

Malaria Bulletin: A Compendium of Current Literature

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ABSTRACTS

1: *Acta Paediatr.* 2008 Feb 27

Congenital malaria in neonates: two case reports and review of the literature.

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Congenital malaria is uncommon in nonendemic countries. We describe two cases involving neonates hospitalized with fever, anaemia and thrombocytopaenia. Thick and thin blood smears were positive for *Plasmodium vivax* (*P. vivax*) and *P. ovale*, respectively. These two cases were discussed regarding the literature and potential implications of HIV coinfection in the mother. Conclusion: Consistent data in the literature suggest that peripheral blood films should be performed in HIV-positive women who travelled to an endemic area or with a history of malaria prior to gestation. With today's travelling patterns, congenital malaria should be considered as an important differential diagnosis of neonatal sepsis.

2: *Acta Trop.* 2008 Mar;105(3):222-8.

Anti-malarial efficacy of pyronaridine and artesunate in combination in vitro and in vivo.

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Pyronaridine is a Mannich base anti-malarial with demonstrated efficacy against drug resistant *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. However, resistance to pyronaridine can develop quickly when it is used alone but can be considerably delayed when it is administered with artesunate in rodent malaria models. The aim of this study was to evaluate the efficacy of pyronaridine in combination with artesunate against *P. falciparum* in vitro and in rodent malaria models in vivo to support its clinical application. Pyronaridine showed consistently high levels of in vitro activity against a panel of six *P. falciparum* drug-sensitive and resistant strains (Geometric Mean IC₅₀=2.24nM, 95% CI=1.20-3.27). In vitro interactions between pyronaridine and artesunate showed a slight antagonistic trend, but in vivo compared to pyronaridine and artesunate administered alone, the 3:1 ratio of the combination, reduced the ED₉₀ of artesunate by approximately 15.6-fold in a pyronaridine-resistant *P. berghei* line and by approximately 200-fold in an artesunate-resistant line of *P. berghei*. Complete cure rates were achieved with doses of the combination above or equal to 8mg/kg per day against *P. chabaudi* AS. These results indicate that the combination had an enhanced effect over monotherapy and lower daily doses of artesunate could be used to obtain a curative effect. The data suggest that the combination of pyronaridine and artesunate should have potential in areas of multi-drug resistant malaria.

3: *Acta Trop.* 2008 Mar;105(3):207-214.

Hyperreactive malarial splenomegaly is associated with low levels of antibodies against red blood cell and *Plasmodium falciparum* derived glycolipids in Yanomami Amerindians from Venezuela.

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The immunological basis of the aberrant immune response in hyperreactive malarial splenomegaly (HMS) is poorly understood, but believed to be associated with polyclonal B cell activation by an unidentified malaria mitogen, leading to unregulated immunoglobulin and autoantibody production. HMS has been previously reported in Yanomami communities in the Upper Orinoco region of the Venezuelan Amazon. To investigate a possible association between antibody responses against *Plasmodium falciparum* and uninfected red blood cell (URBC) glycolipids and splenomegaly, a direct comparison of the parasite versus host anti-glycolipid antibody responses was made in an isolated community of this area. The anti-*P. falciparum* glycolipid (Pfglp) response was IgG3 dominated, whereas the uninfected red blood cell glycolipid (URBCglp) response showed a predominance of IgG1. The levels of IgG1 against Pfglp, and of IgG4 and IgM against URBCglp were significantly higher in women, while the anti-Pfglp or URBCglp IgM levels were inversely correlated with the degree of splenomegaly. Overall, these results suggest differential regulation of anti-parasite and autoreactive responses and that these responses may be linked to the development and evolution of HMS in this population exposed to endemic malaria. The high mortality rates associated with HMS point out that its early diagnosis together with the implementation of malaria control measures in these isolated Amerindian communities are a priority.

4: *Ann Hum Genet.* 2008 Feb 19

Significant Association Between TIM1 Promoter Polymorphisms and Protection Against Cerebral Malaria in Thailand.

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Although cerebral malaria is a major life-threatening complication of *Plasmodium falciparum* infection, its pathophysiology is not well understood. Prolonged activation of the T helper type 1 (Th1) response characterized by the production of pro-inflammatory cytokines such as IFN-gamma and TNF-alpha has been suggested to be responsible for immunopathological process leading to cerebral malaria unless they are downregulated by the anti-inflammatory cytokines produced by the Th2 response. The T cell immunoglobulin and mucin domain (TIM) family of proteins are cell surface proteins involved in regulating Th1 and Th2 immune responses. In this study, the possible association between the polymorphisms of TIM1, TIM3, and TIMD4 genes and the severity of malaria was examined in 478 adult Thai patients infected with *P. falciparum* malaria. The TIM1 promoter haplotype comprising three derived alleles, -1637A (rs7702919), -1549C (rs41297577) and -1454A (rs41297579), which were in complete linkage disequilibrium, was significantly associated with protection against cerebral malaria (OR = 0.41; 95% CI = 0.24-0.71; P= 0.0009). Allele-specific transcription quantification analysis revealed that the level of mRNA transcribed from TIM1 was higher for the protective promoter haplotype than for the other promoter haplotype (P= 0.004). Engagement with TIM1 in combination with T cell receptor stimulation induces anti-inflammatory Th2 cytokine production, which can protect the development of cerebral malaria caused by overproduction of pro-inflammatory Th1 cytokines. The present results suggest that the higher TIM1 expression associated with the protective TIM1 promoter haplotype confers protection against cerebral malaria.

5: *Ann Trop Med Parasitol.* 2008 Mar;102(2):103-18.

Febrile status, malarial parasitaemia and gastro-intestinal helminthiases in schoolchildren resident at different altitudes, in south-western Cameroon.

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In the many areas where human malaria and helminthiases are co-endemic, schoolchildren often harbour the heaviest infections and suffer much of the associated morbidity, especially when co-infected. In one such area, the Buea district, in south-western Cameroon, two cross-sectional surveys, together covering 263 apparently healthy schoolchildren aged 4-12 years, were recently conducted. The prevalences of fever, malarial parasitaemia and intestinal helminth infections, the seroprevalences of anti-*Plasmodium falciparum* IgG and IgE and anti-glycosylphosphatidylinositol (anti-GPI) IgG, plasma concentrations of total IgE, and the incidence of anaemia were all investigated. The mean (S.D.) age of the study children was 7.56 (1.82) years. Overall, 156 (59.3%) of the children were found parasitaemic, with a geometric mean parasitaemia of 565 parasites/ μm^3 . Parasitaemia and fever were significantly associated ($P=0.042$). The children who lived at low altitude, attending schools that lay 400-650 m above sea level, had significantly higher parasitaemias than their high-altitude counterparts ($P<0.01$). At low altitude, the children attending government schools had significantly higher parasitaemias than their mission-school counterparts ($P=0.010$). Of the 31 children (11.9%) found anaemic, 22 (70.4%) had mild anaemia and none had severe anaemia. A significant negative correlation ($r=-0.224$; $P=0.005$) was observed between haemoglobin concentration and level of parasitaemia. Infection with *Plasmodium* appeared to reduce erythrocyte counts ($P=0.045$), a condition that was exacerbated by co-infection with helminths ($P=0.035$). Plasma concentrations of total IgE were higher in the children found to be excreting helminth eggs than in those who appeared helminth-free, while levels of anti-*P. falciparum* IgE were higher in the children with low-grade parasitaemias than in those with more intense parasitaemias. Levels of anti-GPI IgG increased with age and were relatively high in the children who lived at low altitude and in those who were aparasitaemic. The survey results confirm that asymptomatic malarial parasitaemia frequently co-exists with helminth infections in schoolchildren and indicate links with fever, altitude and school type. Immunoglobulin E may play a role in immune protection against helminthiasis whereas anti-GPI antibodies may be important in the development of antimalarial immunity in such children. In Cameroon, as in other areas with endemic malaria, control programmes to reduce the prevalences of infections with intestinal helminths and malarial parasites in schoolchildren, which may effectively reduce the incidence of anaemia, are clearly needed.

6: *Ann Trop Med Parasitol.* 2008 Mar;102(2):95-102.

Complexity of the msp2 locus and the severity of childhood malaria, in south-western Nigeria.

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As the genetic diversity of *Plasmodium falciparum* infections in humans is implicated in the pathogenesis of malaria, the association between *P. falciparum* diversity at the merozoite surface protein-2 (msp2) locus and the severity of childhood malaria was investigated in Ibadan, in south-western Nigeria. The 400 children enrolled had acute uncomplicated malaria (144), cerebral malaria (64),

severe malarial anaemia (67) or asymptomatic infections with *P. falciparum* (125). Nested PCR was used to investigate the *msp2* genotype(s) of the parasites infecting each child. In terms of the complexity of infection and frequency of polyinfection, the children with asymptomatic infection were significantly different from those with uncomplicated malaria or severe malaria. The median number of FC27 alleles detected was higher in the asymptomatic children than in the symptomatic. After controlling for age and level of parasitaemia (with 'asymptomatic infection' as the reference category), a child in whom no FC27 alleles were detected was found to be at five-fold greater risk of uncomplicated malaria, and a child without polyinfection was found to have a three-fold increased risk of severe malarial anaemia and a six-fold increased risk of cerebral malaria. It therefore appears that *msp2* genotypes are associated with asymptomatic carriage and that children with mono-infections are more likely to develop severe malaria than children with polyinfections.

7: *Antimicrob Agents Chemother.* 2008 Feb 11

Malaria Treatment with Atovaquone-Proguanil in Malaria-immune Adults; Implications for Malaria Intervention Trials and for Pre-Exposure Prophylaxis of Malaria.

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Eighty adults in holoendemic Kenya received presumptive treatment with atovaquone/proguanil and were followed closely. The time to first *Plasmodium falciparum* parasitemia was 32 days. This prolonged prophylaxis period has implications for study design when used in malaria intervention trials and cautiously suggests clinical investigation for potential pre-exposure prophylaxis of malaria.

8: *Blood.* 2008 Feb 15;111(4):2007-14.

Inhibition of collagen-induced platelet aggregation by anopheline antiplatelet protein, a saliva protein from a malaria vector mosquito.

Yoshida S, Sudo T, Niimi M, Tao L, Sun B, Kambayashi J, Watanabe H, Luo E, Matsuoka H.

During blood feeding, mosquitoes inject saliva containing a mixture of molecules that inactivate or inhibit various components of the hemostatic response to the bite injury as well as the inflammatory reactions produced by the bite, to facilitate the ingestion of blood. However, the molecular functions of the individual saliva components remain largely unknown. Here, we describe anopheline antiplatelet protein (AAPP) isolated from the saliva of *Anopheles stephensi*, a human malaria vector mosquito. AAPP exhibited a strong and specific inhibitory activity toward collagen-induced platelet aggregation. The inhibitory mechanism involves direct binding of AAPP to collagen, which blocks platelet adhesion to collagen and inhibits the subsequent increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). The binding of AAPP to collagen effectively blocked platelet adhesion via glycoprotein VI (GPVI) and integrin $\alpha(2)\beta(1)$. Cell adhesion assay showed that AAPP inhibited the binding of GPVI to collagen type I and III without direct effect on GPVI. Moreover, intravenously administered recombinant AAPP strongly inhibited collagen-induced platelet aggregation *ex vivo* in rats. In summary, AAPP is a malaria vector mosquito-derived specific antagonist of receptors that mediate the adhesion of platelets to collagen. Our study may provide important insights for elucidating the effects of mosquito blood feeding against host hemostasis.

Applications of Bayesian approach in modelling risk of malaria-related hospital mortality.

Kazembe LN, Chirwa TF, Simbeye JS, Namangale JJ.

ABSTRACT: BACKGROUND: Malaria is a major public health problem in Malawi, however, quantifying its burden in a population is a challenge. Routine hospital data provide a proxy for measuring the incidence of severe malaria and for crudely estimating morbidity rates. Using such data, this paper proposes a method to describe trends, patterns and factors associated with in-hospital mortality attributed to the disease. **METHODS:** We develop semiparametric regression models which allow joint analysis of nonlinear effects of calendar time and continuous covariates, spatially structured variation, unstructured heterogeneity, and other fixed covariates. Modelling and inference use the fully Bayesian approach via Markov Chain Monte Carlo (MCMC) simulation techniques. The methodology is applied to analyse data arising from paediatric wards in Zomba district, Malawi, between 2002 and 2003. **RESULTS AND CONCLUSION:** We observe that the risk of dying in hospital is lower in the dry season, and for children who travel a distance of less than 5 kms to the hospital, but increases for those who are referred to the hospital. The results also indicate significant differences in both structured and unstructured spatial effects, and the health facility effects reveal considerable differences by type of facility or practice. More importantly, our approach shows non-linearities in the effect of metrical covariates on the probability of dying in hospital. The study emphasizes that the methodological framework used provides a useful tool for analysing the data at hand and of similar structure.

10: *Br J Clin Pharmacol.* 2008 Feb 20

Population pharmacokinetics of chloroquine and sulfadoxine and treatment response in children with malaria: suggestions for an improved dose regimen.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT * Both chloroquine (CQ) and sulfadoxine/pyrimethamine (SDx/PYR) remain important drugs in the control of malaria. * The available data on CQ, SDx and PYR are summary pharmacokinetic parameters based on classical/traditional methods, mostly in adults. * No study has described the population pharmacokinetics of a fixed-dose CQ + SDx/PYR combination in children with falciparum malaria. **WHAT THIS STUDY ADDS** * This study presents population pharmacokinetic data on CQ and SDx in children with uncomplicated falciparum malaria. * The study demonstrates that in age-based fixed-dose regimens with CQ and SDx, drug exposures and outcomes may be correctly predicted, although correlation with body weight is poor. * The study proposes dose modification to improve response with the CQ + SDx/PYR combination. **AIMS** To describe the pharmacokinetics of chloroquine (CQ) and sulfadoxine (SDx), and to identify predictors of treatment response in children with malaria given the CQ + SDx and pyrimethamine (PYR) combination. **METHODS** Eighty-six Ugandan children with uncomplicated falciparum malaria, 6 months to 5 years old, were randomly treated with prepacked fixed-dose CQ + SDx/PYR. The youngest children (<24 months) received half strength and the older (>24 months) full strength treatment. The reported day 14 failure rates were 48% and 18%, respectively. Capillary blood (100 µl) applied on to filter paper was collected on eight occasions during 28 days of follow up. Concentrations of CQ and SDx were determined. A population approach was used for the pharmacokinetic analysis. **RESULTS** A two-compartment model adequately described the data for both CQ and SDx. For CQ, the typical apparent clearance (CL/F) and volume of distribution (V(C)/F) values were

estimated to be 2.84 l h(-1) and 230 l. The typical CL/F for SDx was 0.023 l h(-1), while the factor relating its V(C)/F to normalized body weight was 1.6 l kg(-1). Post hoc parameter estimates for both drugs showed lower maximum concentrations (C(max)) and concentration-time curve areas (AUC(0,336 h)) in younger children. The AUC(0,336 h) for SDx and CQ were independently significant factors for prediction of cure. Simulations suggest that giving the higher dose to the youngest children would result in higher CQ and SDx concentrations and improved outcome. CONCLUSIONS The study results suggest that full-strength combination to all children would improve the cure rate.

11: *Bull Math Biol.* 2008 Feb 22

Determining Important Parameters in the Spread of Malaria Through the Sensitivity Analysis of a Mathematical Model.

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We perform sensitivity analyses on a mathematical model of malaria transmission to determine the relative importance of model parameters to disease transmission and prevalence. We compile two sets of baseline parameter values: one for areas of high transmission and one for low transmission. We compute sensitivity indices of the reproductive number (which measures initial disease transmission) and the endemic equilibrium point (which measures disease prevalence) to the parameters at the baseline values. We find that in areas of low transmission, the reproductive number and the equilibrium proportion of infectious humans are most sensitive to the mosquito biting rate. In areas of high transmission, the reproductive number is again most sensitive to the mosquito biting rate, but the equilibrium proportion of infectious humans is most sensitive to the human recovery rate. This suggests strategies that target the mosquito biting rate (such as the use of insecticide-treated bed nets and indoor residual spraying) and those that target the human recovery rate (such as the prompt diagnosis and treatment of infectious individuals) can be successful in controlling malaria.

12: *Cell Microbiol.* 2008 Feb 21

PbCap380, a novel oocyst capsule protein, is essential for malaria parasite survival in the mosquito.

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An essential requisite for transmission of Plasmodium, the causative agent of malaria, is the successful completion of a complex developmental cycle in its mosquito vector. Of hundreds of ookinetes that form in the mosquito midgut, only few transform into oocysts, a loss attributed to the action of the mosquito immune system. However, once oocysts form, they appear to be resistant to mosquito defences. During oocyst development, a thick capsule forms around the parasite and appears to function as a protective cover. Little information is available about the composition of this capsule. Here we report on the identification and partial characterization of the first Plasmodium oocyst capsule protein (PbCap380). Genetic analysis indicates that the gene is essential and that PbCap380(-) mutant parasites form oocysts in normal numbers but are gradually eliminated. As a result, mosquitoes infected with PbCap380(-) parasites do not transmit malaria. Targeting of the oocyst capsule may provide a new strategy for malaria control.

13: *Clin Exp Immunol.* 2008 Feb 14

Antibody responses to a C-terminal fragment of the Plasmodium falciparum blood-stage antigen Pf332 in Senegalese individuals naturally primed to the parasite.

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Previous studies have shown that antibodies from humans exposed continuously to malaria recognize the Plasmodium falciparum asexual blood-stage antigen Pf332. Here we analysed the antibody responses to a C-terminal fragment of Pf332, designated C231, in individuals from Senegal, by measuring the serum levels of immunoglobulin M (IgM), IgG class and subclass and IgE antibodies. IgG antibody reactivity with crude P. falciparum antigen was detected in all the donors, while many of the children lacked or had low levels of such antibodies against C231. The antibody levels increased significantly with age for both crude P. falciparum antigen and C231, and in the older age groups most of the donors displayed antibodies to C231. This was also true for IgM, IgE and IgG subclass reactivity against C231. Moreover, the ratio of IgG1/IgG2 was considerably lower for C231 than for crude P. falciparum antigen, and in age groups 10-14 and 15-19 years the levels of IgG2 against C231 even exceeded that of IgG1. The IgG2/IgG3 ratios suggest that C231 gives similar levels of IgG2 and IgG3, except for children aged 4-9 years, where IgG3 was higher. Raw IgM, IgG class and subclass and IgE antibody levels to C231 tended to be higher in those who did not experience a malaria attack, but following linear multivariate analysis the trends were not significant.

14: *Clin Infect Dis.* 2008 Feb 15;46(4):516-22.

Long-term asymptomatic carriage of Plasmodium falciparum protects from malaria attacks: a prospective study among Senegalese children.

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BACKGROUND: In areas of seasonal malaria transmission, long-term asymptomatic carriage of Plasmodium falciparum throughout the dry season has been primarily studied in terms of the parasites, and the clinical consequences of persistent parasite carriage are unknown. **METHODS:** A prospective study was conducted in Senegal, from 2001 through 2003 among 1356 children living in areas where malaria is endemic, with seasonal transmission occurring from August through December. Cross-sectional parasitological measurements and detection of active malaria attacks were performed. A malaria attack was defined as an axillary temperature ≥ 37.5 degrees C, associated with a parasite density >2500 trophozoites/microL. Children harboring P. falciparum in June who did not have clinical signs were defined as asymptomatic carriers. The association of asymptomatic carriage with parasite densities and with the occurrence of malaria attacks during the rainy season were analyzed separately for the years 2002 and 2003, taking into account potential confounding covariates and use of antimalarial drugs. **RESULTS:** The prevalence of asymptomatic carriage was 32% (332 of 1025 persons) in June 2002 and 23% (208 of 912 persons) in June 2003. Asymptomatic P. falciparum carriers had a significantly higher mean parasite density and a significantly lower probability of developing a malaria attack during the subsequent rainy season than did noncarriers (adjusted odds ratio in 2002, 0.56; $P = .01$; adjusted odds ratio in 2003, 0.50; $P = .01$). **CONCLUSIONS:** These results suggest that in areas

of seasonal transmission, asymptomatic carriage of *P. falciparum* may protect against clinical malaria. Further studies are needed to understand the immune effectors and host susceptibility that could be involved in this phenomenon.

15: *Comp Biochem Physiol B Biochem Mol Biol.* 2008 Mar;149(3):419-427.

Identification and characterization of the receptor for the *Bacillus sphaericus* binary toxin in the malaria vector mosquito, *Anopheles gambiae*.

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The binary toxin (Bin) from *Bacillus sphaericus* exhibits a highly insecticidal activity against *Culex* and *Anopheles* mosquitoes. The cytotoxicity of Bin requires an interaction with a specific receptor present on the membrane of midgut epithelial cells in larvae. A direct correlation exists between binding affinity and toxicity. The toxin binds with high affinity to its receptor in its primary target, *Culex pipiens*, and displays a lower affinity to the receptor in *Anopheles gambiae*, which is less sensitive to Bin. Although the Bin receptor has previously been identified and named Cpml in *C. pipiens*, its structure in *Anopheles* remains unknown. In this study, we hypothesize that the *Anopheles* Bin receptor is an ortholog of Cpml. By screening the *Anopheles* genomic database, we identified a candidate gene (Agm3) which is expressed primarily on the surface of midgut cells in larvae and which functions as a receptor for Bin. A Cpml-like gene is also present in the Bin-refractory species *Aedes aegypti*. Overall, our results indicate that the three mosquito genes examined share a very similar organization and are strongly conserved at the amino acid level, in particular in the NH(2)-terminus, a region believed to contain the ligand binding site, suggesting that relatively few amino acids residues are critical for high affinity binding of the toxin.

16: *Eur Cytokine Netw.* 2008 Feb 26;19(1):9-15

Interleukin-21 is associated with IgG1 and IgG3 antibodies to erythrocyte-binding antigen-175 peptide 4 of *Plasmodium falciparum* in Gabonese children with acute *falciparum* malaria.

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Interleukin-21 (IL-21) is a newly described, typical, four-helix cytokine showing significant homology with IL-2, IL-4 and IL-15. It regulates IgG1 production and co-operates with IL-4 in the production of multiple antibody classes in vivo. IgG1 and IgG3 are critically involved in the development of clinical immunity to *Plasmodium falciparum* malaria. However, the mechanisms driving class-switch recombination towards these specific isotypes remain to be elucidated. Seventy-three children with *P. falciparum*-positive, thick blood smears were recruited from the pediatric wards of the Albert Schweitzer Hospital and the General Hospital in Lambaréné. Children were grouped into two categories according to age: group A (1 to 5 years old) and group B (6 to 16 years old). Patients with severe (severe anemia and/or hyperparasitemia) and mild malaria were enrolled. Prevalence and level of IL-21, total IgG and subclass (IgG1, IgG2, IgG3 and IgG4) titers were determined in plasma by enzyme-linked immunosorbent assay (ELISA). Plasma IL-21 levels correlated with IgG1 and IgG3 levels. Additionally, plasma IL-21 levels correlated with hemoglobin levels in younger children and with parasite density. Here we describe the relationship between IL-21 and antibodies for erythrocyte-binding antigen-175 (EBA-175) peptide 4, a

malaria vaccine candidate in Gabonese children with acute falciparum malaria. This study provides new insights into the field of malaria.

17: *Exp Parasitol.* 2008 Mar;118(3):378-85.

Plasmodium falciparum: Sequence analysis of the gene encoding the C-terminus region of the merozoite surface protein-1, a potential malaria vaccine antigen, in Iranian clinical isolates.

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C-terminal region of merozoite surface protein-1 of *Plasmodium falciparum* (PfMSP-1) isolated from different parts of the world revealed sequence variability, however no data exist on sequence heterogeneity of this region from Iran. To address this question, DNA encoding the carboxyl (C)-terminal region of PfMSP-1 was amplified in 144 Iranian *P. falciparum* clinical isolates, using allele type-specific primers. In this study both MAD20 (88.2%) and K1 (7.6%) types were detected. Sequence analysis of 33 and 92 fragments corresponding to pfmsp-1(42) and pfmsp-1(19) revealed eight (15MAD1-15MAD7 and 15KCH) and five [A1 (E/TSR/L), A2 (Q/KNG/F), A3 (E/KNG/F), A4 (E/TSG/L), and A5 (Q/KNG/L)] distinct haplotypes, respectively. E/TSG/L variant type was the predominant haplotype, and reported only from Thailand and India, but E/KNG/L is widespread in Africa, Asia, and Latin America; but not found among Iranian isolates. In summary, result of this study indicates limited antigenic diversity, and thus support the potential utility of the C-terminal region of PfMSP-1 in designing polyvalent vaccine constructs.

18: *Infect Immun.* 2008 Mar 3

Breadth and magnitude of antibody responses to multiple *Plasmodium falciparum* merozoite antigens are associated with protection from clinical malaria.

Osier FH, Fegan G, Polley SD, Murungi L, Verra F, Tetteh KK, Lowe B, Mwangi T, Bull PC, Thomas AW, Cavanagh DR, McBride JS, Lanar DE, Mackinnon M, Conway DJ, Marsh K.

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Individuals living in malaria-endemic areas are repeatedly exposed to many different malaria parasite antigens. Studies on naturally-acquired antibody-mediated immunity to clinical malaria have largely focused on the presence of responses to individual antigens and their associations with decreased morbidity. We hypothesized that the breadth (number of important targets to which antibodies were made) and magnitude (antibody level measured in a random serum sample) of the antibody response were important predictors of protection from clinical malaria. We analyzed naturally-acquired antibodies to five leading *P. falciparum* merozoite stage vaccine candidate antigens, and schizont extract, in Kenyan children monitored for uncomplicated malaria for 6

months (n=119). Serum antibody levels to apical membrane antigen 1(AMA1), and merozoite surface protein antigens (MSP-1 block 2, MSP-2, MSP-3) were inversely related to the probability of developing malaria, but levels to MSP-119 and erythrocyte binding antigen (EBA-175) were not. The risk of malaria was also inversely associated with increasing breadth of antibody specificities, with none of the children who simultaneously had high antibody levels to five or more antigens experiencing a clinical episode, (17/119, 15%) P=0.0006. Particular combinations of antibodies (AMA1, MSP-2, MSP-3) were more strongly predictive of protection than others. The results were validated in a larger, separate case-control study whose end-point was malaria severe enough to warrant hospital admission (n=387). These findings suggest that under natural exposure, immunity to malaria may result from high titer antibodies to multiple antigenic targets and support the idea of testing combination blood stage vaccines optimized to induce similar antibody profiles.

19: *Infect Immun.* 2008 Feb 19

Childhood schistosomiasis and malaria co-infection: hepatosplenomegaly is associated with low regulatory and Th2 responses to schistosome antigens.

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Hepatosplenomegaly amongst Kenyan schoolchildren has been shown to be exacerbated where there is transmission of both *Schistosoma mansoni* and *Plasmodium falciparum*. This highly prevalent and chronic morbidity often occurs in the absence of ultrasound detectable periportal and choronal fibrosis and maybe due to immunological inflammation. For a cohort of school-aged children, whole blood cultures were stimulated with *S. mansoni* soluble egg antigen (SEA) or soluble worm antigen (SWA). Responses to SWA were found to be predominantly Th2 cytokines, however, they were not significantly associated with either hepatosplenomegaly or infection with *S. mansoni* or *P. falciparum*. In comparison, SEA specific Th2 cytokine responses were low and levels were negatively correlated with *S. mansoni* infection intensities and were lower amongst children who were co-infected with *P. falciparum*. TNFalpha levels in response to stimulation with SEA were high, and a negative association between presentation with hepatomegaly and levels of the regulatory cytokines IL-6 and TGFbeta1, suggests that a possible mechanism for childhood hepatomegaly in malaria/schistosomiasis co-endemic areas is poor regulation of an inflammatory response to schistosome eggs.

20: *Infect Immun.* 2008 Feb 11

The Wheat Germ Cell-Free Based Production of Malaria Proteins for Discovery of Novel Vaccine Candidates.

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One of the major bottlenecks in malaria research has been the difficulty in recombinant protein expression. Here, we report the application of the wheat germ cell-free system for the successful production of malaria proteins. For proof-of-principle, the Pfs25, PfCSP and PfAMA1 proteins were chosen. These genes contain very high A/T sequences and are also difficult to express as recombinant proteins. In our wheat germ cell-free system, both native and codon optimized version of the Pfs25 genes produced equal amount of proteins. PfCSP and PfAMA1 genes without any codon optimization were also expressed. The products were soluble, with yields between 50-200 microg/ml of the translation mixture, indicating that the cell-free system can be used to produce malaria proteins without any prior optimization of their biased codon usage. Biochemical and immunocytochemical analyses of antibodies raised in mice against each protein revealed that every antibody retained its high specificity to the parasite protein in question. The development of parasites in mosquitoes fed patient blood carrying *P. falciparum* gametocytes and supplemented with our mouse anti-Pfs25 sera was strongly inhibited, indicating that both Pfs25-3D7/WG and Pfs25-TBV/WG retained their immunogenicity. Lastly, we carried out a parallel expression assay of proteins of blood stage *P. falciparum*. The PCR products of 124 *P. falciparum* genes chosen from the available database were used directly in a small-scale format of transcription and translation reactions. Autoradiogram testing revealed the production of 93 proteins. Application of this new cell-free based protocol for the discovery of malaria vaccine candidates will be discussed.

21: *Int J Health Geogr.* 2008 Feb 26;7(1):9

Using remote sensing to map larval and adult populations of *Anopheles hyrcanus* (Diptera: Culicidae) a potential malaria vector in Southern France.

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ABSTRACT: **BACKGROUND:** Although malaria disappeared from southern France more than 60 years ago, suspicions of recent autochthonous transmission in the French Mediterranean coast support the idea that the area could still be subject to malaria transmission. The main potential vector of malaria in the Camargue area, the largest river delta in southern France, is the mosquito *Anopheles hyrcanus* (Diptera: Culicidae). In the context of recent climatic and landscape changes, the evaluation of the risk of emergence or re-emergence of such a major disease is of great importance in Europe. When assessing the risk of emergence of vector-borne diseases, it is crucial to be able to characterize the arthropod vector's spatial distribution. Given that remote sensing techniques can describe some of the environmental parameters which drive this distribution, satellite imagery or aerial photographs could be used for vector mapping. **RESULTS:** In this study, we propose a method to map larval and adult populations of *An. hyrcanus* based on environmental indices derived from high spatial resolution imagery. The analysis of the link between entomological field data on *An. hyrcanus* larvae and environmental indices (biotopes, distance to the nearest main productive breeding sites of this species i.e., rice fields) led to the definition of a larval index, defined as the probability of observing *An. hyrcanus* larvae in a given site at least once over a year. Independent accuracy assessments showed a good agreement between observed and predicted values (sensitivity and specificity of the logistic regression model being 0.76 and 0.78, respectively). An adult index was derived from the larval index by averaging the larval index within a buffer around the trap location. This index was highly correlated with observed adult abundance values (Pearson $r = 0.97$, $p < 0.05$). This allowed us to generate predictive maps of *An. hyrcanus* larval and adult populations from the landscape indices. **CONCLUSIONS:** This work shows that it is possible to use high resolution satellite imagery to map malaria vector spatial distribution. It also confirms the potential of remote sensing to help target risk areas, and constitutes a first essential step in assessing the risk of re-emergence of malaria in southern

France.

22: *J Biol Chem.* 2008 Feb 29;283(9):5589-97.

Role of Ca²⁺/Calmodulin-PfPKB Signaling Pathway in Erythrocyte Invasion by Plasmodium falciparum.

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Molecular mechanisms by which signaling pathways operate in the malaria parasite and control its development are promiscuous. Recently, we reported the identification of a signaling pathway in *Plasmodium falciparum*, which involves activation of protein kinase B-like enzyme (PfPKB) by calcium/calmodulin (Vaid, A., and Sharma, P. (2006) *J. Biol. Chem.* 281, 27126-27133). Studies carried out to elucidate the function of this pathway suggested that it may be important for erythrocyte invasion. Blocking the function of the upstream activators of this pathway, calmodulin and phospholipase C, resulted in impaired invasion. To evaluate if this signaling cascade controls invasion by regulating PfPKB, inhibitors against this kinase were developed. PfPKB inhibitors dramatically reduced the ability of the parasite to invade erythrocytes. Furthermore, we demonstrate that PfPKB associates with actin-myosin motor and phosphorylates PfGAP45 (glideosome-associated protein 45), one of the important components of the motor complex, which may help explain its role in erythrocyte invasion.

23: *J Biosoc Sci.* 2008 Mar;40(2):283-96.

Prevention and treatment practices and implications for malaria control in mukono district Uganda.

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Available data in Uganda indicate a resurgence of malaria morbidity and mortality countrywide. This study assessed the burden of malaria, treatment and prevention practices in order to initiate a policy debate on the scaling-up of current interventions. A triangulation of methods using a cross-sectional survey and key informant interviews was used to assess self-reported malaria at a household level in Mukono District, Uganda. A total of 5583 households were surveyed, and a high proportion (2897, 51.9%) reported a person with malaria two weeks prior to the survey. Only 546 households (9.8%) owned and used insecticide-treated nets (ITNs) for malaria prevention. Similarly, only a few households (86, 1.5%) used indoor residual spraying. Self-treatment with home-stocked drugs was high, yet there was low awareness of the effectiveness of expired drugs on malaria treatment. Self-reported malaria was associated with socioeconomic, behavioural and environmental factors, but more especially with household ownership of ITNs. These results will contribute to the current debate on identifying new approaches for scaling-up prevention interventions and effective case management, as well as selection of priority interventions for malaria control in Uganda.

24: *J Ethnopharmacol.* 2008 Feb 28;116(1):74-83.

Antimalarial activity of crude extracts from nine African medicinal plants.

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An ethnobotanical study was conducted in Comores (Ngazidja) about plant species used traditionally for the treatment of various diseases, including malaria. Antimalarial activity of 76 vegetal extracts obtained from 17 species traditionally used to treat malaria symptoms, was evaluated in vitro using *Plasmodium falciparum* chloroquine-resistant strain (W2). Antiproliferative activity was evaluated on human monocytic THP1 cells and the selectivity index of the plant extracts was calculated. The results showed that 10 plant extracts had a moderate activity ($5 < IC(50) \leq 10$ μ g/ml), and 6 a good in vitro activity with $IC(50)$ value ≤ 5 μ g/ml. The highest antiplasmodial activity was found for the MeOH/H₂O leaves extract of *Flueggea virosa* (Roxb. Ex Willd.) Voigt subsp. *virosa* (Euphorbiaceae) ($IC(50)$ =2 μ g/ml), for roots decoction of *Flueggea virosa* ($IC(50)$ =3 μ g/ml) and for chloromethylenic roots extract of *Vernonia colorata* (Willd.) Drake subsp. *grandis* (DC.) C. Jeffrey (Asteraceae) ($IC(50)$ =3 μ g/ml). Three other extracts showed moderate antiplasmodial activity ($IC(50)$ <5 μ g/ml): *Vernonia colorata* (aerial part), *Piper capense* L.f. (Piperaceae), and *Leptadenia madagascariensis* Decne (Asclepiadaceae) chloromethylenic extracts ($IC(50)$ =6 μ g/ml; 7 μ g/ml and 9 μ g/ml, respectively). All the plants tested displayed a low cytotoxicity on THP1 cells.

25: *J Ethnopharmacol.* 2008 Feb 28;116(1):43-8.

Evaluation of Senegalese plants used in malaria treatment: Focus on *Chrozophora senegalensis*.

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An ethnobotanical study was conducted in the Dakar area of Senegal to investigate the species used in the treatment of malaria. Seven plants are principally used: *Cissampelos mucronata*, *Maytenus senegalensis*, *Terminalia macroptera*, *Bidens engleri*, *Ceratotheca sesamoides*, *Chrozophora senegalensis* and *Mitracarpus scaber*. From a bibliographic study, it had been shown that the *Cissampelos mucronata*, *Maytenus senegalensis* and *Terminalia macroptera* have already been studied by several authors, and so only *Bidens engleri*, *Ceratotheca sesamoides*, *Chrozophora senegalensis* and *Mitracarpus scaber* were evaluated in the present study. For each plant, extracts were prepared with different solvents and tested in vitro on two chloroquine-resistant *Plasmodium falciparum* strains. Crude extracts from the leaves and the stems of *Chrozophora senegalensis* showed the best in vitro results. The $IC(50)$ value of an aqueous extract of *Chrozophora senegalensis* was 1.6 μ g/ml without cytotoxicity. The in vivo antiplasmodial activity of *Chrozophora* extracts was determined by both the oral and the intraperitoneal ways. The stages of *Plasmodium* cycle targeted by *Chrozophora* were then studied in vitro. These results could justify the traditional use of this plant in malaria treatment.

26: *J Ethnopharmacol.* 2008 Feb 28;116(1):33-42.

Herbal medicines used in the treatment of malaria in Budiope county, Uganda.

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AIM OF THE STUDY: This study was conducted to document herbal medicines (HMs) used in the treatment of malaria as well as the existing knowledge, attitudes and practices related to malaria recognition, control and treatment in Budiope county, Uganda. METHODS: Data was collected using semi-structured interviews, and open- and close-ended questionnaires. RESULTS: The respondents had a good understanding of malaria, and could recognize it and distinguish it from other fever types. They were also aware that malaria was spread by mosquitoes. Malaria prevalence was high, and affected individuals an average of six times a year. Respondents avoided mosquito bites by using mosquito nets, clearing bush around their homesteads, and burning plant parts to generate smoke. They preferred treating malaria using allopathic medicines because, according to them, they lacked the appropriate traditional knowledge necessary to exploit plants for the treatment of malaria. Secondly, allopathic medicines were believed to be superior to HMs in the treatment of malaria. Twenty-seven species were used for the treatment of malaria. The most frequently mentioned were *Vernonia amygdalina*, *Momordica foetida*, *Zanthoxylum chalybeum*, *Lantana camara* and *Mangifera indica*. Drugs from these plants were prepared from single species as water extracts and were administered in variable doses over varied time periods.

27: *J Immunol.* 2008 Mar 1;180(5):3383-90.

Fine Specificity of Neonatal Lymphocytes to an Abundant Malaria Blood-Stage Antigen: Epitope Mapping of Plasmodium falciparum MSP133.

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Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH 44106.

Cord blood T cells have been reported to respond to a variety of exogenous Ags, including environmental allergens and various viruses and parasites, as demonstrated by enhanced proliferation and cytokine secretion. This finding is evidence that Ags in the maternal environment transplacentally prime and result in fetal development of memory T cells. Some studies suggest these neonatal T cell responses may arise by nonspecific activation of T cells that express TCRs with low binding affinity, thus lacking fine lymphocyte specificity. To address this question, we examined malaria Ag stimulation of human cord and adult blood mononuclear cells in samples from residents of a malaria endemic area in Kenya. We constructed overlapping 18-mer peptides derived from sequences contained in dimorphic alleles of the C-terminal 33-kDa fragment of Plasmodium falciparum merozoite protein 1. This study identified a dominant T cell epitope for one MSP1(33) allele (MAD20) and two T cell epitopes for the second allele (K1); these epitopes were nonoverlapping and allele specific. In a given donor, peptide-specific proliferation and IFN-gamma secretion were highly concordant. However, IL-10 and IL-13 secretion were not correlated. Importantly, the fine specificity of lymphocyte proliferation and cytokine secretion in cord and adult blood mononuclear cells was similar. Cord blood cells obtained from malaria-infected pregnant women were 4-fold more likely to acquire a peptide-specific immune response. We conclude that the fetal malaria response functions in a fully adaptive manner and that this response may serve to help protect the infant from severe malaria during infancy.

28: *J Immunol.* 2008 Feb 15;180(4):2496-503.

Malaria Parasites Require TLR9 Signaling for Immune Evasion by Activating Regulatory T Cells.

Hisaeda H, Tetsutani K, Imai T, Moriya C, Tu L, Hamano S, Duan X, Chou B, Ishida H, Aramaki A, Shen J, Ishii KJ, Coban C, Akira S, Takeda K, Yasutomo K, Torii M, Himeno K.

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Malaria is still a life-threatening infectious disease that continues to produce 2 million deaths annually. Malaria parasites have acquired immune escape mechanisms and prevent the development of sterile immunity. Regulatory T cells (Tregs) have been reported to contribute to immune evasion during malaria in mice and humans, suggesting that activating Tregs is one of the mechanisms by which malaria parasites subvert host immune systems. However, little is known about how these parasites activate Tregs. We herein show that TLR9 signaling to dendritic cells (DCs) is crucial for activation of Tregs. Infection of mice with the rodent malaria parasite *Plasmodium yoelii* activates Tregs, leading to enhancement of their suppressive function. In vitro activation of Tregs requires the interaction of DCs with parasites in a TLR9-dependent manner. Furthermore, TLR9(-/-) mice are partially resistant to lethal infection, and this is associated with impaired activation of Tregs and subsequent development of effector T cells. Thus, malaria parasites require TLR9 to activate Tregs for immune escape.

29: *J Infect Dis.* 2008 Feb 15

Placental Hypoxia during Placental Malaria.

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Background. Placental malaria causes fetal growth retardation (FGR), which has been linked epidemiologically to placental monocyte infiltrates. We investigated whether parasite or monocyte infiltrates were associated with placental hypoxia, as a potential mechanism underlying malarial FGR. Methods. We studied the hypoxia markers hypoxia inducible factor (HIF)-1 α , vascular endothelial growth factor (VEGF), placental growth factor, VEGF receptor 1 and its soluble form, and VEGF receptor 2. We used real-time polymerase chain reaction (in 59 women) to examine gene transcription, immunohistochemistry (in 30 women) to describe protein expression, and laser-capture microdissection (in 23 women) to examine syncytiotrophoblast-specific changes in gene expression. We compared gene and protein expression in relation to malaria infection, monocyte infiltrates, and birth weight. Results. We could not associate any hallmark of placental malaria with a transcription, expression, or tissue-distribution profile characteristic of a response to hypoxia, but we found higher HIF-1 α levels ([Formula: see text]) and lower VEGF levels ([Formula: see text]) in the syncytiotrophoblasts of cases of malaria than in those of asymptomatic control placentas. Conclusions. Our data are inconsistent with a role for placental hypoxia in the pathogenesis of malaria-associated FGR. The laser-capture microdissection study was small, but its results suggest (1)

that malaria affects syncytiotrophoblast-gene transcription and (2) novel potential mechanisms for placental malaria-associated FGR.

30: *J Infect Dis.* 2008 Feb 15;197(4):527-534.

Potent Antimalarial and Transmission-Blocking Activities of Centanamycin, a Novel DNA-Binding Agent.

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Most treatments for malaria target the blood stage of infection in the human host, although few can also block transmission of the parasite to the mosquito. We show here that the compound centanamycin is very effective against blood-stage malarial infections in vitro and in vivo and has profound effects on sexual differentiation of the parasites in mosquitoes. After drug treatment, parasite development is arrested within the midguts of mosquitoes, failing to produce the infective forms that migrate to the salivary glands. The mechanism of parasite death is associated with modification of Plasmodium genomic DNA. We detected DNA damage in parasites isolated from mice 24 h after treatment with centanamycin, and, importantly, we also detected this DNA damage in parasites within mosquitoes that had fed on these mice 10 days earlier. This demonstrates that damage to parasite DNA during blood-stage infection persists from the vertebrate to the mosquito host and provides a novel biochemical strategy to block malaria transmission.

31: *J Infect Dis.* 2008 Feb 15;197(4):519-526.

Antibodies to Pre-erythrocytic Plasmodium falciparum Antigens and Risk of Clinical Malaria in Kenyan Children.

John CC, Tande AJ, Moormann AM, Sumba PO, Lanar DE, Min XM, Kazura JW.

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Background. IgG antibodies to pre-erythrocytic antigens are involved in prevention of infection and disease in animal models of malaria but have not been associated with protection against disease in human malaria. Methods. Levels of IgG antibodies to circumsporozoite protein (CSP), liver-stage antigen type 1 (LSA-1), and thrombospondin-related adhesive protein (TRAP) were measured in 86 children in a malaria-holoendemic area of Kenya. The children were then monitored for episodes of clinical malaria for 52 weeks. Results. Children with high levels of IgG antibodies to CSP, LSA-1, and TRAP had a decreased risk of clinical malaria (adjusted hazard ratio, 0.29; 95% confidence interval 0.10-0.81; [Formula: see text]), a lower incidence of clinical malaria ([Formula: see text]), protection from clinical malaria with a parasite level of ≥ 4000 parasites/ μ L ([Formula: see text]), and a higher hemoglobin level at enrollment ([Formula: see text]), compared with children with lower antibody levels. Protection against malaria morbidity was associated primarily with antibodies to

CSP and LSA-1. Conclusions. Kenyan children with high levels of IgG antibodies to the pre-erythrocytic antigens CSP, LSA-1, and TRAP have a lower risk of developing clinical malaria than children without high levels of these antibodies. The decreased risk of clinical malaria may be mediated in part by prevention of high-density parasitemia.

32: *J Infect Dis.* 2008 Feb 15;197(4):510-8.

Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda.

Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ, Dorsey G.

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Background. In Africa, fever is often treated presumptively as malaria, resulting in misdiagnosis and the overuse of antimalarial drugs. Rapid diagnostic tests (RDTs) for malaria may allow improved fever management. Methods. We compared RDTs based on histidine-rich protein 2 (HRP2) and RDTs based on Plasmodium lactate dehydrogenase (pLDH) with expert microscopy and PCR-corrected microscopy for 7000 patients at sites of varying malaria transmission intensity across Uganda. Results. When all sites were considered, the sensitivity of the HRP2-based test was 97% when compared with microscopy and 98% when corrected by PCR; the sensitivity of the pLDH-based test was 88% when compared with microscopy and 77% when corrected by PCR. The specificity of the HRP2-based test was 71% when compared with microscopy and 88% when corrected by PCR; the specificity of the pLDH-based test was 92% when compared with microscopy and >98% when corrected by PCR. Based on Plasmodium falciparum PCR-corrected microscopy, the positive predictive value (PPV) of the HRP2-based test was high (93%) at all but the site with the lowest transmission rate; the pLDH-based test and expert microscopy offered excellent PPVs (98%) for all sites. The negative predictive value (NPV) of the HRP2-based test was consistently high (>97%); in contrast, the NPV for the pLDH-based test dropped significantly (from 98% to 66%) as transmission intensity increased, and the NPV for expert microscopy decreased significantly (99% to 54%) because of increasing failure to detect subpatent parasitemia. Conclusions. Based on the high PPV and NPV, HRP2-based RDTs are likely to be the best diagnostic choice for areas with medium-to-high malaria transmission rates in Africa.

33: *J Infect Dis.* 2008 Feb 8

Differential Antibody Responses to Plasmodium falciparum Merozoite Proteins in Malawian Children with Severe Malaria.

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Cerebral malaria (CM) and severe malarial anemia (SMA) are 2 major causes of

death in African children infected with *Plasmodium falciparum*. We investigated levels of naturally acquired antibody to conserved and variable regions of merozoite surface protein (MSP)-1 and MSP-2, apical membrane antigen (AMA)-1, and rhoptry-associated protein 1 in plasma samples from 126 children admitted to the hospital with CM, 59 with SMA, and 84 with uncomplicated malaria (UM) in Malawi. Children with SMA were distinguished by very low levels of immunoglobulin (Ig) G to the conserved C-terminus of MSP-1 and MSP-2 and to full-length AMA-1. Conversely, children with CM had significantly higher levels of IgG to the conserved regions of all antigens examined than did children with UM (for MSP-1 and AMA-1, [Formula: see text]; for MSP-2, [Formula: see text]) or SMA (for MSP-1 and MSP-2, [Formula: see text]; for AMA-1, [Formula: see text]). These distinct IgG patterns might reflect differences in age, exposure to *P. falciparum*, and/or genetic factors affecting immune responses.

34: *J Med Chem.* 2008 Feb 28;51(4):1035-1042.

Malaria-Infected Mice Are Cured by Oral Administration of New Artemisinin Derivatives.

Posner GH, Chang W, Hess L, Woodard L, Sinishtaj S, Usera AR, Maio W, Rosenthal AS, Kalinda AS, D'Angelo JG, Petersen KS, Stohler R, Chollet J, Santo-Tomas J, Snyder C, Rottmann M, Wittlin S, Brun R, Shapiro TA.

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In four or five chemical steps from the 1,2,4-trioxane artemisinin, a new series of 23 trioxane dimers has been prepared. Eleven of these new trioxane dimers cure malaria-infected mice via oral dosing at 3 x 30 mg/kg. The clinically used trioxane drug sodium artesunate prolonged mouse average survival to 7.2 days with this oral dose regimen. In comparison, animals receiving no drug die typically on day 6-7 postinfection. At only 3 x 10 mg/kg oral dosing, seven dimers prolong the lifetime of malaria-infected mice to days 14-17, more than double the chemotherapeutic effect of sodium artesunate. Ten new trioxane dimers at only a single oral dose of 30 mg/kg prolong mouse average survival to days 8.7-13.7, and this effect is comparable to that of the fully synthetic trioxolane drug development candidate OZ277, which is in phase II clinical trials.

35: *J Med Chem.* 2008 Feb 16

Design, Synthesis, and Structure-Activity Relationship Studies of 4-Quinolinylnyl- and 9-Acrydinylnylhydrazones as Potent Antimalarial Agents.

Fattorusso C, Campiani G, Kukreja G, Persico M, Butini S, Romano MP, Altarelli M, Ros S, Brindisi M, Savini L, Novellino E, Nacci V, Fattorusso E, Parapini S, Basilico N, Taramelli D, Yardley V, Croft S, Borriello M, Gemma S.
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Malaria is a major health problem in poverty-stricken regions where new antiparasitic drugs are urgently required at an affordable price. We report herein the design, synthesis, and biological investigation of novel antimalarial agents with low potential to develop resistance and structurally based on a highly conjugated scaffold. Starting from a new hit, the designed modifications were performed hypothesizing a specific interaction with free heme and generation of radical intermediates. This approach provided antimalarials with improved potency against chloroquine-resistant plasmodia over known drugs. A number of structure-activity relationship (SAR) trends were identified and among the

analogues synthesized, the pyrrolidinylmethylarylidene and the imidazole derivatives 5r, 5t, and 8b were found as the most potent antimalarial agents of the new series. The mechanism of action of the novel compounds was investigated and their in vivo activity was assessed.

36: *J Nutr.* 2008 Mar;138(3):642-5.

Differential community response to introduction of zinc for childhood diarrhea and combination therapy for malaria in southern Mali.

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Developing effective, affordable, and sustainable delivery strategies for the isolated low-income populations that stand to gain the most from micronutrient interventions has proven difficult. We discuss our experience with implementation of zinc as treatment for diarrhea in children less than 5 y of age over the course of 3 operational research studies in rural Sikasso Region, Mali, West Africa. The initial formative research study highlighted how malaria affects perceptions of diarrhea and its causes and that malaria and diarrhea are not necessarily viewed as distinct conditions. The second-phase pilot introduction demonstrated that, in introducing zinc treatment in malaria-endemic regions, it is especially important that both community- and facility-level providers be trained to manage sick children presenting with multiple symptoms. The third-phase study on large-scale implementation detected that the experience with implementation of new treatments for malaria is distinct from that of diarrhea. To some extent zinc treatment is the solution to a problem that communities may not recognize at all. Interventions to improve case management of sick children must be integrated across diseases and nutritional problems at both the facility and community levels. Operational research can identify points where integration should occur and how it should be carried out. Programs targeting single diseases or single nutritional problems can have a variety of deleterious effects on health systems, no matter how well they are planned.

37: *Malar J.* 2008 Mar 1;7(1):44

Use of antenatal care, maternity services, intermittent presumptive treatment and insecticide treated bed nets by pregnant women in Luwero district, Uganda.

Kiwuwa MS, Mufubenga P.

BACKGROUND: To reduce the intolerable burden of malaria in pregnancy, the Ministry of Health in Uganda improved the antenatal care package by including a strong commitment to increase distribution of insecticide-treated nets (ITNs) and introduction of intermittent preventive treatment with sulphadoxine-pyrimethamine for pregnant women (IPTp-SP) as a national policy in 2000. This study assessed uptake of both ITNs and IPTp-SP by pregnant women as well as antenatal and maternity care use with the aim of optimizing their delivery. **METHODS:** 769 post-partum women were recruited from a rural area of central Uganda with perennial malaria transmission through a cross-sectional, community-based household survey in May 2005. **RESULTS:** Of the 769 women interviewed, antenatal clinic (ANC) attendance was high (94.4%); 417 (57.7%) visiting initially during the 2nd trimester, 242 (33.5%) during the 3rd trimester and 266 (37.1%) reporting aYen4 ANC visits. About 537 (71%) and 272 (35.8%) received one or aYen2 IPTp-SP doses respectively. Only 85 (15.8%) received the first dose of IPTp-SP in the 3rd trimester. ITNs were used by 239 (31.3%) of women during pregnancy and 314 (40.8%) delivered their most recent pregnancy outside a health facility. Post-partum women who lacked post-primary education were more likely not to have attended four or

more ANC visits (odds ratio [OR] 3.3, 95% confidence interval [CI] 1.2-9.3).
CONCLUSION: These findings illustrate the need to strengthen capacity of the district to further improve antenatal care and maternity services utilization and IPTp-SP uptake. More specific and effective community health strategies to improve effective ANC, maternity services utilization and IPTp-SP uptake in rural communities should be undertaken.

38: *Malar J.* 2008 Feb 29;7(1):43

Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya.

Muriu SM, Muturi EJ, Shililu JI, Mbogo CM, Mwangangi JM, Jacob BG, Irungu LW, Mukabana RW, Githure JI, Novak RJ.

ABSTRACT: BACKGROUND: Studies were conducted between April 2004 and February 2006 to determine the blood-feeding pattern of Anopheles mosquitoes in Mwea Kenya. METHODS: Samples were collected indoors by pyrethrum spray catch and outdoors by Centers for Disease Control light traps and processed for blood meal analysis by an Enzyme-linked Immunosorbent Assay. RESULTS: A total of 3,333 blood-fed Anopheles mosquitoes representing four Anopheles species were collected and 2,796 of the samples were assayed, with Anopheles arabiensis comprising 76.2% (n=2,542) followed in decreasing order by Anopheles coustani 8.9% (n=297), Anopheles pharoensis 8.2% (n=272) and Anopheles funestus 6.7% (n=222). All mosquito species had a high preference for bovine (range 56.3-71.4%) over human (range 1.1-23.9%) or goat (0.1-2.2%) blood meals. Some individuals from all the four species were found to contain mixed blood meals. The bovine blood index (BBI) for An. arabiensis was significantly higher for populations collected indoors (71.8%), than populations collected outdoors (41.3%), but the human blood index (HBI) did not differ significantly between the two populations. In contrast, BBI for indoor collected An. funestus (51.4%) was significantly lower than for outdoor collected populations (78.0%) and the HBI was significantly higher indoors (28.7%) than outdoors (2.4%). Anthropophily of An. funestus was lowest within the rice scheme, moderate in unplanned rice agro-ecosystem, and highest within the non-irrigated agro-ecosystem. Anthropophily of An. arabiensis was significantly higher in the non-irrigated agro-ecosystem than in the other agro-ecosystems. CONCLUSIONS: These findings suggest that rice cultivation has an effect on host choice by Anopheles mosquitoes. The study further indicate that zooprophyllaxis may be a potential strategy for malaria control, but there is need to assess how domestic animals may influence arboviruses epidemiology before adapting the strategy.

39: *Malar J.* 2008 Feb 29;7(1):40

Topography-derived wetness indices are associated with household-level malaria risk in two communities in the western Kenyan highlands.

Cohen JM, Ernst KC, Lindblade KA, Vulule JM, John CC, Wilson ML.

ABSTRACT: BACKGROUND: Transmission of Plasmodium falciparum generally decreases with increasing elevation, in part because lower temperature slows the development of both parasites and mosquitoes. However, other aspects of the terrain, such as the shape of the land, may affect habitat suitability for Anopheles breeding and thus risk of malaria transmission. Understanding these local topographic effects may permit prediction of regions at high risk of malaria within the highlands at small spatial scales. METHODS: Hydrologic modelling techniques were adapted to predict the flow of water across the landscape surrounding households in two communities in the western Kenyan highlands. These surface analyses were used to generate indices describing predicted water accumulation in regions surrounding the study area. Households with and without malaria were compared for their proximity to regions of high and low predicted wetness. Predicted wetness and elevation variables were entered

into bivariate and multivariate regression models to examine whether significant associations with malaria were observable at small spatial scales. RESULTS: On average, malaria case households (n=423) were located 280 m closer to regions with very high wetness indices than non-malaria "control" households (n=895) (t=10.35, p<0.0001). Distance to high wetness indices remained an independent predictor of risk after controlling for household elevation in multivariate regression (OR=0.93 [95% confidence interval=0.89-0.96] for a 100 m increase in distance). For every 10 m increase in household elevation, there was a 12% decrease in the odds of the house having a malaria case (OR=0.88 [0.85-0.90]). However, after controlling for distance to regions of high predicted wetness and the community in which the house was located, this reduction in malaria risk was not statistically significant (OR=0.98 [0.94-1.03]). CONCLUSIONS: Proximity to terrain with high predicted water accumulation was significantly and consistently associated with increased household-level malaria incidence, even at small spatial scales with little variation in elevation variables. These results suggest that high wetness indices are not merely proxies for valley bottoms, and hydrologic flow models may prove valuable for predicting areas of high malaria risk in highland regions. Application in areas where malaria surveillance is limited could identify households at higher risk and help focus interventions.

40: *Malar J.* 2008 Feb 29;7(1):39

A census-weighted, spatially-stratified household sampling strategy for urban malaria epidemiology.

Siri JG, Lindblade KA, Rosen DH, Onyango B, Vulule JM, Slutsker L, Wilson ML.

ABSTRACT: BACKGROUND: Urban malaria is likely to become increasingly important as a consequence of the growing proportion of Africans living in cities. A novel sampling strategy was developed for urban areas to generate a sample simultaneously representative of population and inhabited environments. Such a strategy should facilitate analysis of important epidemiological relationships in this ecological context. METHODS: Census maps and summary data for Kisumu, Kenya, were used to create a pseudo-sampling frame using the geographic coordinates of census-sampled structures. For every enumeration area (EA) designated as urban by the census (n = 535), a sample of structures equal to one-tenth the number of households was selected. In EAs designated as rural (n=32), a geographically random sample totalling one-tenth the number of households was selected from a grid of points at 100m intervals. The selected samples were cross-referenced to a geographic information system, and coordinates transferred to handheld global positioning units. Interviewers found the closest eligible household to the sampling point and interviewed the caregiver of a child aged < 10 years. The demographics of the selected sample were compared with results from the Kenya Demographic and Health Survey to assess sample validity. Results were also compared among urban and rural EAs. RESULTS: 4,336 interviews were completed in 473 of the 567 study area EAs from June 2002 through February 2003. EAs without completed interviews were randomly distributed, and non-response was approximately 2%. Mean distance from the assigned sampling point to the completed interview was 74.6 m, and was significantly less in urban than rural EAs, even when controlling for number of households. The selected sample had significantly more children and females of childbearing age than the general population, and fewer older individuals. CONCLUSIONS: This method selected a sample that was simultaneously population-representative and inclusive of important environmental variation. The use of a pseudo-sampling frame and pre-programmed handheld GPS units is more efficient and may yield a more complete sample than traditional methods, and is less expensive than complete population enumeration.

An experimental hut evaluation of Olyset(R) nets against anopheline mosquitoes after seven years use in Tanzanian villages.

Malima RC, Magesa SM, Tunga PK, Mwingira V, Magogo FS, Sudi W, Mosha FW, Curtis CF, Maxwell C, Rowland M.

ABSTRACT: **BACKGROUND:** Long-lasting insecticidal nets (LLINs) are advocated by WHO for protection against malaria. Of the three brands of LLINs currently approved by WHO, Olyset(R) is the only one currently granted full recommendation. With this type of LLIN, the insecticide (permethrin) is incorporated into the polyethylene fibre during manufacture and diffuses from the core to the surface, thereby maintaining surface concentrations. It has not been determined for how long Olyset nets remain protective against mosquitoes in household use. **METHODS:** Examples of Olyset nets, which had been in use in Tanzanian villages for seven years, were tested in experimental huts against naturally entering *Anopheles gambiae* and *Anopheles funestus* mosquitoes. Performance was compared with new Olyset nets, conventionally treated ITNs (either newly treated with alphacypermethrin or taken from local villages after 1.5 years of use) and untreated nets. All nets were artificially holed except for the seven-year Olyset nets, which had developed holes during prolonged domestic use. **RESULTS:** *Anopheles funestus* and *An. gambiae* in NE Tanzania are susceptible to pyrethroids. The new Olyset nets caused high mortality against *An. funestus* (73.9%) and *An. gambiae* (62.7%) in experimental huts. The seven-year Olyset nets caused 58.9% mortality against *An. funestus* and 40.0% mortality against *An. gambiae*. The freshly treated alphacypermethrin nets also caused high mortality against *An. funestus* (70.6%) and *An. gambiae* (72.0%); this decreased to 58.4% and 69.6% respectively after 1.5 years of use. The new Olyset nets inhibited blood-feeding by 40-50%. The 7 year Olyset nets showed no feeding inhibition over that shown by the untreated nets. The alphacypermethrin treated nets failed to inhibit blood-feeding after 1.5 years of use. However in laboratory tunnel tests samples of all types of treated net including the 7 year Olyset inhibited blood-feeding by more than 95%. **CONCLUSIONS:** After seven years of use Olyset nets were still strongly insecticidal. Mosquito mortality decreased by only 20-35% over this period. However, Olyset would not provide personal protection after seven years unless it was in good condition and all holes fully repaired.

***Plasmodium vivax dhfr* and *dhps* mutations in isolates from Madagascar and therapeutic response to sulphadoxine-pyrimethamine.**

Barnadas C, Tichit M, Bouchier C, Ratsimbaoa A, Randrianasolo L, Raherinjafy R, Jahevitra M, Picot S, Menard D.

ABSTRACT: **BACKGROUND:** Four of five *Plasmodium* species infecting humans are present in Madagascar. *Plasmodium vivax* remains the second most prevalent species, but is understudied. No data is available on its susceptibility to sulphadoxine-pyrimethamine, the drug recommended for intermittent preventive treatment during pregnancy. In this study, the prevalence of *P. vivax* infection and the polymorphisms in the *pvdhfr* and *pvdhps* genes were investigated. The correlation between these polymorphisms and clinical and parasitological responses was also investigated in *P. vivax*-infected patients. **METHODS:** *Plasmodium vivax* clinical isolates were collected in eight sentinel sites from the four major epidemiological areas for malaria across Madagascar in 2006/2007. *Pvdhfr* and *pvdhps* genes were sequenced for polymorphism analysis. The therapeutic efficacy of SP in *P. vivax* infections was assessed in Tsiroanomandidy, in the foothill of the central highlands. An intention-to-treat analysis of treatment outcome was carried out. **RESULTS:** A total of 159 *P. vivax* samples were sequenced in the *pvdhfr/pvdhps* genes. Mutant-types in *pvdhfr* gene were found in 71% of

samples, and in pvdhps gene in 16% of samples. Six non-synonymous mutations were identified in pvdhfr, including two novel mutations at codons 21 and 130. For pvdhps, beside the known mutation at codon 383, a new one was found at codon 422. For the two genes, different combinations were ranged from wild-type to quadruple mutant-type. Among the 16 patients enrolled in the sulphadoxine-pyrimethamine clinical trial (28 days of follow-up) and after adjustment by genotyping, 3 (19%, 95% CI: 5%-43%) of them were classified as treatment failure and were pvdhfr 58R/117N double mutant carriers with or without the pvdhps 383G mutation. CONCLUSIONS: This study highlights (i) that genotyping in the pvdhfr and pvdhps genes remains a useful tool to monitor the emergence and the spread of *P. vivax* sulphadoxine-pyrimethamine resistant in order to improve the national antimalarial drug policy, (ii) the issue of using sulphadoxine-pyrimethamine as a monotherapy for intermittent preventive treatment of pregnant women or children.

43: *Malar J.* 2008 Feb 25;7(1):34

Quantitative urban classification for malaria epidemiology in sub-Saharan Africa.

Siri JG, Lindblade KA, Rosen DH, Onyango B, Vulule J, Slutsker L, Wilson ML.

ABSTRACT: BACKGROUND: Although sub-Saharan Africa (SSA) is rapidly urbanizing, the terms used to classify urban ecotypes are poorly defined in the context of malaria epidemiology. Lack of clear definitions may cause misclassification error, which likely decreases the accuracy of continent-wide estimates of malaria burden, limits the generalizability of urban malaria studies, and makes identification of high-risk areas for targeted interventions within cities more difficult. Accordingly, clustering techniques were applied to a set of urbanization- and malaria-related variables in Kisumu, Kenya, to produce a quantitative classification of the urban environment for malaria research. METHODS: Seven variables with a known or expected relationship with malaria in the context of urbanization were identified and measured at the census enumeration area (EA) level, using three sources: a) the results of a citywide knowledge, attitudes and practices (KAP) survey; b) a high-resolution multispectral satellite image; and c) national census data. Principal components analysis (PCA) was used to identify three factors explaining higher proportions of the combined variance than the original variables. A k-means clustering algorithm was applied to the EA-level factor scores to assign EAs to one of three categories: "urban," "peri-urban," or "semi-rural." The results were compared with classifications derived from two other approaches: a) administrative designation of urban/rural by the census or b) population density thresholds. RESULTS: Urban zones resulting from the clustering algorithm were more geographically coherent than those delineated by population density. Clustering distributed population more evenly among zones than either of the other methods and more accurately predicted variation in other variables related to urbanization, but not used for classification. CONCLUSIONS: Effective urban malaria epidemiology and control would benefit from quantitative methods to identify and characterize urban areas. Cluster analysis techniques were used to classify Kisumu, Kenya, into levels of urbanization in a repeatable and unbiased manner, an approach that should permit more relevant comparisons among and within urban areas. To the extent that these divisions predict meaningful intra-urban differences in malaria epidemiology, they should inform targeted urban malaria interventions in cities across SSA.

44: *Malar J.* 2008 Feb 18;7(1):33

Household cost of malaria overdiagnosis in rural Mozambique.

Hume JC, Barnish G, Mangal T, Armazio L, Streat E, Bates I.

ABSTRACT: BACKGROUND: It is estimated that over 70% of patients with suspected malaria in sub-Saharan Africa, diagnose and manage their illness at home without

referral to a formal health clinic. Of those patients who do attend a formal health clinic, malaria overdiagnosis rates are estimated to range between 30-70%. METHODS: This paper details an observational cohort study documenting the number and cost of repeat consultations as a result of malaria overdiagnosis at two health care providers in a rural district of Mozambique. 535 adults and children with a clinical diagnosis of malaria were enrolled and followed over a 21 day period to assess treatment regimen, symptoms, number and cost of repeat visits to health providers in patients misdiagnosed with malaria compared to those with confirmed malaria (determined by positive bloodfilm reading). RESULTS: Diagnosis based solely on clinical symptoms overdiagnosed 23% of children (<16y) and 31% of adults with malaria. Symptoms persisted ($p = 0.023$) and new ones developed ($p < 0.001$) in more adults than children in the three weeks following initial presentation. Adults overdiagnosed with malaria had more repeat visits (67% v 46%, $p = 0.01-0.06$) compared to those with true malaria. There was no difference in costs between patients correctly or incorrectly diagnosed with malaria. Median costs over three weeks were \$0.28 for those who had one visit and \$0.76 for aYen3 visits and were proportionally highest among the poorest ($p < 0.001$) CONCLUSIONS: Overdiagnosis of malaria results in a greater number of healthcare visits and associated cost for adult patients. Additionally, it is clear that the poorest individuals pay significantly more proportionally for their healthcare making it imperative that the treatment they receive is correct in order to prevent wastage of limited economic resources. Thus, investment in accurate malaria diagnosis and appropriate management at primary level is critical for improving health outcomes and reducing poverty.

45: *Malar J.* 2008 Feb 15;7(1):32

Costs and effects of the Tanzanian national voucher scheme for insecticide-treated nets.

Mulligan JA, Yukich J, Hanson K.

ABSTRACT: BACKGROUND: The cost-effectiveness of insecticide-treated nets (ITNs) in reducing morbidity and mortality is well established. International focus has now moved on to how best to scale up coverage and what financing mechanisms might be used to achieve this. The approach in Tanzania has been to deliver a targeted subsidy for those most vulnerable to the effects of malaria while at the same time providing support to the development of the commercial ITN distribution system. In October 2004, with funds from the Global Fund to Fight AIDS Tuberculosis and Malaria, the government launched the Tanzania National Voucher Scheme (TNVS), a nationwide discounted voucher scheme for ITNs for pregnant women and their infants. This paper analyses the costs and effects of the scheme and compares it with other approaches to distribution. METHODS: Economic costs were estimated using the ingredients approach whereby all resources required in the delivery of the intervention (including the user contribution) are quantified and valued. Effects were measured in terms of number of vouchers used (and therefore nets delivered) and treated nets years. Estimates were also made for the cost per malaria case and death averted. Results and conclusions The total financial cost of the programme represents around 5% of the Ministry of Health's total budget. The average economic cost of delivering an ITN using the voucher scheme, including the user contribution, was \$7.57. The cost-effectiveness results are within the benchmarks set by other malaria prevention studies. The Government of Tanzania's approach to scaling up ITNs uses both the public and private sectors in order to achieve and sustain the level of coverage required to meet the Abuja targets. The results presented here suggest that the TNVS is a cost-effective strategy for delivering subsidized ITNs to targeted vulnerable groups.

Sulphadoxine/pyrimethamine versus amodiaquine for treating uncomplicated childhood malaria in Gabon: a randomized trial to guide national policy.

Nsimba B, Guiyedi V, Mabika-Mamfoumbi M, Mourou-Mbina JR, Ngoungou E, Bouyou-Akotet M, Loembet R, Durand R, Le Bras J, Kombila M.

ABSTRACT: **BACKGROUND:** In Gabon, further to the adoption of amodiaquine/artesunate combination (AQ/AS) and of sulphadoxine/pyrimethamine (SP) as malaria first-line treatment and preventive intermittent treatment in pregnant women, respectively, a clinical trial of SP versus AQ was conducted in sub-urban area. This is the first such study carried out in Gabon according to the WHO guideline. **METHODS:** Efficacy of AQ (10 mg/kg/day x 3d) randomly compared with the efficacy of a single dose of SP (25 mg/kg of sulphadoxine/ 1.25 mg/kg of pyrimethamine) were assessed in children under five years with uncomplicated falciparum malaria in sub-urban area according to the 28-day WHO therapeutic efficacy test. In addition, molecular genotyping was performed to distinguish recrudescence from reinfection and to determine the frequency of the dhpsK540E mutation as molecular marker predicting SP-treatment failure. **RESULTS:** The day-28 PCR-treatment failures adjusted for SP and AQ were 11.6% (8/69; 95% IC: 5.5-22.1) and 28.2% (20/71; 95% CI: 17.7-38.7) respectively, indicating that SP was significantly superior than AQ (P= 0.019) in the treatment of uncomplicated childhood malaria and for preventing recurrent infections. Both treatments were safe and well tolerated, with no serious adverse reaction recorded. The dhpsK540E mutation was not found among 76 parasite isolates. **CONCLUSION:** The AQ-resistance level observed in the present study may compromise efficacy and use duration of the AQ/AS combination, the new malaria first-line treatment. Gabonese policy-makers should plan through the country prompted and close surveillance of AQ/AS efficacy to determine whether those new recommendations related to the uncomplicated malaria treatment remain valid.

47: *Matern Child Health J.* 2008 Feb 15

Efficacy of Intermittent Preventive Treatment of Malaria with Sulphadoxine-pyrimethamine in Preventing Anaemia in Pregnancy among Nigerian Women.

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Objective To evaluate the efficacy of intermittent preventive treatment of malaria using sulphadoxine-pyrimethamine (SP) in the prevention of anaemia in women of low parity in a low socio-economic, malaria endemic setting. **Method** The study design was an open randomized control trial comparing anaemia incidence among pregnant women on intermittent presumptive treatment of malaria with SP with those on chloroquine (CQ). A total of 352 primigravid and secondigravid women between 16 and 30 weeks gestation receiving antenatal care at the Primary Health Care Center, Enuwa in Ile-Ife, Osun State, Nigeria were serially recruited and randomly allocated into experimental and control groups of 176 each. The experimental group received SP (to a maximum of three doses depending on the gestational age at enrollment into the study) while the control group had treatment doses of CQ at recruitment and subsequently only if they had symptoms suggestive of malaria. The primary outcome measure was anaemia (haematocrit < 30) at 34 weeks of gestation. **Result** At recruitment and 34 weeks gestation, there was no statistically significant difference between the experimental and control group in terms of socio-demographic characteristics and past medical history. Thirty-three (22.6%) and 52 (37.1%) women in the study and control groups, respectively, had anaemia (protective efficacy 49.5%, p = 0.01). With

multivariate analysis, controlling for the possible confounding effects of education, parity, haemoglobin level at booking and malaria parasitaemia in peripheral blood, the difference in the incidence of anaemia in the two groups remained significant ($p = 0.01$; odds ratio = 0.5; 95% confidence interval = 0.29-0.85). Conclusion The IPT regime with sulphadoxine-pyrimethamine is an effective, practicable strategy to decrease risk of anaemia in women of low parity residing in areas endemic for malaria.

48: *Med Microbiol Immunol*. 2008 Mar;197(1):39-44.

Induction of experimental cerebral malaria is independent of TLR2/4/9.

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The contribution of the Toll-like receptor (TLR) cascade to the pathogenesis of cerebral malaria (CM) is controversially discussed. TLR2 and TLR9 were reported to be involved in the induction of CM in a study while recently TLR signaling was shown to be dispensable for the development of CM. Using *Plasmodium berghei* ANKA (PbA) infection of mice as a model of CM, we demonstrate here that the induction of CM is independent of TLR2, 4 and 9. Using triple TLR2/4/9-deficient mice, we exclude synergistic effects between the single TLRs that have been previously implicated with malaria pathology. In conclusion, this study shows that the activation of the innate immune response and the development of CM is not dependent on the engagement of TLR2/4/9.

49: *Mol Microbiol*. 2008 Mar;67(5):1081-93.

Dissecting the components of quinine accumulation in *Plasmodium falciparum*.

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Although quinine, the active ingredient of chinchona bark, has been used in the treatment of malaria for several centuries, there is little information regarding the interactions of this drug with the human malaria parasite *Plasmodium falciparum*. To better understand quinine's mode of action and the mechanism underpinning reduced responsiveness, we have investigated the factors that contribute to quinine accumulation by parasites that differ in their susceptibility to quinine. Interestingly, passive distribution, in accordance with the intracellular pH gradients, and intracellular binding could account for only a small fraction of the high amount of quinine accumulated by the parasites investigated. The results of trans-stimulation kinetics suggest that high accumulation of quinine is brought about by a carrier-mediated import system. This import system seems to be weakened in parasites with reduced quinine susceptibility. Other data show that polymorphisms within PfCRT are causatively linked with an increased verapamil-sensitive quinine efflux that, depending on the genetic background, resulted in reduced quinine accumulation. The polymorphisms within PfMDR1 investigated did not affect quinine accumulation. Our data are consistent with the model that several factors, including acidotropic trapping, binding to intracellular sites and carrier-mediated import and export transport systems, contribute to steady-state intracellular quinine accumulation.

50: *Nat Chem Biol.* 2008 Mar 2

Synthetic GPI array to study antitoxic malaria response.

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Parasite glycosylphosphatidylinositol (GPI) is an important toxin in malaria disease, and people living in malaria-endemic regions often produce high levels of anti-GPI antibodies. The natural anti-GPI antibody response needs to be understood to aid the design of an efficient carbohydrate-based antitoxin vaccine. We present a versatile approach based on a synthetic GPI glycan array to correlate anti-GPI antibody levels and protection from severe malaria.

51: *Nat Chem Biol.* 2008 Mar;4(3):203-13.

Identification of proteases that regulate erythrocyte rupture by the malaria parasite *Plasmodium falciparum*.

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Newly replicated *Plasmodium falciparum* parasites escape from host erythrocytes through a tightly regulated process that is mediated by multiple classes of proteolytic enzymes. However, the identification of specific proteases has been challenging. We describe here a forward chemical genetic screen using a highly focused library of more than 1,200 covalent serine and cysteine protease inhibitors to identify compounds that block host cell rupture by *P. falciparum*. Using hits from the library screen, we identified the subtilisin-family serine protease PfSU B1 and the cysteine protease dipeptidyl peptidase 3 (DPAP3) as primary regulators of this process. Inhibition of both DPAP3 and PfSUB1 caused a block in proteolytic processing of the serine repeat antigen (SERA) protein SERA5 that correlated with the observed block in rupture. Furthermore, DPAP3 inhibition reduced the levels of mature PfSUB1. These results suggest that two mechanistically distinct proteases function to regulate processing of downstream substrates required for efficient release of parasites from host red blood cells.

52: *Nucleic Acids Res.* 2008 Mar;36(4):1176-86.

A conserved U-rich RNA region implicated in regulation of translation in *Plasmodium* female gametocytes.

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Translational repression (TR) plays an important role in post-transcriptional regulation of gene expression and embryonic development in metazoans. TR also regulates the expression of a subset of the cytoplasmic mRNA population during development of fertilized female gametes of the unicellular malaria parasite, *Plasmodium* spp. which results in the formation of a polar and motile form, the ookinete. We report the conserved and sex-specific regulatory role of either the 3'- or 5'-UTR of a subset of translationally repressed mRNA species as shown by almost complete inhibition of expression of a GFP reporter protein in the female gametocyte. A U-rich, TR-associated element, identified previously in the 3'-UTR

of TR-associated transcripts, played an essential role in mediating TR and a similar region could be found in the 5'-UTR shown in this study to be active in TR. The silencing effect of this 5'-UTR was shown to be independent of its position relative to its ORF, as transposition to a location 3' of the ORF did not affect TR. These results demonstrate for the first time in a unicellular organism that the 5' or the 3'-UTR of TR-associated transcripts play an important and conserved role in mediating TR in female gametocytes.

53: *Parasitol Res.* 2008 Feb 26

Bimodal transmission of cerebral malaria and severe malarial anemia and reciprocal co-existence of sexual and asexual parasitemia in an area of seasonal malaria transmission.

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The parasite dynamics in severe malaria (SM) varies with malaria endemicity. This study was conducted in eastern Sudan, an area of seasonal and unstable malaria transmission. From the beginning of October to the end of December (malaria season) in the years 2000, 2001, and 2003, 99 patients with severe malarial anemia (SMA) and 54 patients with cerebral malaria (CM) were identified. There was marked variation in the incidence of SMA and CM (up to six folds) and in the CM/SMA incidence ratio, over 3 years. In the heavy season of 2003, CM peaked at the beginning of the season and declined within a month at a time that the SMA reached the peak. At diagnosis, the rate of gametocytemia had inclined from approximately 10% to 100% from the beginning to the end of the season. During follow-up, gametocytemia was more associated with SMA than with CM. Paradoxically, the late occurring SMA was associated with early gametocytemia (day 7) and the opposite was true in CM. In conclusion, within the season the transmission of CM and SMA was bimodal, the prevalence of the asexual and sexual parasitemia was reciprocal, and the peaks of transmission and gametocytemia were paradoxical.

54: *Pediatr Infect Dis J.* 2008 Feb 27

Inpatient Mortality in Children With Clinically Diagnosed Malaria As Compared With Microscopically Confirmed Malaria.

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BACKGROUND:: Inpatient treatment for malaria without microscopic confirmation of the diagnosis occurs commonly in sub-Saharan Africa. Differences in mortality in children who are tested by microscopy for *Plasmodium falciparum* infection as compared with those not tested are not well characterized. **METHODS::** A retrospective chart review was conducted of all children up to 15 years of age admitted to Mulago Hospital, Kampala, Uganda from January 2002 to July 2005, with a diagnosis of malaria and analyzed according to microscopy testing for *P. falciparum*. **RESULTS::** A total of 23,342 children were treated for malaria during the study period, 991 (4.2%) of whom died. Severe malarial anemia in 7827 (33.5%) and cerebral malaria in 1912 (8.2%) were the 2 common causes of malaria-related admissions. Children who did not receive microscopy testing had a higher case

fatality rate than those with a positive blood smear (7.5% versus 3.2%, $P < 0.001$). After adjustment for age, malaria complications, and comorbid conditions, children who did not have microscopy performed or had a negative blood smear had a higher risk of death than those with a positive blood smear [odds ratio (OR): 3.49, 95% confidence interval (CI): 2.88-4.22, $P < 0.001$; and OR: 1.59, 95% CI: 1.29-1.96, $P < 0.001$, respectively]. CONCLUSIONS: Diagnosis of malaria in the absence of microscopic confirmation is associated with significantly increased mortality in hospitalized Ugandan children. Inpatient diagnosis of malaria should be supported by microscopic or rapid diagnostic test confirmation.

55: *PLoS Negl Trop Dis.* 2008 Feb 6;2(1):e164.

Helminth Infection and Eosinophilia and the Risk of Plasmodium falciparum Malaria in 1- to 6-Year-Old Children in a Malaria Endemic Area.

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BACKGROUND: Helminth infection is common in malaria endemic areas, and an interaction between the two would be of considerable public health importance. Animal models suggest that helminth infections may increase susceptibility to malaria, but epidemiological data has been limited and contradictory. METHODOLOGY/PRINCIPAL FINDINGS: In a vaccine trial, we studied 387 one- to six-year-old children for the effect of helminth infections on febrile Plasmodium falciparum malaria episodes. Gastrointestinal helminth infection and eosinophilia were prevalent (25% and 50% respectively), but did not influence susceptibility to malaria. Hazard ratios were 1 for gastrointestinal helminth infection (95% CI 0.6-1.6) and 0.85 and 0.85 for mild and marked eosinophilia, respectively (95% CI 0.56-1.76 and 0.69-1.96). Incident rate ratios for multiple episodes were 0.83 for gastro-intestinal helminth infection (95% CI 0.5-1.33) and 0.86 and 0.98 for mild and marked eosinophilia (95% CI 0.5-1.4 and 0.6-1.5). CONCLUSIONS/SIGNIFICANCE: There was no evidence that infection with gastrointestinal helminths or urinary schistosomiasis increased susceptibility to Plasmodium falciparum malaria in this study. Larger studies including populations with a greater prevalence of helminth infection should be undertaken.

56: *PLoS ONE.* 2008 Feb 20;3(2):e1630.

Safety and efficacy of methylene blue combined with artesunate or amodiaquine for uncomplicated falciparum malaria: a randomized controlled trial from burkina faso.

Zoungrana A, Coulibaly B, Sié A, Walter-Sack I, Mockenhaupt FP, Kouyaté B, Schirmer RH, Klose C, Mansmann U, Meissner P, Müller O.

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BACKGROUND: Besides existing artemisinin-based combination therapies, alternative safe, effective and affordable drug combinations against falciparum malaria are needed. Methylene blue (MB) was the first synthetic antimalarial drug ever used, and recent studies have been promising with regard to its revival in malaria therapy. The objective of this study was to assess the safety and efficacy of two MB-based malaria combination therapies, MB-artesunate (AS) and MB-amodiaquine (AQ), compared to the local standard of care, AS-AQ, in Burkina Faso. METHODS AND FINDINGS: Open-label randomised controlled phase II study in 180 children aged 6-10 years with uncomplicated falciparum malaria in Nouna, north-western Burkina Faso. Follow-up was for 28 days and analysis by intention-to-treat. The treatment groups were similar in baseline characteristics and there was only one loss to follow-up. No drug-related serious adverse events and no deaths occurred.

MB-containing regimens were associated with mild vomiting and dysuria. No early treatment failures were observed. Parasite clearance time differed significantly among groups and was the shortest with MB-AS. By day 14, the rates of adequate clinical and parasitological response after PCR-based correction for recrudescence were 87% for MB-AS, 100% for MB-AQ ($p = 0.004$), and 100% for AS-AQ ($p = 0.003$). By day 28, the respective figure was lowest for MB-AS (62%), intermediate for the standard treatment AS-AQ (82%; $p = 0.015$), and highest for MB-AQ (95%; $p < 0.001$; $p = 0.03$). CONCLUSIONS: MB-AQ is a promising alternative drug combination against malaria in Africa. Moreover, MB has the potential to further accelerate the rapid parasite clearance of artemisinin-based combination therapies. More than a century after the antimalarial properties of MB had been described, its role in malaria control deserves closer attention. TRIAL REGISTRATION: ClinicalTrials.gov NCT00354380.

57: *PLoS ONE*. 2008 Feb 13;3(2):e1608.

Pregnancy Outcome and Placenta Pathology in Plasmodium berghei ANKA Infected Mice Reproduce the Pathogenesis of Severe Malaria in Pregnant Women.

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Pregnancy-associated malaria (PAM) is expressed in a range of clinical complications that include increased disease severity in pregnant women, decreased fetal viability, intra-uterine growth retardation, low birth weight and infant mortality. The physiopathology of malaria in pregnancy is difficult to scrutinize and attempts were made in the past to use animal models for pregnancy malaria studies. Here, we describe a comprehensive mouse experimental model that recapitulates many of the pathological and clinical features typical of human severe malaria in pregnancy. We used *P. berghei* ANKA-GFP infection during pregnancy to evoke a prominent inflammatory response in the placenta that entails CD11b mononuclear infiltration, up-regulation of MIP-1 alpha chemokine and is associated with marked reduction of placental vascular spaces. Placenta pathology was associated with decreased fetal viability, intra-uterine growth retardation, gross post-natal growth impairment and increased disease severity in pregnant females. Moreover, we provide evidence that CSA and HA, known to mediate *P. falciparum* adhesion to human placenta, are also involved in mouse placental malaria infection. We propose that reduction of maternal blood flow in the placenta is a key pathogenic factor in murine pregnancy malaria and we hypothesize that exacerbated innate inflammatory responses to Plasmodium infected red blood cells trigger severe placenta pathology. This experimental model provides an opportunity to identify cell and molecular components of severe PAM pathogenesis and to investigate the inflammatory response that leads to the observed fetal and placental blood circulation abnormalities.

58: *PLoS ONE*. 2008 Feb 13;3(2):e1578.

Sulfadoxine-pyrimethamine-based combinations for malaria: a randomised blinded trial to compare efficacy, safety and selection of resistance in Malawi.

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BACKGROUND: In Malawi, there has been a return of Plasmodium falciparum sensitivity to chloroquine (CQ) since sulfadoxine-pyrimethamine (SP) replaced CQ as first line treatment for uncomplicated malaria. When used for prophylaxis, Amodiaquine (AQ) was associated with agranulocytosis but is considered safe for

treatment and is increasingly being used in Africa. Here we compare the efficacy, safety and selection of resistance using SP or CQ+SP or artesunate (ART)+SP or AQ+SP for the treatment of uncomplicated falciparum malaria. **METHODOLOGY AND FINDINGS:** 455 children aged 1-5 years were recruited into a double-blinded randomised trial comparing SP to the three combination therapies. Using intention to treat analysis with missing outcomes treated as successes, and without adjustment to distinguish recrudescence from new infections, the day 28 adequate clinical and parasitological response (ACPR) rate for SP was 25%, inferior to each of the three combination therapies ($p < 0.001$). AQ+SP had an ACPR rate of 97%, higher than CQ+SP (81%) and ART+SP (70%), $p < 0.001$. Nineteen children developed a neutropenia of $\leq 0.5 \times 10^3$ cells/microl by day 14, more commonly after AQ+SP ($p = 0.03$). The mutation pfcr1 76T, associated with CQ resistance, was detected in none of the pre-treatment or post-treatment parasites. The prevalence of the pfmdr1 86Y mutation was higher after treatment with AQ+SP than after SP, $p = 0.002$. **CONCLUSIONS:** The combination AQ+SP was highly efficacious, despite the low efficacy of SP alone; however, we found evidence that AQ may exert selective pressure for resistance associated mutations many weeks after treatment. This study confirms the return of CQ sensitivity in Malawi and importantly, shows no evidence of the re-emergence of pfcr1 76T after treatment with CQ or AQ. Given the safety record of AQ when used as a prophylaxis, our observations of marked falls in neutrophil counts in the AQ+SP group requires further scrutiny. **TRIAL REGISTRATION:** Controlled-Trials.com ISRCTN22075368.

59: *PLoS ONE*. 2008 Feb 13;3(2):e1570.

Evidence-Based Annotation of the Malaria Parasite's Genome Using Comparative Expression Profiling.

Zhou Y, Ramachandran V, Kumar KA, Westenberger S, Refour P, Zhou B, Li F, Young JA, Chen K, Plouffe D, Henson K, Nussenzweig V, Carlton J, Vinetz JM, Duraisingh MT, Winzeler EA.

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A fundamental problem in systems biology and whole genome sequence analysis is how to infer functions for the many uncharacterized proteins that are identified, whether they are conserved across organisms of different phyla or are phylum-specific. This problem is especially acute in pathogens, such as malaria parasites, where genetic and biochemical investigations are likely to be more difficult. Here we perform comparative expression analysis on Plasmodium parasite life cycle data derived from P. falciparum blood, sporozoite, zygote and ookinete stages, and P. yoelii mosquito oocyst and salivary gland sporozoites, blood and liver stages and show that type II fatty acid biosynthesis genes are upregulated in liver and insect stages relative to asexual blood stages. We also show that some universally uncharacterized genes with orthologs in Plasmodium species, Saccharomyces cerevisiae and humans show coordinated transcription patterns in large collections of human and yeast expression data and that the function of the uncharacterized genes can sometimes be predicted based on the expression patterns across these diverse organisms. We also use a comprehensive and unbiased literature mining method to predict which uncharacterized parasite-specific genes are likely to have roles in processes such as gliding motility, host-cell interactions, sporozoite stage, or rhoptry function. These analyses, together with protein-protein interaction data, provide probabilistic models that predict the function of 926 uncharacterized malaria genes and also suggest that malaria parasites may provide a simple model system for the study of some human processes. These data also provide a foundation for further studies of transcriptional regulation in malaria parasites.

60: *PLoS ONE*. 2008 Feb 13;3(2):e1563.

Phase 1 Study of a Combination AMA1 Blood Stage Malaria Vaccine in Malian Children.

Dicko A, Sagara I, Ellis RD, Miura K, Guindo O, Kamate B, Sogoba M, Niambelé MB, Sissoko M, Baby M, Dolo A, Mullen GE, Fay MP, Pierce M, Diallo DA, Saul A, Miller LH, Doumbo OK.

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BACKGROUND: Apical Membrane Antigen-1 (AMA1) is one of the leading blood stage malaria vaccine candidates. AMA1-C1/Alhydrogel(R) consists of an equal mixture of recombinant AMA1 from FVO and 3D7 clones of *P. falciparum*, adsorbed onto Alhydrogel(R). A Phase 1 study in semi-immune adults in Mali showed that the vaccine was safe and immunogenic, with higher antibody responses in those who received the 80 microg dose. The aim of this study was to assess the safety and immunogenicity of this vaccine in young children in a malaria endemic area. **DESIGN:** This was a Phase 1 dose escalating study in 36 healthy children aged 2-3 years started in March 2006 in Donéguébougou, Mali. Eighteen children in the first cohort were randomized 2:1 to receive either 20 microg AMA1-C1/Alhydrogel(R) or Haemophilus influenzae type b Hiberix(R) vaccine. Two weeks later 18 children in the second cohort were randomized 2:1 to receive either 80 microg AMA1-C1/Alhydrogel(R) or Haemophilus influenzae type b Hiberix(R) vaccine. Vaccinations were administered on Days 0 and 28 and participants were examined on Days 1, 2, 3, 7, and 14 after vaccination and then about every two months. Results to Day 154 are reported in this manuscript. **RESULTS:** Of 36 volunteers enrolled, 33 received both vaccinations. There were 9 adverse events related to the vaccination in subjects who received AMA1-C1 vaccine and 7 in those who received Hiberix(R). All were mild to moderate. No vaccine-related serious or grade 3 adverse events were observed. There was no increase in adverse events with increasing dose of vaccine or number of immunizations. In subjects who received the test vaccine, antibodies to AMA1 increased on Day 14 and peaked at Day 42, with changes from baseline significantly different from subjects who received control vaccine. **CONCLUSION:** AMA-C1 vaccine is well tolerated and immunogenic in children in this endemic area although the antibody response was short lived. **TRIAL REGISTRATION:** Clinicaltrials.gov NCT00341250.

61: *PLoS Pathog*. 2008 Feb 15;4(2):e42

Structural Insight into Epitopes in the Pregnancy-Associated Malaria Protein VAR2CSA.

Andersen P, Nielsen MA, Resende M, Rask TS, Dahlbäck M, Theander T, Lund O, Salanti A.

Pregnancy-associated malaria is caused by *Plasmodium falciparum* malaria parasites binding specifically to chondroitin sulfate A in the placenta. This sequestration of parasites is a major cause of low birth weight in infants and anemia in the mothers. VAR2CSA, a polymorphic multi-domain protein of the PfEMP1 family, is the main parasite ligand for CSA binding, and identification of protective antibody epitopes is essential for VAR2CSA vaccine development. Attempts to determine the crystallographic structures of VAR2CSA or its domains have not been successful yet. In this study, we propose 3D models for each of the VAR2CSA DBL domains and we show that regions in the fold of VAR2CSA inter-domain 2 and a PfEMP1 CIDR domain seem to be homologous to the EBA-175 and Pkalpha-DBL fold. This suggests that ID2 could be a functional domain. We also identify regions of VAR2CSA present on the surface of native VAR2CSA by comparing reactivity of plasma

containing anti-VAR2CSA antibodies in peptide array experiments before and after incubation with native VAR2CSA. By this method we identify conserved VAR2CSA regions targeted by antibodies that react with the native molecule expressed on infected erythrocytes. By mapping the data onto the DBL models we present evidence suggesting that the S1+S2 DBL sub-domains are generally surface-exposed in most domains, whereas the S3 sub-domains are less exposed in native VAR2CSA. These results comprise an important step towards understanding the structure of VAR2CSA on the surface of CSA-binding infected erythrocytes.

62: *Proc Biol Sci.* 2008 Feb 20

CD4+T cells do not mediate within-host competition between genetically diverse malaria parasites.

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Ecological interactions between microparasite populations in the same host are an important source of selection on pathogen traits such as virulence and drug resistance. In the rodent malaria model *Plasmodium chabaudi* in laboratory mice, parasites that are more virulent can competitively suppress less virulent parasites in mixed infections. There is evidence that some of this suppression is due to immune-mediated apparent competition, where an immune response elicited by one parasite population suppresses the population density of another. This raises the question whether enhanced immunity following vaccination would intensify competitive interactions, thus strengthening selection for virulence in *Plasmodium* populations. Using the *P. chabaudi* model, we studied mixed infections of virulent and avirulent genotypes in CD4+T cell-depleted mice. Enhanced efficacy of CD4+T cell-dependent responses is the aim of several candidate malaria vaccines. We hypothesized that if immune-mediated interactions were involved in competition, removal of the CD4+T cells would alleviate competitive suppression of the avirulent parasite. Instead, we found no alleviation of competition in the acute phase, and significant enhancement of competitive suppression after parasite densities had peaked. Thus, the host immune response may actually be alleviating other forms of competition, such as that over red blood cells. Our results suggest that the CD4+-dependent immune response, and mechanisms that act to enhance it such as vaccination, may not have the undesirable affect of exacerbating within-host competition and hence the strength of this source of selection for virulence.

63: *Proc Natl Acad Sci U S A.* 2008 Feb 26;105(8):2940-5.

A test of the chromosomal theory of ecotypic speciation in *Anopheles gambiae*.

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The role of chromosomal inversions in speciation has long been of interest to evolutionists. Recent quantitative modeling has stimulated reconsideration of previous conceptual models for chromosomal speciation. *Anopheles gambiae*, the most important vector of human malaria, carries abundant chromosomal inversion polymorphism nonrandomly associated with ecotypes that mate assortatively. Here, we consider the potential role of paracentric inversions in promoting speciation in *A. gambiae* via "ecotypification," a term that refers to differentiation arising from local adaptation. In particular, we focus on the Bamako form, an ecotype characterized by low inversion polymorphism and fixation of an inversion, 2Rj, that is very rare or absent in all other forms of *A. gambiae*. The Bamako

form has a restricted distribution by the upper Niger River and its tributaries that is associated with a distinctive type of larval habitat, laterite rock pools, hypothesized to be its optimal breeding site. We first present computer simulations to investigate whether the population dynamics of *A. gambiae* are consistent with chromosomal speciation by ecotypification. The models are parameterized using field observations on the various forms of *A. gambiae* that exist in Mali, West Africa. We then report on the distribution of larvae of this species collected from rock pools and more characteristic breeding sites nearby. Both the simulations and field observations support the thesis that speciation by ecotypification is occurring, or has occurred, prompting consideration of Bamako as an independent species.

64: *Soc Sci Med.* 2008 Mar;66(5):1106-17.

"He will ask why the child gets sick so often": The gendered dynamics of intra-household bargaining over healthcare for children with fever in the Volta Region of Ghana.

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This paper explores the gendered dynamics of intra-household bargaining around treatment seeking for children with fever revealed through two qualitative research studies in the Volta Region of Ghana, and discusses the influence of different gender and health discourses on the likely policy implications drawn from such findings. Methods used included focus group discussions, in-depth and critical incidence interviews, and Participatory Learning and Action methods. We found that treatment seeking behaviour for children was influenced by norms of decision-making power and 'ownership' of children, access to and control over resources to pay for treatment, norms of responsibility for payment, marital status, household living arrangements, and the quality of relationships between mothers, fathers and elders. However, the implications of these findings may be interpreted from different perspectives. Most studies that have considered gender in relation to malaria have done so within a narrow biomedical approach to health that focuses only on the outcomes of gender relations in terms of the (non-)utilisation of allopathic healthcare. However, we argue that a 'gender transformatory' approach, which aims to promote women's empowerment, needs to include but go beyond this model, to consider broader potential outcomes of intra-household bargaining for women's and men's interests, including their livelihoods and 'bargaining positions'.

65: *Trans R Soc Trop Med Hyg.* 2008 Mar;102(3):259-62.

DEET microencapsulation: a slow-release formulation enhancing the residual efficacy of bed nets against malaria vectors.

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Textile materials treated with synthetic repellents have the potential to provide protection against insect disease vectors but lack the residual activity necessary to achieve a prolonged effect or to be cost-effective. DEET MC is a formulation of DEET (N,N diethyl-m-toluamide) in which the repellent is gradually released from a capsule that binds the repellent. An experiment carried out on DEET-treated mosquito netting showed that the formulation repels, inhibits blood-feeding and kills mosquitoes for a period of at least 6 months under laboratory conditions. Such formulations may have the potential for use on nets against pyrethroid-resistant mosquitoes or on clothing or bedding materials

distributed in disasters, emergencies or refugee camp situations.

66: *Trans R Soc Trop Med Hyg.* 2008 Mar;102(3):263-271.

Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan.

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Species composition, blood meal source, sporozoite infection rate, insecticide resistance and the *kdr* mutations were investigated in the *Anopheles gambiae* complex from 13 sentinel sites in central Sudan. Species identification revealed that 89.5% of 960 specimens were *A. arabiensis*. Of 310 indoor resting females, 88.1% were found to have fed on humans, while 10.6% had fed on bovines. The overall sporozoite infection rate from the five localities tested was 2.3%, ranging from 0 to 5.5%. Insecticide susceptibility bioassay results showed 100% mortality on bendiocarb, 54.6-94.2% on permethrin, 55.4-99.1% on DDT and 76.8-100% on malathion. The *kdr* analysis by PCR and sequencing revealed the presence of the Leu-Phe mutation in both permethrin and DDT bioassays. There was no significant difference in the frequency of *kdr* ($P>0.05$) between dead and surviving specimens. These findings have serious implications for the malaria control programmes in Gezira and Sennar states.

67: *Trans R Soc Trop Med Hyg.* 2008 Feb 28

Malaria in African schoolchildren: options for control.

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Intensified malaria control efforts among young African children may increase disease risks among older children who attend school and whose education may be impaired by malaria. However, there is currently no consensus as to the approach to malaria control in schools, with relevant intervention strategies varying according to patterns of malaria transmission. Life skills messages regarding prevention and accessing prompt treatment are important everywhere. Providing free bed nets to schoolchildren may bring individual and community benefits and should be widely promoted. New approaches to school-based chemoprevention and treatment may also be able to play an important role in school-based malaria control, although these require further investigation.

68: *Trans R Soc Trop Med Hyg.* 2008 Feb 22

Malaria transmission and rice cultivation in Lagdo, northern Cameroon.

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Cross-sectional entomological surveys were carried out during the 2006 dry and rainy seasons in Lagdo, Cameroon to measure the impact of rice cultivation on malaria transmission and to monitor vector susceptibility to insecticides. Adult anopheline mosquitoes were captured on human volunteers and by pyrethrum spray collections. A total of 4740 mosquitoes was collected during the study. *Anopheles arabiensis* was the major species and the main malaria vector in all study sites, followed by *A. funestus*. Malaria transmission was high in the non-irrigated zone of Mayo Mbocki, whereas in the irrigated area of Gounougou it was below detection level during the dry season and high during the rainy season. Insecticide susceptibility tests performed on *A. gambiae* s.l. populations detected resistance to lambda-cyhalothrin and to a lower extent to deltamethrin. All survivors were *A. arabiensis*. None of the surviving mosquitoes carried the kdr mutation, suggesting an alternative resistance mechanism.

69: *Trop Med Int Health*. 2008 Feb 19

Changing pattern of malaria in Bissau, Guinea Bissau.

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Objective To describe the epidemiology of malaria in Guinea-Bissau, in view of the fact that more funds are available now for malaria control in the country. Methods From May 2003 to May 2004, surveillance for malaria was conducted among children less than 5 years of age at three health centres covering the study area of the Bandim Health Project (BHP) and at the outpatient clinic of the national hospital in Bissau. Cross-sectional surveys were conducted in the community in different malaria seasons. Results Malaria was overdiagnosed in both health centres and hospital. Sixty-four per cent of the children who presented at a health centre were clinically diagnosed with malaria, but only 13% of outpatient children who tested for malaria had malaria parasitaemia. Only 44% (963/2193) of children admitted to hospital with a diagnosis of malaria had parasitaemia. The proportion of positive cases increased with age. Among hospitalized children with malaria parasitaemia, those less than 2 years old were more likely to have moderate anaemia (RR = 1.27; 95% CI: 1.02-1.56) (P = 0.03) or severe anaemia (RR = 1.67; 95% CI: 1.25-2.24) (P = 0.0005) than older children. The prevalence of malaria parasitaemia in the community was low (3%, 53/1926). Conclusion In Bissau, the prevalence of malaria parasitaemia in the community is now low and malaria is over-diagnosed in health facilities. Laboratory support will be essential to avoid unnecessary use of the artemisinin combination therapy which is now being introduced as first-line treatment in Bissau with support from the Global Fund.

70: *Trop Med Int Health*. 2008 Feb 19

End-user errors in applying two malaria rapid diagnostic tests in a remote area of Sudan.

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We assessed end-user practice for numerous procedural steps of two types of RDTs: Core Malaria Pftrade mark (the cassette format) and OptiMAL ITtrade mark (the dipstick format). Two types of errors occurred: generic errors common to both types of test and specific errors caused by the test design and manufacturer's instructions. End-user errors were more frequent with OptiMAL ITtrade mark than Core Pftrade mark tests. To improve malaria diagnosis with rapid tests, users require training and better manufacturer's instructions that take into account

local conditions.

71: *Vaccine*. 2008 Mar 4;26(10):1335-43.

Plasmodium berghei merozoite surface protein-9: Immunogenicity and protective efficacy using a homologous challenge model.

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Merozoite surface protein-9 (MSP-9) from *Plasmodium* is considered a promising vaccine candidate due to its location and possible role in erythrocyte invasion. We report the identification and characterization of *Plasmodium berghei* MSP-9 (PbMSP-9) and its properties as an immunogen using a recombinant PbMSP-9 fragment to immunize BALB/c mice. PbMSP-9 was found to harbor erythrocyte binding and serine protease activity. PbMSP-9 formulation in alum was highly immunogenic in BALB/c mice. To evaluate the protective efficacy, immunized mice were submitted to homologous challenge with *P. berghei* NK65 blood-stage parasites. Protection against the parasite challenge was observed in BALB/c mice immunized with the PbMSP-9 formulation. These results suggest for the first time that MSP-9 based immunogens may constitute part of an effective malaria vaccine.

72: *Vaccine*. 2008 Feb 26;26(9):1204-13.

Direct effect of Plasmodium vivax recombinant vaccine candidates AMA-1 and MSP-1(19) on the innate immune response.

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The recombinant apical membrane antigen 1 (AMA-1) and 19-kDa fragment of merozoite surface protein (MSP-1(19)) are the lead candidates for inclusion in a vaccine against blood stages of malaria due to encouraging protective studies in humans and animals. Despite the importance of an efficacious malaria vaccine, vaccine-related research has focused on identifying antigens that result in protective immunity rather than determining the nature of anti-malarial immune effector mechanisms. Moreover, emphasis has been placed on adaptive rather than innate immune responses. In this study, we investigated the effect of *Plasmodium vivax* vaccine candidates Pv-AMA-1 and Pv-MSP-1(19) on the immune response of malaria-naïve donors. Maturation of dendritic cells is altered by Pv-AMA-1 but not Pv-MSP-1(19), as observed by differential expression of cell surface markers. In addition, Pv-AMA-1 induced an increased production of MIP-1 α /CCL3 and decreased production of TARC/CCL17 levels in both dendritic cells (DCs) and peripheral blood mononuclear cells (PBMCs). Finally, a significant pro-inflammatory response was elicited by Pv-AMA-1-stimulated PBMCs. These results suggest that the recombinant vaccine candidate Pv-AMA-1 may play a direct role on innate immune response and might be involved in parasite destruction.