Malaria Bulletin: A Compendium of Current Literature

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The March 2009 issue contains citations and abstracts to 70+ recently published malaria studies. Author email addresses are included when available and the entries are arranged by journal title.

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Journal/Article Index, pages 1-3

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- Malaria morbidity and temperature variation in a low risk Kenyan district: a case of overdiagnosis?

Int J Parasitol. 2009 Mar

- TREP, a novel protein necessary for gliding motility of the malaria sporozoite.
- Genetic analysis of the cytoplasmic domain of the PfRh2b merozoite invasion protein of Plasmodium falciparum.

J Infect Dis. 2009 Mar

- In vivo selection of Plasmodium falciparum parasites carrying the chloroquine-susceptible pfcrt K76 allele after treatment with artemether-lumefantrine in Africa.
- Decreasing efficacy of antimalarial combination therapy in Uganda is explained by decreasing host immunity rather than increasing drug resistance.

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- Early results of integrated malaria control and implications for the management of fever in under-five children at a peripheral health facility: A case study of Chongwe rural health centre in Zambia.
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- Nonradioactive heteroduplex tracking assay for the detection of minority-variant chloroquine-resistant Plasmodium falciparum in Madagascar.
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- TLR9 polymorphisms in African populations: no association with severe malaria, but evidence of cis-variants acting on gene expression.
- FcgammaRIIa (CD32) polymorphism and anti-malarial IgG subclass pattern among Fulani and sympatric ethnic groups living in eastern Sudan.
- The susceptibility of Anopheles lesteri to infection with Korean strain of Plasmodium vivax.
- High sensitivity detection of Plasmodium species reveals positive correlations between infections of different species, shifts in age distribution and reduced local variation in Papua New Guinea.
- Genetic diversity of msp3a and msp1_b5 markers of Plasmodium vivax in French Guiana.
- Feasibility and acceptability of home-based management of malaria strategy adapted to Sudan's conditions using artemisinin-based combination therapy and rapid diagnostic test.
- Glycerol: An unexpected major metabolite of energy metabolism by the human malaria parasite.
- Extended high efficacy of the combination sulphadoxine-pyrimethamine with artesunate in children with uncomplicated falciparum malaria on the Benin coast, West Africa.
- Glatiramer acetate reduces the risk for experimental cerebral malaria: a pilot study.
- AFCo1, a meningococcal B-derived cochleate adjuvant, strongly enhances antibody and T-cell immunity against Plasmodium falciparum merozoite surface protein 4 and 5.
- Efficacy of chloroquine, amodiaquine and sulphadoxine-pyrimethamine for the treatment
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- Isolation and Identification of a Potent Antimalarial and Antibacterial Polyacetylene from Bidens pilosa.

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- Nationwide investigation of the pyrethroid susceptibility of mosquito larvae collected from used tires in Vietnam.

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- C5a enhances dysregulated inflammatory and angiogenic responses to malaria in vitro: potential
- Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria
- A possible mechanism for the suppression of Plasmodium berghei development in the mosquito
- Multi-step polynomial regression method to model and forecast malaria incidence.
- Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers
- High resistance of Plasmodium falciparum to sulphadoxine/pyrimethamine in northern Tanzania

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- Malaria primes the innate immune response due to interferon-{gamma} induced enhancement
- Glycophorin B is the erythrocyte receptor of Plasmodium falciparum erythrocyte-binding ligand, EBL-1.

- Multifunctionality and mechanism of ligand binding in a mosquito anti-inflammatory protein.
- Structural basis for the inhibition of the essential Plasmodium falciparum M1 neutral aminopeptidase.

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- Leucine-Rich Repeat Protein Complex Activates Mosquito Complement in Defense Against Plasmodium Parasites.

Trans R Soc Trop Med Hyg. 2009 Mar

- Insecticide resistance and its association with target-site mutations in natural populations of Anopheles
- Developing national treatment policy for falciparum malaria in Africa: Malawi experience.
- The role of mathematical modelling in malaria elimination and eradication
- Distribution of pyrethroid and DDT resistance and the L1014F kdr mutation in Anopheles gambiae s.l

Trop Med Int Health. 2009 Mar

- In-vivo efficacy of amodiaquine-artesunate in children with uncomplicated Plasmodium falciparum malaria in western Kenya.
- Cost-effectiveness of artesunate for the treatment of severe malaria.
- Performance of OptiMAL-IT compared to microscopy, for malaria detection in Burkina Faso.
- Cross-border malaria control for internally displaced persons: observational results from a pilot programme in eastern Burma/Myanmar.

Vaccine. 2009 Mar

- Comparison of immunogenicity of five MSP1-based malaria vaccine candidate antigens in rabbits.
- Process development for the production of an E. coli produced clinical grade recombinant malaria vaccine for Plasmodium vivax.

Abstracts

Acta Trop. 2009 Mar;109(3):245-6.

Automated detection of haemozoin-containing monocytes for the diagnosis of malaria in microscopically negative cases during pregnancy.

Hänscheid T, Längin M, Codices V, Luty AJ, Adegnika AA, Kremsner PG, Grobusch MP.

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Plasmodium falciparum sequesters in the placenta. Cell-Dyn automated flow cytometric haematology analysers have the capacity to detect haemozoin-containing circulating leukocytes during routine FBC analysis. In Lambaréné, Gabon, 685 FBCs of pregnant women were analysed, yielding 86.8% sensitivity and 78.5% specificity compared to microscopy. In a subset of 37 Cell-Dyn positive but microscopy negative samples, PCR detected five positive cases. This methodology may serve as an adjunct rapid diagnostic tool for malaria during pregnancy, even in microscopically negative cases.

Acta Trop. 2009 Mar;109(3):208-12.

Antibody specificities of children living in a malaria endemic area to inhibitory and blocking epitopes on MSP-1 19 of Plasmodium falciparum.

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Merozoite surface protein-1(19) (MSP-1(19)) specific antibodies which include processing inhibitory, blocking and neutral antibodies have been identified in individuals exposed to Plasmodium falciparum. Here we intend to look at the effect of single and multiple amino acid

substitutions of MSP-1(19) on the recognition by polyclonal antibodies from children living in Igbo-Ora, Nigeria. This would provide us with information on the possibility of eliciting mainly processing inhibitory antibodies with a recombinant MSP-1(19) vaccine. Blood was collected from children in the rainy season and binding of anti-MSP-1(19) antibodies to modified mutants of MSP-1(19) was analysed by ELISA. The MSP-1(19) mutant proteins with single substitutions at positions 22 (Leu-->Arg), 43 (Glu-->Leu) and 53 (Asn-->Arg) and the MSP-1(19) mutant protein with multiple substitutions at positions 27+31+34+43 (Glu-->Tyr, Leu-->Arg, Tyr-->Ser, Glu-->Leu); which had inhibitory epitopes; had the highest recognition. Children recognised both sets of mutants with different age groups having different recognition levels. The percentage of malaria positive individuals (32-80%) with antibodies that bound to the mutants MSP-1(19) containing epitopes that recognize only processing inhibitory and not blocking antibodies, were significantly different from those with antibodies that did not bind to these mutants (21-28%). The amino acid substitutions that abolished the binding of blocking antibodies without affecting the binding of inhibitory antibodies are of particular interest in the design of MSP-1(19) based malaria vaccines. Although these MSP-1(19) mutants have not been found in natural population, their recognition by polyclonal antibodies from humans naturally infected with malaria is very promising for the future use of MSP-1(19) mutants in the design of a malaria vaccine.

Acta Trop. 2009 Mar;109(3):194-8.

Phytochemical licochalcone A enhances antimalarial activity of artemisinin in vitro.

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Resistance to synthetic first-line antimalarial drugs is considered to be a major cause of increased malaria morbidity and mortality. Use of artemisinin-based combination therapies (ACTs) is being encouraged to reduce the malaria mortality in areas of falciparum resistance. Artemisinin is a natural product at times in short supply. With projected rise in demand of artemisinin there is an unmet need for alternate ACTs. Novel compounds that reduce dependence on artemisinin are required. In vitro cultures of Plasmodium falciparum provide a screen system for identifying and evaluating new drug combinations. Interactions of two phytochemicals, artemisinin and licochalcone A, has been studied against synchronized erythrocytic stages of chloroquine-sensitive 3D7 and chloroquine-resistant RKL 303 strains of P. falciparum. These two compounds in combination show synergistic antiplasmodial activity in vitro on these strains. Artemisinin but not licochalcone A interferes with hemozoin formation. Neither of the phytochemicals alone or in combination obstructs sorbitol-induced hemolysis.

Acta Trop. 2009 Mar;109(3):241-4.

Extensive heterozygosity in flanking microsatellites of Plasmodium falciparum Na+/H+ exchanger (pfnhe-1) gene among Indian isolates.

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Plasmodium falciparum Na(+)/H(+) exchanger-1 (pfnhe-1) gene has been proposed to be a possible marker for quinine resistance. Here, we describe the sequence analysis of the flanking microsatellites of the pfnhe-1 gene among 108 Indian P.falciparum isolates. Among the parasite population, a high degree of polymorphism was observed at all the 10 microsatellite loci within +/-40kb region of the pfnhe-1 gene where the number of alleles varied from 2 to 16 with a high expected heterozygosity ranging from 0.43 to 0.91 at these loci. Also, higher levels of heterozygosity have been observed in P.falciparum isolates collected from both low and high transmission and drug resistant areas. Furthermore, there was no association between QN resistance associated DNNND repeats in PFNHE-1 and the flanking microsatellite haplotypes. In

conclusion, the observed high level of microsatellite polymorphism and absence of selective sweep in the flanking +/-40kb region of the pfnhe-1 gene could be an indication that there is no strong selection pressure on this target gene.

Acta Trop. 2009 Mar;109(3):176-80.

Genetic diversity of transmission blocking vaccine candidate (Pvs25 and Pvs28) antigen in Plasmodium vivax clinical isolates from Iran.

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The leading candidates for a Transmission Blocking Vaccine (TBV) in Plasmodium vivax parasite are the ookinete surface protein 25 (Pvs25) and Pvs28, which their phase I clinical trial is ongoing. Therefore, we carried out survey of polymorphisms of the pvs25 and pvs28 genes in P. vivax populations that are circulating in the two malaria areas of contrasting endemicity in Iran, before field application of the TBV. To characterize the polymorphisms of pvs25 and pvs28 genes, 50 isolates were analyzed by sequencing method and their gene structure was compared with parasite populations from India, Bangladesh, Indonesia, Thailand, Mexico and Brazil. Three mutations were detected in pvs25 and pvs28 including Q87K, E97Q, I130T and M52L, T65K, T140S with two and four distinct haplotypes, in comparison with the Sal I sequence type, respectively. Both haplotypes of Pvs25 were found among northern and southern P. vivax isolates; however, only two and three of the Pvs28 variants were observed among the northern and southern isolates, respectively. In conclusion, the present results show the limited sequence polymorphism of the pvs25 and pvs28 genes among field P. vivax population in Iran. These results highly encourage with respect to applicability of Pvs25 and Pvs28-based vaccine against P. vivax infection in the region, where these parasites are prevalent, whether these occur in the temperate or tropical zones.

Am J Trop Med Hyg. 2009 Mar;80(3):492-8.

ADAMTS13 deficiency with elevated levels of ultra-large and active von Willebrand factor in P. falciparum and P. vivax malaria.

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A deficiency in ADAMTS13 (a von Willebrand factor [VWF] cleaving protease) is associated with accumulation of prothrombogenic unusually large VWF multimers (UL-VWF) in plasma. We studied VWF release and proteolysis in patients with symptomatic Plasmodium falciparum or P. vivax malaria on the Indonesian island Sumba. Malaria patients had significantly lower platelet counts and higher VWF concentrations and VWF activation factors than healthy hospital staff controls. The latter indicates that a higher amount of circulating VWF was in a conformation enabling spontaneous platelet binding. In addition, ADAMTS13 activity and antigen levels were reduced in both malaria groups, and this was associated with the appearance of UL-VWF. The mechanism behind this reduction and the role in malaria pathogenesis needs to be further elucidated. In malaria, endothelial cell activation with increased circulating amounts of active and ultra-large VWF, together with reduced VWF inactivation by ADAMTS13, may result in intravascular platelet aggregation, thrombocytopenia, and microvascular disease.

Am J Trop Med Hyg. 2009 Mar;80(3):487-91.

How much malaria occurs in urban Luanda, Angola? A health facility-based assessment.

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We conducted a health facility-based survey of patients with fever during malaria transmission season to determine the proportion with laboratory-confirmed malaria in Luanda, Angola. We enrolled 864 patients at 30 facilities; each underwent a blood film for malaria and a questionnaire. Only 3.6% had a positive blood film. When stratified by distance of the facility to city center (< 15 km and > or = 15 km), the proportions were 1.5% (9/615) and 8.8% (22/249), respectively (P < 0.0001). Of patients traveling outside Luanda in the preceding 3 months, 6.8% (6/88) had malaria, compared with 3.2% (26/776) not traveling (P = 0.13). Children < 5 years of age were less likely to have malaria (2.4%; 12/510) than children ages 5-14 (8.7%; 9/104) and adults (4.0%; 10/250) (P = 0.03). The prevalence of laboratory-confirmed malaria in febrile patients in Luanda is very low, but increases with distance from the urban center. Prevention and treatment should be focused in surrounding rural areas.

Am J Trop Med Hyg. 2009 Mar;80(3):479-86.

Genetic diversity of the malaria vaccine candidate Plasmodium falciparum merozoite surface protein-3 in a hypoendemic transmission environment.

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The N-terminal domain of Plasmodium falciparum merozoite surface protein-3 (PfMSP3) has been excluded from malaria vaccine development largely because of genetic diversity concerns. However, no study to date has followed N-terminal diversity over time. This study describes PfMSP3 variation in a hypoendemic longitudinal cohort in the Peruvian Amazon over the 2003-2006 transmission seasons. Polymerase chain reaction was used to amplify the N-terminal domain in 630 distinct P. falciparum infections, which were allele-typed by size and also screened for sequence variation using a new high-throughput technique, denaturing high performance liquid chromatography. PfMSP3 allele frequencies fluctuated significantly over the 4-year period, but sequence variation was very limited, with only 10 mutations being identified of 630 infections screened. The sequence of the PfMSP3 N-terminal domain is relatively stable over time in this setting, and further studies of its status as a vaccine candidate are therefore warranted.

Am J Trop Med Hyg. 2009 Mar;80(3):475-8.

Short report: Childhood coinfections with Plasmodium falciparum and Schistosoma mansoni result in lower percentages of activated T cells and T regulatory memory cells than schistosomiasis only.

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Flow cytometric analyses were performed to evaluate HLA-DR (+) activated T lymphocytes (Tact; CD3 (+)/CD4 (+)/CD25(medium)) and T regulatory cells (Treg; CD3 (+)/CD4(+)/CD25(high)) in the circulation of children 8-10 years of age living in an area endemic for both Plasmodium falciparum and Schistosoma mansoni in western Kenya. Those children with only S. mansoni had a higher mean percentage of HLA-DR (+) Tact than those who were co-infected with these two intravascular parasites. The proportion of circulating Treg was comparable in children with only schistosomiasis and both schistosomiasis and malaria. However, the mean level of memory Treg (Treg expressing CD45RO (+)) in those with dual infections was lower than in children with schistosomiasis alone. These imbalances in Tact and Treg memory subsets in children infected with both schistosomiasis and malaria may be related to the differential morbidity or course of infection attributed to coinfections with these parasites.

Am J Trop Med Hyg. 2009 Mar;80(3):470-4.

Performance of malaria rapid diagnostic tests as part of routine malaria case management in Kenya.

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Data on malaria rapid diagnostic test (RDT) performance under routine program conditions are limited. We assessed the attributes of RDTs performed by study and health facility (HF) staffs as part of routine malaria case management of patients > or = 5 years of age in Kenya. Expert microscopy was used as our gold standard. A total of 1,827 patients were enrolled; 191 (11.6%) were parasitemic by expert microscopy. Sensitivity and specificity of RDTs performed by study staff were 86.6% (95% confidence interval [CI]: 79.8-93.5%) and 95.4% (95% CI: 93.9-96.9%), respectively. Among tests performed by HF staff, RDTs were 91.7% (95% CI: 80.8-100.0%) sensitive and 96.7% (95% CI: 92.8-100.0%) specific, whereas microscopy was 52.5% (95% CI: 33.2-71.9%) sensitive and 77.0% (95% CI: 67.9-86.2%) specific. Our findings suggest that RDTs perform better than microscopy under routine conditions. Further efforts are needed to maintain this high RDT performance over time.

Am J Trop Med Hyg. 2009 Mar;80(3):460-9.

Community-based promotional campaign to improve uptake of intermittent preventive antimalarial treatment in pregnancy in Burkina Faso.

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Malaria preventive strategies in pregnancy were assessed in a health center randomized trial comparing intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) with and without community based promotional activities in rural Burkina Faso. The study involved 2,240 secundigravidae and secundigravidae and evaluated factors associated with antenatal clinic (ANC) attendance and uptake of IPTp-SP. With promotion, 64.2% completed > or = 3 ANC visits compared with 44.7% without (P = 0.05). Complete uptake of IPTp-SP was 71.8% with and 49.1% without promotion (P = 0.008). The IPTp-SP uptake was lowest in adolescents delivering during high malaria transmission with (29%) or without promotion (30%). Uptake of SP was higher during the low transmission season than in the high transmission season (adjusted odds

ratio = 2.17, 95% confidence interval = 1.59-3.03). Community sensitization increased ANC attendance and IPTp-SP uptake. Adolescents were the most difficult to reach, particularly during the high malaria transmission period. The impact of IPTp-SP will be limited unless this high risk group is protected.

Am J Trop Med Hyg. 2009 Mar;80(3):452-9.

Age-dependent acquisition of protective immunity to malaria in riverine populations of the Amazon Basin of Brazil.

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Five community-based cross-sectional surveys of malaria morbidity and associated risk factors in remote riverine populations in northwestern Brazil showed average parasite rates of 4.2% (thick-smear microscopy) and 14.4% (polymerase chain reaction [PCR]) in the overall population, with a spleen rate of 13.9% among children 2-9 years of age. Plasmodium vivax was 2.8 times more prevalent than P. falciparum, with rare instances of P. malariae and mixed-species infections confirmed by PCR; 9.6% of asymptomatic subjects had parasitemias detected by PCR. Low-grade parasitemia detected by PCR only was a risk factor for anemia, after controlling for age and other covariates. Although clinical and subclinical infections occurred in all age groups, the risk of infection and disease decreased significantly with increasing age, after adjustment for several covariates in multilevel logistic regression models. These findings suggest that the continuous exposure to hypo- or mesoendemic malaria may induce significant anti-parasite and anti-disease immunity in native Amazonians.

Am J Trop Med Hyg. 2009 Mar;80(3):326-35.

Review: Provider practice and user behavior interventions to improve prompt and effective treatment of malaria: do we know what works?

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Effective case management of uncomplicated malaria is a cornerstone of successful malaria control. With current calls for the global elimination of malaria, all strategies to control malaria need to reach the highest achievable level of effective implementation. A systematic literature review of all interventions to improve provider- and/or user-side behavior in the prompt and appropriate treatment of uncomplicated malaria (with appropriate evaluation design and Roll Back Malaria outcome indicators) found 23 studies for review. Only 16 studies targeted providers, nine in the public sector and seven in the private sector. Just four interventions were conducted at national scale. These data suggest that very little is known about what interventions work in improving prompt and effective treatment of malaria. In the context of scaling up effective malaria control and malaria elimination plans and in increasing access to artemisinin combination therapies (ACTs), increased research in this area is crucial.

Antimicrob Agents Chemother. 2009 Mar 23.

Atorvastatin is a Promising Partner for Antimalarial Drugs in Treatment of Plasmodium falciparum Malaria.

Parquet V, Briolant S, Torrentino-Madamet M, Henry M, Almeras L, Amalvict R, Baret E, Fusaï T, Rogier C, Pradines B.

Atorvastatin (AVA) is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. AVA exposure resulted in reduced in vitro growth of 22 P. falciparum strains with IC50s ranging from 2.5 microM to 10.8 microM. A significant positive correlation was found between the strains' responses to AVA and mefloquine (r = 0.553; P = 0.008). We found no correlation between the responses to AVA and chloroquine, quinine, monodesethylamodiaquine, lumefantrine, dihydroartemisinin, atovaquone or doxycycline. These data could suggest different mechanisms of drug uptake and/or mode of action for AVA from other antimalarial drugs. IC50 values for AVA were unrelated to mutations occurring in transport protein genes involved in quinoline antimalarial drug resistance, such as pfcrt, pfmdr1, pfmrp and pfnhe-1. Therefore, AVA can be ruled out as a substrate for these transport proteins (PfCRT, Pgh1 and PfMRP) and is not subject to pH modification induced by PfNHE-1. The absence of in vitro cross-resistance between AVA and chloroquine, quinine, mefloquine, monodesethylamodiaquine, lumefantrine, dihydroartemisinin, atovaquone or doxycycline argues that these antimalarial drugs could potentially be paired with AVA as a malaria treatment strategy. In conclusion, the present observations suggest that AVA is a good candidate for further studies on the use of statins in association known antimarial drugs.

Antimicrob Agents Chemother. 2009 Mar 2.

Dynamics of malaria drug resistance patterns in the Amazon basin region following changes in Peruvian national treatment policy for uncomplicated malaria.

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Monitoring changes in the frequency of drug resistant and sensitive genotypes can facilitate in vivo clinical trials to assess the efficacy of drugs before complete failure occurs. Peru changed its national treatment policy for uncomplicated malaria to artesunate (ART) plus mefloquine (MQ) combination therapy (ACT) in the Amazon basin in 2001. We genotyped isolates collected in 1999 and isolates collected in 2006-2007 for mutations in the genes Plasmodium falciparum dihydrofolate reductase (Pfdhfr) and dihydropteroate synthase (Pfdhps), multi-drug resistance gene-1 (Pfmdr-1), chloroquine resistance transporter (Pfcrt), and Ca(2+-)ATPase (PfATPase6); these have been shown to be involved in resistance to sulfadoxine-pyrimethamine (SP), MQ, chloroquine (CQ) and possibly ART, respectively. Microsatellite haplotypes around the Pfdhfr, Pfdhps, Pfcrt and Pfmdr-1 loci also were determined. There was a significant decline in the highly SP resistant Pfdhfr and Pfdhps genotypes from 1999 to 2006. Conversely, a CQ resistant Pfcrt genotype increased in frequency during the same period. Among five different Pfmdr-1 allelic forms noted in 1999, two genotypes increased in frequency while one genotype decreased by 2006. We also noted previously undescribed polymorphisms in the PfATPase6 gene as well as an increase in the frequency of a deletion mutant during this period. In addition, microsatellite analysis revealed that Pfdhfr, Pfdhps, and Pfcrt resistant genotypes each have evolved from a single founder haplotype while Pfmdr-1 genotypes have evolved from at least two independent

haplotypes. Importantly, this study demonstrates that the Peruvian triple mutant Pfdhps genotypes are very similar to those found in other parts of South America.

Antimicrob Agents Chemother. 2009 Mar;53(3):888-95.

Selection of Plasmodium falciparum multidrug resistance gene 1 alleles in asexual stages and gametocytes by artemether-lumefantrine in Nigerian children with uncomplicated falciparum malaria.

Happi CT, Gbotosho GO, Folarin OA, Sowunmi A, Hudson T, O'Neil M, Milhous W, Wirth DF, Oduola AM.

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We assessed Plasmodium falciparum mdr1 (Pfmdr1) gene polymorphisms and copy numbers as well as P. falciparum Ca(2+) ATPase (PfATPase6) gene polymorphisms in 90 Nigerian children presenting with uncomplicated falciparum malaria and enrolled in a study of the efficacy of artemether-lumefantrine (AL). The nested PCR-restriction fragment length polymorphism and the quantitative real-time PCR methodologies were used to determine the alleles of the Pfmdr1 and PfATPase6 genes and the Pfmdr1 copy number variation, respectively, in patients samples collected prior to treatment and at the reoccurrence of parasites during a 42-day follow-up. The Pfmdr1 haplotype 86N-184F-1246D was significantly associated ($P < 0.00001$) with treatment failures and was selected for among posttreatment samples obtained from patients with newly acquired or recrudescing infections ($P < 0.00001$; $chi(2) = 36.5$) and in gametocytes (log rank statistic = 5; $P = 0.0253$) after treatment with AL. All pre- and posttreatment samples as well as gametocytes harbored a single copy of the Pfmdr1 gene and the wild-type allele (L89) at codon 89 of the PfATPase6 gene. These findings suggest that polymorphisms in the Pfmdr1 gene are under AL selection pressure. Pfmdr1 polymorphisms may result in reduction in the therapeutic efficacy of this newly adopted combination treatment for uncomplicated falciparum malaria in Saharan countries of Africa.

Antimicrob Agents Chemother. 2009 Mar;53(3):1100-6.

In vitro and in vivo properties of ellagic acid in malaria treatment.

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Malaria is one of the most significant causes of infectious disease in the world. The search for new antimalarial chemotherapies has become increasingly urgent due to the parasites' resistance to current drugs. Ellagic acid is a polyphenol found in various plant products. In this study, antimalarial properties of ellagic acid were explored. The results obtained have shown high activity in vitro against all Plasmodium falciparum strains whatever their levels of chloroquine and mefloquine resistance (50% inhibitory concentrations ranging from 105 to 330 nM). Ellagic acid was also active in vivo against Plamodium vinckei petteri in suppressive, curative, and prophylactic murine tests, without any toxicity (50% effective dose by the intraperitoneal route inferior to 1 mg/kg/day). The study of the point of action of its antimalarial activity in the erythrocytic cycle of Plasmodium falciparum demonstrated that it occurred at the mature trophozoite and young schizont stages. Moreover, ellagic acid has been shown to potentiate the activity of current antimalarial drugs such as chloroquine, mefloquine, artesunate, and atovaquone. This study also proved the antioxidant activity of ellagic acid and, in contrast, the inhibitory effect of the antioxidant compound N-acetyl-l-cysteine on its antimalarial efficacy. The possible mechanisms of action of ellagic acid on P. falciparum are discussed in light of the results.

Ellagic acid has in vivo activity against plasmodia, but modification of the compound could lead to improved pharmacological properties, principally for the oral route.

BMC Public Health. 2009 Mar 23;9(1):85.

Pathways to malaria persistence in remote central Vietnam: a mixed-method study of health care and the community.

Morrow M, Nguyen QA, Caruana S, Biggs BA, Doan NH, Nong TT.

BACKGROUND: There is increasing interest in underlying socio-cultural, economic, environmental and health-system influences on the persistence of malaria. Vietnam is a Mekong regional 'success story' after dramatic declines in malaria incidence following introduction of a national control program providing free bed-nets, diagnosis and treatment. Malaria has largely retreated to pockets near international borders in central Vietnam, where it remains a burden particularly among impoverished ethnic minorities. In these areas commune and village health workers are lynchpins of the program. This study in the central province of Quang Tri aimed to contribute to more effective malaria control in Vietnam by documenting the non-biological pathways to malaria persistence in two districts. METHODS: Multiple and mixed (qualitative and quantitative) methods were used. The formative stage comprised community meetings, observation of bed-net use, and focus group discussions and semi-structured interviews with health managers, providers and community. Formative results were used to guide development of tools for the assessment stage, which included a provider quiz, structured surveys with 160 community members and 16 village health workers, and quality check of microscopy facilities and health records at district and commune levels. Descriptive statistics and chi-square analysis were used for quantitative data. RESULTS: The study's key findings were the inadequacy of bed-nets (only 45% of households were fully covered) and sub-optimal diagnosis and treatment at local levels. Bed-net insufficiencies were exacerbated by customary sleeping patterns and population mobility. While care at district level seemed good, about a third of patients reportedly self-discharged early and many were lost to follow-up. Commune and village data suggested that approximately half of febrile patients were treated presumptively, and 10 village health workers did not carry artesunate to treat the potentially deadly and common P. falciparum malaria. Some staff lacked diagnostic skills, time for duties, and quality microscopy equipment. A few gaps were found in community knowledge and reported behaviours. CONCLUSIONS: Malaria control cannot be achieved through community education alone in this region. Whilst appropriate awareness-raising is needed, it is most urgent to address weaknesses at systems level, including bed-net distribution, health provider staffing and skills, as well as equipment and supplies.

BMC Public Health. 2009 Feb 24;9:67.

A 10 year study of the cause of death in children under 15 years in Manhiça, Mozambique.

Sacarlal J, Nhacolo AQ, Sigaúque B, Nhalungo DA, Abacassamo F, Sacoor CN, Aide P, Machevo S, Nhampossa T, Macete EV, Bassat Q, David C, Bardají A, Letang E, Saúte F, Aponte JJ, Thompson R, Alonso PL.

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BACKGROUND: Approximately 46 million of the estimated 60 million deaths that occur in the world each year take place in developing countries. Further, this mortality is highest in Sub-Saharan Africa, although causes of mortality in this region are not well documented. The objective of this study is to describe the most frequent causes of mortality in children under 15 years of age in the demographic surveillance area of the Manhiça Health Research Centre, between 1997 and 2006, using the verbal autopsy tool. METHODS: Verbal autopsy interviews for

causes of death in children began in 1997. Each questionnaire was reviewed independently by three physicians with experience in tropical paediatrics, who assigned the cause of death according to the International Classification of Diseases (ICD-10). Each medical doctor attributed a minimum of one and a maximum of 2 causes. A final diagnosis is reached when at least two physicians agreed on the cause of death. RESULTS: From January 1997 to December 2006, 568,499 person-year at risk (pyrs) and 10,037 deaths were recorded in the Manhiça DSS. 3,730 deaths with 246,658 pyrs were recorded for children under 15 years of age. Verbal autopsy interviews were conducted on 3,002 (80.4%) of these deaths. 73.6% of deaths were attributed to communicable diseases, non-communicable diseases accounted for 9.5% of the defined causes of death, and injuries for 3.9% of causes of deaths. Malaria was the single largest cause, accounting for 21.8% of cases. Pneumonia with 9.8% was the second leading cause of death, followed by HIV/AIDS (8.3%) and diarrhoeal diseases with 8%. CONCLUSION: The results of this study stand out the big challenges that lie ahead in the fight against infectious diseases in the study area. The pattern of childhood mortality in Manhiça area is typical of developing countries where malaria, pneumonia and HIV/AIDS are important causes of death.

BMC Res Notes. 2009 Mar 6;2:36.

Macrophage migration inhibitory factor is associated with mortality in cerebral malaria patients in India.

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BACKGROUND: Macrophage migration inhibitory factor (MIF) is a multifunctional cytokine implicated in the pathogenesis of a number of human diseases including inflammatory neurological diseases. Its role in the pathogenesis of cerebral malaria is unknown. Cerebral malaria is a life-threatening complication of falciparum malaria with approximately 20%-30% of patients dying despite appropriate anti-malarial treatment. The reason for this cerebral malaria mortality is still unknown although host proinflammatory factors have been shown to be evidently important. The current study investigated the role of circulating MIF in the pathogenesis and outcomes of cerebral malaria. FINDINGS: Three categories of subjects contributed to this study: healthy controls subjects, mild malaria patients, and cerebral malaria patients. The cerebral malaria patients were further grouped into cerebral malaria survivors and cerebral malaria non-survivors. MIF levels in the peripheral blood plasma, obtained at the time of enrollment, were measured using standard ELISA methods. In logistic regression on cerebral malaria patients, log MIF levels were found to be significantly associated with fatal outcome (odds ratio 4.0; 95%CI 1.6, 9.8; p = 0.003). In multinomial logistic regression log MIF levels were found to be significantly associated with patient category (p = 0.004). CONCLUSION: This study suggests that elevated levels of MIF in the peripheral blood of cerebral malaria patients may be associated with fatal outcomes.

Cell Host Microbe. 2009 Mar 19;5(3):273-84.

Two mosquito LRR proteins function as complement control factors in the TEP1-mediated killing of Plasmodium.

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Plasmodium development within Anopheles mosquitoes is a vulnerable step in the parasite transmission cycle, and targeting this step represents a promising strategy for malaria control. The thioester-containing complement-like protein TEP1 and two leucine-rich repeat (LRR) proteins, LRIM1 and APL1, have been identified as major mosquito factors that regulate parasite loads. Here, we show that LRIM1 and APL1 are required for binding of TEP1 to parasites. RNAi silencing of the LRR-encoding genes results in deposition of TEP1 on Anopheles tissues, thereby depleting TEP1 from circulation in the hemolymph and impeding its binding to Plasmodium. LRIM1 and APL1 not only stabilize circulating TEP1, they also stabilize each other prior to their interaction with TEP1. Our results indicate that three major antiparasitic factors in mosquitoes jointly function as a complement-like system in parasite killing, and they reveal a role for LRR proteins as complement control factors.

Clin Vaccine Immunol. 2009 Mar;16(3):312-9.

Relationship between human immunodeficiency virus type 1 coinfection, anemia, and levels and function of antibodies to variant surface antigens in pregnancy-associated malaria.

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Human immunodeficiency virus type 1 (HIV-1) coinfection decreases antibodies to variant surface antigens implicated in pregnancy-associated malaria (VSA-PAM) caused by Plasmodium falciparum. The effect of HIV-1 on antibody functions that may protect mothers from pregnancy-associated malaria is unknown. Sera from multigravid pregnant women with malaria and HIV-1 coinfection (n=58) or malaria alone (n=29) and from HIV-1-infected (n=102) or -uninfected (n=54) multigravidae without malaria were analyzed for anti-VSA-PAM antibodies by flow cytometry, the ability to inhibit adhesion to chondroitin sulfate A, or to opsonize CS2-infected erythrocytes for phagocytosis by THP-1 cells. In women with malaria, anti-VSA-PAM levels correlated better with opsonic activity (r=0.60) than with adhesion-blocking activity (r=0.33). In univariate analysis, HIV-1 coinfection was associated with lower opsonic activity but not adhesion-blocking activity or anti-VSA-PAM levels. Malaria-infected women with anemia (hemoglobin levels of <11.0 g/dl) had lower opsonic activity than nonanemic women (P=0.007) independent of HIV-1 status. By multivariate analysis, in malaria-infected women, anemia (but not HIV status) was associated with opsonic activity. In women without malaria, opsonic activity was not associated with either anemia or HIV-1 status. In multigravid pregnant women with malaria, impaired serum opsonic activity may contribute to anemia and possibly to the decreased immunity to pregnancy-associated malaria associated with HIV-1.

Clin Vaccine Immunol. 2009 Mar;16(3):293-302.

Prediction of merozoite surface protein 1 and apical membrane antigen 1 vaccine efficacies against Plasmodium chabaudi malaria based on prechallenge antibody responses.

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For the development of blood-stage malaria vaccines, there is a clear need to establish in vitro measures of the antibody-mediated and the cell-mediated immune responses that correlate with

protection. In this study, we focused on establishing correlates of antibody-mediated immunity induced by immunization with apical membrane antigen 1 (AMA1) and merozoite surface protein 1(42) (MSP1(42)) subunit vaccines. To do so, we exploited the Plasmodium chabaudi rodent model, with which we can immunize animals with both protective and nonprotective vaccine formulations and allow the parasitemia in the challenged animals to peak. Vaccine formulations were varied with regard to the antigen dose, the antigen conformation, and the adjuvant used. Prechallenge antibody responses were evaluated by enzyme-linked immunosorbent assay and were tested for a correlation with protection against nonlethal P. chabaudi malaria, as measured by a reduction in the peak level of parasitemia. The analysis showed that neither the isotype profile nor the avidity of vaccine-induced antibodies correlated with protective efficacy. However, high titers of antibodies directed against conformation-independent epitopes were associated with poor vaccine performance and may limit the effectiveness of protective antibodies that recognize conformation-dependent epitopes. We were able to predict the efficacies of the P. chabaudi AMA1 (PcAMA1) and P. chabaudi MSP1(42) (PcMSP1(42)) vaccines only when the prechallenge antibody titers to both refolded and reduced/alkylated antigens were considered in combination. The relative importance of these two measures of vaccine-induced responses as predictors of protection differed somewhat for the PcAMA1 and the PcMSP1(42) vaccines, a finding confirmed in our final immunization and challenge study. A similar approach to the evaluation of vaccine-induced antibody responses may be useful during clinical trials of Plasmodium falciparum AMA1 and MSP1(42) vaccines.

Genome Res. 2009 Mar;19(3):452-9.

Two duplicated P450 genes are associated with pyrethroid resistance in Anopheles funestus, a major malaria vector.

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Pyrethroid resistance in Anopheles funestus is a potential obstacle to malaria control in Africa. Tools are needed to detect resistance in field populations. We have been using a positional cloning approach to identify the major genes conferring pyrethroid resistance in this vector. A quantitative trait locus (QTL) named rp1 explains 87% of the genetic variance in pyrethroid susceptibility in two families from reciprocal crosses between susceptible and resistant strains. Two additional QTLs of minor effect, rp2 and rp3, were also detected. We sequenced a 120-kb BAC clone spanning the rp1 QTL and identified 14 protein-coding genes and one putative pseudogene. Ten of the 14 genes encoded cytochrome P450s, and expression analysis indicated that four of these P450s were differentially expressed between susceptible and resistant strains. Furthermore, two of these genes, CYP6P9 and CYP6P4, which are 25 and 51 times overexpressed in resistant females, are tandemly duplicated in the BAC clone as well as in laboratory and field samples, suggesting that P450 gene duplication could contribute to pyrethroid resistance in An. funestus. Single nucleotide polymorphisms (SNPs) were identified within CYP6P9 and CYP6P4, and genotyping of the progeny of the genetic crosses revealed a maximum penetrance value f(2) = 1, confirming that these SNPs are valid resistance markers in the laboratory strains. This serves as proof of principle that a DNA-based diagnostic test could be designed to trace metabolic resistance in field populations. This will be a major advance for insecticide resistance management in malaria vectors, which requires the early detection of resistance alleles.

Health Econ. 2009 Mar 19.

Concentration and drug prices in the retail market for malaria treatment in rural Tanzania.

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The impact of market concentration has been little studied in markets for ambulatory care in the developing world, where the retail sector often accounts for a high proportion of treatments. This study begins to address this gap through an analysis of the consumer market for malaria treatment in rural areas of three districts in Tanzania. We developed methods for investigating market definition, sales volumes and concentration, and used these to explore the relationship between antimalarial retail prices and competition.The market was strongly geographically segmented and highly concentrated in terms of antimalarial sales. Antimalarial prices were positively associated with market concentration. High antimalarial prices were likely to be an important factor in the low proportion of care-seekers obtaining appropriate treatment.Retail sector distribution of subsidised antimalarials has been proposed to increase the coverage of effective treatment, but this analysis indicates that local market power may prevent such subsidies from being passed on to rural customers. Policymakers should consider the potential to maintain lower retail prices by decreasing concentration among antimalarial providers and recommending retail price levels. Copyright (c) 2009 John Wiley & Sons, Ltd.

Int J Biometeorol. 2009 Mar 5.

Malaria morbidity and temperature variation in a low risk Kenyan district: a case of overdiagnosis?

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Diagnosis of malaria using only clinical means leads to overdiagnosis. This has implications due to safety concerns and the recent introduction of more expensive drugs. Temperature is a major climatic factor influencing the transmission dynamics of malaria. This study looked at trends in malaria morbidity in the low risk Kenyan district of Nyandarua, coupled with data on temperature and precipitation for the years 2003-2006. July had the highest number of cases (12.2% of all cases) followed by August (10.2% of all cases). July and August also had the lowest mean maximum temperatures, 20.1 and 20.2 degrees C respectively. April, July and August had the highest rainfall, with daily means of 4.0, 4.3 and 4.9 mm, respectively. Observation showed that the coldest months experienced the highest number of cases of malaria. Despite the high rainfall, transmission of malaria tends to be limited by low temperatures due to the long duration required for sporogony, with fewer vectors surviving. These cold months also tend to have the highest number of cases of respiratory infections. There is a possibility that some of these were misdiagnosed as malaria based on the fact that only a small proportion of malaria cases were diagnosed using microscopy or rapid diagnostic tests. We conclude that overdiagnosis may be prevalent in this district and there may be a need to design an intervention to minimise it.

Int J Parasitol. 2009 Mar;39(4):489-96.

TREP, a novel protein necessary for gliding motility of the malaria sporozoite.

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The invasive stages of parasites of the protozoan phylum Apicomplexa have the capacity to traverse host tissues and invade host cells using a unique type of locomotion called gliding motility. Gliding motility is powered by a sub-membranous actin-myosin motor, and the force generated by the motor is transduced to the parasite surface by transmembrane proteins of the apicomplexan-specific thrombospondin-related anonymous protein (TRAP) family. These proteins possess short cytoplasmic tails that interact with the actin-myosin motor via the glycolytic enzyme aldolase. Gliding motility of the Plasmodium sporozoite, the stage of the malaria parasite that is transmitted by the mosquito to the mammalian host, depends on the TRAP protein. We describe a second protein, herein termed TREP, which also plays a role in the gliding motility of the Plasmodium sporozoite. TREP is a transmembrane protein that possesses a short cytoplasmic tail typical of members of the TRAP family of proteins, as well as a large extracellular region that contains a single thrombospondin type 1 repeat domain. TREP transcripts are expressed predominantly in oocyst stage sporozoites. Plasmodium berghei sporozoites harbouring a disrupted TREP gene have a highly diminished capacity to invade mosquito salivary glands and display a severe defect in gliding motility. We conclude that the gliding motility of the Plasmodium sporozoite in the mosquito depends on at least two proteins, TRAP and TREP.

Int J Parasitol. 2009 Mar;39(4):399-405.

Genetic analysis of the cytoplasmic domain of the PfRh2b merozoite invasion protein of Plasmodium falciparum.

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Apicomplexan parasites employ multiple adhesive ligands for recognition and entry into host cells. The Duffy binding-like (DBL) and the reticulocyte binding protein-like (RBL) families are central to the invasion of erythrocytes by the malaria parasite. These type-1 transmembrane proteins are composed of large ectodomains and small conserved cytoplasmic tail domains. The cytoplasmic tail domain of the micronemal DBL protein EBA-175 is required for a functional ligand-receptor interaction, but not for correct trafficking and localisation. Here we focus on the cytoplasmic tail domain of the rhoptry-localised Plasmodium falciparum RBL PfRh2b. We have identified a conserved sequence of six amino acids, enriched in acidic residues, in the cytoplasmic tail domains of RBL proteins from Plasmodium spp. Genetic analyses reveal that the entire cytoplasmic tail and the conserved motif within the cytoplasmic tail are indispensable for invasion P. falciparum. Site-directed mutagenesis of the conserved moiety reveals that changes in the order of the amino acids of the conserved moiety, but not the charge of the sequence, can be tolerated. Shuffling of the motif has no effect on either invasion phenotype or PfRh2b expression and trafficking. Although the PfRh2b gene can be readily disrupted, our results suggest that modification of the PfRh2b cytoplasmic tail results in strong dominant negative activity, highlighting important differences between the PfRh2b and EBA-175 invasion ligands.

J Infect Dis. 2009 Mar 1; 199(5):750-7.

In vivo selection of Plasmodium falciparum parasites carrying the chloroquine-susceptible pfcrt K76 allele after treatment with artemether-lumefantrine in Africa.

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BACKGROUND: Artemether-lumefantrine (AL) is a major and highly effective artemisinin-based combination therapy that is becoming increasingly important as a new first-line therapy against Plasmodium falciparum malaria. However, recrudescences occurring after AL treatment have been reported. Identification of drug-specific parasite determinants that contribute to treatment failures will provide important tools for the detection and surveillance of AL resistance.

METHODS: The findings from a 42-day follow-up efficacy trial in Tanzania that compared AL with sulfadoxine-pyrimethamine (SP) were analyzed to identify candidate markers for lumefantrine tolerance/resistance in the chloroquine resistance transporter gene (pfcrt) and multidrug resistance gene 1 (pfmdr1). The findings were corroborated in vitro with genetically modified isogenic P. falciparum parasite lines. RESULTS: Treatment with AL selected for the chloroquine-susceptible pfcrt K76 allele (P < .0001) and, to a lesser extent, the pfmdr1 N86 (P = .048) allele among recurrent infections. These genotypes were not selected during SP treatment. No pfmdr1 gene amplifications were observed. Isogenic pfcrt-modified parasite lines demonstrated a 2-fold increase in susceptibility to lumefantrine, which was directly attributable to the K76T mutation. CONCLUSIONS: Our findings suggest that the pfcrt K76T mutation is a drug-specific contributor to enhanced P. falciparum susceptibility to lumefantrine in vivo and in vitro, and they highlight the benefit of using AL in areas affected by chloroquine-resistant P. falciparum malaria.

J Infect Dis. 2009 Mar 1;199(5):758-65.

Decreasing efficacy of antimalarial combination therapy in Uganda is explained by decreasing host immunity rather than increasing drug resistance.

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BACKGROUND: Improved control efforts are reducing the burden of malaria in Africa but may result in decreased antimalarial immunity. METHODS: A cohort of 129 children aged 1-10 years in Kampala, Uganda, were treated with amodiaquine plus sulfadoxine-pyrimethamine for 396 episodes of uncomplicated malaria over a 29-month period as part of a longitudinal clinical trial. RESULTS: The risk of treatment failure increased over the course of the study from 5% to 21% (hazard ratio [HR], 2.4 per year [95% confidence interval {CI}, 1.3-4.3]). Parasite genetic polymorphisms were associated with an increased risk of failure, but their prevalence did not change over time. Three markers of antimalarial immunity were associated with a decreased risk of treatment failure: increased age (HR, 0.5 per 5-year increase [95% CI, 0.2-1.2]), living in an area of higher malaria incidence (HR, 0.26 [95% CI, 0.11-0.64]), and recent asymptomatic parasitemia (HR, 0.06 [95% CI, 0.01-0.36]). In multivariate analysis, adjustment for recent asymptomatic parasitemia, but not parasite polymorphisms, removed the association between calendar time and the risk of treatment failure (HR, 1.5 per year [95% CI, 0.7-3.4]), suggesting that worsening treatment efficacy was best explained by decreasing host immunity. CONCLUSION: Declining immunity in our study population appeared to be the primary factor underlying decreased efficacy of amodiaquine plus sulfadoxine-pyrimethamine. With improved malaria-control efforts, decreasing immunity may unmask resistance to partially efficacious drugs.

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Malar J. 2009 Mar 17;8(1):49.

Early results of integrated malaria control and implications for the management of fever in under-five children at a peripheral health facility: A case study of Chongwe rural health centre in Zambia.

Chanda P, Hamainza B, Mulenga S, Chalwe V, Msiska C, Chizema-Kawesha E.

ABSTRACT: BACKGROUND: Zambia has taken lead in implementing integrated malaria control so as to attain the National Health Strategic Plan goal of "reducing malaria incidence by 75% and

under-five mortality due to malaria by 20% by the year 2010". The strategic interventions include the use of long-lasting insecticide-treated nets and indoor residual spraying, the use of artemisinin-based combination therapies (ACT) for the treatment of uncomplicated malaria, improving diagnostic capacity (both microscopy and rapid diagnostic tests), use of intermittent presumptive treatment for pregnant women, research, monitoring and evaluation, and behaviour change communication. Financial barriers to access have been removed by providing free malaria prevention and treatment services. METHODS: Data involving all under-five children reporting at the health facility in the first quarter of 2008 was evaluated prospectively. Malaria morbidity, causes of non-malaria fever, prescription, patterns treatment patterns and referral cases were evaluated RESULTS: Malaria infection was found only in 0.7% (10/1378), 1.8% (251378) received anti-malarial treatment, no severe malaria cases and deaths occurred among the under-five children with fever during the three months of the study in the high malaria transmission season. 42.5% (586/1378) of the cases were acute respiratory infections (non-pneumonia), while 5.7% (79/1378) were pneumonia. Amoxicillin was the most prescribed antibiotic followed by septrin. CONCLUSION: Malaria related OPD visits have reduced at Chongwe rural health facility. The reduction in health facility malaria cases has led to an increase in diagnoses of respiratory infections. These findings have implications for the management of non-malaria fevers in children under the age of five years.

Malar J. 2009 Mar 16;8(1):48.

The efficacy and safety of a new fixed-dose combination of amodiaquine and artesunate in young African children with acute uncomplicated Plasmodium falciparum.

Sirima SB, Tiono AB, Gansane A, Diarra A, Ouedraogo A, Konate AT, Kiechel JR, Morgan CC, Olliaro PL, Taylor WR.

BACKGROUND: Artesunate (AS) plus amodiaquine (AQ) is one artemisinin-based combination (ACT) recommended by the WHO for treating Plasmodium falciparum malaria. Fixed-dose AS/AQ is new, but its safety and efficacy are hitherto untested. METHODS: A randomized, open-label trial was conducted comparing the efficacy (non-inferiority design) and safety of fixed (F) dose AS (25 mg) / AQ (67.5 mg) to loose (L) AS (50 mg) + AQ (153 mg) in 750, P. falciparum-infected children from Burkina Faso aged 6 months to 5 years. Dosing was by age. Primary efficacy endpoint was Day (D) 28, PCR-corrected, parasitological cure rate. Recipients of rescue treatment were counted as failures and new infections as cured. Documented, common toxicity criteria (CTC) graded adverse events (AEs) defined safety. RESULTS: Recruited and evaluable children numbered 750 (375/arm) and 682 (90.9%), respectively. There were 8 (AS/AQ) and 6 (AS+AQ) early treatment failures and one D7 failure (AS+AQ). Sixteen (AS/AQ) and 12 (AS+AQ) patients had recurrent parasitaemia (PCR new infections 10 and 6, respectively). Fourteen patients per arm required rescue treatment for vomiting/spitting out study drugs. Efficacy rates were 92.1% in both arms: AS/AQ=315/342 (95% CI: 88.7-94.7) vs. AS+AQ=313/340 (95% CI: 88.6-94.7). Non-inferiority was demonstrated at two-sided a=0.05: [increment] (AS+AQ - AS/AQ) = 0.0% (95% CI: -4.1% to 4.0%). D28, Kaplan Meier PCR-corrected cure rates (all randomized children) were similar: 93.7% (AS/AQ) vs. 93.2% (AS+AQ) [increment] = -0.5 (95% CI -4.2 to 3.0%). By D2, both arms had rapid parasite (F & L, 97.8% aparasitaemic) and fever (97.2% [F], 96.0% [L] afebrile) clearances. Both treatments were well tolerated. Drug-induced vomiting numbered 8/375 (2.1%) and 6/375 (1.6%) in the fixed and loose arms, respectively (p=0.59). One patient developed asymptomatic, CTC grade 4 hepatitis (AST 1052, ALT 936). Technical difficulties precluded the assessment and risk of neutropaenia for all patients. CONCLUSIONS: Fixed dose AS/AQ was efficacious and well tolerated. These data support the use of this new fixed dose combination for treating P. falciparum malaria with continued safety monitoring. Trial registration Current Controlled Trials ISRCTN07576538.

Malar J. 2009 Mar 16;8(1):47.

Nonradioactive heteroduplex tracking assay for the detection of minority-variant chloroquine-resistant Plasmodium falciparum in Madagascar.

Juliano JJ, Randrianarivelojosia M, Ramarosandratana B, Ariey F, Mwapasa V, Meshnick SR.

BACKGROUND: Strains of Plasmodium falciparum genetically resistant to chloroquine (CQ) due to the presence of pfcrt 76T appear to have been recently introduced to the island of Madagascar. The prevalence of such resistant genotypes is reported to be low (< 3%) when evaluated by conventional PCR. However, these methods are insensitive to low levels of mutant parasites present in patients with polyclonal infections. Thus, the current estimates may be an under representation of the prevalence of the CQ-resistant P. falciparum isolates on the island. Previously, minority variant chloroquine resistant parasites were described in Malawian patients using an isotopic heteroduplex tracking assay (HTA), which can detect pfcrt 76T-bearing P. falciparum minority variants in individual patients that were undetectable by conventional PCR. However, as this assay required a radiolabeled probe, it could not be used in many resource-limited settings. METHODS: This study describes a digoxigenin (DIG)-labeled chemiluminescent heteroduplex tracking assay (DIG-HTA) to detect pfcrt 76T-bearing minority variant P. falciparum. This assay was compared to restriction fragment length polymorphism (RFLP) analysis and to the isotopic HTA for detection of genetically CQ-resistant parasites in clinical samples. RESULTS: Thirty one clinical P. falciparum isolates (15 primary isolates and 16 recurrent isolates) from 17 Malagasy children treated with CQ for uncomplicated malaria were genotyped for the pfcrt K76T mutation. Two (11.7%) of 17 patients harboured genetically CQ-resistant P. falciparum strains after therapy as detected by HTA. RFLP analysis failed to detect any pfcrt K76T-bearing isolates. CONCLUSIONS: These findings indicate that genetically CQ-resistant P. falciparum are more common than previously thought in Madagascar even though the fitness of the minority variant pfcrt 76T parasites remains unclear. In addition, HTAs for malaria drug resistance alleles are promising tools for the surveillance of anti-malarial resistance. The use of a non-radioactive label allows for the use of HTAs in malaria endemic countries.

Malar J. 2009 Mar 16;8(1):46.

Anopheles pseudowillmori is the predominant malaria vector in Motuo County, Tibet Autonomous Region.

Song W, Jia-Yun P, Xue-Zhong W, Shui-Sen Z, Guo-Qing Z, Qian L, Lin-Hua T.

BACKGROUND: Malaria is endemic in Linzhi Prefecture in the Tibet Autonomous Region (TAR), but the vector for malaria transmission had never been identified. METHODS: Adult Anopheles spp. were collected in Motuo County, Linzhi Prefecture on the Sino-Indian border in July and August, 2007. Multiplex PCR was adopted for species identification, and a nested PCR approach was used to detect sporozoites in the salivary glands of the mosquitoes. RESULTS: 3,675 mosquitoes of the Anopheles maculatus group were collected and processed for species identification. Among them, 3,602 (98.0%) were Anopheles pseudowillmori and 73 (2.0%) were Anopheles willmori. The Plasmodium vivax SSUrDNA fragment was amplified in two of 360 pooled An. pseudowillmori samples. CONCLUSION: The local An. maculatus group comprises the species An. pseudowillmori and An. willmori. Anopheles pseudowillmori is considered the sole malaria vector in Motuo County in Linzhi Prefecture.

Malar J. 2009 Mar 13;8(1):44.

TLR9 polymorphisms in African populations: no association with severe malaria, but evidence of cis-variants acting on gene expression.

Campino S, Forton J, Auburn S, Fry A, Diakite M, Richardson A, Hull J, Jallow M, Sisay-Joof F, Pinder M, Molyneux ME, Taylor TE, Rockett K, Clark TG, Kwiatkowski DP.

BACKGROUND: During malaria infection the Toll-like receptor 9 (TLR9) is activated through induction with plasmodium DNA or another malaria motif not yet identified. Although TLR9 activation by malaria parasites is well reported, the implication to the susceptibility to severe malaria is not clear. The aim of this study was to assess the contribution of genetic variation at TLR9 to severe malaria. METHODS: This study explores the contribution of TLR9 genetic variants to severe malaria using two approaches. First, an association study of four common single nucleotide polymorphisms was performed on both family- and population-based studies from Malawian and Gambian populations (n>6000 individual). Subsequently, it was assessed whether TLR9 expression is affected by cis-acting variants and if these variants could be mapped. For this work, an allele specific expression (ASE) assay on a panel of HapMap cell lines was carried out. RESULTS: No convincing association was found with polymorphisms in TLR9 for malaria severity, in either Gambian or Malawian populations, using both case-control and family based study designs. Using an allele specific expression assay it was observed that TLR9 expression is affected by cis-acting variants, these results were replicated in a second experiment using biological replicates. CONCLUSION: By using the largest cohorts analysed to date, as well as a standardized phenotype definition and study design, no association of TLR9 genetic variants with severe malaria was found. This analysis considered all common variants in the region, but it is remains possible that there are rare variants with association signals. This report also shows that TLR9 expression is potentially modulated through cis-regulatory variants, which may lead to differential inflammatory responses to infection between individuals.

Malar J. 2009 Mar 13;8(1):43.

FcgammaRIIa (CD32) polymorphism and anti-malarial IgG subclass pattern among Fulani and sympatric ethnic groups living in eastern Sudan.

Nasr A, Iriemenam NC, Giha HA, Balogun HA, Anders RF, Troye-Blomberg M, Elghazali G, Berzins K.

BACKGROUND: A SNP at position 131, in the FcgammaRIIa gene, affects the binding of the different IgG subclasses and may influence the clinical variation seen in patients with falciparum malaria. This study confirms and extends previous findings, analysing the FcgammaRIIa (CD32) polymorphism in relation to the IgG subclass distribution seen among two sympatric tribes living in eastern Sudan, characterized by marked differences in susceptibility to Plasmodium falciparum malaria. METHODS: Two hundred and fifty Fulani subjects living in an area of meso-endemic P. falciparum malaria infection were genotyped for the FcgammaRIIa-131 polymorphism. For comparison, 101 non-Fulani donors - (Masaleit, Hausa and Four) - living in the same study area, were genotyped. The levels of plasma antibodies (IgG and subclasses) to four malaria antigens (AMA-1, MSP 2 - 3D7 & FC27, Pf332-C231) were measured using indirect enzyme-linked immunosorbent assays. RESULTS: The FcgammaRIIa-H/H131 genotype was found to be significantly more prevalent in the Fulani as compared to the non-Fulani ethnic groups (36.0% for Fulani versus 17.8% for non-Fulani, adjusted OR 3.10, 95% CI 1.61- 5.97, P value < 0.001). The Fulani showed lower anti-malarial IgG1 and IgG3 antibody levels as compared to the non-Fulani and higher levels of IgG2 antibodies. CONCLUSION: The FcgammaRIIa-H/H131 genotype and H131 allele is at higher frequency in the Fulani ethnic group. The H/H131 genotype was consistently associated with higher levels of anti-malarial IgG2 and IgG3 antibodies, while the R/R131 genotype was associated with higher levels of IgG1 antibodies.

Malar J. 2009 Mar 12;8(1):42.

The susceptibility of Anopheles lesteri to infection with Korean strain of Plasmodium vivax.

Joshi D, Choochote W, Park MH, Kim JY, Kim TS, Suwonkerd W, Min GS.

BACKGROUND: Following its recent re-emergence, malaria has gained renewed attention as a serious infectious disease in Korea. Three species of the Hyrcanus group, Anopheles lesteri, Anopheles sinensis and Anopheles pullus, have long been suspected malaria vectors. However, opinions about their vector ability are controversial. The present study was designed with the aim of determining the susceptibility of these mosquitoes to a Korean isolate of Plasmodium vivax. Also, An. sinensis is primarily suspected to be vector of malaria in Korea, but in Thailand, the same species is described to have less medical importance. Therefore, comparative susceptibility of Thai and Korean strains of An. sinensis with Thai strain of P. vivax may be helpful to understand whether these geographically different strains exhibit differences in their susceptibility or not. METHODS: The comparative susceptibility of An. lesteri, An. sinensis and An. pullus was studied by feeding laboratory-reared mosquitoes on blood from patients carrying gametocytes from Korea and Thailand. RESULTS: In experimental feeding with Korean strain of P. vivax, oocysts developed in An. lesteri, An. Sinensis and An. pullus. Salivary gland sporozoites were detected only in An. lesteri and An. sinensis but not in An. pullus. Large differences were found in the number of sporozoites in the salivary glands, with An. lesteri carrying much higher densities, up to 2,105 sporozoites in a single microscope field of 750X560 uM, whereas a maximum of 14 sporozoites were found in any individual salivary gland of An. sinensis. Similar results were obtained from a susceptibility test of two different strains of An. sinensis to Thai isolate of P. vivax, and differences in vector susceptibility according to geographical variation were not detected. CONCLUSIONS: The high sporozoite rate and sporozoite loads of An. lesteri indicate that this species is highly susceptible to infection with P. vivax. Anopheles sinensis appears to have a markedly reduced ability to develop salivary gland infection, whilst in An. pullus, no sporozoites were found in the salivary glands. Provided that the survival rate of An. lesteri is sufficiently high in the field, it would be a highly competent vector of vivax malaria.

Malar J. 2009 Mar 11;8:41.

High sensitivity detection of Plasmodium species reveals positive correlations between infections of different species, shifts in age distribution and reduced local variation in Papua New Guinea.

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BACKGROUND: When diagnosed by standard light microscopy (LM), malaria prevalence can vary significantly between sites, even at local scale, and mixed species infections are consistently less common than expect in areas co-endemic for Plasmodium falciparum, Plasmodium vivax and Plasmodium malariae. The development of a high-throughput molecular species diagnostic assay now enables routine PCR-based surveillance of malaria infections in large field and intervention studies, and improves resolution of species distribution within and between communities. METHODS: This study reports differences in the prevalence of infections with all four human malarial species and of mixed infections as diagnosed by LM and post-PCR ligase detection reaction-fluorescent microsphere (LDR-FMA) assay in 15 villages in the central Sepik area of Papua New Guinea. RESULTS: Significantly higher rates of infection by P. falciparum, P. vivax, P. malariae and Plasmodium ovale were observed in LDR-FMA compared to LM diagnosis

($p < 0.001$). Increases were particularly pronounced for P. malariae (3.9% vs 13.4%) and P. ovale (0.0% vs 4.8%). In contrast to LM diagnosis, which suggested a significant deficit of mixed species infections, a significant excess of mixed infections over expectation was detected by LDR-FMA ($p < 0.001$). Age of peak prevalence shifted to older age groups in LDR-FMA diagnosed infections for P. falciparum (LM: 7-9 yrs 47.5%, LDR-FMA: 10-19 yrs 74.2%) and P. vivax (LM: 4-6 yrs 24.2%, LDR-FMA: 7-9 yrs 50.9%) but not P. malariae infections (10-19 yrs, LM: 7.7% LDR-FMA: 21.6%). Significant geographical variation in prevalence was found for all species (except for LM-diagnosed P. falciparum), with the extent of this variation greater in LDR-FMA than LM diagnosed infections (overall, 84.4% vs. 37.6%). Insecticide-treated bednet (ITN) coverage was also the dominant factor linked to geographical differences in Plasmodium species infection prevalence explaining between 60.6% - 74.5% of this variation for LDR-FMA and 81.8% - 90.0% for LM (except P. falciparum), respectively. CONCLUSION: The present study demonstrates that application of molecular diagnosis reveals patterns of malaria risk that are significantly different from those obtained by standard LM. Results provide insight relevant to design of malaria control and eradication strategies.

Malar J. 2009 Mar 11;8(1):40.

Genetic diversity of msp3a and msp1_b5 markers of Plasmodium vivax in French Guiana.

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BACKGROUND: Reliable molecular typing tools are required for a better understanding of the molecular epidemiology of Plasmodium vivax. The genes msp3a and msp1_block5 are highly polymorphic and have been used as markers in many P. vivax population studies. These markers were used to assess the genetic diversity of P. vivax strains from French Guiana (South America) and to develop a molecular typing protocol. METHODS: A total of 120 blood samples from 109 patients (including 10 patients suffered from more than one malaria episode, samples were collected during each episode) with P. vivax infection were genotyped. All samples were analysed by msp3a PCR-RFLP and msp1_b5 gene sequencing was performed on 57 samples. Genotyping protocol applied to distinguish between new infection or relapse from heterologus hypnozoites and treatment failure or relapse from homologus hypnozoites was based on analysing first msp3a by PCR-RFLP and secondly, only if the genotypes of the two samples are identical, on sequencing the msp1_b5 gene. RESULTS: msp3a alleles of three sizes were amplified by PCR: types A, B and C. Eleven different genotypes were identified among the 109 samples analysed by msp3a PCR-RFLP. In 13.8% of cases, a mixed genotype infection was observed. The sequence of msp1_b5 gene revealed 22 unique genotypes and 12.3% of cases with mixed infection. In the 57 samples analysed by both methods, 45 genotypes were found and 21% were mixed. Among ten patients with two or three malaria episodes, the protocol allowed to identify five new infections or relapses from heterologous hypnozoites and six treatment failures of relapses from homologous hypnozoites. CONCLUSION: The study showed a high diversity of msp3a and msp1_b5 genetic markers among P. vivax strains in French Guiana with a low polyclonal infection rate. These results indicated that the P. vivax genotyping protocol presented has a good discrimination power and can be used in clinical drug trials or epidemiological studies.

Malar J. 2009 Mar 9;8(1):39.

Feasibility and acceptability of home-based management of malaria strategy adapted to Sudan's conditions using artemisinin-based combination therapy and rapid diagnostic test.

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ABSTRACT: BACKGROUND: Malaria remains a major public health problem especially in sub-Saharan Africa. Despite the efforts exerted to provide effective anti-malarial drugs, still some

communities suffer from getting access to these services due to many barriers. This research aimed to assess the feasibility and acceptability of home-based management of malaria (HMM) strategy using artemisinin-based combination therapy (ACT) for treatment and rapid diagnostic test (RDT) for diagnosis. METHODS: This is a study conducted in 20 villages in Um Adara area, South Kordofan state, Sudan. Two-thirds (66%) of the study community were seeking treatment from heath facilities, which were more than 5km far from their villages with marked inaccessibility during rainy season. Volunteers (one per village) were trained on using RDTs for diagnosis and artesunate plus sulphadoxine-pyrimethamine for treating malaria patients, as well as referral of severe and non-malaria cases. A system for supply and monitoring was established based on the rural health centre, which acted as a link between the volunteers and the health system. Advocacy for the policy was done through different tools. Volunteers worked on non-monetary incentives but only a consultation fee of One Sudanese Pound (equivalent to US$0.5). Pre- and post-intervention assessment was done using household survey, focus group discussion with the community leaders, structured interview with the volunteers, and records and reports analysis. Results and Discussion The overall adherence of volunteers to the project protocol in treating and referring cases was accepted that was only one of the 20 volunteers did not comply with the study guidelines. Although the use of RDTs seemed to have improved the level of accuracy and trust in the diagnosis, 30% of volunteers did not rely on the negative RDT results when treating fever cases. Almost all (94.7%) the volunteers felt that they were satisfied with the spiritual outcome of their new tasks. As well, volunteers have initiated advocacy campaigns supported by their village health committees which were found to have a positive role to play in the project that proved their acceptability of the HMM design. The planned system for supply was found to be effective. The project was found to improve the accessibility to ACTs from 25% to 64.7% and the treatment seeking behaviour from 83.3% to 100% before- and after the HMM implementation respectivly. CONCLUSIONS: The evaluation of the project identified the feasibility of the planned model in Sudan's condition. Moreover, the communities as well as the volunteers found to be satisfied with and supportive to the system and the outcome. The problem of treating other febrile cases when diagnosis is not malaria and other non-fever cases needs to be addressed as well.

Malar J. 2009 Mar 6;8(1):38.

Glycerol: An unexpected major metabolite of energy metabolism by the human malaria parasite.

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BACKGROUND: Malaria is a global health emergency, and yet our understanding of the energy metabolism of the principle causative agent of this devastating disease, Plasmodium falciparum, remains rather basic. Glucose was shown to be an essential nutritional requirement nearly 100 years ago and since this original observation, much of the current knowledge of Plasmodium energy metabolism is based on early biochemical work, performed using basic analytical techniques (e.g. paper chromatography), carried out almost exclusively on avian and rodent malaria. Data derived from malaria parasite genome and transcriptome studies suggest that the energy metabolism of the parasite may be more complex than hitherto anticipated. This study was undertaken in order to further characterize the fate of glucose catabolism in the human malaria parasite, P. falciparum. METHODS: Products of glucose catabolism were determined by incubating erythrocyte-freed parasites with D-[1-13C] glucose under controlled conditions and metabolites were identified using 13C-NMR spectroscopy. RESULTS: Following a 2 h incubation of freed-P. falciparum parasites with 25 mM D-[1-13C] glucose (n = 4), the major metabolites identified included; [3-13C] lactate, [1,3-13C] glycerol, [3-13C] pyruvate, [3-13C] alanine and [3-13C] glycerol-3-phosphate. Control experiments performed with uninfected erythrocytes incubated under identical conditions did not show any metabolism of D-[1-13C] glucose to glycerol or glycerol-3-phosphate. DISCUSSION: The identification of glycerol as a major glucose metabolite confirms the view that energy metabolism in this parasite is more complex than previously proposed. It is hypothesized here that glycerol production by the malaria

parasite is the result of a metabolic adaptation to growth in O2-limited (and CO2 elevated) conditions by the operation of a glycerol-3-phosphate shuttle for the re-oxidation of assimilatory NADH. Similar metabolic adaptations have been reported previously for other microaerobic/anaerobic organisms, such as yeast, rumen protozoa and human parasitic protozoa. CONCLUSIONS: These data highlight the need to re-evaluate the carbon and redox balance of this important human pathogen, ultimately leading to a better understanding of how the parasite is able to adapt to the variable environments encountered during parasite development and disease progression.

Malar J. 2009 Mar 3;8:37.

Extended high efficacy of the combination sulphadoxine-pyrimethamine with artesunate in children with uncomplicated falciparum malaria on the Benin coast, West Africa.

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BACKGROUND: A study carried out in 2003-2005 in Southern Benin showed a day-28 sulphadoxine-pyrimethamine (SP) monotherapy failure rate greater than 40%, while for SP combined with artesunate (SP-AS) the failure rate was 5.3%. Such a large difference could be explained by the relatively short 28-day follow-up period, with a substantial number of recurrent infections possibly occurring after day 28. This paper reports the treatment outcome observed in the same study cohort beyond the initial 28-day follow-up. METHODS: After the 28-day follow-up, children treated with either chloroquine alone (CQ), SP or SP-AS, were visited at home twice a week until day 90 after treatment. A blood sample was collected if the child had fever (axillary temperature > or =37.5 degrees C). Total clinical failure for each treatment group was estimated by combining all the early treatment failures and late clinical failures that occurred over the whole follow-up period, i.e. from day 0 up to day 90. Pre-treatment randomly selected blood samples were genotyped for the dhfr gene (59) and the dhps gene (437 and 540) point mutations related to SP resistance. RESULTS: The PCR-corrected clinical failure at day 90 was significantly lower in the SP-AS group (SP-AS: 2.7%, SP alone: 38.2%; CQ: 41.1%) (Log-Rank p < 0,001). The most prevalent haplotype was dhfr Arg-59 with the dhps Gly-437 mutant and the dhps 540 wild type (85.5%). The dhps 540 mutation could be found in only three (8.3%) samples. CONCLUSION: Combining artesunate to SP dramatically increased the treatment efficacy, even when extending the follow-up to day 90 post-treatment, and despite the high percentage of failures following treatment with SP alone. Such a good performance may be explained by the low prevalence of the dhps 540 mutation, by the rapid parasite clearance with artesunate and by the level of acquired immunity.

Malar J. 2009 Feb 27;8:36.

Glatiramer acetate reduces the risk for experimental cerebral malaria: a pilot study.

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BACKGROUND: Cerebral malaria (CM) is associated with high mortality and morbidity caused by a high rate of transient or persistent neurological sequelae. Studies on immunomodulatory and neuroprotective drugs as ancillary treatment in murine CM indicate promising potential. The

current study was conducted to evaluate the efficacy of glatiramer acetate (GA), an immunomodulatory drug approved for the treatment of relapsing remitting multiple sclerosis, in preventing the death of C57Bl/6J mice infected with Plasmodium berghei ANKA. METHODS AND RESULTS: GA treatment led to a statistically significant lower risk for developing CM (57.7% versus 84.6%) in treated animals. The drug had no effect on the course of parasitaemia. The mechanism of action seems to be an immunomodulatory effect since lower IFN-gamma levels were observed in treated animals in the early course of the disease (day 4 post-infection) which also led to a lower number of brain sequestered leukocytes in treated animals. No direct neuro-protective effect such as an inhibition of apoptosis or reduction of micro-bleedings in the brain was found. CONCLUSION: These findings support the important role of the host immune response in the pathophysiology of murine CM and might lead to the development of new adjunctive treatment strategies.

Malar J. 2009 Feb 27;8(1):35.

AFCo1, a meningococcal B-derived cochleate adjuvant, strongly enhances antibody and T-cell immunity against Plasmodium falciparum merozoite surface protein 4 and 5.

Bracho G, Zayas C, Wang L, Coppel R, Perez O, Petrovsky N.

BACKGROUND: Whilst a large number of malaria antigens are being tested as candidate malaria vaccines, a major barrier to the development of an effective vaccine is the lack of a suitable human adjuvant capable of inducing a strong and long lasting immune response. In this study, the ability of AFCo1, a potent T and B cell adjuvant based on cochleate structures derived from meningococcal B outer membrane proteoliposomes (MBOMP), to boost the immune response against two Plasmodium falciparum antigens, merozoite surface protein 4 (MSP4) and 5 (MSP5), was evaluated. METHODS: Complete Freund's adjuvant (CFA), which is able to confer protection against malaria in animal MSP4/5 vaccine challenge models, was used as positive control adjuvant. MSP4 and 5-specific IgG, delayed-type hypersensitivity (DTH), T-cell proliferation, and cytokine production were evaluated in parallel in mice immunized three times intramuscularly with MSP4 or MSP5 incorporated into AFCo1, synthetic cochleate structures, CFA or phosphate buffered saline. RESULTS: AFCo1 significantly enhanced the IgG and T-cell response against MSP4 and MSP5, with a potency equivalent to CFA, with the response being characterized by both IgG1 and IgG2a isotypes, increased interferon gamma production and a strong DTH response, consistent with the ability of AFCo1 to induce Th1-like immune responses. CONCLUSIONS: Given the proven safety of MBOMP, which is already in use in a licensed human vaccine, AFCo1 could assist the development of human malaria vaccines that require a potent and safe adjuvant.

Malar J. 2009 Feb 26;8:34.

Efficacy of chloroquine, amodiaquine and sulphadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria: revisiting molecular markers in an area of emerging AQ and SP resistance in Mali.

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BACKGROUND: To update the National Malaria Control Programme of Mali on the efficacy of chloroquine, amodiaquine and sulphadoxine-pyrimethamine in the treatment of uncomplicated falciparum malaria. METHODS: During the malaria transmission seasons of 2002 and 2003, 455 children--between six and 59 months of age, with uncomplicated malaria in Kolle, Mali, were randomly assigned to one of three treatment arms. In vivo outcomes were assessed using WHO standard protocols. Genotyping of msp1, msp2 and CA1 polymorphisms were used to distinguish

reinfection from recrudescent parasites (molecular correction). RESULTS: Day 28 adequate clinical and parasitological responses (ACPR) were 14.1%, 62.3% and 88.9% in 2002 and 18.2%, 60% and 85.2% in 2003 for chloroquine, amodiaquine and sulphadoxine-pyrimethamine, respectively. After molecular correction, ACPRs (cACPR) were 63.2%, 88.5% and 98.0% in 2002 and 75.5%, 85.2% and 96.6% in 2003 for CQ, AQ and SP, respectively. Amodiaquine was the most effective on fever. Amodiaquine therapy selected molecular markers for chloroquine resistance, while in the sulphadoxine-pyrimethamine arm the level of dhfr triple mutant and dhfr/dhps quadruple mutant increased from 31.5% and 3.8% in 2002 to 42.9% and 8.9% in 2003, respectively. No infection with dhps 540E was found. CONCLUSION: In this study, treatment with sulphadoxine-pyrimethamine emerged as the most efficacious on uncomplicated falciparum malaria followed by amodiaquine. The study demonstrated that sulphadoxine-pyrimethamine and amodiaquine were appropriate partner drugs that could be associated with artemisinin derivatives in an artemisinin-based combination therapy.

Malar J. 2009 Feb 25;8:33.

Physical and chemical stability of expired fixed dose combination artemether-lumefantrine in uncontrolled tropical conditions.

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BACKGROUND: New artemisinin combination therapies pose difficulties of implementation in developing and tropical settings because they have a short shelf-life (two years) relative to the medicines they replace. This limits the reliability and cost of treatment, and the acceptability of this treatment to health care workers. A multi-pronged investigation was made into the chemical and physical stability of fixed dose combination artemether-lumefantrine (FDC-ALU) stored under heterogeneous, uncontrolled African conditions, to probe if a shelf-life extension might be possible. METHODS: Seventy samples of expired FDC-ALU were collected from private pharmacies and malaria researchers in seven African countries. The samples were subjected to thin-layer chromatography (TLC), disintegration testing, and near infrared Raman spectrometry for ascertainment of active ingredients, tablet integrity, and chemical degradation of the tablet formulation including both active ingredients and excipients. RESULTS: Seventy samples of FDC-ALU were tested in July 2008, between one and 58 months post-expiry. 68 of 70 (97%) samples passed TLC, disintegration and Raman spectrometry testing, including eight samples that were post-expiry by 20 months or longer. A weak linear association (R2 = 0.33) was observed between the age of samples and their state of degradation relative to brand-identical samples on Raman spectrometry. Sixty-eight samples were retested in February 2009 using Raman spectrometry, between eight and 65 months post-expiry. 66 of 68 (97%) samples passed Raman spectrometry retesting. An unexpected observation about African drug logistics was made in three batches of FDC-ALU, which had been sold into the public sector at concessional pricing in accordance with a World Health Organization (WHO) agreement, and which were illegally diverted to the private sector where they were sold for profit. CONCLUSION: The data indicate that FDC-ALU is chemically and physically stable well beyond its stated shelf-life in uncontrolled, tropical conditions. While these data are not themselves sufficient, it is strongly suggested that a re-evaluation of the two-year shelf-life by drug regulatory authorities is warranted.

Planta Med. 2009 Mar 4.

Isolation and Identification of a Potent Antimalarial and Antibacterial Polyacetylene from Bidens pilosa.

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Diseases caused by malaria parasites and pathogenic bacteria were thought to be on the brink of eradication in the 1950-1960s, but they have once again become a serious threat to mankind as a result of the appearance of multidrug resistant strains. The spread of these multidrug resistant organisms has prompted a worldwide search for new classes of effective antimalarial and antibacterial drugs. Natural products have been recognized as highly important candidates for this purpose. Our attention has focused on the herbal plant BIDENS PILOSA, a weed common throughout the world, as one of the target plants in the search for new active compounds, owing to its empirical use in the treatment of infectious diseases and to pharmaco-chemical studies of its crude extract. We report the isolation of two new compounds of B. PILOSA, the linear polyacetylenic diol 1 and its glucoside 2 which have previously been isolated from different plants. Compound 1 exhibited highly potent antimalarial and antibacterial properties IN VITRO as well as potent antimalarial activity by way of intravenous injection IN VIVO, thereby representing a promising new class of drugs potentially effective in the treatment of malarial and bacterial diseases. We suspect that discovery of these compounds in B. PILOSA in appreciable quantity is because the Fijian tradition of using the fresh plant for extraction rather than the Asian tradition of using dried plants (1 is unstable in the dried state) was followed.

PLoS Negl Trop Dis. 2009;3(3):e391.

Nationwide investigation of the pyrethroid susceptibility of mosquito larvae collected from used tires in Vietnam.

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Pyrethroid resistance is envisioned to be a major problem for the vector control program since, at present, there are no suitable chemical substitutes for pyrethroids. Cross-resistance to knockdown agents, which are mainly used in mosquito coils and related products as spatial repellents, is the most serious concern. Since cross-resistance is a global phenomenon, we have started to monitor the distribution of mosquito resistance to pyrethroids. The first pilot study was carried out in Vietnam. We periodically drove along the national road from the north end to the Mekong Delta in Vietnam and collected mosquito larvae from used tires. Simplified susceptibility tests were performed using the fourth instar larvae of Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus. Compared with the other species, Ae. aegypti demonstrated the most prominent reduction in susceptibility. For Ae. aegypti, significant increases in the susceptibility indices with a decrease in the latitude of collection points were observed, indicating that the susceptibility of Ae. aegypti against d-allethrin was lower in the southern part, including mountainous areas, as compared to that in the northern part of Vietnam. There was a significant correlation between the susceptibility indices in Ae. aegypti and the sum of annual pyrethroid use for malaria control (1998-2002). This might explain that the use of pyrethroids as residual treatment inside houses and pyrethroid-impregnated bed nets for malaria control is attributable to low pyrethroid susceptibility in Ae. aegypti. Such insecticide treatment appeared to have been intensively administered in the interior and along the periphery of human habitation areas where, incidentally, the breeding and resting sites of Ae. aegypti are located. This might account for the strong selection pressure toward Ae. aegypti and not Ae. albopictus.

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C5a enhances dysregulated inflammatory and angiogenic responses to malaria in vitro: potential implications for placental malaria.

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BACKGROUND: Placental malaria (PM) is a leading cause of maternal and infant mortality. Although the accumulation of parasitized erythrocytes (PEs) and monocytes within the placenta is thought to contribute to the pathophysiology of PM, the molecular mechanisms underlying PM remain unclear. Based on the hypothesis that excessive complement activation may contribute to PM, in particular generation of the potent inflammatory peptide C5a, we investigated the role of C5a in the pathogenesis of PM in vitro and in vivo. METHODOLOGY AND PRINCIPAL FINDINGS: Using primary human monocytes, the interaction between C5a and malaria in vitro was assessed. CSA- and CD36-binding PEs induced activation of C5 in the presence of human serum. Plasmodium falciparum GPI (pfGPI) enhanced C5a receptor expression (CD88) on monocytes, and the co-incubation of monocytes with C5a and pfGPI resulted in the synergistic induction of cytokines (IL-6, TNF, IL-1beta, and IL-10), chemokines (IL-8, MCP-1, MIP1alpha, MIP1beta) and the anti-angiogenic factor sFlt-1 in a time and dose-dependent manner. This dysregulated response was abrogated by C5a receptor blockade. To assess the potential role of C5a in PM, C5a plasma levels were measured in malaria-exposed primigravid women in western Kenya. Compared to pregnant women without malaria, C5a levels were significantly elevated in women with PM. CONCLUSIONS AND SIGNIFICANCE: These results suggest that C5a may contribute to the pathogenesis of PM by inducing dysregulated inflammatory and angiogenic responses that impair placental function.

PLoS ONE. 2009;4(3):e4912. http://www.plosone.org/home.action - Free full-text articles are online

Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children.

Lovegrove FE, Tangpukdee N, Opoka RO, Lafferty EI, Rajwans N, Hawkes M, Krudsood S, Looareesuwan S, John CC, Liles WC, Kain KC.

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BACKGROUND: Limited tools exist to identify which individuals infected with Plasmodium falciparum are at risk of developing serious complications such as cerebral malaria (CM). The objective of this study was to assess serum biomarkers that differentiate between CM and non-CM, with the long-term goal of developing a clinically informative prognostic test for severe malaria. METHODOLOGY/PRINCIPAL FINDINGS: Based on the hypothesis that endothelial activation and blood-brain-barrier dysfunction contribute to CM pathogenesis, we examined the endothelial regulators, angiopoietin-1 (ANG-1) and angiopoietin-2 (ANG-2), in serum samples from P. falciparum-infected patients with uncomplicated malaria (UM) or CM, from two diverse populations--Thai adults and Ugandan children. Angiopoietin levels were compared to tumour necrosis factor (TNF). In both populations, ANG-1 levels were significantly decreased and ANG-2 levels were significantly increased in CM versus UM and healthy controls ($p<0.001$). TNF was significantly elevated in CM in the Thai adult population ($p<0.001$), but did not discriminate well between CM and UM in African children. Receiver operating characteristic curve analysis showed that ANG-1 and the ratio of ANG-2:ANG-1 accurately discriminated CM patients from UM in both populations. Applied as a diagnostic test, ANG-1 had a sensitivity and specificity of 100% for distinguishing CM from UM in Thai adults and 70% and 75%, respectively, for Ugandan children. Across both populations the likelihood ratio of CM given a positive test (ANG-1<15 ng/mL) was 4.1 (2.7-6.5) and the likelihood ratio of CM given a negative test was 0.29 (0.20-0.42). Moreover, low ANG-1 levels at presentation predicted subsequent mortality in children with CM ($p = 0.027$). CONCLUSIONS/SIGNIFICANCE: ANG-1 and the ANG-2/1 ratio are promising clinically

informative biomarkers for CM. Additional studies should address their utility as prognostic biomarkers and potential therapeutic targets in severe malaria.

PLoS ONE. 2009;4(3):e4676. http://www.plosone.org/home.action - Free full-text articles are online

A possible mechanism for the suppression of Plasmodium berghei development in the mosquito Anopheles gambiae by the microsporidian Vavraia culicis.

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BACKGROUND: Microsporidian parasites of mosquitoes offer a possible way of controlling malaria, as they impede the development of Plasmodium parasites within the mosquito. The mechanism involved in this interference process is unknown. METHODOLOGY: We evaluated the possibility that larval infection by a microsporidian primes the immune system of adult mosquitoes in a way that enables a more effective anti-Plasmodium response. To do so, we infected 2-day old larvae of the mosquito Anopheles gambiae with one of 4 isolates of the microsporidian Vavraia culicis and reared one group as an uninfected control. Within each treatment, we fed half the adult females on a mix of P. berghei ookinetes and blood and inoculated the other half with a negatively charged CM-25 Sephadex bead to evaluate the mosquitoes' melanisation response. CONCLUSIONS: The microsporidian-infected mosquitoes were less likely to harbour oocysts (58.5% vs. 81.8%), harboured fewer oocysts (8.9 oocysts vs. 20.7 oocysts) if the malaria parasite did develop and melanised the Sephadex bead to a greater degree (73% vs. 35%) than the controls. While the isolates differed in the number of oocysts and in the melanisation response, the stimulation of the immune response was not correlated with either measure of malaria development. Nevertheless, the consistent difference between microsporidian-infected and –uninfected mosquitoes--more effective melanisation and less successful infection by malaria--suggests that microsporidians impede the development of malaria by priming the mosquito's immune system.

PLoS ONE. 2009;4(3):e4726. http://www.plosone.org/home.action - Free full-text articles are online

Multi-step polynomial regression method to model and forecast malaria incidence.

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Malaria is one of the most severe problems faced by the world even today. Understanding the causative factors such as age, sex, social factors, environmental variability etc. as well as underlying transmission dynamics of the disease is important for epidemiological research on malaria and its eradication. Thus, development of suitable modeling approach and methodology, based on the available data on the incidence of the disease and other related factors is of utmost importance. In this study, we developed a simple non-linear regression methodology in modeling and forecasting malaria incidence in Chennai city, India, and predicted future disease incidence with high confidence level. We considered three types of data to develop the regression methodology: a longer time series data of Slide Positivity Rates (SPR) of malaria; a smaller time series data (deaths due to Plasmodium vivax) of one year; and spatial data (zonal distribution of P. vivax deaths) for the city along with the climatic factors, population and previous incidence of the disease. We performed variable selection by simple correlation study, identification of the initial relationship between variables through non-linear curve fitting and used multi-step methods for induction of variables in the non-linear regression analysis along with applied Gauss-Markov models, and ANOVA for testing the prediction, validity and constructing the confidence intervals. The results execute the applicability of our method for different types of data, the

autoregressive nature of forecasting, and show high prediction power for both SPR and P. vivax deaths, where the one-lag SPR values plays an influential role and proves useful for better prediction. Different climatic factors are identified as playing crucial role on shaping the disease curve. Further, disease incidence at zonal level and the effect of causative factors on different zonal clusters indicate the pattern of malaria prevalence in the city. The study also demonstrates that with excellent models of climatic forecasts readily available, using this method one can predict the disease incidence at long forecasting horizons, with high degree of efficiency and based on such technique a useful early warning system can be developed region wise or nation wise for disease prevention and control activities.

PLoS ONE. 2009;4(3):e4708. http://www.plosone.org/home.action - Free full-text articles are online

Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in Western Kenya.

Ogutu BR, Apollo OJ, McKinney D, Okoth W, Siangla J, Dubovsky F, Tucker K, Waitumbi JN, Diggs C, Wittes J, Malkin E, Leach A, Soisson LA, Milman JB, Otieno L, Holland CA, Polhemus M, Remich SA, Ockenhouse CF, Cohen J, Ballou WR, Martin SK, Angov E, Stewart VA, Lyon JA, Heppner DG, Withers MR; MSP-1 Malaria Vaccine Working Group.

Collaborators: Wasunna KM, Tornieporth N, Dubois MC, de Kock E, Tiono A, Eckels K, Miller RS, Robinson S, Khan F, Potter R, Pichyangkul S, Gettyacamin M, Fukuda M.

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OBJECTIVE: The antigen, falciparum malaria protein 1 (FMP1), represents the 42-kDa C-terminal fragment of merozoite surface protein-1 (MSP-1) of the 3D7 clone of P. falciparum. Formulated with AS02 (a proprietary Adjuvant System), it constitutes the FMP1/AS02 candidate malaria vaccine. We evaluated this vaccine's safety, immunogenicity, and efficacy in African children. METHODS: A randomised, double-blind, Phase IIb, comparator-controlled trial.The trial was conducted in 13 field stations of one mile radii within Kombewa Division, Nyanza Province, Western Kenya, an area of holoendemic transmission of P. falciparum. We enrolled 400 children aged 12-47 months in general good health.Children were randomised in a 1ratio1 fashion to receive either FMP1/AS02 (50 microg) or Rabipur(R) rabies vaccine. Vaccinations were administered on a 0, 1, and 2 month schedule. The primary study endpoint was time to first clinical episode of P. falciparum malaria (temperature >/=37.5 degrees C with asexual parasitaemia of >/=50,000 parasites/microL of blood) occurring between 14 days and six months after a third dose. Case detection was both active and passive. Safety and immunogenicity were evaluated for eight months after first immunisations; vaccine efficacy (VE) was measured over a six-month period following third vaccinations. RESULTS: 374 of 400 children received all three doses and completed six months of follow-up. FMP1/AS02 had a good safety profile and was well-tolerated but more reactogenic than the comparator. Geometric mean anti-MSP-1(42) antibody concentrations increased from1.3 microg/mL to 27.3 microg/mL in the FMP1/AS02 recipients, but were unchanged in controls. 97 children in the FMP1/AS02 group and 98 controls had a primary endpoint episode. Overall VE was 5.1% (95% CI: -26% to +28%; p-value = 0.7). CONCLUSIONS: FMP1/AS02 is not a promising candidate for further development as a monovalent malaria vaccine. Future MSP-1(42) vaccine development should focus on other formulations and antigen constructs. TRIAL REGISTRATION: ClinicalTrials.gov NCT00223990.

PLoS ONE. 2009;4(2):e4569. http://www.plosone.org/home.action - Free full-text articles are online

High resistance of Plasmodium falciparum to sulphadoxine/pyrimethamine in northern Tanzania and the emergence of dhps resistance mutation at Codon 581.

Gesase S, Gosling RD, Hashim R, Ord R, Naidoo I, Madebe R, Mosha JF, Joho A, Mandia V, Mrema H, Mapunda E, Savael Z, Lemnge M, Mosha FW, Greenwood B, Roper C, Chandramohan D.

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BACKGROUND: Sulphadoxine-pyrimethamine (SP) a widely used treatment for uncomplicated malaria and recommended for intermittent preventive treatment of malaria in pregnancy, is being investigated for intermittent preventive treatment of malaria in infants (IPTi). High levels of drug resistance to SP have been reported from north-eastern Tanzania associated with mutations in parasite genes. This study compared the in vivo efficacy of SP in symptomatic 6-59 month children with uncomplicated malaria and in asymptomatic 2-10 month old infants. METHODOLOGY AND PRINCIPAL FINDINGS: An open label single arm (SP) standard 28 day in vivo WHO antimalarial efficacy protocol was used in 6 to 59 months old symptomatic children and a modified protocol used in 2 to 10 months old asymptomatic infants. Enrolment was stopped early (87 in the symptomatic and 25 in the asymptomatic studies) due to the high failure rate. Molecular markers were examined for recrudescence, re-infection and markers of drug resistance and a review of literature of studies looking for the 581G dhps mutation was carried out. In symptomatic children PCR-corrected early treatment failure was 38.8% (95% CI 26.8-50.8) and total failures by day 28 were 82.2% (95% CI 72.5-92.0). There was no significant difference in treatment failures between asymptomatic and symptomatic children. 96% of samples carried parasites with mutations at codons 51, 59 and 108 in the dhfr gene and 63% carried a double mutation at codons 437 and 540. 55% carried a third mutation with the addition of a mutation at codon 581 in the dhps gene. This triple: triple haplotype maybe associated with earlier treatment failure. CONCLUSION: In northern Tanzania SP is a failed drug for treatment and its utility for prophylaxis is doubtful. The study found a new combination of parasite mutations that maybe associated with increased and earlier failure. TRIAL REGISTRATION: ClinicalTrials.gov NCT00361114.

Proc Natl Acad Sci U S A. 2009 Mar 18.

Malaria primes the innate immune response due to interferon-{gamma} induced enhancement of toll-like receptor expression and function.

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Malaria-induced sepsis is associated with an intense proinflammatory cytokinemia for which the underlying mechanisms are poorly understood. It has been demonstrated that experimental infection of humans with Plasmodium falciparum primes Toll-like receptor (TLR)-mediated proinflammatory responses. Nevertheless, the relevance of this phenomenon during natural infection and, more importantly, the mechanisms by which malaria mediates TLR hyperresponsiveness are unclear. Here we show that TLR responses are boosted in febrile patients during natural infection with P. falciparum. Microarray analyses demonstrated that an extraordinary percentage of the up-regulated genes, including genes involving TLR signaling, had sites for IFN-inducible transcription factors. To further define the mechanism involved in malaria-mediated "priming," we infected mice with Plasmodium chabaudi. The human data were

remarkably predictive of what we observed in the rodent malaria model. Malaria-induced priming of TLR responses correlated with increased expression of TLR mRNA in a TLR9-, MyD88-, and IFNgamma-dependent manner. Acutely infected WT mice were highly susceptible to LPS-induced lethality while TLR9(-/-), IL12(-/-) and to a greater extent, IFNgamma(-/-) mice were protected. Our data provide unprecedented evidence that TLR9 and MyD88 are essential to initiate IL12 and IFNgamma responses and favor host hyperresponsiveness to TLR agonists resulting in overproduction of proinflammatory cytokines and the sepsis-like symptoms of acute malaria.

Proc Natl Acad Sci U S A. 2009 Mar 11.

Glycophorin B is the erythrocyte receptor of Plasmodium falciparum erythrocyte-binding ligand, EBL-1.

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In the war against Plasmodium, humans have evolved to eliminate or modify proteins on the erythrocyte surface that serve as receptors for parasite invasion, such as the Duffy blood group, a receptor for Plasmodium vivax, and the Gerbich-negative modification of glycophorin C for Plasmodium falciparum. In turn, the parasite counters with expansion and diversification of ligand families. The high degree of polymorphism in glycophorin B found in malaria-endemic regions suggests that it also may be a receptor for Plasmodium, but, to date, none has been identified. We provide evidence from erythrocyte-binding that glycophorin B is a receptor for the P. falciparum protein EBL-1, a member of the Duffy-binding-like erythrocyte-binding protein (DBL-EBP) receptor family. The erythrocyte-binding domain, region 2 of EBL-1, expressed on CHO-K1 cells, bound glycophorin B(+) but not glycophorin B-null erythrocytes. In addition, glycophorin B(+) but not glycophorin B-null erythrocytes adsorbed native EBL-1 from the P. falciparum culture supernatants. Interestingly, the Efe pygmies of the Ituri forest in the Democratic Republic of the Congo have the highest gene frequency of glycophorin B-null in the world, raising the possibility that the DBL-EBP family may have expanded in response to the high frequency of glycophorin B-null in the population.

Proc Natl Acad Sci U S A. 2009 Mar 10;106(10):3728-33.

Multifunctionality and mechanism of ligand binding in a mosquito anti-inflammatory protein.

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The mosquito D7 salivary proteins are encoded by a multigene family related to the arthropod odorant-binding protein (OBP) superfamily. Forms having either one or two OBP domains are found in mosquito saliva. Four single-domain and one two-domain D7 proteins from Anopheles gambiae and Aedes aegypti (AeD7), respectively, were shown to bind biogenic amines with high affinity and with a stoichiometry of one ligand per protein molecule. Sequence comparisons indicated that only the C-terminal domain of AeD7 is homologous to the single-domain proteins from A. gambiae, suggesting that the N-terminal domain may bind a different class of ligands. Here, we describe the 3D structure of AeD7 and examine the ligand-binding characteristics of the N- and C-terminal domains. Isothermal titration calorimetry and ligand complex crystal structures show that the N-terminal domain binds cysteinyl leukotrienes (cysLTs) with high affinities (50-60 nM) whereas the C-terminal domain binds biogenic amines. The lipid chain of the cysLT binds in a

hydrophobic pocket of the N-terminal domain, whereas binding of norepinephrine leads to an ordering of the C-terminal portion of the C-terminal domain into an alpha-helix that, along with rotations of Arg-176 and Glu-268 side chains, acts to bury the bound ligand.

Proc Natl Acad Sci U S A. 2009 Feb 24;106(8):2537-42.

Structural basis for the inhibition of the essential Plasmodium falciparum M1 neutral aminopeptidase.

McGowan S, Porter CJ, Lowther J, Stack CM, Golding SJ, Skinner-Adams TS, Trenholme KR, Teuscher F, Donnelly SM, Grembecka J, Mucha A, Kafarski P, Degori R, Buckle AM, Gardiner DL, Whisstock JC, Dalton JP.

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Plasmodium falciparum parasites are responsible for the major global disease malaria, which results in >2 million deaths each year. With the rise of drug-resistant malarial parasites, novel drug targets and lead compounds are urgently required for the development of new therapeutic strategies. Here, we address this important problem by targeting the malarial neutral aminopeptidases that are involved in the terminal stages of hemoglobin digestion and essential for the provision of amino acids used for parasite growth and development within the erythrocyte. We characterize the structure and substrate specificity of one such aminopeptidase, PfA-M1, a validated drug target. The X-ray crystal structure of PfA-M1 alone and in complex with the generic inhibitor, bestatin, and a phosphinate dipeptide analogue with potent in vitro and in vivo antimalarial activity, hPheP[CH(2)]Phe, reveals features within the protease active site that are critical to its function as an aminopeptidase and can be exploited for drug development. These results set the groundwork for the development of antimalarial therapeutics that target the neutral aminopeptidases of the parasite.

Science. 2009 Mar 5.

Leucine-Rich Repeat Protein Complex Activates Mosquito Complement in Defense Against Plasmodium Parasites.

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Leucine-rich repeat-containing proteins are central to host defense in plants and animals. We show that in the mosquito Anopheles gambiae, two such proteins that antagonize malaria parasite infections, LRIM1 and APL1C, circulate in the hemolymph as a high-molecular-weight complex held together by disulfide bridges. The complex interacts with the complement C3-like protein, TEP1, promoting its cleavage or stabilization, and its subsequent localization on the surface of midgut-invading Plasmodium berghei parasites, targeting them for destruction. LRIM1 and APL1C are members of a protein family with orthologs in other disease vector mosquitoes and appear to be important effectors in innate mosquito defenses against human pathogens.

Trans R Soc Trop Med Hyg. 2009 Mar 18.

Insecticide resistance and its association with target-site mutations in natural populations of Anopheles gambiae from eastern Uganda.

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Insecticide resistance in Anopheles gambiae threatens the success of malaria vector control programmes in sub-Saharan Africa. In order to manage insecticide resistance successfully, it is essential to assess continuously the target mosquito population. Here, we collected baseline information on the distribution and prevalence of insecticide resistance and its association with target-site mutations in eastern Uganda. Anopheles gambiae s.l. adults were raised from wild-caught larvae sampled from two ecologically distinct breeding sites and exposed to WHO discriminating concentrations of DDT, permethrin, deltamethrin, bendiocarb and malathion. Survival rates to DDT were as high as 85.4%, alongside significant resistance levels to permethrin (38.5%), reduced susceptibility to deltamethrin, but full susceptibility to bendiocarb and malathion. Using molecular diagnostics, susceptible and resistant specimens were further tested for the presence of knockdown resistance (kdr) and acetylcholinesterase 1 resistance (ace-1(R)) alleles. While ace-1(R) and kdrL1014F ('kdr west') alleles were absent, the kdr L1014S ('kdr east') allele was present in both populations. In A. gambiae s.s., L1014S was closely associated with DDT and, to a lesser degree, with permethrin resistance. Intriguingly, the association between DDT resistance and the presence of L1014S is consistent with a co-dominant effect, with heterozygous individuals showing an intermediate phenotype.

Trans R Soc Trop Med Hyg. 2009 Mar 12.

Developing national treatment policy for falciparum malaria in Africa: Malawi experience.

Malenga G, Wirima J, Kazembe P, Nyasulu Y, Mbvundula M, Nyirenda C, Sungani F, Campbell C, Molyneux M, Bronzan R, Dodoli W, Ali D, Kabuluzi S.

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The emergence and spread across sub-Saharan Africa of Plasmodium falciparum resistant to the inexpensive antimalarials chloroquine and sulfadoxine-pyrimethamine has worsened the health and hampered the socio-economic development of affected countries, a situation that calls for urgent review of malaria treatment policies in these countries. The Roll Back Malaria (RBM) initiative promotes strong partnerships for implementing effective malaria control measures. The development of clear policies to guide such implementation at country level offers a way of assessing the achievement of set milestones in this collaborative venture. In this article we describe the policy development process for the treatment of falciparum malaria in Africa, based on experience in Malawi, where the first-line drug treatment was recently changed from sulfadoxine-pyrimethamine to an artemisinin combination therapy.

Trans R Soc Trop Med Hyg. 2009 Feb 24.

Distribution of pyrethroid and DDT resistance and the L1014F kdr mutation in Anopheles gambiae s.l. from Burkina Faso (West Africa).

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This study reports on the distribution of pyrethroid and DDT resistance and the L1014F knockdown resistance (kdr) mutation in Anopheles gambiae s.l. populations from 21 localities in three different climatic zones of Burkina Faso from August to October 2006. The susceptibility of these populations was assessed by bioassay using DDT (4%), permethrin (1%) and deltamethrin (0.05%). Anophelesgambiae were resistant to both permethrin and DDT in the Sudanian regions but were susceptible in the central and sahelian areas and susceptible to deltamethrin at all sites except Orodara, although mortality values in some populations were close to the resistance threshold. The kdr frequency varied from 0.4 to 0.97 in populations from the Sudanian region and was lower in populations from the Sudano-sahelian and sahelian areas (0.047 to 0.54). Compared to the last survey of kdr in An. gambiae populations conducted in 2000, the kdr frequency did not differ in the S form but had increased in the M form (0.6), with an extended distribution into the Sudano-sahelian region. The frequency of kdr was also found to have increased in An. arabiensis populations (0.28), where it was formerly reported in only a single specimen. These results have practical significance for malaria vector control programs.

Trop Med Int Health. 2009 Mar;14(3):294-300.

In-vivo efficacy of amodiaquine-artesunate in children with uncomplicated Plasmodium falciparum malaria in western Kenya.

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OBJECTIVES: To assess the efficacy of amodiaquine-artesunate in an area with high chloroquine resistance in western Kenya. METHODS: Twenty-eight day in-vivo efficacy trial of amodiaquine-artesunate in 103 children aged 6-59 months in western Kenya with smear-confirmed uncomplicated Plasmodium falciparum malaria. RESULTS: The 28-day uncorrected adequate clinical and parasitological response (ACPR) was 69.0%, with 15.5% Late Clinical Failure and 15.5% Late Parasitologic Failure rates. The PCR-corrected 28-day ACPR was 90.2%. Clinical risk factors for recurrent infection (recrudescences and reinfections) were lower axillary temperature at enrollment and low weight-for-age Z-score. The presence of single nucleotide polymorphisms pfcrt 76T and pfmdr1 86Y at baseline was associated with increased risk of recurrent infections, both reinfections and recrudescences. CONCLUSION: Although artemether-lumefantrine (Coartem) is the first line ACT in Kenya, amodiaquine-artesunate is registered as an option for treatment of uncomplicated P. falciparum and remains an effective alternative to Coartem in western Kenya. Continued amodiaquine monotherapy in the private sector may jeopardize the future use of amodiaquine-artesunate as an alternative artemisinin-based combination therapy.

Trop Med Int Health. 2009 Mar;14(3):332-7.

Cost-effectiveness of artesunate for the treatment of severe malaria.

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OBJECTIVE: To explore the cost-effectiveness of artesunate against quinine based principally on the findings of a large multi-centre trial carried out in Southeast Asia. METHODS: Trial data were used to compare mortality of patients with severe malaria, treated with either artesunate or quinine. This was combined with retrospectively collected cost data to estimate the incremental cost per death averted with the use of artesunate instead of quinine. RESULTS: The incremental cost per death averted using artesunate was approximately 140 USD. Artesunate maintained this high level of cost-effectiveness also when allowing for the uncertainty surrounding the cost and effectiveness assessments. CONCLUSION: This analysis confirms the vast superiority of artesunate for treatment of severe malaria from an economic as well as a clinical perspective.

Trop Med Int Health. 2009 Mar;14(3):338-40.

Performance of OptiMAL-IT compared to microscopy, for malaria detection in Burkina Faso.

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OBJECTIVE: To compare the performance of OptiMAL-IT, a rapid diagnostic test for malaria, with that of microscopy in Burkina Faso. METHOD: Finger-prick blood samples of 464 children attending hospital for suspected malaria were tested for malaria by microscopy and OptiMAL-IT. RESULTS: The sensitivity and specificity of OptiMAL-IT were 98.7% (CI 95% = 97.6-99.8) and 96.2% (CI 95% = 94.3-98.1) respectively, with a high positive likelihood ratio (25.97). CONCLUSION: OptiMAL-IT can be considered a good method to diagnose malaria in Burkina Faso, particularly in remote areas with little or no access to microscopy services.

Trop Med Int Health. 2009 Feb 24.

Cross-border malaria control for internally displaced persons: observational results from a pilot programme in eastern Burma/Myanmar.

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Objectives To document the feasibility of a cross-border community based integrated malaria control programme implemented by internally displaced persons in eastern Burma/Myanmar. Methods This pilot study was conducted from February 2003 through January 2005 in seven villages of displaced ethnic Karen. Interventions comprised early diagnosis of Plasmodium falciparum and treatment with mefloquine and artesunate, distribution of long-lasting insecticide treated nets (LLITNs), and educational messages. The primary outcome measure was P. falciparum prevalence during bi-annual universal screenings with the Paracheck-Pf((R)) (Orchid Biomedical Systems, Goa, India) device. Secondary outcomes were P. falciparum incidence and

process indicators related to net use and malaria knowledge, attitudes and practices (KAP). Results P. falciparum prevalence in original programme areas declined from 8.4% [95% confidence interval (CI) 8.3-8.6] at baseline to 1.1% (95% CI 1.1-1.2) in the final screening. Annual incidence in original areas declined from 232 to 70 cases/1000/year [incidence rate ratio 0.30 (95% CI 0.24-0.39)]. The proportion of household members sleeping under a LLITN improved from 0% to 89% and malaria KAP improved in all areas. Conclusions Integrated malaria control organized and implemented by displaced persons is feasible in eastern Burma/Myanmar. The decline in P. falciparum prevalence and incidence suggest that it may be possible to reduce the burden of disease and the reservoir of malaria in eastern Burma/Myanmar, with implications for malaria control in the greater Mekong region.

Vaccine. 2009 Mar 4;27(10):1651-60.

Comparison of immunogenicity of five MSP1-based malaria vaccine candidate antigens in rabbits.

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A number of laboratories around the world are producing Plasmodium falciparum erythrocyte-stage vaccine candidates in the pursuit of a vaccine against clinical malaria disease. These candidates are often based on the same parasite protein. Rigorous clinical development and testing of multiple candidates is limited by available resources, which underscores the need to conduct comparative studies of the different vaccine candidates. The purpose of this study was to compare five different candidate proteins all based on P. falciparum merozoite surface protein-1 (MSP1). After investigators submitted their candidates, basic protein profiles were evaluated in a blinded fashion by an independent laboratory, and groups of rabbits were immunized with the proteins. Sera obtained from the rabbits were compared for antibody titers by ELISA and for functional activity by an in vitro parasite growth inhibition assay (GIA) activity, again in a blinded fashion. In selected cases the fine specificity of the antibodies was assessed. Significant differences in immunogenicity as well as the functional activity of antibodies induced by the various vaccine candidates were noted. Data from this study can assist in making decisions for further clinical development of MSP1-based candidates, and this process sets a precedent for future comparisons of malaria vaccine candidates.

Vaccine. 2009 Feb 25;27(9):1448-53.

Process development for the production of an E. coli produced clinical grade recombinant malaria vaccine for Plasmodium vivax.

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The global eradication of malaria will require the development of vaccines to prevent infection cause by Plasmodium vivax in addition to Plasmodium falciparum. In an attempt to contribute to this effort we have previously reported the cloning and expression of a vaccine based on the circumsporozoite protein of P. vivax. The synthetic vaccine encodes for a full-length molecule encompassing the N-terminal and C-terminal regions flanking a chimeric repeat region representing VK210 and VK247, the two major alleles of P. vivax CSP. The vaccine, designated vivax malaria protein 001 (VMP001), was purified to >95% homogeneity using a three-column purification scheme and had low endotoxin levels and passed the rabbit pyrogenicity assay. The protein is recognized by monoclonal antibodies directed against the two repeat motifs, as well as

polyclonal antibodies. Immunization with VMP001 induced high titer antibodies in mice using Montanide ISA 720. We currently have more than 10,000 doses of purified bulk and 1800 vials of formulated bulk vaccine available for clinical testing and VMP001 is currently undergoing further development as a candidate vaccine to prevent malaria in humans.