosts to a level below the necessary basic reproductive rate of 1.0 for a pathogen to persist. To evaluate whether herd immunity may protect against West Nile virus (Flaviviridae) transmission, we sampled adult house sparrows in 23 study sites in Colorado after a West Nile virus outbreak in 2003, and determined the prevalence of neutralizing antibodies (immunity) in each site. Preliminary results indicate that immunity ranged from 7%-54%. Transmission activity in each of these study sites was then monitored in 2004 to determine whether herd immunity may have had a dampening effect on transmission, and whether a definitive level of seroprevalence in the house sparrow population can be defined as conferring herd immunity.

### REAL-TIME REVERSE TRANSCRIPTION LOOP MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP) ASSAY AS A RAPID DIAGNOSTIC TOOL FOR EMERGING VIRUSES

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Loop Mediated Isothermal Amplification (LAMP) is a novel method of gene amplification that amplifies the nucleic acid with high specificity, efficiency and rapidity under isothermal condition employing a set of six specially designed primers that recognize eight distinct sequence of the target. The whole procedure is very simple and rapid wherein the amplification can be obtained in less than one hour (20-30 min) by incubating all the reagents in a single tube with reverse transcriptase and Bst DNA polymerase at 63°C. The detection of gene amplification could be accomplished by agarose gel electrophoresis as well as by real-time monitoring in an inexpensive turbidimeter. Being an isothermal amplification, LAMP does not require any thermal cycler and thus can be performed even with heating block and/or water bath. In addition, the gene amplification can be visualized either as turbidity in the form of white precipitate or by employing a fluorescent intercalating dye through UV lamp. We have developed and evaluated one-step single tube real-time accelerated reverse transcription loop mediated isothermal amplification (RT-LAMP) assays for rapid detection of some of the recently emerged human viral pathogens viz; West Nile, SARS and Dengue viruses. On comparison to conventional RT-PCR, RT-LAMP assay demonstrated 10 to 100 fold more sensitivity with a detection limit of 0.01 to 10 PFU of virus in all these cases. Thus the RT-LAMP assay reported here has the advantages of rapid amplification, simple operation and easy detection with potential usefulness for clinical diagnosis and surveillance of viral diseases in developing countries.

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INTRA-HOST GENETIC DIVERSITY IN ENZOOTICALLY TRANSMITTED WEST NILE VIRUS

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RNA virus populations exist within a host as a genetically heterogeneous mixture of variants that differ in varying degrees from a consensus sequence. This mutant spectrum, known as a quasispecies, is increasingly recognized as critical to the biology and evolution of RNA viruses. The quasispecies structure of arthropod-transmitted viruses is poorly defined, and the preservation of genetic diversity throughout a complex transmission cycle has not been documented. We therefore sought to describe the quasispecies structure of West Nile virus (WNV), and to determine whether particular non-consensus genotypes are present in spatially and temporally linked infections. WNV was sampled from ten infected birds and ten infected mosquito pools collected in or near Brookhaven township on Long Island, NY during the peak of the 2003 WNV transmission season. A 1,961 bp fragment comprising the 3’ 1,159 bp of the WNV envelope coding region and the 5’ 802 bp of the ns1 coding region was amplified, cloned, and 20 clones per infection were sequenced using overlapping primers. Intra-host genetic diversity was generally low. Mosquito-derived WNV sequences, however, were more genetically diverse within each host than avian-derived WNV. Limited evidence of preservation of the mutant spectrum throughout the transmission cycle was found in the samples studied.

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A STRUCTURE-FUNCTION MAP OF DOMAIN III OF THE ENVELOPE PROTEIN OF WEST NILE VIRUS

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Antibodies have been shown to play a critical role in protection from infection from West Nile virus (WNV). To elucidate the structural basis of antibody-mediated protection and develop potential WNV-specific immunotherapeutics a panel of 30 monoclonal antibodies (mAbs) to the WNV virus E protein was generated after immunizing with recombinant protein. Using a yeast expression system that displays either the ectodomain of E or domain III alone, 12 of the antibodies were mapped specifically to domain III. Four of the mAbs that mapped to sites within domain III exhibited strong neutralizing activity in vitro by plaque reduction assay. Two of these were studied in vivo as potential therapeutic agents in a mouse model of WNV infection. These mAbs were strongly protective with improvement in survival even when administered six days after infection, results that were superior to that observed with immune (Omrix™) human γ-globulin. Using the yeast expression system and a library of domain III mutants, individual amino acid residues that are essential for the binding of the neutralizing and non-neutralizing antibodies have been identified. Additional functional studies are being performed to assess which mAbs most efficiently induce complement-mediated lysis of infected cells. The comparison of in vitro and in vivo functional and protection assays with structural mapping data should provide an enhanced picture of the protective epitopes on domain III of the WNV E protein.

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MECHANISMS OF CD8+ T CELL MEDIATED CONTROL OF WNV INFECTION IN NEURONS

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West Nile virus (WNV) is an emerging neurotropic flavivirus that causes severe neurologic disease in a subset of individuals. We have previously shown that embryonic stem cell-derived neurons (ESDN) are vulnerable to WNV infection and die via apoptosis (Shrestha et al, Journal of Virology 2003, 77:13203). To understand how the immune system affects the control of WNV infection and fate of infected neurons in vivo, we have utilized an infection model in adult C57BL/6 mice. Mice that lack CD8+ T cells had increased mortality, WNV burden, and neuronal degeneration in the brain and spinal cord (Shrestha et al., Journal of Virology 2004, in press). Although our data suggested that CD8+ T cells control WNV infection in the central nervous system, the mechanism remained unclear: CD8+ T cells can limit viral infection both by cytolytic (e.g., perforin, NKGD2, or Fas ligand) and non-cytolytic (e.g., interferon and tumor necrosis factor) mechanisms. To elucidate the mechanism of CD8+ T cell-mediated control, congenic deficient mice were utilized. Mice that lack either perforin or Fas ligand demonstrated increased vulnerability to infection: this was reflected by increased mortality, ~50 to 100-fold higher viral burdens in the CNS, and...
delayed clearance from the spleen and the brain. Histopathology of brains from deficient mice showed increased numbers of inflammatory cells in close apposition to infected neurons. To gain additional insight, an ex vivo system that reconstitutes T cell interaction with neurons was developed. Treatment of ESDN with wild type WNV-specific CD8+ memory T cells reduced the production of infectious WNV ~1,000-fold. Interestingly, pre-treatment of ESDN with IFN γ, which is normally produced by activated CD8+ T cells, had little inhibitory effect on virus production. These data suggest that CD8+ T cells control WNV in the neurons by a cytolytic mechanism. To confirm this hypothesis, additional neuronal infection studies are underway with periforin, Fas ligand, and IFN γ-deficient WNV-specific memory T cells.

WEST NILE VIRUS STRAINS VARY IN VIRULENCE FOR HOUSE SPARROWS (PASSER DOMESTICUS)

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The observation of avian mortality associated with West Nile virus (WNV) infection has become a hallmark epidemiological feature in the recent emergence of this pathogen in Israel and North America. To determine if phenotypic differences exist among different WNV isolates, we exposed house sparrows (Passer domesticus) to low passage, Lineage 1 WNV strains from North America (NY99), Kenya (KEN), and Australia (KUN; a.k.a. Kunjin virus). House sparrows inoculated with NY99 and KEN experienced similar mortality rates and viremia profiles. KUN elicited significantly lower-titered viremia when compared to the other strains and induced no mortality. This study suggests that natural mortality in house sparrows due to Old World strains of West Nile virus may be occurring where the KEN strain occurs.

EFFICACY AND SAFETY OF COARTEM (ARTEMETHER-LUMEFANTRINE) IN SUPERVISED AND NON-SUPERVISED PATIENTS WITH UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA. A RANDOMIZED CLINICAL TRIAL IN MBARARA, UGANDA

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Coartem is a promising fixed formulation of artemether and lumefantrine used to treat uncomplicated Plasmodium falciparum (P.f.) malaria. A randomised clinical trial was conducted in western Uganda with the objectives of confirming the efficacy and safety of 6-dose Coartem when administered under supervised conditions, and to compare this efficacy with that of Coartem given to an unsupervised group of patients. Only patients with a confirmed Pf mono-infection, a simple symptomatic malaria, weighing more than 10 Kgs and not pregnant were included. They were randomised either in a group receiving directly observed treatment and a fatty meal, or in a group receiving the first dose in the study site and finishing the treatment at home. Follow-up was 28 days. Failure was defined either as Early Treatment Failure (WHO definitions), as the reappearance of parasites after day 3, or as the presence of non parasitological criteria such as intake of antimalarials during follow up. The distinction between recrudescence and reinfection was done by PCR. Reinfections were excluded from the analysis. A total of 5410 patients were screened of which 957 were included. Thirty-eight (4.0%) patients were lost to follow-up and 35 (3.5%) were excluded from analysis, mainly reinfections confirmed by PCR. In the supervised group, there were 3 failures, all non-parasitological (Cure Rate = 98.1% [95% CI: 96.8% - 99.7%]). In the non-supervised group, there were 5 failures, all non-parasitological (Cure Rate= 98.1% [95% CI: 96.8% - 99.7%]). Only 1.4% (13 patients) had adverse events definitely or probably related to Coartem. Eight serious adverse events occurred but none was likely to be related to Coartem. Coartem is safe and efficacious even in unsupervised conditions. The use of this combination as first-line treatment of uncomplicated malaria could significantly contribute to the control of malaria.

RANDOMIZED COMPARISON OF SIX DOSES OF ARTEMETHER-LUMEFANTRINE AND QUININE-DOXYCYCLINE IN THE TREATMENT OF PLASMODIUM FALCIPARUM MALARIA IN THE WESTERN AMAZON

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Artemether-lumefantrine (A-L) is the first and only fixed artemisinin-based combination therapy for acute uncomplicated Plasmodium falciparum (P.f.) malaria. A open, randomized comparison of 6 doses of A-L with quinine-doxycline (Q-D) was conducted in 59 patients (26 years) with acute uncomplicated P.f. malaria from the western Amazon. Q-D is the recommended first-line antimalarial regimen in that region. For patients in the A-L group, 4 tablets (1 tablet = artemether 20 mg plus lumefantrine 120 mg) were taken initially, followed by 4 tablets 8 hours (h) later. During the next two days, dosing continued at 4 tablets every 12 h. Patients in the Q-D group took quinine 500 mg every 8 h for 3 days and doxycline 100 mg every 12 h for 5 days. Both drugs were initiated on the same day. Patients were followed up for 6 days. Demographic and baseline characteristics were comparable between groups. In total, 56 patients (27 A-L and 29 Q-D patients) completed the study. By Day 3, the parasitemia of all A-L patients had disappeared compared to 51.2% of Q-D patients. Complete resolution of parasitemia in the Q-D group was not achieved before Day 6. Median parasite clearance time was significantly faster in the A-L treatment group (2 days) compared with the Q-D group (3 days) (p<0.0001). No significant difference was noted between the 2 groups with regard to fever clearance. One patient in the Q-D group discontinued the study early because of treatment ineffectiveness. Adverse events were reported by 91.5% of study participants with no apparent difference between treatment groups. Most adverse events were mild and/or not considered related to study treatment. One patient in the Q-D group discontinued therapy because of vomiting and 1 patient in the A-L group withdrew consent. In conclusion, the 6-dose regimen of A-L provides a highly effective, well-tolerated, and convenient treatment for falciparum malaria in a highly drug-resistant area of South America.
TREATMENT OF UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA IN ZAMBIA

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Because of high chloroquine treatment failure rates for uncomplicated falciparum malaria, Zambia adopted Coartem, an artemisinin-based combination (ACT). Although Coartem is known to be highly efficacious, concerns have arisen regarding patient compliance, given the complexity of the new dosage regimen. A descriptive study was conducted in January 2004 to measure the level of compliance to the six dose regimen in adult malaria patients seeking treatment at health facilities and to determine whether Coartem was being correctly administered by health care workers (HCW).

Compliance was defined as follows: a) Total: Verbal confirmation of completion of all doses in the presence of an empty blister pack and a correct description of how the doses were taken, b) Probable: Confirmation of completion of all doses in the absence of a blister pack and correct description of how the doses were taken, c) Non-Compliance: Presence of tablets on the blister pack on Day 3 and or inability to explain how the doses were supposed to be taken. At the point of prescription, HCW performance was assessed based on their use of key educational messages. Patient age, education level, sex and distance to the health centre were assessed as possible risk factors for non-compliance. All patients clinically suspected of having uncomplicated falciparum malaria visiting health centres during the peak malaria transmission season in target districts in Chongwe, Kabwe, Chibombo, Kalomo and Livingstone were eligible. Of 568 patients enrolled, 536 completed the study; the rest were lost to follow up. Full compliance was observed in 64%, 18% were probably compliant and 21% were definitely non-compliant. Of 85 HCW observed at the dispensing point, 23.5% were rated as excellent, 29.4% good, 24.7% fair and 22.5% poor at giving the key messages needed to encourage the correct use of Coartem. The dosing regimen for Coartem is more complex than previously used antimalarial regimens and other currently available ACTs. Under field conditions both patient use and HCW dispensing compliance may be low, thus potentially negating Coartem’s excellent efficacy. The finding that at least 18% of patients were non-compliant emphasizes the need for improved HCW communication and caretaker/patient understanding of the necessity of taking the full course in order to fully benefit from the treatment.

EXAMINING THE TREATMENT OPTIONS FOR FALCIPARUM MALARIA IN AFGHAN REFUGEE CAMPS: EFFICACY AND POTENTIAL FOR TRANSMISSION REDUCTION OF CHLOROQUINE OR SULPHADOXINE-PYRIMETHAMINE ALONE AND IN COMBINATION WITH PRIMAQUINE OR ARTESUNATE

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Although chloroquine resistance has been documented in many areas of Pakistan since the 1980s, it remains, in combination with primaquine, the national 1st line treatment for falciparum malaria. The gametocidal drug primaquine is used locally, and internationally in epidemic response, to lower transmission. We examined the treatment efficacy of chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) and their primaquine (CQ+PQ, SP+PQ) and artesunate (CQ+AS, SP+AS) combinations, for uncomplicated falciparum malaria in the Afghan refugee camps in Pakistan. Gametocyte carriage after treatment was also investigated. 355 patients were randomized to the 6 arms and followed for 28 days. 308 patients completed the trial. Baseline characteristics were comparable between treatment groups. CQ monotherapy achieved only 23% clinical cure and 21% parasitological cure. CQ+AS gave an improved cure rate (72% clinical and parasitological cure) over CQ alone (P<0.001). Some resistance to SP was seen with 10% clinical failure and 7% parasitological failure. SP+AS achieved 100% clinical cure with only 1 patient (2%) failing by parasitological outcomes. The likelihood of patients carrying gametocytes on or after day 7 is slightly reduced by co-treatment with primaquine (CQ versus CQ+PQ, odds ratio(OR)=8.9, P<0.001, SP versus SP+PQ, OR not significant). The artesunate combinations have a far greater impact on gametocyte carriage (CQ versus CQ+AS, OR=33.9, P<0.001, SP versus SP+AS, OR=45.4, P<0.001).

Clinical trial to compare the efficacy of intrarectal versus intravenous quinine in the treatment of childhood cerebral malaria

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Cerebral malaria is the most lethal form of complicated malaria and is a major cause of morbidity and mortality in children. Antimalarial drugs remain the only intervention that affects outcome. Quinine remains the most effective treatment for severe malaria. Intrarectal treatment is a non-aggressive, painless and easy treatment that could decrease the morbidity and mortality associated with severe malaria. Intrarectal quinine has been found to have good efficacy and safety in the management of childhood cerebral malaria. This randomized double-blind placebo controlled clinical trial study was undertaken to: 1) compare the efficacy of intrarectal versus intra-venous quinine in the treatment of childhood cerebral malaria; and 2) document adverse effects and evaluate the safety of intrarectal versus intravenous quinine. Patients recruited were children 6 months to 5 years in the Acute Care Unit and Stanfield ward. Patients with cerebral malaria randomized to treatment with either intrarectal or intravenous quinine. Outcome measures included fever clearance time, parasite clearance time, coma recovery time, time to sit unsupported, time to begin oral intake, duration of intervention and death. Adverse drug events and neurological sequelae were also documented. SPSS statistical package was used for data analysis. Categorical data analysed by frequencies, cross tabulations and chi-squared tests. Quantitative continuous data analysed by means and student’s t-test. Two-tailed p-values of < 0.05 were significant. Kaplan Meier, linear and logistic regression analyses done. 110 children recruited between September 2003 - January 2004. The coma recovery time, fever clearance time, parasite clearance time, time to begin oral intake, time to sit unsupported and duration of intervention were similar in the two treatment arms. Intrarectal quinine well tolerated and no adverse events were documented. In conclusion, intrarectal quinine is as effective and as safe as intravenous quinine in the treatment of childhood cerebral malaria.
firmed falciparum malaria. UNHCR have authorized this protocol for the refugee camps, and it is being introduced during the 2004 falciparum season. Use of artesunate combinations rather than co-treatment with primaquine maybe more effective at reducing transmission, the international recommendations for treatment policy during epidemic response should be re-visited.

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A SINGLE CENTRE OPEN PHASE IV STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF CHLORPROGUANIL-DAPSONE WHEN DOSED BY DEFINED HEIGHT BANDS

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Chlorproguanil-dapsone (CD) is a potent, low-cost antolute antimalarial with Regulatory approval in the UK and many African countries. This study was conducted to examine the safety and efficacy of CD when dosed by height (instead of weight). This was a single-centre, open-label study in adults and children with uncomplicated \textit{P.falciparum} malaria. All subjects received CD (target doses 2.0 and 2.5 mg/kg respectively), administered according to the subject’s height, once daily; on Days 0, 1 and 2. Subjects returned for follow-up visits on Days 3, 7 and 14. The height-dosing table was developed using data from WHO Demographic and Health Surveys. Safety was assessed by monitoring adverse events (AEs) and clinical laboratory data throughout the study. Efficacy was assessed by the number of subjects with treatment success or early or late treatment failure. 155 subjects were enrolled, of whom 154 received CD and were included in analyses of efficacy and safety. Compliance was high, with 148(96%) subjects being fully compliant and taking all 3 doses of CD successfully. 132(85%) subjects completed the study and 23(15%) subjects withdrew prematurely. Reasons for withdrawal were: lack of efficacy 8(5%), deviation from protocol 7(5%), withdrawal of consent 2(1%), AEs 2(1%) and other 4(3%). CD was generally well tolerated, with the majority of AEs being mild or moderate in intensity. 3(2%) subjects had serious AEs (SAEs) following treatment with CD, including 1 subject with severe anaemia. 4(3%) subjects had treatment-limiting AEs. The most commonly reported treatment-related AEs were raised methaemoglobin 29(19%) and reduced haemoglobin 25(16%). The primary efficacy analysis was based on 1996 WHO definitions for adequate response, modified to also consider as failures subjects with drug-related, treatment-limiting AEs or haemoglobin <7g/dL. In this analysis, 128(86%) subjects overall were considered adequate responders. Based on the latest WHO definition of treatment failure, 139(93%) subjects had adequate clinical and parasitological response; no subjects developed clinically severe malaria. In conclusion, in this small pilot study CD was generally well tolerated, with no new or unexpected AEs seen. The efficacy results were comparable to those seen in previous studies dosed by weight. Dosing by height may be a practical alternative to dosing by weight but larger studies are required to confirm this.

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A RANDOMIZED 4-ARM EFFECTIVENESS TRIAL OF AMODIAQUINE, AMODIAQUINE+ARTESUNATE, AMODIAQUINE+SULFADOXINE-PYRIMETHAMINE AND ARTEMETHER-LUMEFANTRINE IN TANZANIAN CHILDREN

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The crisis in treating uncomplicated malaria in East Africa is well known. Chloroquine has failed and sulfadoxine-pyrimethamine (SP) is failing. Amodiaquine+artesunate, amodiaquine+sulfadoxine-pyrimethamine and artemether-lumefantrine have been shown to be efficacious and safe in efficacy trials in Africa. There is almost no effectiveness data on these drug combinations. An open-label randomized effectiveness trial was set up to test these combinations against monotherapy in an outpatient setting. Muheza district in Tanzania has some of the highest recorded levels of drug-resistance in Africa. Children under 5 with slide-proven uncomplicated malaria whose guardians give consent are randomized to one of the three drug combinations or to monotherapy. Drugs are prescribed by the study team, and taken unsupervised at home. Guardians bring children back on days 14 and 28, or when they become unwell. Primary outcome is day 14 slide clearance, read blind to treatment allocation. A pilot study randomized children to either SP (Tanzanian first line treatment) or amodiaquine (AQ, second-line treatment). By day 14 following treatment, 33/83 (39.8%) of children who took SP and 23/88 (26%) of those who took AQ were slide positive. AQ was therefore used as the monotherapy comparison arm in the main study. To date over 1500 children have been randomized in the main study, which is due to stop recruiting in September 2004; follow-up is over 90%. After 1000 children had been randomized an interim safety analysis demonstrated 101/245 (41%) of children receiving AQ had parasites by day 14. This arm was stopped by the DSMB. PCR genotyping is ongoing to differentiate recrudescence from re-infections. The final day 14 and 28 slide and clinical failure rates for this trial will be presented, along with initial PCR-corrected data.

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PREVALENCE OF ANEMIA, MALARIA AND INTESTINAL HELMINTHS IN PREGNANT WOMEN IN IQUITOS, PERU

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Anemia, malaria and intestinal helminths are three common coexisting and potentially serious conditions of pregnant women in many developing countries. WHO recommends anthelmintic treatment of pregnant women after the first trimester in areas where the prevalence of hookworm infection is over 20-30%. Anthelmintic treatment is in addition to routine iron supplementation and malaria preventive and treatment measures, where and when appropriate. The objective of these studies was to assess the prevalence of anemia, malaria and intestinal helminth infection in second-trimester pregnant women in a highly endemic area (Iquitos, Peru). Anemia...
was assessed by Hemocue, malaria by thick and thin smears and intestinal helminths by the Kato-Katz method. A total of 1042 second-trimester pregnant women were recruited between April and December 2003. The following prevalences were obtained: anemia (<11mg/dl) 47.3%, malaria (P. vivax and P. falciparum) 0.19%, Ascaris 63.9%, Trichuris 82.2%, hookworm 47.2%. Other less frequent parasites found included Hymenolepis nana, Strongyloides stercoralis and Enterobius vermicularis. More anemic women were infected with one or more helminth infections than non-anemic women (p<0.05). Important proportions of pregnant women suffer from co-morbid conditions that require prenatal treatment — including antihelminthic treatment. A comprehensive evaluation of the local parasite disease burden should inform preventive treatment measures to be included in routine prenatal care programs.

RANDOMIZED CONTROLLED TRIAL OF MEBENDAZOLE PLUS IRON SUPPLEMENTATION VERSUS PLACEBO PLUS IRON SUPPLEMENTATION DURING PREGNANCY

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Iron supplementation is regularly included in prenatal care programs. In highly hookworm-endemic areas, WHO recommends antihelminthic treatment of pregnant women. The additional antihelminthic-attributable benefit on reducing maternal anemia or on reducing low infant birthweight has not previously been measured. This study was undertaken to determine the benefit (in terms of maternal anemia and infant birthweight) of a single dose antihelminthic treatment administered to second-trimester pregnant women, over and above iron supplementation. A randomized controlled trial was conducted between April 2003 and June 2004 in Iquitos Peru. Baseline and longitudinal data on anemia (Hemocue; <11mg/dl) and helminths (Kato-Katz; <1mg/ml) and anemia were obtained at recruitment (during the second trimester), during the third trimester and at delivery. Birthweight was measured electronically (± 20g) at birth (if in-hospital) or within 2-3 days (if at home). Between April and December 2003, a total of 1042 second-trimester pregnant women were randomized into two groups: iron supplementation plus single dose mebendazole and iron supplementation plus single dose placebo. Baseline data confirmed a high prevalence of soil-transmitted helminth infection (90.7%) and anemia (47.3%). Treatment status will be known the first week of June 2004, when all follow-up has been completed and blinding can be lifted. Results from the intention-to-treat analysis will be presented. In conclusion, this trial will demonstrate that PGN infections in infants can occur at high rates and supports a treatment strategy for children under two years of age. In addition, the age specific jump in prevalence suggests a potential intervention point.

FUNCTIONAL SIGNIFICANCE OF LOW INTENSITY POLYPARASITIC HELMINTH INFECTIONS IN CHILDHOOD ANEMIA

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Epidemiologic studies acknowledge the high prevalence of concurrent multiple species infection in helminth endemic regions, but studies assessing the health impact of multiple concurrent infections are lacking. This study was undertaken to assess the impact of low intensity polyhelminthic infections on prevalent anemia in school age children. We hypothesize that low intensity polyparasitism will be associated with anemia. This cross-sectional study, among 632 school-aged children, was carried out in rural rice farming villages in Leyte, The Philippines. Burden due to 4 helminths:
Ascaris lumbricoides, Trichuria trichiura, Necator americanus, and S. japonicum were measured in three stools, each read in duplicate by the Kato-Katz method. Infection intensity was defined using WHO criteria as follows: uninfected, low, and > Moderate (M+) intensity. 4 profiles of polyparasite helminth infection were defined as follows: multiple low (>2 low), polyparasite I (1 M+), polyparasite II (2 M+) and polyparasite III (>3 M+). The reference category consists of uninfected children, or those with 1 low intensity infection. Anemia was defined as hemoglobin level < 11 mg/dL. A logistic regression model was used to quantify the association between the 4 infection profiles and prevalent anemia. After confounder adjustment, multiple low intensity infection was associated with 5 fold higher odds of prevalent anemia (90% CI: 1.1 - 28). The prevalent odds of anemia for children in polyparasite I, polyparasite II and polyparasite III infection categories relative to uninfected children were 4.1 (0.96-23), 6.2(1.4-30), and 8(1.7, 39) respectively. In conclusion, low intensity polyparasite infection is associated with significantly higher prevalent anemia. This is the first study to quantify the functional significance of polyparasite low intensity infections in children, an important finding due to the high prevalence of this pattern of infection. A major strength of this study is adjustment for key confounders such as SES, age, sex and nutritional status.

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Th2 CYTOKINES AND RESISTANCE TO HUMAN HOOKWORM INFECTION

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Cytokine and proliferative responses to Necator americanus infection were measured during a treatment-reinfection study in a highly endemic area of Papua New Guinea. There was a mixed Th1/Th2 cytokine response to hookworm antigen. Specific IFN-γ responses were lower in heavy infections and increased after treatment, suggesting parasite-induced immunosuppression. Resistance was associated with parasite-specific IL-5 production: the intensity of reinfection was significantly negatively correlated with pretreatment IL-5 responses. The IL5, IL4 and IL13 genes lie in the Th2 gene cluster on chromosome 5q31. Polymorphisms in this region were typed and association with infection and NDVI data are easy to use, affordable and available with world-wide coverage.

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ECOLOGICAL COVARIATES OF ASCARIS LUMBRICOIDES INFECTION IN KWAZULU-NATAL/SOUTH AFRICA - A GIS BASED STUDY

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Geographic information systems (GIS) are increasingly being used for the prediction of parasitic diseases and for research into their environmental epidemiology. However, yet there have been few GIS based studies into the ecology of geohelminth infection. This study examines associations of selected ecological factors with Ascaris lumbricoides infection in order to assess their potential for disease prediction and to further investigate A. lumbricoides ecology. Approximately 750 primary schoolchildren from a small area in rural KwaZulu-Natal were examined for geohelminth infection at baseline and re-examined 3 and 29 weeks after treatment with 400 mg albendazole. We recorded the geographical positions of their homes and interviewed the pupils in order to assess their relevant behavior and socioeconomic background. This information was combined with remotely sensed, cartographic and other data to form a GIS database.

Prevalence maps and spatial statistics revealed considerable spatial clustering of infection in the relatively small study area. Multivariate models showed a strong positive association of infection and re-infection with vegetation density as characterized by the normalized difference vegetation index (NDVI) and a strong negative association of re-infection with the exposure of the soil surface to the sun as estimated from digital elevation models. Infection and re-infection were only moderately positively associated with the clay content of the soil. Socio-economic and behavioral variables, although correlated with A. lumbricoides infection, did not appear to confound the above associations in the demographically homogenous study area. Spatial analysis of the model residuals suggested that since the models accounted for most of the spatial pattern, the model standard errors should not be affected by spatial clustering. We conclude that NDVI has a high potential for the prediction of ascariasis because it is strongly associated with infection and NDVI data are easy to use, affordable and available with world-wide coverage.

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GNATHOSTOMIA: AN EMERGING PARASITIC DISEASE IN SINALOA, MEXICO

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Human gnathostomiasis is acquired by ingestion of raw freshwater fish infected with the third stage larvae of at least four species of the genus Gnathostoma. It has been proposed that G. bineculeatum may be responsible for human gnathostomiasis in America. The state of Sinaloa is located on the Northwest Pacific coast of Mexico, where the population eats raw freshwater fish as “cebiche”. The consumption has been increasing since 1989, when the first cases of gnathostomiasis were detected. More than 3000 cutaneous, 3 ocular and 5 acute cases with visceral injury have been recorded in Sinaloa. Cutaneous and ocular cases were found only in the capital city of Culiacan, whereas the acute cases were identified in the southern part of the state To identify the source of infection, fish, birds, mammals, amphibians and reptiles were captured for over 10 years. Third stage larvae were found in six out of thirty fish species. The species with the highest number of larvae were E. picta (664), D. latifrons (538) and G. maculatus (70), all captured in a lagoon in southern Sinaloa, where five acute human cases were diagnosed. Four turtles captured in this area were also infected. Eight out of 15 species of ichthyophagous birds were infected. In contrast, two mammals (19 Procyon lotor and 12 Didelphis virginiana) as well as two amphibians (Rana catesbeiana and R. pipiens) were not infected. To identify Gnathostoma species, the larvae isolated from fish and birds were treated with the conventional protocols for extraction, amplification and automatic sequencing of ITS2 ribosomal DNA and 28S ribosomal RNA. In accordance with the high similarity of the ITS2 sequences, the larvae were classified as G. bineculeatum. Our results show that in Sinaloa fish, birds and reptiles, are the natural hosts for G. bineculeatum. To date, all infected fish were
found in southern Sinaloa, whereas almost all human cases of cutaneous and ocular disease were registered in the city of Culiacan. Whether the source of human infection in Culiacan is from fish imported from the South or from an unidentified source in lakes or dams close to Culiacan remains to be clarified. The high percentage of parasitized birds (53%) is clearly related to their fish diet. Their feeding and migration habits could be contributing to the spread of this zoonosis. Our results will be useful in establishing the preventive control measures in other endemic areas with similar socio economic conditions.

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**BURDEN OF INVASIVE DISEASE CAUSED BY *HAEMOPHILUS INFLUENZAE* TYPE B IN BAMAKO, MALI**

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Few sub-Saharan African countries have bacteriologically-confirmed, population-based data to quantify the burden of invasive *Haemophilus influenzae* type b (Hib) disease and help guide decision makers pondering whether to introduce Hib immunization for infants. A clinical bacteriology laboratory was established in Hôpital Gabriel Touré, which serves the pediatric population of Bamako, Mali. Children 0-15 years of age with high fever (axillary temperature > 39°C) or clinical syndromes compatible with invasive bacterial disease (meningitis, pneumonia, empyema, peritonitis, etc.) were eligible. Parental consent was sought for all children; assent was also required for children 13 years and older. Two to 4 ml of blood or relevant body fluid were collected and inoculated into Ped Plus® medium for automated culture. Body fluids were also inoculated directly onto solid media. Hib isolates were confirmed by standard clinical microbiologic techniques. Antibiograms were generated by disk diffusion. From June 1, 2002 to May 31, 2003, 2499 children admitted to HGT with high fever or a clinical syndrome compatible with invasive Hib disease were cultured including 950 0-11 month olds, 661 1-4 year olds and 438 were 5-15 year olds. Hib was isolated from 98 Bamako children, 48 from blood alone and 40 from CSF (alone or with a positive blood culture). Fully 96 of the 98 cases (98%) occurred in children < 5 years of age (incidence 43.7/105) and 72 (73.5%) in infants age 0-11 months (incidence 147.6/105). The peak incidence occurred in children < 5 years of age (73.5% of cases, p < 0.001). Among 12 culture-positive gastric aspirates, 3 were positive by LJ only; 10 were positive by MODS only; and 8 were positive by both. Among 12 culture-positive gastric NPAs, 1 was positive by LJ only; 4 were positive by MODS only; and 7 were positive by both. Of 4 culture-positive stools done by both methods, one was positive by LJ only; 2 were positive by MODS only; and one was positive by both. Of the 18 specimens that were culture-positive by one method but not the other, 14/18 (22.2%) were culture-positive by LJ only and 4/18 (77.8%) were culture-positive by MODS only. Mean time from inoculation of plates to isolation of MTB for culture-positive specimens was 25.9 ± 10.5 days for LJ and 10.7 ± 4.5 days for MODS. Use of the MODS technique resulted in higher yield and faster recovery of MTB from these high risk children.

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**ISOLATION OF MYCOBACTERIUM TUBERCULOSIS FROM PERUVIAN CHILDREN BY MYCOBROTH OBSERVATION-DIRECT SUSCEPTIBILITY (MODS) METHOD**

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The diagnosis of pediatric TB in developing countries is severely limited because children rarely produce smear-positive sputum, and isolation of *M. tuberculosis* (MTB) by traditional Lowenstein-Jensen (LJ) culture is inefficient and slow, requiring 4-6 weeks. The Mycobroth Observation Direct Susceptibility (MODS) technique is an easy and transferable method, using direct microscopic observation of early MTB colony formation in Middlebrook 7H11 broth in the wells of tissue culture plates. In adults, this simple approach results in faster isolation and sensitivity testing of MTB compared to LJ cultures.

We compared rates of MTB isolation from multiple gastric aspirate, nasopharyngeal aspirate, and stool specimens collected from 165 children ages 0-12 years with clinical evidence of TB (based on a Stegen-Toledo score ≥ 5 points) evaluated at the Instituto de Salud del Niño in Lima, Peru. Overall, 15 of 165 subjects (9%) were culture-positive in at least one specimen (10/59 [17%] of children with ≥ 7 ST points, vs. 5/106 (4.7%) of children with 5 or 6 points, p < 0.009). Among 21 culture-positive gastric aspirates, 3 were positive by LJ only; 10 were positive by MODS only; and 8 were positive by both. Among 12 culture-positive gastric NPAs, 1 was positive by LJ only; 4 were positive by MODS only; and 7 were positive by both. Of 4 culture-positive stools done by both methods, one was positive by LJ only; 2 were positive by MODS only; and one was positive by both. Of the 18 specimens that were culture-positive by one method but not the other, 4/18 (22.2%) were culture-positive by LJ only and 14/18 (77.8%) were culture-positive by MODS only. Mean time from inoculation of plates to isolation of MTB for culture-positive specimens was 25.9 ± 10.5 days for LJ and 10.7 ± 4.5 days for MODS. Use of the MODS technique resulted in higher yield and faster recovery of MTB from these high risk children.

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**A COMPARATIVE STUDY OF CURE RATES OF DOTS AND NON-DOTS INTERVENTION STRATEGY IN NEW SPUTUM POSITIVE PULMONARY TUBERCULOSIS PATIENTS TREATED AT DTC PATNA AND DTC BHAGALPUR, BIHAR, INDIA**

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A DOTS strategy was implemented for the treatment of pulmonary tuberculosis in Patna, India. This study was undertaken to evaluate the therapeutic efficacy of DOTS with NON-DOTS intervention strategy in new sputum positive pulmonary tuberculosis patients at District TB Center, Patna. The treatment of 1786 new pulmonary tuberculosis patients who were registered and received treatment with a DOTS strategy from January to December 2002 was compared to 218 new patients who were prescribed anti-tubercular medicines from April 2002 to March 2003 at District TB Center, Bhagalpur without implementing DOTS strategy.
cough of ≥3 weeks duration and without any previous history of tuberculosis were tested by three sputum smears taken on spot, next morning sample and next day spot sample and at least two samples were required to be positive for M. tuberculosis for patients to be identified as new sputum positive cases. Registered patients at DTC Patna had intermittent drug regimen positive for M. tuberculosis for patients to be identified as new sputum positive cases.

EARLY INDUCTION OF COTRIMOXAZOLE RESISTANT PNEUMOCOCCI AMONG ZAMBIAN INFANTS RECEIVING EMPIRIC PROPHYLAXIS

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WHO currently advises that infants exposed to HIV at birth receive cotrimoxazole (CTM) prophylaxis for at least one year. It is unknown whether this policy could adversely impact antibiotic resistance patterns for common pediatric pathogens. We report preliminary results from an ongoing 3 yr. longitudinal study of HIV-exposed infants receiving CTM prophylaxis (cases), and age-matched HIV-unexposed infants (controls), and their mothers to determine the impact of CTM on antibiotic resistant pneumococci. We screened mother/infant pairs from Ndola, Zambia for pneumococcal colonization with nasopharyngeal swabs, pneumococcal antibiotic resistance was determined via E-tests. Our primary outcome was CTM resistance (combined intermediate and high-level resistance per NCCLS guidelines). We calculated relative risks (RR) and 95% confidence intervals (95% CI) for infants and mothers prior to CTM prophylaxis at age six weeks (baseline) and after receiving prophylaxis for six weeks (age 3 months).

In April 2004, 3 children from the village of Los Delfines near Iquitos, Peru died. One had renal and respiratory failure; one had abdominal pain and cutaneous and gingival hemorrhages; no information was available for the third. At the same time, 12 people from Los Delfines presented with undifferentiated fever to the Hospital Regional in Iquitos. These findings prompted an outbreak investigation. Cases were those with undifferentiated fever with onset between April 25 to May 2, a time period with a clear epidemic curve.

AN OUTBREAK OF LEPTOSPIROSIS IN A NEW RURAL COMMUNITY IN THE PERUVIAN AMAZON DUE TO ANTHROPOGENIC ENVIRONMENTAL CHANGES

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In April 2004, 3 children from the village of Los Delfines near Iquitos, Peru died. One had renal and respiratory failure; one had abdominal pain and cutaneous and gingival hemorrhages; no information was available for the third. At the same time, 12 people from Los Delfines presented with undifferentiated fever to the Hospital Regional in Iquitos. These findings prompted an outbreak investigation. Cases were those with undifferentiated fever with onset between April 25 to May 2, a time period with a clear epidemic curve.
ated liver, 36 without, were screened for IgM antibodies against *Leptospira*, dengue, oropouche, yellow fever, hantavirus, arenavirus, Group C and mayaro viruses. Urine (32 samples), throat swabs (23) and whole blood (30) samples were taken primarily from febrile patients and screened for *Leptospira* in addition to other agents. The majority of those enrolled (67%) were 0-15 years, 47 (70%) of whom were febrile. An IgM ELISA test using sonicates of 6 pathogenic *Leptospira* found that 47/103 (46%) were positive. The microscopic agglutination test (MAT) was used to confirm the IgM ELISA. Of these, 34 (33%) were from febriles, 13 (13%) from asymptomatics. Subjects in the age-group 6-10 (59%) and 11-15 (60%) had the highest percentages of IgM positive cases. Cases decreased with age although fewer individuals over 30 were tested. Viral serology results were negative, except for an IgM titer for Carapicus virus, a Group C virus, in four patients (6%). Study of potential reservoirs for *Leptospira* in the locale of Los Delfines including pigs, dogs, marsupials and domestic rats confirmed a high level of exposure and active excretion. Environmental water sampling of effluent from a pig farm to a recreational and bathing area demonstrated a gradient of pathogenic leptospires. Leptospirosis was the major cause of this outbreak of febrile illness outbreak, and was due to anthropogenic environmental changes leading to transmission from peridomestic animals.

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**CLINICAL SPECTRUM OF PULMONARY INVOLVEMENT IN LEPTOSPIROSIS IN AN ENDEMIC REGION, WITH QUANTIFICATION OF LEPTOSPIRAL BURDEN**

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Pulmonary involvement in leptospirosis remains poorly recognized in the endemic setting despite reports of recent outbreaks and epidemic disease. A prospective, population-based study was carried out to identify febrile patients exposed to *Leptospira* in urban and rural contexts in Iquitos, Peru. Evidence of exposure to *Leptospira* was obtained by serology. Leptospirosis was confirmed in cases with pulmonary involvement by demonstration of the organism by culture and/or on-site performance of a quantitative real time PCR assay. Of 633 consecutively enrolled febrile patients, 321 (50.7%) had IgM or high-titer anti-leptospiral antibodies. Seven patients with history only of urban exposure to leptospires had severe pulmonary manifestations; of these, 5 patients died, 4 with pulmonary hemorrhage, 1 with acute respiratory distress syndrome and multiorgan failure. A real time, quantitative PCR assay showed high levels of leptospiremia in most fatal cases (2 x 10^6 leptospires/mL); one patient from whom tissues were obtained at autopsy had 2 x 10^7 leptospires/kg of lung, kidney, and muscle. This study demonstrates the under-diagnosis of leptospirosis in a highly endemic region, including recognition of grave pulmonary complications. Pulmonary involvement in leptospirosis was present in urban but not rural areas. A quantitative, real time PCR assay provided rapid diagnosis of leptospirosis in severe cases. Presumptive treatment for leptospirosis should be initiated immediately in the appropriate epidemiological and clinical context. Public health measures to control this disease in endemic regions need to be initiated at the governmental level.

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**TEMPORAL GENOTYPE DISTRIBUTION THROUGHOUT THE HIGH MALARIAL TRANSMISSION SEASON IN CHILDREN OF MISSIRA, MALI**

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Recent studies have demonstrated that human infections with *Plasmodium falciparum* malaria can involve over 5 distinct genotypes within the Block 2 region of the *msp1* gene. These genotypes are morphologically identical and share a high degree of sequence similarity, making differentiation by traditional means difficult. We have only recently developed a sensitive, specific assay that combines the quantitation capabilities of real-time PCR with the ability of capillary electrophoresis to separate by amplicon size, which allows for both identification and quantitation of these different genotypes of *P. falciparum*. Using this technique, we have analyzed genotype data obtained from filter paper blots during a prospective cohort study in the village of Missira in Mali, West Africa, beginning late in the dry season and continuing through the period of the transmission season (April-November). Here we describe the genetic diversity of the *msp1* gene within the *P. falciparum* population in Missira, as well as the genotype distribution during the above described study period. The implications of the association between the genotype population and disease outcome of children with the disease, will also be discussed. (ACMCI P abstract)

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**EXPRESSION AND CHARACTERISATION OF A SOLUBLE AND REFOLDED RECOMBINANT DOMAIN OF PLASMODIUM FALCIPARUM ERYTHROCYTE MEMBRANE PROTEIN 1**

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Immunity to malarial disease develops during late childhood to early adulthood and is partly attributed to antibodies against variant parasite antigens expressed on the surface of infected erythrocytes. The principal target of these antibodies is the variant and adhesive antigen *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 is a large multi-domain protein, encoded by var genes, and mediates adhesion of infected erythrocytes to host endothelial cells, contributing to disease pathogenesis. Some members of this family of proteins bind to platelet glycoprotein IV (CD36) and intercellular adhesion molecule-1 (ICAM-1) on endothelial cells. We have expressed the Duffy-binding like (DBL) 4γ-domain, encoded by A4var, from the A4 parasite line which adheres to CD36 and ICAM-1. The DBL4γ domain of PfEMP1 is cysteine rich (12 cysteine residues), yet was expressed as a soluble his-tag fusion protein in E. coli. It was purified in a three-step process using Ni-IDA, anion-exchange and size-exclusion chromatography. The recombinant domain fragment reacted strongly with antibodies in serum from malaria-exposed individuals but was not recognised by serum from non-exposed individuals. Furthermore, this material was found to be a stable monomer in which no free cysteines could be detected using a combination of alkylation and RPL-MS methods, and may be suitable for crystallization to examine DBL domain structure. We have observed exposure-specific recognition of the domain by serum antibodies and we are currently assessing whether antibodies to this recombinant PfEMP1 domain can be used as a marker of immunity. The use of the recombinant refolded
domain and a reduced and alkylated form allows us to examine and compare antibody responses to linear and conformational epitopes, which may be of functional or antigenic importance.

964
**Cd36 Plays an Important Role in Conferring Protective Immunity Against Mice Infected with Plasmodium Chabaudi Chabaudi As Malaria**

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Previous in vitro studies have shown that Cd36 is a major receptor involved in clearance of non-opsonised Plasmodium falciparum-infected erythrocytes (PEs) by monocytes/macrophages (Mfs). Based on these data, the objective of this study was to determine the role of Cd36 in innate immunity to Plasmodium chabaudi chabaudi A5 malaria. We hypothesized that Cd36 is required for innate control of P. chabaudi malaria and that Cd36-/- (KO) mice would develop severe and fatal malaria compared to Cd36 +/- (WT) mice. WT and KO mice were infected with 10⁶ parasites intraperitoneally. Blood smears from each mouse were taken daily to monitor infection. For in-vitro phagocytosis assays, WT and KO Mfs were isolated and plated onto glass coverslips. Mfs were incubated with erythrocytes infected with PCC A5 for 2 hours. Following incubation, Mfs were stained with Diff-Quik stain. Serum from WT and KO-infected mice were also collected on appropriate days and assayed for TNF-α and TGF-β. In vitro phagocytosis assays revealed that WT Mfs ingested significantly more P. chabaudi infected erythrocytes than KO Mfs. In vivo survival studies showed that Cd36 KO-infected mice had higher mortality rate than Cd36 WT infected mice (63% vs. 20%; p<0.01). The infection peaked earlier and with higher parasite densities in Cd36 KO mice compared to Cd36 WT mice. TNF-α levels were higher but TGF-β levels were lower in KO mice than WT mice, supporting a contributing role for unbalanced cytokine responses in severe and fatal malaria in this model. Cd36 KO Mfs are unable to control parasitaemia and have dysregulated cytokine responses to infection, resulting in higher mortality. Cd36 plays an important role in innate control of P. chabaudi malaria in mice. (ACMCIP abstract)

965
**Functional Insights into the Double EGF Domains of Plasmodium Falciparum MSP-1 and Related Proteins**

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An understanding of structural and functional constraints on the C-terminal double epidermal growth factor (EGF)-like modules of merozoite surface protein 1 (MSP-1) and related proteins is of importance to the development of these molecules as malaria vaccines and drug targets. This MSP-1 double EGF module is considered an important target of merozoite invasion into erythrocytes. Recently, we have shown that both EGF-like modules of MSP1 interact with the band 3 receptor as well as intact erythrocytes in a sialic acid-dependent and chymotrypsin-sensitive mechanism during Plasmodium falciparum invasion into erythrocytes. MSP1 is essentially composed of a pair of consecutive epidermal growth factor (EGF)-like modules. A number of three-dimensional structural studies of MSP1 have shown that the two EGF-like domains adopt an unusual U-shaped conformation rather than a more commonly observed elongated conformation. Immunization studies using recombinant MSP1 in a murine malaria model showed both EGF-like domains are necessary as immunogen to have effective protection against Plasmodium yoelii challenge in mice. Here, we report the mapping of band 3-binding sites in MSP1 and show that each EGF-like module of MSP1 interacts directly with the band 3 receptor as well as intact erythrocytes in solution. Neither the N-terminal 83-kDa secondary processing product (MSP1) nor its 19-kDa secondary processing product (MSP1), interact with band 3 by a sialic acid-independent and chymotrypsin-sensitive mechanism during Plasmodium falciparum invasion into erythrocytes. MSP1 is essentially composed of a pair of consecutive epidermal growth factor (EGF)-like modules. A number of three-dimensional structural studies of MSP1 have shown that the two EGF-like domains adopt an unusual U-shaped conformation rather than a more commonly observed elongated conformation. Immunization studies using recombinant MSP1 in a murine malaria model showed both EGF-like domains are necessary as immunogen to have effective protection against Plasmodium yoelii challenge in mice. Here, we report the mapping of band 3-binding sites in MSP1 and show that each EGF-like module of MSP1 interacts directly with the band 3 receptor as well as intact erythrocytes in solution. Neither the N-terminal 83-kDa secondary processing product of MSP1 nor the 19-kDa secondary processing product of MSP1 interacted with band 3. When each EGF-like module of MSP1 interacted with band 3 receptor, interaction between native MSP1 and intact erythrocytes was blocked. A quantitative analysis of the interactions suggests that both EGF-like modules are crucial for the direct binding of MSP1 to and/or MSP1 to the band 3 receptor. (ACMCIP abstract)

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**Crystal Structure of EBA-175 RII: Structure, Function and a Model for Erythrocyte Binding**

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EBA-175 is a Plasmodium falciparum ligand that binds the erythrocyte receptor glycophorin A during merozoite invasion of erythrocytes. RII, a 73 kDa extracellular domain that is the erythrocyte binding domain of EBA-175,
also represents the DBL domain family that is present in the \( ebl \) and \( var \) gene family of proteins. We had determined the structure of RII to 2.25 Å resolution, and found that the elongated RII molecule forms a dimer displaying two prominent channels at the center of the dimer implicated in glycoporphin A glycan binding. We selectively mutated the residues that appear to be involved in receptor binding and dimerization, a single residue at a time. We found that a few specific residues involved in dimer formation or putative glycoporphin A glycan binding are required for erythrocyte binding. Thus a single residue change of those specific residues can abolish the ability of RII to bind erythrocytes. Based on the structure and functional analysis, we propose a model for erythrocyte binding. Our structural studies facilitate the elucidation of the mechanism of erythrocyte invasion as well as allow the rational design of receptor blockade therapy and vaccines. (ACMCIP abstract)

**PUNICA GRANATUM AS A HUMAN USE, WIDESPECTRUM PROPHYLACTIC AGAINST MALARIA AND VIRAL DISEASES IN INDIA.**

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The eastern province of Orissa is among India's worst endemic zone. Koraput district has the most resistant type \( Plasmodium falciparum \) and \( P. vivax \) strains causing very high incidence of systemic malaria and cerebral malaria. Two villages were selected for this study: Badamput has 173 adult inhabitants living in 35 households; and Gunthaguda and 118 adult inhabitants living 26 households. In either village at least 7 persons would be found afflicted with Mlr. on any day of the year and 10 % with measles & chickenpox between Feb. to April. A programme for whole village prophylaxis was launched under the aegis of district administration through Indian Red Cross Society's Ayurvedic charitable dispensary. *Punica granatum* (PG) is a fruit, dermis of which has been used by Ayurveda & in modern herbal formulations for other than anti-Mlr or anti-viral. Its dermis has been reported to have therapeutic efficacy against Mlr. Pulverised powder of sun dried dermis, hand filled @ 500mg / gelatin capsules of No 1 size, is given orally with water as vehicle. The capsules are termed OMARIA - P [Orissa malaria research indigenous attempt - prevention]. Every inhabitant of village Badamput was given single dose of 1000mg of PG powder ( 500mg x 2 gelatin cap.) daily in morning after food consecutively for 7 days as 3 consecutive months starting 07 / 07 / 2003 to 13 / 07 / 2003 from 07 / 08 / 03 to 13 / 08 / 03 and again between 07 / 09 / 03 to 13 / 09 / 03. A booster dose of 1000mg for 4 day duration was given between 07 / 02 / 2004 to 10 / 02 / 04. Every inhabitant of village Gunthaguda was given identical prophylaxis from 09 / 12 / 2003 to 2003 / 15 / 12 / 2003 from 09 / 01 / 2004 to 15 / 01 / 04 and 09 / 02 / 2004 to 15 / 02 / 04 ; booster dose from 07 / 03 / 04 to 11 / 03 / 04 ( on villager's demand infants included - extra mural ). Drug compliance at 90% Mlr. affliction ( including fever of all etiology ) reduced to < 2 % of gross population within 2 weeks post introduction of OMARIA-P in both the villages. Since 08 / 2003 no case of SM,CM, Measles,Chicken Pox, Conjunctitis & Herpes have been reported [ pregnancy included ; infant excluded ]. The district administration has since 04 / 2004 extended the prophylactic use programme to other villages. Empty gelatin capsules gifted by m/s Sunil Synchem

**21ST CENTURY TECHNOLOGIES FOR VACCINE DEVELOPMENT, USING MALARIA AS A MODEL**

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We are evaluating and optimizing promising 21st century vaccine technologies for the development of vaccines against a range of public health threats, using the *Plasmodium* spp parasite as a model, with single antigen and multi-antigen combinations. We are focusing on platform technologies that have the potential to induce a broad range of immune responses, including CD8⁺ T cell responses required for protection against an intracellular pathogen such as *Plasmodium*. Using homologous and heterologous immunization strategies, we have evaluated plasmid DNA, recombinant poxvirus, recombinant adenovirus, and Alphavirus replicon particle vaccines for their capacity to induce broad and sustained cellular and antibody mediated immune responses and confer long-term protection against pathogen challenge. Data demonstrate that the different vaccine platforms vary in their capacity to prime for induction of antigen-specific immune responses and boost for recall of vaccine-induced immune responses. In addition, the fine specificity and breadth of the induced immune responses and the maintenance of cell populations capable of conferring long-term protective immunity depends on the specific combination of priming and boosting agent as well as the vaccination regimen. Based on data generated in the murine model, we have selected promising vaccine platform technologies and combinations for further evaluation and prioritization in swine and nonhuman primate models. In this manner, we have implemented a systematic and comprehensive vaccine development plan that builds on data generated with multiple vaccine technology platforms in animal models and transitions to clinical evaluation. It is anticipated that our studies will improve the prospects for developing effective vaccines against existing, emerging and re-emerging pathogens that threaten public health. (ACMCIP abstract)

**IMPROVED CROSS-STRAIN RECOGNITION OF POLYMORPHIC PLASMODIUM FALCIPARUM ISOLATES BY ANTIBODIES INDUCED WITH SHUFFLED PIEMP-1 CIDRI-1 A VACCINE ANTIGENS**

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The PIEMP-1 (*Plasmodium falciparum* Erythrocyte Membrane Protein-1) CIDR-1α domain is a prime target for the development of a malaria vaccine because it is exposed on the surface of the infected erythrocyte, antibodies to variant surface antigens are associated with resistance to disease, and immunization of *Aotus* monkeys provides protection in a strain-specific manner. The CIDR-1α domain also mediates attachment of the infected erythrocyte to endothelial cells using CD36 as its receptor and in this way the infected erythrocyte evades destruction in the spleen. Cross-strain protection by immunization with CIDR-1αs is currently limited by the antigenic polymorphism of CIDR-1α from different *P. falciparum* strains. To
develop a novel PIEMP-1 vaccine that induces cross-strain protection, we have used DNA shuffling to generate libraries of chimeric CIDR-1α antigens. Shuffled clones from these libraries had incorporated sequence diversity from a number of parental P. falciparum isolates throughout the chimeric recombinant polypeptides. Mice were immunized with shuffled CIDR-1αs and parental CIDR-1αs using both protein immunization and DNA immunization procedures. Sera from immunized mice were then tested for reactivity with CIDR-1αs from various P. falciparum strains displayed on the surface of CHO cells using a whole cell ELISA approach. Several shuffled CIDR-1α vaccine antigens induced antibodies with extended cross-strain recognition of CIDR-1αs compared to parental CIDR-1α vaccine antigens. Repeated shuffling and screening of selected chimeric CIDR-1α vaccine antigens that induce cross-reactive antibodies is being done to further improve immunity across P. falciparum strains. (ACMCIF abstract)

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VAR2CSA, A NOVEL VACCINE CANDIDATE AGAINST PREGNANCY-ASSOCIATED MALARIA

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Pregnancy-associated malaria (PAM) is a major cause of maternal anaemia and perinatal morbidity and mortality in endemic areas. PAM is characterised by the accumulation of Plasmodium falciparum parasitised erythrocytes in the intervillos space of the placenta, where the parasites adhere to low-sulphated chondroitin sulphate proteoglycans (CSA). We have previously reported that a single, uniquely structured var gene (var2csa) is upregulated in P. falciparum isolates in response to CSA-selection. We now show that VAR2CSA is expressed on the surface of different CSA selected parasites and that it cannot be detected in the unselected parental lines. We also report that IgG VAR2CSA levels are significantly higher in women than in men, that IgG levels against VAR2CSA are higher in multigravidae compared to primigravidae and that the presence of VAR2CSA antibodies is predictive of favourable birth outcomes. Sequence analysis of different placental isolates show that this particular VSA is very conserved and common. This work is a major step toward the development of a vaccine that could protect pregnant women against PAM.

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IMMUNOGENICITY OF A MULTI-ALLELE, RECOMBINANT AMA1-BASED BLOOD STAGE MALARIA VACCINE IN HUMANS

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Apical Membrane Antigen 1 (AMA1), a polymorphic merozoite surface protein, is a leading candidate for a blood stage malaria vaccine. In a Phase 1 trial in 30 naive volunteers, details of which are presented elsewhere in this forum, an equal mixture (AMA1-C1) of recombinant proteins representing two different AMA1 alleles (FVO and 3D7) expressed in Pichia pastoris adsorbed on Alhydrogel® were safe and well-tolerated. Here, we report on the detailed characterization of the immune responses to AMA1 in the three dose groups (5 ug, 20 ug and 80 ug per vaccination) on serum samples collected up to day 194 of the study. Anti-AMA1 IgGs were detected by ELISA in 15/28 (54%) volunteers two weeks after the second immunization and in 17/19 (90%) two weeks after the third immunization (GM 408 U/ml, range undetectable-4084 U/ml). There was a dose-related increase in antibody titers. IgG titers to the two different proteins were strongly correlated (Spearman rank test, p<0.05). Sera from vaccinated volunteers with ELISA titers >50 U/ml reacted with blood-stage Plasmodium falciparum parasites by IFA showing a distribution consistent with native parasite AMA1. Subclass analysis of sera with high IgG titers two weeks after the second vaccination detected anti-AMA1 specific IgG1 and IgG3 as the predominant subclasses. Sera from vaccinated individuals cross-reacted with a third allelic variant of AMA1, designated L32, in ELISA assays, showing titers that were similar to those against AMA1-FVO and slightly lower than AMA1-3D7 (Wilcoxon Signed Rank Test, p<0.05). Taken together, these data indicate that AMA1-C1 primes naive individuals successfully for specific antibodies and support the further development of this vaccine.

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IMMUNOGENICITY AND PROTECTIVE EFFICACY OF TWO PLASMODIUM FALCIPARUM LSA3 POLYPEPTIDES IN AOTUS MONKEYS

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The immunogenicity and protective efficacy of two polypeptides from Plasmodium falciparum Liver Stage Antigen-3 (LSA-3), covering regions previously identified as relevant to protection, were assessed in Aotus monkeys. Two long synthetic peptides 100-222 from the non-repetitive N-terminal region, and 501-596 representative of the repeat region 2 (R2), containing numerous B and T cell epitopes were used to immunize two groups of Aotus lemurinus griseimembra. One group of Aotus was immunized with peptide 100-222 alone, and the second group was immunized with a mix of 100-222 plus 501-596 peptides. Monkeys were injected subcutaneously with 3 doses of 50 µg of each peptide mixed with AS02 adjuvant. Control animals received PBS with the same adjuvant. After immunization, animals were challenged intravenously with 105 P. falciparum sporozoites. Parasitemia was assessed by two different methods both, thick blood smear and immunocapture of parasite Lactic Dehydrogenase (pLDH). Protection was achieved in all immunized monkeys, as compared to the controls and the non-immunized animal. Monkeys immunized with either peptide 100-222 or 501-596 exhibited effector memory T-cell responses in terms of antigen-driven Lympho-proliferation and/or specific gamma-interferon (γ-IFN) secretion detectable by ELISPOT assays. γ-IFN was produced in response to: i) the recombinant proteins overlapping long peptide sequences, ii) NRI, NRII, RE synthetic peptides covering part of 100-222 sequence iii) P. falciparum sporozoite extract. ELISA antibodies ranged 1,900 to 1,270,000. These results confirm the vaccine potential of LSA-3 indicated previously in chimpanzees and in Aotus with particular formulations. They also confirm the value of the new Aotus model recently developed for the pre-clinical assessment of pre-erythrocytic stage vaccines
IMMUNOGENICITY OF PLASMODIUM FALCIPARUM MSP1: EFFECTS OF IMMUNE GENE KNOCKOUTS ON ADJUVANTS’ EFFICACY

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Adjuvants often play a critical role in determining the efficacy of many types of vaccines. The biological activities of adjuvants can often be diverse and may affect many cell types in different manners. We have previously studied the dependency of several adjuvants on IFN-γ and IL-4 to potentiate antibody response to a P. falciparum MSP1 vaccine. Unique requirements for these two cytokines for adjuvanticity were demonstrated. In this report, we further examine several popular adjuvant formulations, including ISA720, MF59, Qs21, and proprietary formulations of emulsified and aqueous monophosphoryl Lipid A, to determine their adjuvanticity in a number of murine immune knockout models (eg. IL-6, ICAM-1, IL-12b, TNFrs1a/b, CD80/86, IFN-γ, IL-4) using the P. falciparum MSP1 as an immunogen. ELISA antibody titers, isotypes, as well as ELISPOT (IFN-γ and IL-4) were used as readout for immunogenicity. Our results showed a complex picture of reliance of different immune mediators by various adjuvant formulations. Separate requirements for immune mediators were observed for the potentiation of antibody versus cellular responses, suggesting more than one cell type as targets of adjuvanticity. The use of oil-type emulsion (ISA720) influenced the effects of immune mediators on other immunological adjuvants and thus it has properties other than the classic depot effects. We will present results of selected KO mouse strains to illustrate adjuvants’ dependency and to demonstrate the ability to overcome such reliance by further reformulation. We will also illustrate comparisons of the immunogenicity of MSP1 induced by adjuvants across various KO mouse strains in order to gauge each formulation’s degree of “promiscuity” in the potentiation of immune responses under different restrictive environments.

TOLL-LIKE RECEPTOR (TLR5) MEDIATED IMMUNE RESPONSES IN HUMAN SKIN BY ONCHOCERCA VOLVULUS ANTIGENS: POTENTIAL CONTRIBUTION TO THE PATHOGENESIS OF DERMATITIS

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The major non-ocular effect of infection with the filarial parasite Onchocerca volvulus is onchoceral skin disease (OSD) characterized by dermatitis with intense itching, skin depigmentation, and skin atrophy. To further understand the pathogenesis of onchoceral dermatitis, we investigated the expression of the Toll-like-receptors (TLRs) TLR2 and TLR4, which act as pattern recognition receptors initiating innate immune responses against a wide array of pathogens. Using primary cultures of differentiated human keratinocytes exposed to either O. volvulus antigens or polymyxin-absorbed O. volvulus antigens, we analyzed the regulation of TLR2 and TLR4 by RT-PCR, Western blot analysis, and immunohistochemistry. We found that O. volvulus and their Wolbachia antigens are potent activators of TLR4 (but not of TLR2) mRNA expression. As shown by immunoblot analyses, none of these TLRs were expressed at the protein level on normal unchallenged cultured keratinocytes. In contrast, O. volvulus antigens induced a potent up-regulation of both TLR2 and TLR4 expression comparable to that seen in response to LPS / IFN-γ. More importantly, immunohistochemical analysis of intact human skin after in situ exposure to the filarial antigens revealed a differential expression and up-regulation of TLRs with TLR2 seen in the stratum granulosum and stratum corneum and TLR4 in the stratum basale of the epidermis. We also found that TLR4 signalling pathways are both CD14-dependent and CD14-independent. In addition, we have also analyzed the pattern of cytokine production by the keratinocytes after exposure to O. volvulus antigens. Our data suggest that signalling through TLR2 and TLR4 may contribute significantly to the pathiology of onchocercal dermatitis. (ACMCI abstract)

ABILITY OF REPLICATION-DEFICIENT ADENOVIRUS 35 AND 5 VECTORS TO PRIME AND BOOST PRE-ERYTHROCYTIC IMMUNITY AGAINST PLASMODIUM FALCIPARUM IN RHESUS MACAQUES

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The circumsporozoite protein (CS) of Plasmodium falciparum has been demonstrated to be a viable candidate antigen for pre-erythrocytic vaccine development. A set of experiments was designed to study the ability of CS-expressing, recombinant, non-replicative Adenovirus 35 and 5 vectors (rAd35-CS and rAd5-CS, Crucell, Netherlands) to improve CD8+ and CD4+ T cell responses to CS in rhesus macaques. Adenovirus 35 seroprevalence in the human population, particularly in malaria endemic areas, is significantly lower than Ad5, and rAd35 vectors expressing foreign antigens have been shown to efficiently bypass Ad5 pre-existing immunity. Experiments tested prime-boost schedules of rAd35-CS at 0.3 and 0.6 months versus a prime of rAd5-CS and a boost of rAd35-CS at 6 months. All vaccines were safe. Immune responses were measured by ELISA, ELISPOT, and intracellular cytokine staining with flow cytometry. We will present comprehensive data and discuss their implications for subsequent clinical trials. (ACMCI abstract)

EARLY INNATE IMMUNE RESPONSES TO FILARIAL PARASITES AND ENDOSYMBIOTIC WOLBACHIA ARE PREDOMINANTLY DEPENDENT ON TOLL-LIKE RECEPTOR 2

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Lymphatic filariasis is caused by infection with Brugia malayi, B. timori and Wuchereria bancrofti and is a major health problem in tropical regions of the world with over 120 million infected individuals. The pathological consequences of infection are primarily acute and chronic lymphedema of the extremities (elephantiasis) and hydrocele in men, however, not all infected individuals manifest severe disease. Wolbachia bacteria are found in most filarial parasites of importance to human health (B. malayi, W. bancrofti, O. volvulus). The obligate symbiotic organisms are concentrated in intracytoplasmic vacuoles within the hypodermal lateral cords and female reproductive organs, transmitted in a matri-linear fashion and play an important role in reproduction and development of the nematode. Early studies of innate immune activation by the filarial worms or worm extracts showed a predominantly TLR4 dependence in animal models as well as in vitro stimula-
tion of neutrophils and macrophages. Using human TLR transected HEK cell lines as well as mouse strains deficient in TLR2, TLR4 and MyD88, we show that inflammatory responses to soluble filarial extracts containing Wolbachia are instead primarily dependent on TLR2. Responses are also dependent on MyD88. There is a relatively minor contribution of TLR4. Differences in the design and reagents employed in these studies are discussed. Dominant negative transfection studies confirm the TLR dependence and signaling pathways activated by the Wolbachia containing extracts. The impact of variable bacteria content on select TLR binding and activation will be presented. These finding have important implications for future studies aimed at identifying host factors that contribute to the pathogenesis of clinical filarial diseases.

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HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS PRODUCE THE MONOCYTE CHEMOATTRACTANTS MCP-1 AND MIP-1 β IN RESPONSE TO WOLBACHIA SURFACE PROTEIN
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We have shown that antibody responses to Wolbachia Surface Protein (WSP) are associated with the presence of lymphedema or hydrocele and that Wolbachia bacteria can be found in association with human macrophages/giant cells in biopsy specimens collected from patients experiencing filarial adenolymphangitis. In order to further characterize the types of immune responses mounted to Wolbachia by persons with lymphatic filariasis, we analyzed cytokines produced in response to WSP in vitro. Peripheral blood mononuclear cells (PBMC) from 26 individuals living in Leogane, Haiti, an area endemic for lymphatic filariasis, were selected to represent the different parasitological and clinical outcomes of filarial infection. PBMC were cultured in vitro either with PHA, Brugia pahangi adult worm antigen, or synthetic WSP composed of overlapping 24-mer peptides. Cell culture supernatants were harvested at 48 hours and analyzed for the presence of 17 different cytokines using a multiplex array technology. All PHA-stimulated cell cultures produced IL-2, IL-4 and IFN-γ demonstrating that the cells were viable and capable of producing cytokines. Consistent with previous studies showing a polarization of the immune responses in persons with lymphatic filariasis, individuals with lymphedema, in general, produced higher levels of cytokines in response to worm antigen than asymptomatic microfilaremic individuals. Interestingly, 50 and 59% of WSP-stimulated cell cultures produced the inflammatory CC chemokines MCP-1 and MIP-1 β. Although levels of MIP-1 β were similar among the different groups, individuals with lymphedema produced significantly higher levels of MCP-1 than asymptomatic microfilaremic individuals. These results provide further support for an association between immune reactivity to Wolbachia and the presence of chronic filarial disease and suggest that activation of monocytes/macrophages may play an important role in the recognition of Wolbachia antigens.

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EOSINOPHIL CHEMOTAXIS TO THE INITIAL SITE OF FILARIAL INFECTION: ASSESSMENT USING A NOVEL IN-VITRO MODEL OF SKIN/FILARIAL INTERACTION
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Eosinophils have been shown to surround helminth parasites in the tissues and to deposit eosinophil granule proteins on their surface. To explore the mechanism of selective recruitment of eosinophils to the sites of filarial depo-
**PATHOLOGICAL CHANGES IN LYMPHATICS INFECTED WITH Wuchereria bancrofti**

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Lymphatic vessels from male patients with active infection of Wuchereria bancrofti, including nodules containing degenerating adult worms, were examined using regular histochemical stains, as well as immunohistochemical reagents identifying general lymphocyte, macrophage and endothelial phenotype antigens, a scanning electron microscopic analysis was also carried out. More than 24 specimens were screened and certain samples from this group selected for detailed studies. A very characteristic lymphangectasia was seen in all specimens that involved alterations to the surface endothelial surface and the intimal layers. Increased vascularity of the walls of the lymphatics was seen in virtually all specimens and these vessels were often located close to endothelial cell layer and the lumen. Many of the endothelial cells lining these dilated vessels were altered in morphology and many surface projections, folds and valve like structures were seen in these vessels. These changes in vessel anatomy were present in all the observed lymphatic tissues. In samples where the adult worms were known to be alive and healthy, mild infiltrations of lymphocytes, macrophages and plasma cells were seen in the walls of these vessels in the immediate vicinity of the parasites; eosinophils in small numbers were present, but only in locations considered to be very close to the adult worms. Degenerating worms were associated with a significant, chronic cellular response characteristic of a parasite granuloma with a well organized anatomy. These finding support the concept that considerable, non-inflammatory tissue changes are associated with living adult worms that these proceed the more well known inflammatory responses associated with adult worm death and destruction.

**AN EPIDEMIOLOGIC ASSESSMENT OF FILARIAL, INTESTINAL HELMINTH AND MYCOBACTERIAL COINFECTION IN SOUTH INDIA: BCG VACCINATION PROTECTS FROM HOOKWORM INFECTION, BUT HELMINTHS FAIL TO INFLUENCE SUBSEQUENT RESPONSES TO MYCOBACTERIA TUBERCULOSIS**

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To determine if pre-existing helminth infection (Th2-dominated) alters the ability to mount a Th1-dominated immune response to *Mycobacteria tuberculosis*, a cross-sectional study was conducted in five communities in the Chingleput district of southern India near Chennai. All community members (n=5096) aged 6-65 were screened for the presence of intestinal helminths and circulating filarial antigens as well as PPD reactivity (cut-off of ≥ 12 mm), active tuberculosis and history of BCG vaccination (presence of scar). Among the 5096, 47% were PPD positive, 32% had intestinal helminth infection (primarily hookworm), 9% were circulating filarial antigen positive and 0.5% had culture confirmed active TB. After controlling for confounding factors, in a multivariate analysis, we found that only age and BCG vaccination status were significantly associated with PPD reactivity: PPD reactivity increased linearly with age; persons aged 50-65 were 6.8 times more likely to be PPD reactive than persons aged 6-14. Persons who had been vaccinated with BCG were 1.9 times more likely to be PPD positive than those who had never been vaccinated. Interestingly and contrary to the proposed hypothesis, neither intestinal helminth infection nor filarial infection were associated with diminished frequencies of PPD positivity; however, the presence of a BCG scar (from vaccination at birth) was associated with less prevalent hookworm infection (OR = 0.82, 95% CI: 0.72-0.94). Ultimately, these findings indicate that neither preexisting hookworm nor filarial infection play a role in altering the response to PPD and consequently, both systemic and intestinal helminth infections may not have a significant effect on the delayed hypersensitivity response to *Mycobacterium tuberculosis*.
ANTIGEN TARGETING TO LYSOSONAL VESICLES INCREASES ANTIBODY RESPONSE TO A DENGUE DNA VACCINE IN RHESUS MACAQUES

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A dengue virus type 2 DNA vaccine (D2MEL) expressing pre-membrane (prM) and envelope (E) genes fused to the lysosome targeting sequences was constructed. The vaccine construct expressed antigen that was localized in lysosomal vesicles of transfected cells and produced higher antibody responses in vaccinated mice compared to constructing only the prM and E genes (D2ME) (Virology 290:74-82). To determine if this observation can be extended to non human primates, D2ME and D2MEL were tested in rhesus macaques. To ascertain if a vaccine construct using sequences from a low passage clinical isolate is more immunogenic compared to that with sequences from lab adapted virus, D2ME and D2MEL constructed from new guinea C strain (ngc) and a Philippine isolate (Phi) of dengue-2 virus were also tested. Monkeys (n=3) were immunized intramuscularly with 1 mg DNA (D2ME-ngc, D2ME-Phi, D2MEL-ngc or D2MEL-Phi) at 0, 1 and 5 months. Antibody responses were determined by using serially diluted sera in a plaque reduction neutralization assay. At 6 months, all vaccinated animals (except 2 that had received D2ME-ngc) had virus neutralizing antibodies. The mean 50% virus neutralization titer (PRNT₅₀) for groups vaccinated with D2ME-Phi, D2MEL-Phi and D2MEL-ngc were 105, 369 and 158 respectively. The one animal which developed placebo, and in the second study to receive PEGASYS® (6 µg/kg) or placebo, on day one after the onset of viremia. Serial daily viremia levels were measured, and convalescent D2 virus neutralizing antibody titers were determined. Compared to placebo, Roferon®-A temporarily suppressed D2 virus replication and delayed the time to peak viremia by a median of 2 days. Mean peak serum viremia levels and area under the curve (AUC) virus concentrations were not different between the two groups. This finding led to a study of pegylated IFN-α. PEGASYS® produced a 1-log drop in mean daily viremia levels over 4 days when compared to placebo. Peak viremia levels were not significantly affected, but D2 virus AUC and elimination t½ trended lower in the PEGASYS® group. There were no significant differences in D2 virus PRNT₁₀ between two groups at 30 and 90 days post-infection. Rapid identification of dengue viremic patients early in illness may provide an opportunity to suppress viremia and ameliorate subsequent disease severity. A single injection of PEGASYS®, or a combination of PEGASYS® and Roferon®-A, early in dengue illness should be further investigated.

A RANDOMIZED, PLACEBO-CONTROLLED, STUDY OF NON-PEGYLATED AND PEGYLATED FORMS OF RECOMBINANT HUMAN INTERFERON-α-2A FOR SUPPRESSION OF DENGUE VIREMIA IN RHESUS MACAQUES

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Infection with any dengue viruses can produce a spectrum of disease, ranging from a mild febrile illness, to classic dengue fever, to the most severe form, dengue hemorrhagic fever (DHF). There is growing evidence that infection with any dengue viruses can produce a spectrum of disease, ranging from a mild febrile illness, to classic dengue fever, to the most severe form, dengue hemorrhagic fever (DHF). Rapid identification of dengue viremic patients early in illness may provide an opportunity to suppress viremia and ameliorate subsequent disease severity. A single injection of PEGASYS®, or a combination of PEGASYS® and Roferon®-A, early in dengue illness should be further investigated.

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REFINEMENT AND VALIDATION OF AN IMMUNOCYTOMETRIC ASSAY PERMITTING RAPID MEASUREMENT OF ANTI-DENGUE NEUTRALIZING ANTIBODIES

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Dengue infection continues to be a growing international public health concern with vaccine development a principal public health goal. The primary correlate of immunity is thought to be neutralizing antibodies. Thus, a prerequisite for comparing and optimizing vaccine candidates is the ability to precisely measure neutralizing antibody responses. Currently, the plaque reduction neutralization test (PRNT) is the gold standard. However, this test has many limitations, including its laborious and time-consuming performance and reliance on laboratory adapted viral strains. For those reasons, we have developed an assay capable of measuring neutralizing antibodies to low-passage dengue viruses within 24 hours. This assay uses a human cell line transfected to express a dendritic cell pathogen-capturing lectin, DC-SIGN, which renders the cells highly susceptible to infection. Infection is measured by immunocytometric assay using antibodies against dengue antigens approximately 20 hours after inoculation of cells with dengue virus that was pre-incubated with test serum or non-immune serum. We have previously reported successful proof-of-principle with this technique using neutralizing monoclonal antibodies and selected positive control sera. We have now expanded our previous work with sera from several experimental animal infections to demonstrate serotype specific antibody-mediated neutralizing activity in this assay equivalent to that seen in conventional PRNTs using Vero cells. We also show that this novel assay can measure neutralization of low-passage, non-adapted virus isolates. Finally, because the cells in which infection is measured in this assay constitutively express FcγRII in addition to the putative dengue receptor DC-SIGN, we show that antibody-mediated enhancement of infection, as well as neutralization, can be observed in some circumstances. We are continuing our studies to assess whether in vitro enhancement measured in this assay correlates with disease outcome from natural infections.
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A PHASE 2 STUDY OF THE SAFETY AND IMMUNOGENICITY OF CHIMERIVAX™-JE VACCINE IN HEALTHY ADULTS

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ChimeriVax™-JE (ChV-JE) is an experimental live, attenuated, genetically engineered virus prepared by replacing the prM-E genes of yellow fever 17D vaccine virus with corresponding genes of Japanese Encephalitis virus. This randomized, controlled study includes a cross-over double-blind stage during which 201 subjects received two vaccinations 28 days apart, one of ChV-JE and one of diluent placebo. Subsequently, ~100 subjects received an open label ChV-JE vaccination 6 months after the double-blind vaccinations. There have been no deaths and three serious adverse events: two were deemed unrelated to vaccination while one “acute viral illness” was possibly related to vaccination. The subject reported episodic diarrhea and fever 8 days following vaccination, was admitted to the hospital for re-hydration, and recovered within 5 days. One diluent recipient had an oral temperature of >38.1°C in the 28 days following vaccination. There were no differences in number and frequency of adverse events experienced by subjects following ChV-JE or diluent administration. The most common treatment-related adverse events were transient constitutional symptoms and injection site complaints. The majority (>80%) of adverse events were mild in severity. No viremia was seen in any of the subjects 14 days following vaccination. Of the 200 subjects in the intention-to-treat population, 191 (97.0%) seroconverted at day 28 following single dose vaccination. Seroconversion was defined as an appearance of neutralizing antibody titer to the homologous virus when not present before vaccination, or a four-fold rise in neutralizing antibody titer between pre- and post-immunization samples. The geometric mean neutralizing antibody titer rose from 59 by day 14 following vaccination, to 317 by day 28. The results from this study so far provide additional support for the hypothesis that ChV-JE is a safe and immunogenic vaccine.

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CONTINUING SPREAD OF USUTU VIRUS, IN CENTRAL EUROPE

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Usutu virus (USUV), a member of the mosquito-borne clade within the genus Flavivirus, has been responsible for avian mortality in Austria since 2001. USUV activity has now been recognized during 3 consecutive years. As with West Nile virus, dead bird surveillance is an excellent tool for monitoring USUV activity. In total, USUV has been found in 132 birds with at least two independent methods, usually RT-PCR and immunohistochemistry. The affected birds belong to 8 different species, but more than 90% of all positives were Eurasian blackbirds. In 2001, a few local episodes of bird mortality were recognized in Vienna and adjacent villages. In 2002 and 2003 the areas with USUV-associated bird die-offs gradually extended to the South and East and to a lesser degree to the North and West. Currently the distribution area of USUV comprises the city of Vienna, 11 (out of 25) districts of Lower Austria and 3 (out of 9) districts of Burgenland and covers approximately 3500 square kilometers in the East of Austria. So far USUV cases have only been noticed during the months July through September, a time period which we currently define as USUV season. In addition, USUV has been identified in several mosquito species. These data demonstrate that USUV has established an efficient bird-mosquito transmission cycle in Eastern Austria. We expect further extension of the affected areas and an involvement of neighboring countries, like Slovakia and Hungary soon. Although USUV belongs to the Japanese Encephalitis group of mosquito-borne flaviviruses, which includes several members with considerable pathogenicity for humans, USUV has not been associated with human diseases in Austria, so far.

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YELLOW FEVER IMMUNIZATION COVERAGE IN HOST COMMUNITIES FOR INTERNALLY DISPLACED PERSONS - LIBERIA, 2004

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A yellow fever (YF) outbreak was declared in Liberia in February of 2004. Immunization campaigns occurred in internal displacement camps and host communities (HCs) in Bong County in February and March. A retrospective study was conducted to determine whether immunization coverage in HCs was adequate following the campaigns. Ten of 21 HCs were selected; population estimates were obtained from health workers and town chiefs. Coverage was estimated using the lot quality assurance method under World Health Organization guidelines. Based on a campaign goal of 80% and precision of 8%, minimum sample size was 150 households (15 per HC). One person (or proxy) randomly chosen from each household was surveyed. The decision value for inadequate coverage was 4 unimmunized persons per HC. Coverage in the overall population was estimated by taking a weighted average of the coverage in each HC. One person was surveyed in each of 158 households; 87 (55%) were female. Fifteen (9%) were 6 mo-4 yr, 33 (21%) were 5-14 yr, 79 (50%) were 15-44 yr, and 31 (20%) were 45 yr. Median household size was 10 persons (range: 1-32). The number of unimmunized persons met the decision value in one HC. During the campaign, 89% (95% confidence interval CI: 0.84-0.94) of the overall population was immunized. Of these, 38% (95% CI: 0.28-0.49) had been previously immunized. Total coverage in the overall population was 96% (95% CI: 0.93-0.99). In conclusion, coverage goals were met in the overall population of the ten HCs, but not in one remote HC. This study showed that the campaign was effective and immunity is likely high in this population. To maintain high immunity, YF immunization should be included in routine schedules and accessible at remote sites.

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HCV INFECTION IN RURAL EGYPTIAN CHILDREN

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In spite of its high prevalence in rural Egyptian communities, information about the incidence and natural history of hepatitis C virus (HCV) infection, in Egyptian pediatric populations is limited. The present study exam-
ines the rates of HCV transmission (both vertical and horizontal) as well as the natural history of early HCV infection in a cohort of 2287 rural Egyptian children living in the Nile Delta. Mothers of participating children were enrolled during pregnancy. After delivery, 2-4 ml blood samples, questionnaire histories, and physical examinations were obtained for participating children at 2 and 6 months as well as years 1, 2, 3, 4, and 5 after birth. Serum specimens were evaluated for antibodies to HCV (anti-HCV) and HCV-RNA. During study follow-up 11 children were diagnosed with HCV infections; 7 of these were perinatally acquired, while 4 were community-acquired cases. The rate of confirmed HCV maternal-infant infection was 3.15% (95% CI [1.39 6.66]); 7 of the 222 infants born to HCV-RNA positive mothers were HCV infected on their initial study visit. Four out of 1244 initially HCV negative children became subsequently infected from community sources (incidence of 1.48 per 1,000 person years). Two of the four community-acquired HCV cases presented with no obvious risk factors aside from the fact that they were born to HCV positive mothers; the remaining two cases with HCV negative mothers reported histories of extended hospitalization. Of the 11 HCV infected children, 1 (9%) cleared the virus and 4 (36%) presented with hepatosplenomegaly (one child had a congenital liver defect and experienced symptoms prior to infection with HCV). Updated results including risk factors for HCV infection, morbidity, viral clearance, and HCV genomic sequence comparisons of mother-infant pairs will be presented.

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COMPUTERIZED VISUAL DIAGNOSTIC SYSTEM FOR INTERNATIONAL AND TROPICAL DERMATOLOGY

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A Visual Diagnosis Reference System (VDRS) has been designed to address visual diagnosis in international and tropical dermatology. The VDRS functions as both a computerized diagnostic and a therapeutic reference system. The current system, known as VisualDx, is designed to address dermatologic disease in the context of a patient’s presenting problems by topic (e.g., Fever and a Rash), or body location, and has been adapted to address dermatologic conditions as they relate to specific geographic locations (“International Dermatology”). Unlike an atlas, which has a one-dimensional index, the VDRS allows multi-axial searches by multiple key patient findings (symptoms, occupation, past medical history, country of origin, recent travel, etc.) as well as the morphology and distribution of the skin lesions. The VDRS then groups images by diagnostic “visual stack”, resorting the images based on patient presentation. This allows more efficient image matching and provides a window into unusual case presentations, rather than the “classic” images found in textbooks. The “International Dermatology” module includes over 4000 geographic relationships to over 100 geographically related diseases represented by over 800 images. These geographic knowledge relationships will allow the clinician to search by any country in the world and immediately see the diagnoses to be considered if the patient has traveled to, emigrated from, or lives in, a particular region. Other international medical databases utilize powerful text based approaches but lack a visual component to assist in diagnosis or are limited by design around a pathophysiologic grouping (i.e. infectious disease). The International module of this VDRS is designed to aid clinicians in visual diagnosis of cases of classic travel-related illnesses (i.e. leishmaniasis), diseases that are common to residents from specific locations (i.e. leprosy), and many diseases that span the globe (i.e. scabies and impetigo) but may be seen frequently among travelers and immigrants.

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TELEDIAGNOSIS AND INTERNET-BASED TOOLS TO ENHANCE THE DIAGNOSIS OF PARASITIC DISEASES


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DPDx was developed by the Division of Parasitic Diseases, CDC to assist and strengthen the laboratory diagnosis of parasitic diseases and to provide distance-based educational training. DPDx provides diagnostic assistance through telediagnosis, which is based on the exchange of images captured from clinical specimens that may contain suspected parasites. To date, forty-seven public health laboratories (in 46 states and Puerto Rico), in addition to one site at MERTU-Guatemala, have received grants to acquire a digital camera that can be connected to a microscope and a computer, with internet access. This allows rapid screening of difficult cases and the dissemination of information and results. This is particularly valuable in outbreak situations. Between 2002 and 2003, there was an increase of approximately 30% in telediagnosis consults. This indicates a positive trend towards the use of telediagnosis for parasitic diseases. Distance-based education and training are also available through the DPDx Web site (http://www.dpd.cdc.gov/dpx). The DPDx web site has become a very useful resource for diagnostic parasitology (3,915,541 hits and 108,617 visitor sessions per month that averaged 9 minutes and 30 seconds [based on WebTrends reporting software for the period of July 1, 2003-December 31, 2003]). The site provides laboratorians worldwide with information on the biology of parasites, diagnostic tests relevant for each disease, procedures to follow in the laboratory, and images and video clips of parasites. Training opportunities include: monthly case studies on laboratory diagnosis of parasitic diseases sent to a LISTSERV with over 1,000 members; a Continuing Education Unit titled “Laboratory Diagnosis of Infections Caused by Coccidian Parasites”; wet lab workshops on the identification of intestinal and bloodborne parasites; and a CD-ROM with similar content to the Web site but which also includes CDC manuals and a foodborne and intestinal parasites quiz for self-testing.

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PREVALENCE OF HYPERTENSION IN WOMEN IN ACCRA, GHANA - RESULTS OF THE WHSA

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Hypertension (HTN) is a silent killer. The complications of undiagnosed hypertension include myocardial infarction, stroke and renal failure. The Women’s Health Study of Accra (WHSA) is a cross-sectional survey of the burden of disease of a representative sampling of women aged 18 and higher. This study included a household survey (HHS) of 3174 women for self-reporting histories, and physical examinations were obtained for participating women. After delivery, 2-4 ml blood samples, questionnaire histories, and physical examinations were obtained for participating women at 2 and 6 months as well as years 1, 2, 3, 4, and 5 after birth. Serum specimens were evaluated for antibodies to HCV (anti-HCV) and HCV-RNA. During study follow-up 11 children were diagnosed with HCV infections; 7 of these were perinatally acquired, while 4 were community-acquired cases. The rate of confirmed HCV maternal-infant infection was 3.15% (95% CI [1.39 6.66]); 7 of the 222 infants born to HCV-RNA positive mothers were HCV infected on their initial study visit. Four out of 1244 initially HCV negative children became subsequently infected from community sources (incidence of 1.48 per 1,000 person years). Two of the four community-acquired HCV cases presented with no obvious risk factors aside from the fact that they were born to HCV positive mothers; the remaining two cases with HCV negative mothers reported histories of extended hospitalization. Of the 11 HCV infected children, 1 (9%) cleared the virus and 4 (36%) presented with hepatosplenomegaly (one child had a congenital liver defect and experienced symptoms prior to infection with HCV). Updated results including risk factors for HCV infection, morbidity, viral clearance, and HCV genomic sequence comparisons of mother-infant pairs will be presented.

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ory of HTN and taking medications from a doctor (p < 0.01) but not from a pharmacist, herbalist or traditional healer. There also was a significant association with socioeconomic status of the residential area, work status, marital status, and education. There was no correlation with employment status, ethnicity, religion, income, body mass index, chest pain, shortness of breath, or family history of HTN, stroke or myocardial infarction. This study identified a very high prevalence of hypertension (45%) in adult women residing in an urban environment in a developing country. Approximately half of the women were unaware that they had HTN. A strong public health initiative is critical to increase awareness of the prevalence of HTN, to encourage routine bp measurements and to take appropriate preventive and treatment measures to reduce the risk of the serious sequelae of this disease.

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INFORMED CONSENT IN PERSONS WITH LIMITED LITERACY AND LIMITED EXPERIENCE WITH HEALTH PROFESSIONALS

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Across developed countries in North America, Europe and Asia, the informed consent process is almost totally dependent on literacy. Potential subjects are frequently asked to read consent forms 10-20 pages or more in length, which may focus more on limiting the responsibilities of the investigator and the legal liability of the institution than on the medical/public health questions under study. As a result, the understanding of participants in clinical studies is frequently marginal, even in developed countries. Unfortunately, most of these problems are exacerbated in developing countries where the persons at greatest risk (who should be included in studies of disease control strategies) frequently have limited literacy and limited understanding of modern medicine. To address this problem, we have used: 1) standardized presentations by in-country investigators in dialect on videotape, 2) demonstrations of proposed procedures such as fingersticks, venipunctures or Holter monitoring on the videotape, 3) group question and answer sessions for communities or groups of potential subjects in clinical environments with the study team following videotape presentations, 4) individual discussions between study representatives and potential subjects (that are videotaped), 5) conversion of the videotaped record of the study discussion and the consent transaction to a DVD in order to provide a permanent record, and 6) focus groups to identify areas of incomplete or inadequate understanding. This strategy provides consistent information about the disease being studied and the nature of the study from someone who understands the culture in which the study is taking place. It also addresses the need to provide a permanent record of the consent transaction (which can be audited by a funding agency) without insisting that an illiterate subject sign a consent form that he/she cannot read. Conversely, the major disadvantage of this strategy is that it requires considerable time, beginning with visits to the chief, elders, women’s council, youth council and the community at large before approaching individual subjects.

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APRAISAL OF SNAKEBITE INCIDENCE IN SENEGAL, WEST AFRICA

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Surveys were carried out in four savanna areas of Senegal. The studied zones had different climatic, economic and demographic characteristics. Two types of studies were performed: on the one hand, a retrospective survey carried out in health centers and, on the other, household surveys in the corresponding areas. Questions involved the circumstances of the bite, symptoms and treatment. The retrospective survey reported very few cases of snakebites over the prospective period. In the first zone, Mbour, a suburban area (population density by 100 inhabitants per km²), primarily occupied by fruit plantations (mangos, oranges, lemons) and gardens, the annual incidence of snakebites (i.e. all snakebites including those without any symptoms) reached 85 bites per 100,000 and morality was 3.7 deaths per 100,000. In the study zone of Niakhar, located in the groundnut culture area of Senegal which is also highly populated (130 inhabitants per km²), the incidence of snakebites was 30 bites per 100,000 and mortality was close to 1.5 deaths per 100,000 a year. The area of Niorto is a mixed agricultural area (food producing and revenue agriculture) and less populated (100 inhabitants per km²). The annual incidence was roughly of 320 per 100,000 and the mortality 3.8% per 100,000. Finally, the fourth area, Bandafassi in the extreme South-East of Senegal is a mountainous zone exclusively occupied by bush and food producing agriculture, and scantily populated (20 inhabitants per km²). The incidence was 905 per 100,000 and the mortality 11 deaths per 100,000. The population at risk involved males from 15 to 45 years. Pastoral work (agriculture and breeding) was at the origin of the majority of the accidents. The recourse to the traditional practitioners was systematic, which explained why the data from the health centers were not relevant enough to evaluate the importance of the envenomings.

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HEPATIC CYSTIC ECHINOCOCCOSIS AND AMEBIC ABSCESS OF THE LIVER: A DIAGNOSTIC DILEMMA IN THE MIDDLE EAST?

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Hepatic cystic echinococcosis (CE) and amebic liver abscess (ALA) are both prevalent in Kuwait, the former being endemic while the latter is imported primarily from the Indian sub-continent. Following a clinical examination, imaging studies, usually an ultrasound (US) or computerized tomography (CT), are requested. Both modalities are excellent at identifying space-occupying lesions but there are limitations in establishing their etiology. Serodiagnosis for CE and ALA is recommended but interpretation of the titers, as we illustrate, may prove problematic in a country where foreigners are in a substantial majority.

We elucidate the dilemma faced by physicians as both hepatic CE and ALA serodiagnosis are being increasingly requested together and in substantial numbers. We first describe 4 patients in whom both tests were ordered simultaneously, emphasizing the difficulties and pitfalls in diagnosis. Of 232 samples for concurrent CE and ALA serology, 115 (49.5%) were from Arabs. Of the 23 (9.91%) positive for hepatic echinococcosis, 19 (82.6%) were from Arabs but only 3 (13.04%) from the Indian subcontinent (P < 0.0001). In contrast 105 (45%) samples for ALA from non-Arabs, the titer was significant in 86 (37%) patients, 54 (62.1%) from the Indian subcontinent and 26 (30.23%) Arabs (P < 0.0001). Thus apart from the clinical presentations, the origin of the patients is a significant indicator of the possible etiology of the space-occupying lesion. We highlight attendant problems such requests create in terms of the level of the clinical acumen, laboratory time, manpower and the costs involved. We suggest guidelines for
physicians to arrive at a reasonable clinical diagnosis before requesting both serologies. We also show that apart from CE and ALA, other clinical conditions give rise to similar hepatic lesions. Bacterial and fungal abscesses and hepatomas were identified and perhaps such a list of differential diagnoses is important in endemic areas.

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ETIOLOGIES OF FEBRILE ILLNESSES IN LA MERCEDE, PERU, 2000-2004

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Between January 2001 and January 2004, surveillance was conducted in La Merced, Peru, a city of 31,000 population, located in the cloud forest (high jungle) on the eastern slope of the Andes Mountains at 750 meters elevation. Patients aged 5-years or older who presented to treatment facilities with fever for less than 5 days and gave informed consent were enrolled in the febrile syndromic surveillance study. We sought to determine the etiologic agents responsible for their illnesses. Patients were administered a questionnaire that included a description of the clinical illness and demographic information. Sera collected from patients were tested for evidence of dengue, yellow fever, Venezuelan equine encephalitis, Oropouche, and Mayaro viruses. Leptospira, Brucella, spotted fever group (SFG) rickettsia and Coxiella burnetii. Infections were confirmed in patients who demonstrated a 4-fold or greater increase in antibody titer from the acute phase to the convalescent phase of illness. Acute and convalescent sera from 357 patients were available for testing from a total of 439 patients admitted to the study. Seventy-five (21%) were confirmed as dengue fever, 32(9%) leptospirosis, 23 (6%) rickettsial spotted fever, 10 (3%) Q fever. Other less frequent etiologies included brucellosis and Oropouche fever.

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THE EFFECT OF HOST IRON STATUS ON HOOKWORM PATHOGENESIS

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Hookworm infection affects nearly 1 billion people worldwide, leading to poor growth, anemia, increased fetal and maternal morbidity/mortality, and decreased work productivity. Iron deficiency is the most prevalent nutritional deficiency worldwide and occurs in many of the same geographic areas as hookworm infection. In order to characterize the effect of host iron status on the natural history of hookworm infection, we compared the pathogenesis of disease in laboratory animals fed either high or low iron diets. The golden Syrian hamster (Mesocricetus auratus) is a fully permissive host for the human hookworm Ancylostoma ceylanicum, and laboratory infection in this model is characterized by inoculum dependent growth delay and anemia similar to that seen in humans. Groups of 15 hamsters were fed either a low iron pellet diet (6-10 ppm of iron in the form of ferric citrate) or a control iron diet (200 ppm iron). After 3 weeks, 10 animals from each diet group were infected by oral gavage with 125 third stage larvae of A. ceylanicum. Animals were followed post-infection for evidence of hookworm disease, which included measurement of weight, blood hemoglobin, and serum iron. Five animals from each diet group were sacrificed at day 10 post-infection and day 20, and adult parasite worm burdens were recorded. Fecal egg excretion was measured on day 20 post-infection. In the animals fed the low iron diet, pre-existing iron deficiency was not associated with further growth delay and anemia following hookworm infection. Host iron deficiency was also associated with substantially reduced numbers of worms recovered at day 20 post-infection (P=0.003). These findings suggest that iron may be among the first host factors required for hookworm development in vivo. Further investigation of the relationship between host iron stores and parasite development may provide insight into the pathogenesis of hookworm infection, as well as identify novel targets for vaccine and antihelminthic development.

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HAEMOLYSIS IN BLOOD FEEDING HELMINTHS

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Hookworms feed on blood but the mechanisms by which they lyse ingested erythrocytes are unknown. Here we show that Ancylostoma caninum, the common dog hookworm, expresses a detergent soluble, haemolytic factor. Activity was identified in both adult and larval stages, was heat-stable and unaffected by the addition of protease inhibitors, metal ions, chelators and reducing agents. Trypsin ablated lysis indicating that the haemolysin is a protein. A closely migrating doublet of hookworm proteins with apparent molecular weights of 55-60 kDa bound to erythrocyte membrane after lysis of cells using both unlabeled and biotinylated detergent-solubilized hookworm extracts. In addition, separation of detergent-soluble parasite extracts using strong cation-exchange chromatography, resulted in purification of 55-60 kDa proteins with trypsin-sensitive haemolytic activity. Erythrocytes lysed with particulate, buffer-insoluble worm extracts were observed using scanning electron microscopy and appeared as red cell ghosts with approximately 100 nm diameter pores formed in the cell membranes. Red blood cell ghosts remained visible indicating that lysis was likely caused by pore formation and followed by osmotic disruption of the cell. Molecular characterisation of the haemolytic protein is underway. We also, however, took a gene-first approach to identifying haemolysins from hookworms, by scanning the expressed sequence tag (EST) datasets. A family of mRNAs encoding proteins similar to amoebapores, pore-forming proteins of Entamoeba histolytica, was identified from A. caninum and the nematode blood fluke, Schistosoma mansoni. Recombinant proteins were expressed in insect cells and antisera raised for functionality studies, immunolocalisation and efficacy in vaccine trials. Much attention is now being focused on gut derived or “hidden” antigens as anti-helminth vaccines - haemolysins and haemoglobin-degrading proteases are therefore viable candidates for new vaccine therapies and drug design.
THE HUMAN HOOKWORM VACCINE INITIATIVE (HHVI): PROGRESS IN THE DEVELOPMENT AND TESTING OF A RECOMBINANT HOOKWORM VACCINE

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Human hookworm infection is a leading global cause of iron-deficiency anemia (IDA) and malnutrition affecting 740 million people in the developing countries of the tropics. Currently, the major approach to hookworm control relies on frequent and periodic deworming with benzimidazole anthelmintics (BZAs). Through resolution 54:19, the WHO has proposed administering BZAs in the schools to 75% of school-age children at-risk for acquiring soil-transmitted helminth (STH) infections (e.g., ascariasis, trichuriasis, hookworm) by 2010 (www.who.int/wormcontrol). However, unlike ascariasis and trichuriasis in which the heaviest worm burdens occur in this age group, the IDA of hookworm disease is a significant cause of morbidity among all children, including preschool children, as well as women of reproductive age and other adults. Therefore, programs that target school-age children will miss important vulnerable populations, including 44 million hookworm-infected pregnant women. Also of concern, is the possibility of emerging BZA resistance. As an alternative or complementary approach for hookworm control, the HHVI is developing a first-generation recombinant vaccine comprised of an alum-formulated recombinant antigen, ASP-2, produced in yeast. ASP-2 is an abundant protein secreted by infective hookworm larvae. Human investigations conducted in Brazil (Minas Gerais State) and China (Hainan) identified a subset of individuals with anti-ASP-2 antibody responses who harbor low intensity hookworm infections. Subsequently it was confirmed that laboratory animals actively vaccinated with adjuvant-formulated ASP-2 acquire reduced hookworm burdens relative to adjuvant-injected controls, following larval challenge with either *Ancylostoma caninum* or *A. ceylanicum*. It was further shown that antisera to ASP-2 inhibits hookworm larval invasion in *in vitro* Process development and cGMP pilot manufacture of Na-ASP-2 from *N. americanus* has been completed. The X-ray crystal structure of Na-ASP-2 has been solved. Additional plans are underway to formulate Na-ASP-2 with a second antigen from adult hookworms, including a hookworm hemoglobinase. Following the completion of toxicity and formal stability studies, an IND for Na-ASP-2 will be filed with plans to commence Phase 1 clinical trials. These studies will be followed by Phase 2b pilot efficacy studies in Brazil and Africa. Clinical endpoints unique to helminth infections will be examined.

IMMUNODIAGNOSIS OF STRONGYLOIDIASIS BY A SIMPLIFIED DOT BLOT METHOD USING A RECOMBINANT LARVAL ANTIGEN (NIE)

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Strongyloides stercoralis is an intestinal nematode parasite of a global distribution. Most infected individuals have few or no symptoms. Strongyloidiasis is of primary medical importance and fatal disease can occur in infected people who become immunosuppressed or immunocompromised throughout the administration of steroids or because of co-infection with HTLV-1. Because chronic infection is difficult to diagnose by standard stool examination, reliable serodiagnosis is important. We have previously described use of the recombinant NIE antigen by ELISA for the detection of specific antibody in humans infected with *S. stercoralis*. The specificity and sensitivity using rNIE based ELISA results were 94% and 87.5%, respectively. Based on this dot blot showed that 65 of 71 (91.5%) *S. stercoralis* infected human sera reacted in this format. Only 2 of 31 (7%) sera from normal reacted with the NIE dot blot. Moreover importantly, cross-reactivity occurred with only one of 24 sera from patients with various filarial infections. Thus, a rapid, qualitative dot blot has been developed for diagnosis of *S. stercoralis* with specificity and sensitivity being equal to that seen in more conventional ELISA based assay. (ACMICP abstract)

IDENTIFICATION OF NOVEL ANTHELMINTICS FROM RNAI-BASED FUNCTIONAL GENOMICS SCREENS

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Divergence Inc. is utilizing a combination of comparative genomics and rapid gene-function assessment to provide new targets for the selection of novel and safe anthelmintics including macrofilaricides. Using the complete *Caenorhabditis elegans* genome and the large number of publicly available parasitic nematode ESTs, we have identified genes present in nematodes that lack homologs in host genomes. RNAi knockouts have narrowed the set to genes likely to be essential to parasite survival. Products of these genes are currently being evaluated for utility in screens to identify novel anthelmintics. Using this methodology, a compound (DC3746) and several analogs that mimic the substrates of two essential nematode enzymes were identified. These enzymes work sequentially in a biochemical pathway that is not found in vertebrates. Current best compounds in the DC3746 class inhibit the life-cycle of *C. elegans* at 5 μM and have shown activity against the Strongyloids parasite *Trichostongyulus colubriformis in vitro* and against the plant parasite *Meloidogyne incognita* in greenhouse assays. Toxicity is not seen at high doses to select fungi, arthropods, and plants. DC7651, and other molecules in its class, mimic the natural substrate of a third Divergence enzyme target. *In vitro*, DC7651 and other molecules in its class are lethal to the filarial nematode *Brugia malayi* microfilariae and adults. The activity on filarial adult stages is particularly noteworthy given the lack of macrofilaricidal activity of many current anthelmintics. Activity is also seen against the nematodes *C. elegans*, *Haemonchus contortus*, *Teladorsagia circumcincta*, *T. colubriformis*, *Meloidogyne incognita*, *Heteroderia glycines*, and *Belonolaimus longicaudatus*, indicating broad nematidical activity. DC7651 is a synthetic derivative of a naturally occurring plant metabolite with a favorable toxicity profile. Divergence is currently working to develop both the DC3746 and the DC7651 class of chemicals to optimize commercially viable formulations for each, and to further evaluate spectrum of activity. The chemical identity of DC7651 and its target will be discussed.
INBREEDING IN ASCARIS: IMPLICATIONS FOR SPREAD OF DRUG RESISTANCE

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Large-scale programs for treatment of human geohelminths impose strong selection for anthelmintic resistance. It is often argued that recessive resistance alleles will spread very slowly in parasitic nematode populations, since they will rarely be present in the homozygous state, and will therefore be largely invisible to selection. We present some less reassuring conclusions emerging from analysis of Ascaris microsatellites. Since male and female worms mate in the human gut, eggs expelled from people carrying few worms are expected to be more closely related than those expelled from people carrying many worms. Thus, we might expect that the level of inbreeding in parasites in low-transmission regions will be higher than in parasites in areas of high transmission. To test this prediction we genotyped 14 microsatellite markers in 1019 parasites expelled from people living in eight villages in Jiri, Nepal, in which prevalence ranged from 26-78%. We calculated the inbreeding coefficient of nematodes in villages with varying levels of parasite transmission within this study region. We also compared the Nepalese data to that obtained from a region of intense transmission in Guatemala (~80% infected) in an effort to identify broad trends in transmission intensity and Ascaris population genetics. We observed a significant negative correlation between prevalence and inbreeding (r=0.61, p<0.02), confirming our predictions. At the highest levels of inbreeding observed (F=0.4) resistance alleles will spread approximately 30 times faster than when random mating occurs, since homozygous genotypes will be overrepresented and exposed to selection. We conclude that resistance alleles will spread most rapidly in low transmission regions, or more worrying, at the tail-end of successful control programs when transmission is maintained at low levels.

DISTRIBUTION OF BARTONELLA INFECTION IN COLONIES OF BLACK-TAILED PRAIRIE DOGS

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Black-tailed prairie dogs (PD) have experienced extensive habitat losses but remain widely distributed throughout the Great Plains. During the summer of 2003, we analyzed PDs from 20 colonies in Boulder County, CO for evidence of Bartonella infection. Blood samples were collected from 318 PDs and later cultured on rabbit blood agar. Bartonella isolates were identified on the basis of bacterial morphology and PCR amplification of a region of the citrate synthase (gltA) gene. Overall prevalence of Bartonella infection among PDs was 9.1% (29/318) and was significantly higher in juveniles (17.7%; 11/62) than in adults (3.1%; 8/256). Infected PDs were found in 10 of the 20 colonies, and the prevalence of the infection varied from 0 to 33.3% per colony. Levels of bacteremia varied from 40 to >1000 colony-forming units per 1 ml of blood. Sequence analysis of gltA gene amplicons from 29 isolates resulted in the identification of 4 unique genetic variants. Phylogenetic comparisons showed that all variants were closely related to each other (similarity 0.6% to 2.4%) and clustered in a clade that appears to be specific for PDs but is closely related to Bartonella isolates obtained from other Sciuridae species. Bartonella washoensis, which has been associated with human cardiac illness, is also among the squirrel isolates to which the PD isolates appear closely related. Within each PD colony, the number of genetic variants varied from 1 to 4. We hypothesize that the diversity of Bartonella isolates can serve as a marker for measuring the geographical and ecological isolation of PD colonies. Our finding of B. washoensis-related bacteria in a high proportion of PD colonies living in close proximity to residential areas suggests the need to investigate the potential significance of these bacteria as sources of human illness.

BARTONELLA IN SMALL MAMMALS FROM DHAKA, BANGLADESH LINKED TO BARTONELLA IN AMERICA AND EUROPE

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This investigation represents the first identification of Bartonella in an urban center in South Asia. We have investigated the prevalence and diversity of Bartonella infections among small mammals in Dhaka. Bartonella were isolated from mammal blood and confirmed by PCR amplification of a region of the citrate synthase (gltA) gene followed by sequencing the resulting amplicon. 42.3% (85/201) tested samples were found Bartonella-positive. Prevalence of Bartonella infection by species was: Bandicota bengalensis (BB) - 61.8% (47/76), R. rattus (RR) - 33.3% (33/99), Suncus murinus (SM) - 35.7% (5/14), and Mus musculus - 0 (0/12). Eleven unique gltA sequence variants, which were clustered into 5 phylogenetic groups, were identified among 85 isolates. Sequences from the shrew SM belonged to one separate group. The other groups include sequences obtained from both Bandicota and Rattus rats. Sequences from 37 BB and from 2 RR were closely related, but not identical to Bartonella elizabethae, a bacterium isolated from a human patient in Massachusetts. Six sequences from RR were identical to the sequence of an isolate found in RR from Portugal. Four sequences from BB isolates were identical to the sequence of Bartonella ribosomal from a domestic rat in France. Nineteen sequences (16 from RR and 3 from BB) were identical to the sequence of strain from a domestic rat in Louisiana. The identity of some isolates from Bangladeshi rodents with Bartonella strains obtained from rats in different continents supports the hypothesis of an Asian origin of Rattus-associated bartonellae. Findings of a high proportion of domestic rats infected with B. elizabethae-related bacteria in Bangladesh suggest the potential public health risk of acquiring this infection.

ISOLATION AND IDENTIFICATION OF A NOVEL BARTONELLA SPECIES FROM A FEBRILE PATIENT IN THAILAND

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Undifferentiated febrile illness is a common problem in tropical countries and often eludes specific diagnosis. We describe a patient enrolled in a study of patients presenting to rural hospitals in Thailand designed to characterize the agents causing fever, including Bartonella species. The patient is...
a 38-year-old shopkeeper in northeast Thailand who presented with 3 weeks of fatigue, myalgia and headache and 6 days of fever. He had an axillary temperature of 38.2 °C, hypotension, petechial rash and a white blood cell count of 23,400/mm³; HIV antibody test was positive. Routine hospital testing and malaria smear, as well as testing of acute and convalescent sera by MAT for leptospirosis and ELISA for dengue and rickettsial infections identified no cause for the fever. Since culturing of the patient blood on heart infusion agar supplemented with rabbit blood for 60 days did not yield growth, the blood was then co-cultivated with Vero E6 cells. After 7 days, the suspension was found to be positive by PCR amplification of a *Bartonella*-specific fragment of the *gltA* gene. The inoculated Vero E6 cells were sub-cultured on rabbit blood agar, and very small colonies appeared after day 17 with little increase out to day 60. Growth rate increased after several sequential sub-culturing passages of the collected bacterial suspension. Gram staining revealed small rod-shaped gram-negative bacteria. Sequence analyses of *gltA* and 16S rRNA genes demonstrated that the strain belongs to the genus *Bartonella*. Based on comparisons with sequences of the same genes for all known *Bartonella* species, we consider the strain to be a novel *Bartonella* species.

**1007 VALUE DIAGNOSIS OF THE THIN SMEAR IN HUMAN BARTONELLOSIS (CARRION’S DISEASES)**

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Human bartonellosis is a re-emerging disease in Peru. Over the past 5 years, more than 20,000 cases, 200 deaths and new areas of transmission on the coast, hIGHLands and forest have been reported. Thin smear is the method used for diagnosis due to its ease of use, rapidity, and low cost. The objective of this study was to determine the sensitivity (S), specificity (E), positive predictive value (PPV), and negative predictive value (NPV) of thin smear in the diagnosis of bartonellosis. The study was performed at the Hospital of Caraz, Ancash Department, Peru between July 2002 and October 2003. Inclusion criteria for enrollment were: 1) fever for 5 days without apparent focus of infection, or 2) eruptive or nodular lesion, and 3) no antibiotic use during the past 5 days. Blood samples were collected for thin smear and culture. PCR for *Bartonella bacilliformis* (Bb) was performed on contaminated cultures. Subjects with a Bb positive culture or PCR were considered a confirmed case. 778 patients were enrolled, 624 with febrile syndrome and 154 with eruptive bartonellosis. Bb was isolated from 347 (45%) cultures and 5 (0.6%) contaminated cultures were PCR positive. Of the 352 confirmed cases of bartonellosis, 84 (24%) were thin smear positive. For those with acute bartonellosis, the S of thin smear differed depending on age ≥15 vs < 15 years (11% vs 39%; p<0.0001), presence or absence of pallor (53% vs 9.6%; p<0.00001) or jaundice (64.7% vs 18.6%; p<0.00001); temperature > 37 °C vs ≤ 37 °C (35% vs 21%; p<0.000); sedimentation velocity (SV) ≥30 or < 30 (51% vs 13%; p<0.00001); and hemoglobin ≥12 vs <12 g/dL (10% vs 34%; p<0.00001). Among those with eruptive bartonellosis, the S, E, VPP and VPN were 8%, 99%, 80%, and 70%, respectively. In conclusion, the S of thin smear for the diagnosis of bartonellosis is low and depends on the clinical form of the disease, age of the patient, pallor, temperature at the time of sample collection, jaundice, SV, and hemoglobin. We suggest beginning treatment for bartonellosis based on clinical criteria.

**1008 MICROSATELLITE MAPPING OF MYCOBACTERIA LEPRAE IN HUMAN INFECTION- A NEW TOOL FOR UNDERSTANDING TRANSMISSION OF LEPROSY**

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Mycobacteria leprae can not be grown in vitro and this has hampered work on understanding transmission patterns. Here we describe the development and use of a new molecular tool to analyse *M. leprae* from human samples. To investigate genetic diversity of the bacterial population, we measured the copy number of simple sequence repeats, or microsatellites, in *Mycobacterium leprae* from patients living in and around Hyderabad, India. Three microsatellite loci containing trinucleotide or dinucleotide repeats were amplified from infected tissues, and the copy number established by sequence analysis. Extensive diversity was observed in a cross-sectional survey of 33 patients, but closely related profiles were found for members of a multiscase family likely to share a common transmission source. Sampling of multiple tissues from single individuals demonstrated identical microsatellite profiles in skin, nasal cavity and bloodstream, but revealed differences at one or more loci for *M. leprae* present in nerves. Microsatellite mapping of *M. leprae* provides a useful tool to track short transmission chains. Comparison of skin and nerve lesions suggests that the evolution of disease within an individual involves expansion of multiple distinct sub-populations of *M. leprae*.

**1009 RISK FACTORS FOR BURULI ULCER DISEASE (MYCOBACTERIUM ULCERANS INFECTION): A CASE-CONTROL STUDY IN GHANA**


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Morbidity due to Buruli ulcer disease (BUD), a cutaneous infection caused by *Mycobacterium ulcerans*, has been increasingly recognized in rural West Africa. The source and mode of transmission remain unknown. To identify risk factors for BUD, we conducted a case-control study in three endemic districts of Ghana. We enrolled case-patients with clinically diagnosed BUD and obtained skin biopsies. We confirmed *M. ulcerans* infection by ≥1 positive test: histopathology, culture, polymerase chain reaction, and Zielh-Neelsen staining from lesion swab. Among 121 confirmed case-patients, males were significantly more likely than females to have lesions on the trunk (25% vs. 6%, p=0.009); however, most case-patients had leg (49%) or arm (36%) lesions. Multivariable conditional logistic regression modeling among 116 age- and community-matched cases and controls identified the following BUD risk and protective factors: wading in a river or stream (OR = 2.86; 95% CI 1.3, 6.2; p=0.0081), wearing a top while farming (OR = 0.24; 95% CI 0.089, 0.633; p=0.0040), sharing indoor living space with livestock (OR = 0.36; 95% CI 0.15, 0.88; p=0.0254), and bathing with toilet soap (OR = 0.41; 95% CI 0.20, 1.00; p=0.0509). BUD was not signifi-
cantly associated with penetrating injuries (p=0.139), insect bites near water bodies (p=0.842), BCG vaccination (p=0.329), or HIV infection (p=0.992). Our findings support the hypothesis that BUD is an environmentally acquired infection strongly associated with exposure to river areas. Exposed skin may facilitate transmission, consistent with the predominance of trunk lesions in males and the protective effect of clothing and soap. Until transmission is better defined, control strategies in endemic areas could focus on covering exposed skin while farming.

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RELIABILITY AND VALIDITY OF THE BURULI ULCER FUNCTIONAL LIMITATION SCORE (BUFLS) QUESTIONNAIRE


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The reliability and validity of the earlier developed functional limitation questionnaire was assessed, to enable the measurement of the nature and severity of the limitations caused by Buruli ulcer. The questionnaire consisted of 19 items of daily activities. Of 638 former Buruli ulcer patients (of a total of 678 individuals examined) sufficient items (=13) were applicable to calculate a score. To determine the validity, the functional limitation scores of the 638 individuals were compared with the global impression of the limitations, range of motion, and the social impact (change of occupation or education) as a result of Buruli ulcer. To determine inter observer reliability in 107 participants the functional limitation score was reassessed between one and three weeks by another interviewer and interpreter. Both global impression and range of motion correlated well with the functional limitation scores (ρ=0.66 and ρ=0.61). Inter observer reliability of 107 participants as measured by the intra class correlation coefficient was very good (ICC of 0.86). The functional limitation scores measured in the second assessment were significantly higher than in the first assessment. This should be taken into account when the functional limitation score is used for the individual patient. The Buruli ulcer functional limitation score (BUFLs) can be used for between group comparisons of endpoints in clinical trials and in the planning of resources.

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HERVES TRANSPOSABLE ELEMENT MOVEMENT IN POPULATIONS OF ANOPHELES GAMBAE

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The Herves element is related to the Hermes and Jobo elements from Musca domestica and Drosophila melanogaster, respectively, and was discovered in Anopheles gambiae s.s. Herves elements are also present in other members of the An. gambiae species complex including An. merus and An. arabiensis. Natural populations of An. gambiae s.s., An. merus, and An. arabiensis were examined for the presence of Herves, its copy number and variability with respect to insertion sites. In all populations and species examined Herves appears to be actively transposing with the levels of activity varying by species. Similarities and differences between populations sampled in Mozambique and Tanzania will be described. Herves has also been developed into a functional insect gene vector. The implications of the natural history of Herves on the idea of using Class II transposable elements as vectors for introducing and spreading transgenes through populations of Anopheles gambiae will be discussed.

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INTERSPECIFIC NUCLEOTIDE VARIABILITY WITHIN THE ANOPHELES GAMBAE SPECIES COMPLEX

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Although numerous campaigns for malaria vector control have reduced the burden of malaria in some sub-saharan countries during the last century, none has reached the goal of eradicating the disease and the malaria situation is worsening in tropical Africa. Malaria is still responsible for more than one million of children deaths every year, and innovative, specific and selective vector control strategies are urgently needed. Anopheles gambiae Giles s.s. and An. arabiensis Patton, two members of the An. gambiae species complex, contribute to the maintenance of malaria transmission in most endemic areas. The Anopheles gambiae genome sequence, together with the recent development of molecular tools for genome-wide analysis, promises new insights into the biology of the malaria vector. We investigated nucleotide polymorphism in candidate genes that affects phenotypes such as anthropophily or parasite permissiveness. Interspecific divergence was measured in An. gambiae s.s., An. arabiensis and An. melas, the later belongs to the An. gambiae species complex and has only a minor role in malaria transmission. The numerous polymorphisms we found in the mosquito genes should be useful to undertake association studies. Genome-wide analysis based on SNP markers should allow discovering genetic factors associated with complex traits such as permissiveness to Plasmodium and identifying phenotypic markers linked to vectorial capacity and competence.

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GEOGRAPHIC DIFFERENTIATION AT MULTIPLE LOCI IN ANOPHELES GAMBAE POPULATIONS FROM SAO TOME AND PRINCIPE (WEST AFRICA)

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In recent years a surprising finding has been the indication that Anopheles gambiae s.s. includes distinct “chromosomal forms” identified with non-Linnean names describing their locality or habitat, Mopti, Bamako, Forest, Savanna, and Bissau. Molecular studies of these “forms” have confirmed the existence of genetic discontinuity in An. gambiae s.s. The M and S molecular forms are differentiated at the rDNA (X chromosome) by diagnostic sites

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in the intergenic (IGS) region. These forms generally correspond to the Mopii and Savanna chromosomal forms, respectively. A parallel screen of variants in the internal transcribed spacer region (ITS) found three distinct ITS variants (Type I, II, and III). The first two are in complete linkage with the S and M molecular forms, respectively. Type III was only found in a small sample from the population of Sao Tome, belonging to the M form. More recently variation in the intron I of the voltage-gated sodium gene has been found to co-segregate with the ITS and IGS site that define the M and S molecular forms. The finding of an unique ITS variant (Type III) on Sao Tome, an island 300 km off the coast of West Africa, stimulated us to take a deeper look at the pattern of genetic differentiation between and within 3 populations on this island and the neighboring island of Principe. The aims were to verify the existence of the ITS-Type III haplotype, previously described only on a few individuals from a population, and to attempt to understand its evolutionary origin. The same set of individuals was also screened for variation at the Intron I region of the voltage-gated channel gene and for variation at the mtDNA ND5 gene. We interpret these data in an expanded phylogeographic context, which include populations from continental Africa for both DNA regions. We also compare our results with the ones from a previous microsatellite survey. The results are of relevance in understanding levels of genetic differentiation and genetic isolation of these populations and in providing baseline data on multiple loci for future control interventions.

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MOLECULAR ORGANIZATION OF THE ANOPHELES GAMBIAE 2La INVERSION BREAKPOINTS

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The 2La inversion is polymorphic in Anopheles gambiae s.s. and is fixed in An. arabiensis. An. merus has a cytologically identical but presumably different fixed inversion 2La. The correspondence between the An. gambiae PEST (2La+/+) genome sequence and the polytene chromosome complement allows identification of sequences at the inversion breakpoints. Here we report the first successful cloning and characterization of a chromosomal inversion breakpoint in An. gambiae s.s. We have been cloning and characterizing the 2La breakpoints from the SUA (2La/a) and Bamako (2La/a) strains of An. gambiae, and from An. merus (2La/a') and An. arabiensis (2La/a). We have also developed a PCR-based diagnostic of the 2La inversion that allows “molecular karyotyping” on a broad array of developmental stages and both sexes where polytene chromosomes cannot be analyzed directly. Inter-specific comparative analysis of the sequences surrounding the breakpoints has been focusing on identification of insertions/deletions, genes, transposable elements and other repetitive DNA sequences, and on estimation of the nucleotide polymorphism and divergence. The breakpoints molecular organization and the 2La inversion origin and evolution will be discussed.

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CLONING AND MAPPING OF THE RanGAP HOMOLOG AS A PUTATIVE MEIOTIC DRIVER IN MOSQUITO Aedes aegypti

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‘Meiotic drive’ is a phenomenon that subverts meiosis to favor gametes carrying a particular gene. It has been reported in several organisms including plants, insects and vertebrates. Segregation distorter, the meiotic driver in Drosophila melanogaster, is the best characterized drive system and was determined to be caused by a 3' truncated version of RanGAP. RanGAP is a GTPase activator for Ran, a protein critical to nuclear transport. The product of the distorter allele causes a failure of chromatin condensation at a responder locus during spermatogenesis. Meiotic drive has previously been reported in two mosquito species, Aedes aegypti and Culex pipiens. In Ae. aegypti, the meiotic drive gene product targets a locus tightly linked with the female determining sex allele. This causes fragmentation of the female-determining chromosome during meiosis resulting in a decreased number of female progeny. As part of our efforts to identify and characterize the meiotic drive gene system in mosquitoes, here we report the cloning and mapping of the RanGAP homolog from Ae. aegypti. We used a degenerate primer PCR assay to identify a BAC genomic clone containing the gene. In addition, we used RT-PCR with an Ae. aegypti specific RanGAP primer to isolate and characterize a partial cDNA sequence. Efforts to obtain complete genomic and cDNA sequences, and to evaluate the potential of RanGAP as a drive system in Ae. aegypti are ongoing.

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PHYSICAL MAPPING OF THE ANOPHELES GAMBIAE GENOME ASSEMBLY: UPDATE

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The genome of Anopheles gambiae, the major vector of malaria in Africa, was sequenced by a whole-genome shotgun approach. Physical mapping of the genome was conducted by in situ hybridization of about 2000 bacterial artificial chromosome (BAC) clones on ovarian nurse cell polytene chromosomes. As a result, 149 scaffolds were assigned to chromosomes. However, approximately 16% of the assembled An. gambiae genome was represented in scaffolds that did not have a chromosomal location assigned. The most poorly mapped parts of the An. gambiae genome in its first publication were the heterochromatic regions of the chromosomes. In this paper we present an updated physical map of the Anopheles gambiae genome assembly. Physical mapping of Bacterial Artificial Chromosome (BAC) clones containing repetitive DNA sequences and results of in situ hybridization of repetitive DNA, with C_T=1, allowed us to localize heterochromatic regions on An. gambiae ovarian nurse cell polytene chromosomes. Using cDNA for in situ hybridization we placed 16 unmapped scaffolds of lengths between 50kb and 600kb in heterochromatic regions of the genome. In addition, 7 scaffolds mapped to euchromatic chromosome regions. About 7Mb have been added to the mapped part of the genome and 23 scaffolds have been oriented. The identification of polymorphic regions of chromosomes was another difficulty faced assembling the A. gambiae genome. An additional in silico analysis of scaffold sequences allowed us to localize 64 polymorphic scaffolds with total size 7.6Mb within the genome. The analysis of BAC end sequences has identified BAC clones that span 23 physical gaps between scaffolds. The sequencing of these clones will close these gaps and therefore improve the quality of the An. gambiae genome assembly.
THE GENETICS OF PLASMODIUM FALCIPARUM RESISTANCE LOCI IN A NATURAL ANOPHELES GAMBIAE POPULATION

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High-throughput microsatellite genotyping of pedigrees generated from wild caught female mosquitoes from Mali, West Africa is being used to carry out a genetic screen of the natural vector population for naturally occurring malaria-resistance alleles. At present, 25 microsatellite loci can be typed in as few as 5 PCR reactions, allowing for efficient analysis of pedigrees with hundreds of individuals. To date, we have published 2 resistance loci and have since identified others. One example is Pfin1, a semi-dominant malaria resistance locus located on the left arm of chromosome 2 that explains 97% of the parasite-free mosquitoes present in the pedigree. Finer scale mapping using microsatellite and SNP markers, with the goal of positional cloning the gene(s) underlying Pfin1, has narrowed the area of interest to a ~5 MB region on 2L. The interval contains a number of mosquito immune genes by the criterion of transcriptional screening on microarrays, including some serine protease relatives. In addition, microsatellite mapping in wild pedigrees will address the genomic distribution and population frequency of resistance alleles in nature.