

Malaria Bulletin: A Compendium of Current Literature

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Abstracts

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Natural Plasmodium infections in Brazilian wild monkeys: Reservoirs for human infections?

de Castro Duarte AM, Malafronte Rdos S, Cerutti C Jr, Curado I, de Paiva BR, Maeda AY, Yamasaki T, Summa ME, Neves Ddo V, de Oliveira SG, Gomes Ade C.

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Four hundred and forty-eight samples of total blood from wild monkeys living in areas where human autochthonous malaria cases have been reported were screened for the presence of Plasmodium using microscopy and PCR analysis. Samples came from the following distinct ecological areas of Brazil: Atlantic forest (N=140), semideciduous Atlantic forest (N=257) and Cerrado (a savannah-like habitat) (N=51). Thick and thin blood smears of each specimen were examined and Plasmodium infection was screened by multiplex polymerase chain reaction (multiplex PCR). The frequency of Plasmodium infections detected by PCR in *Alouatta guariba clamitans* in the São Paulo Atlantic forest was 11.3% or 8/71 (5.6% for *Plasmodium malariae* and 5.6% for *Plasmodium vivax*) and one specimen was positive for *Plasmodium falciparum* (1.4%); *Callithrix* sp. (N=30) and *Cebus apella* (N=39) specimens were negative by PCR tests. Microscopy analysis was negative for all specimens from the Atlantic forest. The positivity rate for *Alouatta caraya* from semideciduous Atlantic forest was 6.8% (16/235) in the PCR tests (5.5, 0.8 and 0.4% for *P. malariae*, *P. falciparum* and *P. vivax*, respectively), while *C. apella* specimens were negative. Parasitological examination of the samples using thick smears revealed Plasmodium sp. infections in only seven specimens, which had few parasites (3.0%). Monkeys from the Cerrado (a savannah-like habitat) (42 specimens of *A. caraya*, 5 of *Callithrix jacchus* and 4 of *C. apella*) were negative in both tests. The parasitological prevalence of *P. vivax* and *P. malariae* in wild monkeys from Atlantic forest and semideciduous Atlantic forest and the finding of a positive result for *P. falciparum* in *Alouatta* from both types of forest support the hypothesis that monkeys belonging to this genus could be a potential reservoir. Furthermore, these findings raise the question of the relationship between simian and autochthonous human malaria in extra-Amazonian regions.

Acta Trop. 2008 Aug;107(2):174-8.

Building small dams can decrease malaria: A comparative study from Sundargarh District, Orissa, India.

Sharma SK, Tyagi PK, Upadhyay AK, Haque MA, Adak T, Dash AP.

National Institute of Malaria Research (NIMR), Field Station, Sector 5, Rourkela 769002, Sundargarh District, Orissa, India.

The adverse health effect of environmental changes brought about with the construction of large and small dams has often been reported. Here, we present results of a 5-year (2001-2005) study documenting the positive effect of such developmental projects in reducing malaria in an area where malaria transmission is mainly due to the highly efficient anthropophilic vector *Anopheles fluviatilis* with some contribution from *Anopheles culicifacies*. The former breeds exclusively in the slow-flowing streams and the latter breeds in a variety of habitats. The study was conducted in San Dulakudar village and comparisons were made with two control villages situated near the stream with similar topography and malaria transmission pattern. Epidemiological data was collected through longitudinal

weekly surveillance and cross-sectional surveys in all the study villages. The mean annual malaria incidence rates due to Plasmodium falciparum in children of 1-5 years age group during 2001 before construction of dam was 1304.3 and 785.7 cases/1000 population in dam site village and control villages, respectively. However, after construction of dam, there was gradual reduction in the malaria cases in dam site village and during 2005 the incidence was significantly reduced to 181.8 ($P < 0.01$) whereas it was increased to 1000 in control villages without any significant change in comparison to baseline year ($P > 0.05$). A significant reduction in malaria incidence and parasite rate was also recorded in all the age groups in dam site village without registering any significant change in control villages. The construction of a small dam in the study village altered the water flow above and below the dam thereby making it unfavourable for the breeding of *A. fluviatilis* which in turn brought about significant impact on malaria transmission.

Acta Trop. 2008 Aug;107(2):145-9.

The influence of food on the pharmacokinetics of piperazine in healthy Vietnamese volunteers.

Hai TN, Hietala SF, Van Huong N, Ashton M.

Pharmaceutical Unit, Department of Malaria Treatment and Research, National Institute of Malariology, Parasitology and Entomology (NIMPE), BC10200 Tu liem, Hanoi, Viet Nam.

The combination piperazine and dihydroartemisinin is emerging as first line treatment of uncomplicated falciparum malaria in Southeast Asia. The aim of this study was to determine the influence of a standard Vietnamese meal on the single-dose pharmacokinetics of piperazine when administered in combination with dihydroartemisinin, and to gain extended data on the terminal half-life of piperazine in healthy Vietnamese volunteers. Subjects were randomly assigned to take a single oral dose of piperazine phosphate (640mg)+dihydroartemisinin (80mg) together with a standardized Vietnamese meal ($n=16$) or to remain fasting for 4h following drug intake ($n=16$). Frequent blood sampling was conducted during 36h, followed by weekly samples for 7 weeks. The pharmacokinetic parameters of piperazine were determined by noncompartmental analysis. The median (80% central range) AUC(0-last) was 11.5 (6.9-17.3)hmg/L in fed and 13.9 (2.8-19.3)hmg/L in fasting subjects, indicating a considerable variability in exposure in both groups. The estimated overall oral clearance was 0.27 (0.12-1.49)L/(hkg), the volume of distribution during the terminal elimination phase was 230 (102-419)L/kg and estimated terminal half-life was 18 (5-93) days. This study did not demonstrate a significant impact of a standardized Vietnamese meal on the oral absorption of piperazine.

Acta Trop. 2008 Aug;107(2):99-105.

Natural infectivity of Anopheles species from the Pacific and Atlantic Regions of Colombia.

Gutiérrez LA, Naranjo N, Jaramillo LM, Muskus C, Luckhart S, Conn JE, Correa MM.

Grupo de Microbiología Molecular, Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia.

Malaria is an important public health problem in Colombia. Among the major vectors in Colombia, *Anopheles albimanus* is recognized for its importance on the Pacific Coast where it is the predominant species; it is also found in the Atlantic Coast, although its vectorial role in this region is not clear. We examined the occurrence of *An. albimanus* in four localities of the Pacific and three of the Atlantic Coast. Morphological identification of problematic

specimens was confirmed by a molecular assay. All identified mosquitoes at these sites, including *An. albimanus*, were also tested for malaria parasite infection. From 12,189 anophelines collected, 6370 were from the Pacific Coast, and corresponded to 99% *An. albimanus*, 0.8% *Anopheles neivai*, and three other species at <0.2%. From the Atlantic Coast we identified 5819 specimens with 61% *An. albimanus*, 36% *Anopheles triannulatus* s.l. and five other species at <2%. In both coasts, species present at lower percentages included several incriminated as vectors in neighboring countries. Six Pacific Coast specimens were infected with malaria parasites: four *An. albimanus*, two with *Plasmodium vivax* VK247, one with *P. vivax* VK210 and one with *Plasmodium falciparum*; two *An. neivai* with *P. falciparum*. Our data support the continued predominance of *An. albimanus* in the Pacific Coast, and demonstrate that this species is the most abundant in the Atlantic Coast as well.

Acta Trop. 2008 Aug;107(2):121-7.

A dried blood sample on filter paper is suitable for detecting *Plasmodium falciparum* gametocytes by reverse transcription polymerase chain reaction.

Maeno Y, Nakazawa S, Dao le D, Yamamoto N, Giang ND, Van Hanh T, Thuan le K, Taniguchi K.

Department of Virology and Parasitology, Fujita Health University School of Medicine, 1-98 Kutsukake, Toyoake, Aichi 470-1192, Japan.

The detection of gametocytes in human peripheral blood is one of the most important measures in a malaria survey. We attempted to detect gametocytes of *Plasmodium falciparum* by reverse transcription polymerase chain reaction (RT-PCR) of dried blood on filter paper. On field samples analysis, the specific RT-PCR products for region 3 of *pfg377* mRNA were observed in 67 of 131 *falciparum* malaria patients. The minimum detection level of RT-PCR-positive samples was 0.03gametocytes/mul on quantitative real-time RT-PCR. Gametocyte positive rate was not dependent on sex or age. A higher frequency of gametocytes was found in single *P. falciparum* infection than in mixed species infection ($P<0.01$). In this study, 47 of the 131 patients were asymptomatic. Eighteen of these 47 patients showed *pfg377* mRNA expression. Moreover, four alleles of region 3 of *pfg377* were detected in *pfg377* mRNA-positive patients and 13 of 67 *pfg377* mRNA-positive patients carried more than one gametocyte-producing clone. These results suggest that dried blood on filter paper is a useful for a molecular epidemiologic study of malaria transmission and gametocyte-targeted control.

Acta Trop. 2008 Jul 12.

Transstadial and horizontal transfer of bacteria within a colony of *Anopheles gambiae* (Diptera: Culicidae) and oviposition response to bacteria-containing water.

Lindh JM, Borg-Karlson AK, Faye I.

Department of Genetics, Microbiology and Toxicology, Stockholm University, Stockholm S-10691, Sweden.

In a paratransgenic approach, genetically modified bacteria are utilized to kill the parasite in the vector gut. A critical component for paratransgenics against malaria is how transgenic bacteria can be introduced and then kept in a mosquito population. Here, we investigated transstadial and horizontal transfer of bacteria within an *Anopheles gambiae* mosquito colony with the focus on spiked breeding sites as a possible means of introducing bacteria to mosquitoes. A *Pantoea stewartii* strain, previously isolated from *An. gambiae*, marked with a green fluorescent protein (GFP), was introduced to mosquitoes in different life stages. The following life stages or older mosquitoes in the case of adults were

screened for bacteria in their guts. In addition to *P. stewartii* other bacteria were isolated from the guts: these were identified by 16S rRNA sequence analysis and temporal temperature gradient gel electrophoresis (TTGE). Bacteria were transferred from larvae to pupae but not from pupae to adults. The mosquitoes were able to take up bacteria from the water they emerged from and transfer the same bacteria to the water they laid eggs in. *Elizabethkingia meningoseptica* was more often isolated from adult mosquitoes than *P. stewartii*. A bioassay was used to examine *An. gambiae* oviposition responses towards bacteria-containing solutions. The volatiles emitted from the solutions were sampled by headspace-solid phase microextraction (SPME) and identified by gas chromatography and mass spectrometry (GC-MS) analysis. *P. stewartii* but not *E. meningoseptica* mediated a positive oviposition response. The volatiles emitted by *P. stewartii* include indole and 3-methyl-1-butanol, which previously have been shown to affect *An. gambiae* mosquito behaviour. *E. meningoseptica* emitted indole but not 3-methyl-1-butanol, when suspended in saline. Taken together, this indicates that it may be possible to create attractive breeding sites for distribution of genetically modified bacteria in the field in a paratransgenic approach against malaria. Further research is needed to determine if the bacteria are also transferred in the same way in nature.

2 - *Antimicrob Agents Chemother.* 2008 Aug;52(8):2966-9.

The antimalarial trioxaquine DU1301 alkylates heme in malaria-infected mice.

Bousejra-El Garah F, Claparols C, Benoit-Vical F, Meunier B, Robert A.

Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse Cedex 4, France.

The in vivo alkylation of heme by the antimalarial trioxaquine DU1301 afforded covalent heme-drug adducts that were detected in the spleens of *Plasmodium* sp.-infected mice. This result indicates that the alkylation capacities of trioxaquinines in mammals infected with *Plasmodium* strains are similar to that of artemisinin, a natural antimalarial trioxane-containing drug.

3 - *Arch Insect Biochem Physiol.* 2008 Jul 9.

Characterization of *Anopheles minimus* CYP6AA3 expressed in a recombinant baculovirus system.

Boonsuepsakul S, Luepromchai E, Rongnoparut P.

Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand.

Metabolism by cytochrome P450 monooxygenases is a major mechanism implicated in resistance of insects to insecticides, including pyrethroids. We previously isolated the cytochrome P450 CYP6AA3 from deltamethrin-selected resistant strain of *Anopheles minimus* mosquito, a major malaria vector in Thailand. In the present study, we further investigated the role of CYP6AA3 enzyme in deltamethrin metabolism in vitro. The CYP6AA3 was expressed in *Spodoptera frugiperda* (Sf9) insect cells via baculovirus-mediated expression system. The enzymatic activity of CYP6AA3 in deltamethrin metabolism was characterized after being reconstituted with *An. minimus* NADPH-cytochrome P450 reductase and a NADPH-regenerating system. The contribution of CYP6AA3 responsible for deltamethrin metabolism was determined by measurement of deltamethrin disappearance following the incubation period and deltamethrin-derived compounds were detected using combined gas chromatography mass spectrometry analysis. 3-Phenoxybenzaldehyde was a major product of CYP6AA3-mediated deltamethrin metabolism. Deltamethrin degradation and formation of metabolites were NADPH-dependent and inhibited by piperonyl butoxide. Deltamethrin was catalyzed by CYP6AA3 with an apparent $K(m)$ of 80.0 +/-

2.0 and V(max) of 60.2 +/- 3.6 pmol/min/pmol P450. Furthermore, deltamethrin cytotoxicity assays by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypan blue dye exclusion were examined in Sf9 insect cells, with and without expression of CYP6AA3. Results revealed that CYP6AA3 could play a role in detoxifying deltamethrin in the cells. Thus, the results of this study support the role of CYP6AA3 in deltamethrin metabolism. Arch. Insect Biochem. Physiol. 2008. (c) 2008 Wiley-Liss, Inc.

4 - *BMC Evol Biol.* 2008 Jul 30;8(1):223.

Selection shapes malaria genomes and drives divergence between pathogens infecting hominids versus rodents.

Prugnolle F, McGee K, Keebler J, Awadalla P.

ABSTRACT: BACKGROUND: Malaria kills more people worldwide than all inherited human genetic disorders combined. To characterize how the parasites causing this disease adapt to different host environments, we compared the evolutionary genomics of two distinct groups of malaria pathogens in order to identify critical properties associated with infection of different hosts: those parasites infecting hominids (*Plasmodium falciparum* and *P. reichenowi*) versus parasites infecting rodent hosts (*P. yoelii yoelii*, *P. berghei*, and *P. chabaudi*). Adaptation by the parasite to its host is likely highly critical to the evolution of these species. RESULTS: Our comparative analysis suggests that patterns of molecular evolution in the hominid parasite lineage are generally similar to those of the rodent lineage but distinct in several aspects. The most rapidly evolving genes in both lineages are those involved in host-parasite interactions as well as those that show the lowest expression levels. However, we found that, similar to their respective mammal host lineages, parasite genomes infecting hominids are generally less constrained, evolving at faster rates, and accumulating more deleterious mutations than those infecting murids, which may reflect an historical lower effective size of the hominid lineage and relaxed host-driven selective pressures. CONCLUSIONS: Our study highlights for the first time the differences in trends and rates of evolution in *Plasmodium* lineages infecting different hosts and emphasizes the potential importance of the variation in effective size between lineages to explain variation in selective constraints among genomes.

5 - *BMC Health Serv Res.* 2008 Jul 31;8(1):165.

From strategy development to routine implementation: the cost of Intermittent Preventive Treatment in Infants for malaria control.

Manzi F, Hutton G, Schellenberg J, Tanner M, Alonso P, Mshinda H, Schellenberg D.

ABSTRACT: BACKGROUND: Achieving the Millennium Development Goals for health requires a massive scaling-up of interventions in Sub Saharan Africa. Intermittent Preventive Treatment in infants (IPTi) is a promising new tool for malaria control. Although efficacy information is available for many interventions, there is a dearth of data on the resources required for scaling up of health interventions. METHOD: We worked in partnership with the Ministry of Health and Social Welfare (MoHSW) to develop an IPTi strategy that could be implemented and managed by routine health services. We tracked health system and other costs of (1) developing the strategy and (2) maintaining routine implementation of the strategy in five districts in southern Tanzania. Financial costs were extracted and summarized from a costing template and semi-structured interviews were conducted with key informants to record time and resources spent on IPTi activities. RESULTS: The estimated financial cost to start-up and run IPTi in the whole of Tanzania in 2005 was US\$1,486,284. Start-up costs of US\$36,363 were incurred at the national level, mainly on the development of Behaviour Change Communication (BCC) materials, stakeholders' meetings and other

consultations. The annual running cost at national level for intervention management and monitoring and drug purchase was estimated at US\$459,096. Start-up costs at the district level were US\$7,885 per district, mainly expenditure on training. Annual running costs were US\$170 per district, mainly for printing of BCC materials. There was no incremental financial expenditure needed to deliver the intervention in health facilities as supplies were delivered alongside routine vaccinations and available health workers performed the activities without working overtime. The economic cost was estimated at 23 US cents per IPTi dose delivered. CONCLUSION: The costs presented here show the order of magnitude of expenditures needed to initiate and to implement IPTi at national scale in settings with high Expanded Programme on Immunization (EPI) coverage. The IPTi intervention appears to be affordable even within the budget constraints of Ministries of Health of most sub-Saharan African countries.

6 - *BMC Infect Dis.* 2008 Jul 31;8(1):105.

Molecular epidemiology of drug-resistant malaria in western Kenya highlands.

Zhong D, Afrane Y, Githeko A, Cui L, Menge DM, Yan G.

ABSTRACT: BACKGROUND: Since the late 1980s a series of malaria epidemics has occurred in western Kenya highlands. Among the possible factors that may contribute to the highland malaria epidemics, parasite resistance to antimalarials has not been well investigated. METHODS: Using parasites from highland and lowland areas of western Kenya, we examined key mutations associated with *Plasmodium falciparum* resistance to sulfadoxine - pyrimethamine and chloroquine, including dihydrofolate reductase (*pfdhfr*) and dihydropteroate synthetase (*pfdhps*), chloroquine resistance transporter gene (*pfcr1*), and multi-drug resistance gene 1 (*pfmdr1*). RESULTS: We found that >70% of samples harbored 76T *pfcr1* mutations and over 80% of samples harbored quintuple mutations (51I/59R/108N *pfdhfr* and 437G/540E *pfdhps*) in both highland and lowland samples. Further, we did not detect significant difference in the frequencies of these mutations between symptomatic and asymptomatic malaria volunteers, and between highland and lowland samples. CONCLUSIONS: These findings suggest that drug resistance of malaria parasites in the highlands could be contributed by the mutations and their high frequencies as found in the lowland. The results are discussed in terms of the role of drug resistance as a driving force for malaria outbreaks in the highlands.

7 - *Cell.* 2008 Jul 11;134(1):48-61.

Comment in:

Cell. 2008 Jul 11;134(1):20-2.

Exported proteins required for virulence and rigidity of *Plasmodium falciparum*-infected human erythrocytes.

Maier AG, Rug M, O'Neill MT, Brown M, Chakravorty S, Szestak T, Chesson J, Wu Y, Hughes K, Coppel RL, Newbold C, Beeson JG, Craig A, Crabb BS, Cowman AF.

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A major part of virulence for *Plasmodium falciparum* malaria infection, the most lethal parasitic disease of humans, results from increased rigidity and adhesiveness of infected host red cells. These changes are caused by parasite proteins exported to the erythrocyte using novel trafficking machinery assembled in the host cell. To understand these unique modifications, we used a large-scale gene knockout strategy combined with functional screens to identify proteins exported into parasite-infected erythrocytes and involved in remodeling these cells. Eight genes were identified encoding proteins required for export of the

parasite adhesin PfEMP1 and assembly of knobs that function as physical platforms to anchor the adhesin. Additionally, we show that multiple proteins play a role in generating increased rigidity of infected erythrocytes. Collectively these proteins function as a pathogen secretion system, similar to bacteria and may provide targets for antivirulence based therapies to a disease responsible for millions of deaths annually.

Cell. 2008 Jul 11;134(1):20-2.

Comment on:

Cell. 2008 Jul 11;134(1):48-61.

Deconstructing export of malaria proteins.

Bonnefoy S, Ménard R.

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The virulence of the malaria parasite *Plasmodium falciparum* is mediated by parasite proteins exported to the surface of infected erythrocytes. In this issue, Maier et al. (2008) report a screen of malaria parasite genes predicted to be involved in parasite protein export and trafficking within the host erythrocyte and discover that many more than expected are essential for parasite survival in vitro.

8 - *Cell Host Microbe*. 2008 Jul 17;4(1):52-62.

Duffy antigen receptor for chemokines mediates trans-infection of HIV-1 from red blood cells to target cells and affects HIV-AIDS susceptibility.

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Duffy antigen receptor for chemokines (DARC) expressed on red blood cells (RBCs) influences plasma levels of HIV-1-suppressive and proinflammatory chemokines such as CCL5/RANTES. DARC is also the RBC receptor for *Plasmodium vivax*. Africans with DARC -46C/C genotype, which confers a DARC-negative phenotype, are resistant to *vivax* malaria. Here, we show that HIV-1 attaches to RBCs via DARC, effecting trans-infection of target cells. In African Americans, DARC -46C/C is associated with 40% increase in the odds of acquiring HIV-1. If extrapolated to Africans, approximately 11% of the HIV-1 burden in Africa may be linked to this genotype. After infection occurs, however, DARC-negative RBC status is associated with slower disease progression. Furthermore, the disease-accelerating effect of a previously described CCL5 polymorphism is evident only in DARC-expressing and not in DARC-negative HIV-infected individuals. Thus, DARC influences HIV/AIDS susceptibility by mediating trans-infection of HIV-1 and by affecting both chemokine-HIV interactions and chemokine-driven inflammation.

Cell Host Microbe. 2008 Jul 17;4(1):40-51.

Erythrocyte binding protein PfrH5 polymorphisms determine species-specific pathways of *Plasmodium falciparum* invasion.

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Some human malaria *Plasmodium falciparum* parasites, but not others, also cause disease in Aotus monkeys. To identify the basis for this variation, we crossed two clones that differ in Aotus nancymae virulence and mapped inherited traits of infectivity to erythrocyte invasion by linkage analysis. A major pathway of invasion was linked to polymorphisms in a putative erythrocyte binding protein, PFRH5, found in the apical region of merozoites. Polymorphisms of PFRH5 from the A. nancymae-virulent parent transformed the nonvirulent parent to a virulent parasite. Conversely, replacements that removed these polymorphisms from PFRH5 converted a virulent progeny clone to a nonvirulent parasite. Further, a proteolytic fragment of PFRH5 from the infective parasites bound to A. nancymae erythrocytes. Our results also suggest that PFRH5 is a parasite ligand for human infection, and that amino acid substitutions can cause its binding domain to recognize different human erythrocyte surface receptors.

9 - *Clin Infect Dis.* 2008 Jul 15;47(2):151-7.

The relationship between age and the manifestations of and mortality associated with severe malaria.

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BACKGROUND: The reported case-fatality rate associated with severe malaria varies widely. Whether age is an independent risk factor is uncertain. **METHODS:** In a large, multicenter treatment trial conducted in Asia, the presenting manifestations and outcome of severe malaria were analyzed in relation to age. **RESULTS:** Among 1050 patients with severe malaria, the mortality increased stepwise, from 6.1% in children (age, <10 years) to 36.5% in patients aged >50 years ($P<0.001$). Compared with adults aged 21-50 years, the decreased risk of death among children (adjusted odds ratio, 0.06; 95% confidence interval, 0.01-0.23; $P<0.001$) and the increased risk of death among patients aged >50 years (adjusted odds ratio, 1.88; 95% confidence interval, 1.01-3.52; $P<0.001$) was independent of the variation in presenting manifestations. The incidence of anemia and convulsions decreased with age, whereas the incidence of hyperparasitemia, jaundice, and renal insufficiency increased with age. Coma and metabolic acidosis did not vary with age and were the strongest predictors of a fatal outcome. The number of severity signs at hospital admission also had a strong prognostic value. **CONCLUSION:** Presenting syndromes in severe malaria depend on age, although the incidence and the strong prognostic significance of coma and acidosis are similar at all ages. Age is an independent risk factor for a fatal outcome of the disease.

10 - *Clin Pharmacol Ther.* 2008 Jul 9.

Availability and Choice of Antimalarials at Medicine Outlets in Ghana: The Question of Access to Effective Medicines for Malaria Control.

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Although national and international efforts to combat malaria have intensified over the years, problems with availability, distribution, and choice of antimalarials at medicine outlets in Africa continue to exist. This article

presents the results of an indicator-based assessment of availability and choice of antimalarials at 130 licensed medicine outlets in Ghana. We also discuss how the choice of an antimalarial to dispense conforms to recommendations of the national policy for malaria therapy. Data were obtained through face-to-face interviews, by reviewing facility records, and by observing the practices of dispensing staff in the medicine outlets. Antimalarials recommended in the policy were not readily available in the most accessible medicine outlets. Few outlets adhered to the policy when choosing antimalarials. Interventions targeting medicine outlets should be initiated to improve availability and access to effective medicines in order to support the national program for malaria control. *Clinical Pharmacology & Therapeutics* advance online publication 09 July 2008. doi:10.1038/clpt.2008.130.

11 - *Clin Vaccine Immunol.* 2008 Jul 16.

LEVELS OF SOLUBLE CD163 AND MALARIA SEVERITY IN GHANAIAN CHILDREN.

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CD163 is an acute phase-regulated monocyte/macrophage membrane receptor expressed late in inflammation. It is involved in the haptoglobin-mediated removal of free hemoglobin (Hb) from plasma and has been identified as a natural soluble plasma glycoprotein with potential anti-inflammatory properties, possibly linked to the individual's haptoglobin (Hp) phenotype. High levels of soluble CD163 (sCD163) in a malaria episode may therefore down-regulate inflammation and curb disease severity. In order to verify this, the relationships between sCD163 levels, malaria severity and selected inflammatory mediators (TNF-alpha, IL6, IL10) were assessed by ELISA in plasma samples obtained from pediatric malaria patients with uncomplicated malaria (UM, N=38), cerebral malaria (CM, N=52) and severe malarial anemia (SA, N=55) during two consecutive malaria transmission seasons (2002 and 2003). Median sCD163 levels were higher in UM (11.9 microg/mL) than in SA (7.7 microg/mL, P = 0.010) and CM (8.0 microg/mL, P = 0.031). Levels of sCD163 were also higher in all patient groups as compared to a group of 81 age-matched healthy controls. The higher sCD163/TNF-alpha ratio in UM, coupled with the fact that sCD163 levels correlated with TNF-alpha levels in UM but not in CM and SA, suggests inflammatory dys-regulation in the complicated cases. The study showed that sCD163 levels are elevated during acute malaria. High sCD163 levels in UM patients may be due to the induction of higher anti-inflammatory responses enabling them to avoid disease complications, or that UM patients simply lost their CD163 receptors from macrophages in inflammatory sites while complicated malaria groups still had their receptors attached to activated macrophages, reflecting on-going and higher inflammation associated with complicated malaria.

12 - *Eur J Clin Microbiol Infect Dis.* 2008 Aug;27(8):725-32.

High frequency of Plasmodium falciparum CICNI/SGEAA and CVIET haplotypes without association with resistance to sulfadoxine/pyrimethamine and chloroquine combination in the Daraweesh area, in Sudan.

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Estimation of the prevalence of the molecular markers of sulfadoxine/pyrimethamine (SP) and chloroquine (CQ) resistance and validation of the association of mutations with resistance in different settings is needed for local policy guidance and for contributing to a global map for anti-malarial drug resistance. In this study, malaria patients treated with SP alone (60) and SP with CQ (194) had a total treatment failure (TF) of 35.4%, with no difference between the two arms. The polymerase chain reaction-enzyme-linked immunosorbent assay (PCR-ELISA) method was used to identify polymorphisms in 15 loci in the *dhfr*, *dhps* and *pfprt* genes in a subset of 168 infections. The results revealed a similar frequency of all single nucleotide polymorphisms (SNPs) in the two arms, except *dhps* 581G, which was over-represented in infections that failed to respond to SP alone (TF). In all infections, a high frequency of *dhfr* CICNI haplotype (51I and 108N) was found, but without discrimination between the adequate clinical and parasitological response (ACPR, 75.6%) and TF (82.9%). Similarly, the *dhps* SGEAA haplotype (437G and 540E) (ACPR, 60.5%; TF, 65.9%) and the combined CICNI/SGEAA haplotype (ACPR, 50%; TF 55%) were not associated with TF. In contrast to other studies in Africa, the triple 51I/59R/108N mutation was rare (0.6%). In addition, the *pfprt* CVIET haplotype (93%) was found to be associated with the CICNI/SGEAA haplotype. Finally, these data represent a baseline for SP resistance molecular markers needed before the deployment of SP/artesunate combination therapy in the Sudan.

13 - *Gene*. 2008 Aug 1;419(1-2):35-42.

Plasmodium vivax: Sequence polymorphism and effect of natural selection at apical membrane antigen 1 (PvAMA1) among Indian population.

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Present study describes the characterization of apical membrane antigen 1 (PvAMA1) polymorphisms among Indian *Plasmodium vivax* isolates. The partial PvAMA1 gene (covering domain I and domain II regions) sequenced from sixty-one (n=61) isolates in this study resulted into 49 haplotypes. Comparison with the previously available PvAMA1 sequences in the GenBank database revealed that 45 of these were new haplotypes that have never been reported till date. For further analyses, we also included 11 previously reported PvAMA1 sequences from India available in the database. Thus genetic diversity and effect of natural selection were analyzed both at domain I and domain II of this promising malaria vaccine candidate among 72 Indian *P. vivax* isolates. Non-synonymous mutations were found at 25 codons (16 at domain I and 9 at domain II) where 17 codons were dimorphic while rest of them (8 codons) were trimorphic. Thus codon polymorphisms were observed to be more at domain I as compared to domain II. Although the difference between the rate of non-synonymous (dN) and synonymous (dS) mutations was positive (dN-dS, 0.002+/-0.004SE) at domain II, it was not significantly different from each other (P=0.272), indicating tendency of stronger diversifying selection at this domain. The dN-dS difference for domain I (-0.006+/-0.009SE, P=0.268) and for entire 900 bp region (-0.002+/-0.005E, P=0.320) being negative and statistically insignificant suggests the role of both positive as well as purifying selection. Three-dimensional distributions of all polymorphic residues were mapped on a modeled PvAMA1 structure. Results suggested that almost all of the observed polymorphisms were located at one surface of the antigen. In conclusion, PvAMA1 antigen displays high diversity among Indian isolates with more diversifying selection at domain II. The result has significant value in malaria vaccine development using this antigen.

Mother-to-child transmission of HIV-1: association with malaria prevention, anaemia and placental malaria.

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Objectives Malaria infection may impact on mother-to-child transmission (MTCT) of HIV-1. Prevention of malaria in pregnancy could thus potentially affect MTCT of HIV. We studied the impact of intermittent preventive treatment during pregnancy (IPTp) on HIV-1 MTCT in southern Mozambique. Methods A total of 207 HIV-positive Mozambican pregnant women were enrolled in the study as part of a randomized placebo-controlled trial of two-dose sulfadoxine-pyrimethamine (SP) IPTp in women receiving single-dose nevirapine to prevent MTCT of HIV. HIV RNA viral load, maternal anaemia and peripheral and placental malaria were assessed at delivery. Infant HIV status was determined by DNA polymerase chain reaction (PCR) at 1 month of age. Results There were 19 transmissions of HIV in 153 mother-infant pairs. IPTp with SP did not have a significant impact on MTCT (11.8% in the SP group vs. 13.2% in the placebo group; $P=0.784$) or on maternal HIV RNA viral load [16 312 (interquartile range {IQR} 4076-69 296) HIV-1 RNA copies/mL in the SP group vs. 18 274 (IQR 5471-74 104) copies/mL in the placebo group; $P=0.715$]. In multivariate analysis, maternal HIV RNA viral load [adjusted odds ratio (AOR) 19.9; 95% confidence interval (CI) 2.3-172; $P=0.006$] and anaemia (haematocrit $<33\%$; AOR 7.5; 95% CI 1.7-32.4; $P=0.007$) were independent risk factors for MTCT. Placental malaria was associated with a decrease in MTCT (AOR 0.23; 95% CI 0.06-0.89; $P=0.034$). Conclusions IPTp with SP was not associated with a significant impact on MTCT of HIV. Maternal anaemia was an independent risk factor for MTCT.

15 - *Infect Immun.* 2008 Aug;76(8):3628-31.

Effector CD8+ T lymphocytes against liver stages of Plasmodium yoelii do not require gamma interferon for antiparasite activity.

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The protective immune response against liver stages of the malaria parasite critically requires CD8(+) T cells. Although the nature of the effector mechanism utilized by these cells to repress parasite development remains unclear, a critical role for gamma interferon (IFN-gamma) has been widely assumed based on circumstantial evidence. However, the requirement for CD8(+) T-cell-mediated IFN-gamma production in protective immunity to this pathogen has not been directly tested. In this report, we use an adoptive transfer strategy with circumsporozoite (CS) protein-specific transgenic T cells to examine the role of CD8(+) T-cell-derived IFN-gamma production in Plasmodium yoelii-infected mice. We show that despite a marginal reduction in the expansion of naive IFN-gamma-deficient CS-specific transgenic T cells, their antiparasite activity remains intact. Further, adoptively transferred IFN-gamma-deficient CD8(+) T cells were as efficient as their wild-type counterparts in limiting parasite growth in naive mice. Taken together, these studies demonstrate that IFN-gamma secretion by CS-specific CD8(+) T cells is not essential to protect mice against live sporozoite challenge.

Infect Immun. 2008 Aug;76(8):3817-23.

The oligomerization domain of C4-binding protein (C4bp) acts as an adjuvant, and the fusion protein comprised of the 19-kilodalton merozoite surface protein 1 fused with the murine C4bp domain protects mice against malaria.

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Highly purified protein antigens are usually poor immunogens; in practice, adjuvants are needed to obtain satisfactory immune responses. *Plasmodium yoelii* 19-kDa merozoite surface protein 1 (MSP1(19)) is a weak antigen, but mice vaccinated with this antigen in strong adjuvants can survive an otherwise lethal parasite challenge. Fusion proteins comprising this antigen fused to the oligomerization domain of the murine complement inhibitor C4-binding protein (C4bp) and a series of homologues have been produced. These C4bp domains acted as adjuvants for the fused antigen; the MSP1(19)-murine C4bp fusion protein induced protective immunity in BALB/c mice. Because this fusion protein also induced antibodies against circulating murine C4bp, distantly related C4bp oligomerization domains fused to the same antigen were tested. These homologous domains did not induce antibodies against murine C4bp and, surprisingly, induced higher antibody titers against the antigen than the murine C4bp domain induced. These results demonstrate a new adjuvantlike effect of C4bp oligomerization domains.

Infect Immun. 2008 Jul 21.

Host Biomarkers and Biological Pathways that are Associated with the Expression of Experimental Cerebral Malaria in Mice.

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Cerebral malaria (CM) is a primary cause of malaria-associated deaths in young African children. Yet there are no diagnostic tools available that could be used to predict which of the children infected with *Plasmodium falciparum* malaria will progress into CM. We used the *P. berghei* ANKA murine model of experimental cerebral malaria (ECM) and high density oligonucleotide microarray analyses to identify host molecules that are strongly associated with the clinical symptoms of ECM. Comparative expression analyses were performed in the ECM susceptible phenotype C57BL/6 mice and in the ECM resistant phenotypes CD8 knockout and perforin knockout mice on the C57BL/6 background and in BALB/c mice that allowed the identification of over 200 host molecules (a majority previously not identified) with altered expression patterns in the brain that are strongly associated with the manifestation of ECM. Among these host molecules, brain samples from ECM mice had a significantly higher protein expression of the p21, metallothionein and hemoglobin alpha 1 molecules by western blot analysis, suggesting their possible utility as prognostic biomarkers of CM in humans. We

suggest that the higher expression of hemoglobin alpha 1 in the brain may be associated with ECM and could be a source of excess heme, a molecule that is considered to trigger the pathogenesis of CM. Our studies greatly enhance the repertoire of host molecules for use as diagnostics and novel therapeutics in CM.

16 - *Int J Biometeorol.* 2008 Jul 17.

The effect of water turbidity on the near-surface water temperature of larval habitats of the malaria mosquito *Anopheles gambiae*.

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Water temperature is an important determinant in many aquatic biological processes, including the growth and development of malaria mosquito (*Anopheles arabiensis* and *A. gambiae*) immatures. Water turbidity affects water temperature, as suspended particles in a water column absorb and scatter sunlight and hence determine the extinction of solar radiation. To get a better understanding of the relationship between water turbidity and water temperature, a series of semi-natural larval habitats (diameter 0.32 m, water depth 0.16 m) with increasing water turbidity was created. Here we show that at midday (1300 hours) the upper water layer (thickness of 10 mm) of the water pool with the highest turbidity was on average 2.8 degrees C warmer than the same layer of the clearest water pool. Suspended soil particles increase the water temperature and furthermore change the temperature dynamics of small water collections during daytime, exposing malaria mosquito larvae, which live in the top water layer, longer to higher temperatures.

17 - *Int J Parasitol.* 2008 Aug;38(10):1099-109.

Frequent recombination events generate diversity within the multi-copy variant antigen gene families of *Plasmodium falciparum*.

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The human malaria parasite *Plasmodium falciparum* utilises a mechanism of antigenic variation to avoid the antibody response of its human host and thereby generates a long-term, persistent infection. This process predominantly results from systematic changes in expression of the primary erythrocyte surface antigen, a parasite-produced protein called PfEMP1 that is encoded by a repertoire of over 60 var genes in the *P. falciparum* genome. var genes exhibit extensive sequence diversity, both within a single parasite's genome as well as between different parasite isolates, and thus provide a large repertoire of antigenic determinants to be alternately displayed over the course of an infection. Whilst significant work has recently been published documenting the extreme level of diversity displayed by var genes found in natural parasite populations, little work has been done regarding the mechanisms that lead to sequence diversification and heterogeneity within var genes. In the course of producing transgenic lines from the original NF54 parasite isolate, we cloned and characterised a parasite line, termed E5, which is closely related to but distinct from 3D7, the parasite used for the *P. falciparum* genome nucleotide sequencing project. Analysis of the E5 var gene repertoire, as well as that of the surrounding rif and stevor multi-copy gene families, identified examples of frequent recombination events within these gene families, including an example of a duplicative transposition which indicates that recombination events play a significant role in the generation of diversity within the antigen encoding genes of *P. falciparum*.

Int J Parasitol. 2008 Aug;38(10):1083-97.

Histone lysine methyltransferases and demethylases in Plasmodium falciparum.

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Dynamic histone lysine methylation, regulated by methyltransferases and demethylases, plays fundamental roles in chromatin structure and gene expression in a wide range of eukaryotic organisms. A large number of SET-domain-containing proteins make up the histone lysine methyltransferase (HKMT) family, which catalyses the methylation of different lysine residues with relatively high substrate specificities. Another large family of Jumonji C (JmjC)-domain-containing histone lysine demethylases (JHDMs) reverses histone lysine methylation with both lysine site and methyl-state specificities. Through bioinformatic analysis, at least nine SET-domain-containing genes were found in the malaria parasite *Plasmodium falciparum* and its sibling species. Phylogenetic analysis separated these putative HKMTs into five subfamilies with different putative substrate specificities. Consistent with the phylogenetic subdivision, methyl marks were found on K4, K9 and K36 of histone H3 and K20 of histone H4 by site-specific methyl-lysine antibodies. In addition, most SET-domain genes and histone methyl-lysine marks displayed dynamic changes during the parasite asexual erythrocytic cycle, suggesting that they constitute an important epigenetic mechanism of gene regulation in malaria parasites. Furthermore, the malaria parasite and other apicomplexan genomes also encode JmjC-domain-containing proteins that may serve as histone lysine demethylases. Whereas prokaryotic expression of putative active domains of four *P. falciparum* SET proteins did not yield detectable HKMT activity towards recombinant *P. falciparum* histones, two protein domains expressed *in vitro* in a eukaryotic system showed HKMT activities towards H3 and H4, respectively. With the discovery of these *Plasmodium* SET- and JmjC-domain genes in the malaria parasite genomes, future efforts will be directed towards elucidation of their substrate specificities and functions in various cellular processes of the parasites.

18: *Int J Qual Health Care.* 2008 Jul 11.

Content of antenatal care services in secondary health care facilities in Nigeria: implication for quality of maternal health care.

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OBJECTIVE: /st> To assess the contents of antenatal care and to relate the findings to the adequacy of maternal health care. DESIGN: /st> Cross-sectional study. SETTING: /st> Public secondary health-care facilities. PARTICIPANTS: /st> Pregnant women. INTERVENTIONS: /st> Three hundred and ninety consecutive pregnant women attending 12 selected secondary health facilities were recruited proportionate to the client load recorded for each facility during the year preceding the study. Interviews were conducted using the antenatal care exit interview form of the Safe Motherhood Needs Assessment package. MAIN OUTCOME MEASURES: /st> Antenatal care services provided to pregnant women in current pregnancy. RESULTS: /st> Blood pressure measurement, abdominal palpation and detection of foetal heart rate were provided to all participants. Three hundred and eighty-six (99%) were reached with at least one educational message. One hundred and sixty-seven (42.8%) had haemoglobin or packed cell volume estimated, whereas 168 (43.1%) had urine checked for protein, at least once during antenatal

visits. Routine iron and folate supplements, and malaria prophylaxis were, respectively, given to 142 (36.4%) and 25 (6.4%). CONCLUSIONS: The antenatal care service as provided had reasonable capacity for intervention against pre-eclampsia and some foetal problems, and could contribute to delivery in a health facility and by a health worker. Capacity to address the possible effects of severe anaemia and malaria in pregnancy was lacking. Equipping health-care facilities with capacity to detect anaemia and proteinuria as well as ensuring that iron and folate supplements, and malaria prophylaxis are given to all pregnant women would help to meet national guidelines and improve quality of service.

19 - *J Acquir Immune Defic Syndr.* 2008 Jul 23.

Imported Malaria in HIV-Infected Patients Enrolled in the ANRS CO4 FHDH Study.

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From the *INSERM, U720, Paris, F-75013 France; daggerUPMC Univ Paris 06, UMR S270, F-75013, Paris, France; double daggerAssistance Publique-Hôpitaux de Paris, Groupe hospitalier Bichat Claude Bernard, Service de parasitologie, Paris, F-75018 France; section signRouen University Hospital, Service de maladies infectieuses et tropicales, Rouen, France; ||Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Tenon, Service de maladies infectieuses et tropicales, Paris, F-75020 France; paragraph signCentre Hospitalier de Tourcoing, Service universitaire des maladies infectieuses et du voyage, Tourcoing, France; #Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Pitié-Salpêtrière, Service de maladies infectieuses et tropicales, Paris, F-75013 France; and **Assistance Publique-Hôpitaux de Paris, Groupe hospitalier Bichat Claude Bernard, Service de maladies infectieuses et tropicales, Paris, F-75018 France.

BACKGROUND:: To describe episodes of imported malaria in human immunodeficiency virus type 1-infected patients and to study the risk factors for severe Plasmodium falciparum malaria. METHODS:: Patients enrolled in the French Hospital Database on HIV who were diagnosed with a first episode of malaria between 1996 and 2003 were included. The severity of P. falciparum imported malaria was graded with World Health Organization criteria. Geographic areas were classified according to P. falciparum chemoresistance. Risk factors for severe malaria were identified with logistic regression. RESULTS:: We studied 190 patients infected by P. falciparum in 178 cases. All but four of the patients were infected in sub-Saharan Africa, and half were returning from a country with a high P. falciparum chloroquine resistance. Their median age was 37.5 years, and 57% came from a country endemic with malaria. The median CD4 cell count was 299/mm, and the median plasma human immunodeficiency virus type 1 RNA load was 4.5 log₁₀ copies/mL. Sixty-five (36.5%) episodes of P. falciparum malaria were severe. Severe imported malaria was associated with CD4 cells/mm <350 (odds ratio = 2.58; 95% confidence interval: 1.19 to 5.57). The risk of severe malaria was lower in patients returning from a country with a high prevalence of chemoresistance (odds ratio = 0.50; 95% confidence interval: 0.25 to 0.99). CONCLUSIONS:: Severe imported malaria in human immunodeficiency virus type 1-infected patients is associated with decreased CD4 cell count. The risk seems lower when P. falciparum infection was acquired in areas of high prevalence of chemoresistance.

20 - *J Environ Sci Health B.* 2008 Aug;43(6):484-9.

Placental transfer of DDT in mother-infant pairs from Northern Thailand.

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The present study objective was to investigate ratios and correlation coefficients between dichlorodiphenyltrichloroethane (DDT) compounds in cord and maternal sera of mother-infant pairs from northern Thailand. The study site was located in Chiang Dao District of Chiang Mai Province which was an agricultural and former malaria endemic area. DDT compounds were analyzed in 88 cord and maternal serum samples using gas chromatography-electron capture detection (GC-ECD). *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) was the major component and detected in every cord and maternal serum samples with geometric means of 1,255 and 1,793 ng(-1) lipids, respectively. *p,p'*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) was detected at 89.8 and 100% of cord and maternal serum samples, respectively. The second and third highest levels detected were *p,p'*-DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and *p,p'*-DDT, respectively. The ratios between cord and maternal sera for *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD that were less than 1 had high correlation coefficients (ratio = 0.70, $r = 0.82$ for *p,p'*-DDE, ratio = 0.62, $r = 0.66$ for *p,p'*-DDT, and ratio = 0.79, $r = 0.78$ for *p,p'*-DDD). The high correlation coefficients indicate that cord serum levels of DDT compounds could be accurately estimated from maternal serum levels. It can be concluded that cord serum levels of *p,p'*-DDE, *p,p'*-DDT, and *p,p'*-DDD were approximately 70%, 62%, and 79% of maternal serum levels, respectively. Furthermore, our findings can be applied in public health to monitor and evaluate risk among infants from high DDT exposure area.

21 - *J Immunol.* 2008 Aug 1;181(3):1988-2000.

Identification of residues in the Cmu4 domain of polymeric IgM essential for interaction with Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1).

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The binding of nonspecific human IgM to the surface of infected erythrocytes is important in rosetting, a major virulence factor in the pathogenesis of severe malaria due to *Plasmodium falciparum*, and IgM binding has also been implicated in placental malaria. Herein we have identified the IgM-binding parasite ligand from a virulent *P. falciparum* strain as PfEMP1 (TM284var1 variant), and localized the region within this PfEMP1 variant that binds IgM (DBL4beta domain). We have used this parasite IgM-binding protein to investigate the interaction with human IgM. Interaction studies with domain-swapped Abs, IgM mutants, and anti-IgM mAbs showed that PfEMP1 binds to the Fc portion of the human IgM H chain and requires the IgM Cmu4 domain. Polymerization of IgM was shown to be crucial for the interaction because PfEMP1 binding did not occur with mutant monomeric IgM molecules. These results with PfEMP1 protein have physiological relevance because infected erythrocytes from strain TM284 and four other IgM-binding *P. falciparum* strains showed analogous results to those seen with the DBL4beta domain. Detailed investigation of the PfEMP1 binding site on IgM showed that some of the critical amino acids in the IgM Cmu4 domain are equivalent to those regions of IgG and IgA recognized by Fc-binding proteins from bacteria, suggesting that this region of Ig molecules may be of major functional significance in host-microbe interactions. We have therefore shown that PfEMP1 is an Fc-binding protein of malaria parasites specific for polymeric human IgM, and that it shows functional similarities with Fc-binding proteins from pathogenic bacteria.

J Immunol. 2008 Jul 15;181(2):1454-9.

Systemic release of high mobility group box 1 protein during severe murine influenza.

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Hypercytokinemia is gaining recognition as the mechanism of fatality from influenza. No work to date has addressed the role of high mobility group box 1 protein (HMGB1) in influenza, the parallel being that in other severe proinflammatory cytokine syndromes (e.g., sepsis and malaria) levels of circulating HMGB1 are elevated and may correlate with death. Using a commercially available ELISA for HMGB1, we found that HMGB1 was not increased in the plasma of influenza virus-infected mice (A/Japan/305/57) on day 7 post infection, about the time of peak mortality, and peak levels of HMGB1 in the plasma did not occur until relatively late in infection, on day 9 post infection. In keeping with the late peak of HMGB1 being unassociated with mortality, administration of ethyl pyruvate, which inhibits active secretion but not passive release of HMGB1, to influenza virus-infected mice, did not affect their survival. Further work is required to determine whether influenza virus infection induces passive release of HMGB1, and whether HMGB1 neutralization with a specific Ab would improve survival.

J Immunol. 2008 Jul 15;181(2):1420-8.

Malaria-specific and nonspecific activation of CD8+ T cells during blood stage of Plasmodium berghei infection.

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Cerebral malaria is one of the severe complications of Plasmodium falciparum infection. Studies using a rodent model of Plasmodium berghei ANKA infection established that CD8(+) T cells are involved in the pathogenesis of cerebral malaria. However, it is unclear whether and how Plasmodium-specific CD8(+) T cells can be activated during the erythrocyte stage of malaria infection. We generated recombinant Plasmodium berghei ANKA expressing OVA (OVA-PbA) to investigate the parasite-specific T cell responses during malaria infection. Using this model system, we demonstrate two types of CD8(+) T cell activations during the infection with malaria parasite. Ag (OVA)-specific CD8(+) T cells were activated by TAP-dependent cross-presentation during infection with OVA-PbA leading to their expression of an activation phenotype and granzyme B and the development to functional CTL. These highly activated CD8(+) T cells were preferentially sequestered in the brain, although it was unclear whether these cells were involved in the pathogenesis of cerebral malaria. Activation of OVA-specific CD8(+) T cells in RAG2 knockout TCR-transgenic mice during infection with OVA-PbA did not have a protective role but rather was pathogenic to the host as shown by their higher parasitemia and earlier death when compared with RAG2 knockout mice. The OVA-specific CD8(+) T cells, however, were also activated during infection with wild-type parasites in an Ag-nonspecific manner, although the levels of activation were much lower. This nonspecific activation occurred in a TAP-independent manner, appeared to require NK cells, and was not by itself pathogenic to the host.

Upgrading the flow-cytometric analysis of anti-Leishmania immunoglobulins for the diagnosis of American tegumentary leishmaniasis.

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We have previously described a flow cytometry-based assay to detect anti-live *Leishmania (Viania) braziliensis* promastigote antibodies (FC-ALPA) with prominent performance of FC-ALPA to diagnosis American tegumentary leishmaniasis (ATL). However, the laboriousness to work with live parasites represented the major drawback for using FC-ALPA in routine clinical laboratory. Herein, we have presented an upgraded technology using fixed *Leishmania (Leishmania) amazonensis* promastigotes as antigen (FC-AFPA). Our data demonstrated that FC-AFPA-IgG displays outstanding performance for ATL diagnosis with high sensitivity (99%) and specificity (100%). Moreover, Likelihood Ratio indicated that positive results (LR+) has an infinite times more chance to come from ATL than from non-infected individuals (NI). Despite the high frequency of cross-reactivity with putative ATL co-endemic diseases, including visceral leishmaniasis, Chagas disease and malaria, FC-AFPA-IgG showed remarkable potential for differential diagnosis with other dermatological illnesses such as leprosy and sporotrichosis. FC-AFPA-IgG subclasses analysis revealed that LTA is characterized by IgG1>IgG3>IgG2 = IgG4 anti-L. *amazonensis* profiling, electing FC-AFPA-IgG1 and IgG3 with better performances to diagnosis ATL diagnosis. Additionally, FC-AFPA-IgG3 showed to be a better diagnostic tool in endemic areas for malarial disease. Despite the substantial advance to work with fixed promastigotes that contributes to its higher sensitivity, the lower specificity of FC-AFPA represented the major flaws as compared to FC-ALPA, suggesting that further improvement is still required to minimize the cross-reactivity with trypanosomatidae infections. Perspectives for using a flow cytometry multiplex based methodology to simultaneously assess anti-L. *amazonensis*, anti-L. *chagasi* and anti-*Trypanosoma cruzi* IgG reactivity is currently under investigation.

alpha(+)-Thalassemia Protects against Anemia Associated with Asymptomatic Malaria: Evidence from Community-Based Surveys in Tanzania and Kenya.

Veenemans J, Andang'o PE, Mbugi EV, Kraaijenhagen RJ, Mwaniki DL, Mockenhaupt FP, Roewer S, Olomi RM, Shao JF, Meer JW, Savelkoul HF, Verhoef H.

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Background. In hospital-based studies, alpha(+)-thalassemia has been found to protect against severe, life-threatening falciparum malaria.

alpha(+)-Thalassemia does not seem to prevent infection or high parasite densities but rather limits progression to severe disease-in particular, severe malarial anemia. We assessed to what extent alpha(+)-thalassemia influences the association between mild, asymptomatic Plasmodium falciparum infection and hemoglobin concentration. Methods. The study was based on 2 community-based surveys conducted among afebrile children (0.5-8 years old; [Formula: see text]) in Kenya and Tanzania. Results. Among children

without inflammation (whole-blood C-reactive protein concentration ≤ 10 mg/L), *P. falciparum* infection was associated with only small reductions in hemoglobin concentration, and effects were similar across alpha-globin genotypes. By contrast, the reduction in hemoglobin concentration associated with *P. falciparum* infection accompanied by inflammation was larger and strongly depended on genotype (normal, -21.8 g/L; heterozygous, -16.7 g/L; and homozygous, -4.6 g/L). Relative to children with a normal genotype, this difference in effect was 5.1 g/L (95% confidence interval [CI], -1.0 to 11.1 g/L) for heterozygotes and 17.2 g/L (95% CI, 8.3 to 26.2 g/L) for homozygotes (estimates are adjusted for study site, age, height-for-age z score, and iron deficiency). Conclusions. $\alpha(+)$ -Thalassemia limits the decline in hemoglobin concentration that is associated with afebrile infections, particularly those that are accompanied by inflammation.

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Factors Determining the Heterogeneity of Malaria Incidence in Children in Kampala, Uganda.

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Background. Malaria risk may be heterogeneous in urban areas of Africa. Identifying those at highest risk for malaria may lead to more targeted approaches to malaria control. **Methods.** A representative sample of 558 children aged 1-10 years were recruited from a census population in a single parish of Kampala and followed up for 2 years. Malaria was diagnosed when a child presented with a new episode of fever and a thick blood smear positive for parasites. Multivariate analysis was used to identify independent predictors of malaria incidence. **Results.** A total of 695 episodes of uncomplicated malaria were diagnosed after 901 person years of follow-up. Sick cell trait (relative risk [RR], 0.68 [95% confidence interval {CI}, $0.52-0.90$]), glucose-6-phosphate dehydrogenase deficiency in female children (RR, 0.48 [95% CI, $0.31-0.75$]), and use of an insecticide-treated bed net (RR, 0.52 [95% CI, $0.32-0.83$]) were associated with a lower risk of malaria. The distance of the subject's residence from a swamp bordering the parish showed a strong "dose-response" relationship; living in the swamp was the strongest predictor of malaria risk (RR, 3.94 [95% CI, $2.61-5.97$]). **Conclusion.** Malaria incidence was highly heterogeneous in this urban cohort of children. Malaria control interventions in urban areas should target populations living in pockets of high malaria risk.

J Infect Dis. 2008 Jul 28.

Variants in the Toll-Like Receptor Signaling Pathway and Clinical Outcomes of Malaria.

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Federal da Bahia, Salvador, Brazil.

Background. Malaria is one of the most significant infectious diseases in the world and is responsible for a large proportion of infant deaths. Toll-like receptors (TLRs), key components of innate immunity, are central to countering infection. Variants in the TLR-signaling pathway are associated with susceptibility to infectious diseases. **Methods.** We genotyped single nucleotide polymorphisms (SNPs) of the genes associated with the TLR-signaling pathway in patients with mild malaria and individuals with asymptomatic Plasmodium infections by means of polymerase chain reaction. **Results.** Genotype distributions for the TLR-1 I602S differed significantly between patients with mild malaria and persons with asymptomatic infection. The TLR-1 602S allele was associated with an odds ratio (OR) of 2.2 ([Formula: see text]; [Formula: see text]) for malaria among patients with mild malaria due to any Plasmodium species and 2.1 ([Formula: see text]; [Formula: see text]) among patients with mild malaria due to Plasmodium falciparum only. The TLR-6 S249P SNP showed an excess of homozygotes for the TLR-6 249P allele in asymptomatic persons, compared with patients with mild malaria due to any Plasmodium species (OR 2.1; 95% confidence interval [CI], 1.1-4.2; [Formula: see text]; [Formula: see text]), suggesting that the TLR-6 249S allele may be a risk factor for malaria (OR, 2.0; 95% CI, 1.1-3.7; [Formula: see text]; [Formula: see text]). The TLR-9 -1486C allele showed a strong association with high parasitemia ([Formula: see text]). **Conclusions.** Our findings indicate that the TLR-1 and TLR-6 variants are significantly associated with mild malaria, whereas the TLR-9-1486C/T variants are associated with high parasitemia. These discoveries may bring additional understanding to the pathogenesis of malaria.

J Infect Dis. 2008 Jul 15;198(2):163-6.

Decreased susceptibility to Plasmodium falciparum infection in pregnant women with iron deficiency.

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Iron plus folate supplementation increases mortality and morbidity among children in areas of malaria endemicity in Africa, but the effects of supplementation on pregnant women in malaria-endemic areas remain unclear. In northeastern Tanzania, where malaria and iron deficiency are common, we found that placental malaria was less prevalent (8.5% vs. 47.3% of women; $P < .0001$) and less severe (median parasite density, 4.2% vs. 6.3% of placental red blood cells; $P < .04$) among women with iron deficiency than among women with sufficient iron stores, especially during the first pregnancy. Multivariate analysis revealed that iron deficiency ($P < .0001$) and multigravidity ($P < .002$) significantly decreased the risk of placental malaria. Interventional trials of iron and folate supplementation during pregnancy in malaria-endemic regions in Africa are urgently needed to ascertain the benefits and risks of this intervention.

24 - *J Mol Biol.* 2008 Jul 16.

Active Transcription is Required for Maintenance of Epigenetic Memory in the Malaria Parasite Plasmodium falciparum.

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The most severe form of human malaria is caused by the protozoan parasite *Plasmodium falciparum*. The primary antigenic and virulence determinant expressed on the surface of infected red blood cells is PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), a protein that mediates adhesion and sequestration of the parasites in deep tissue vascular beds. Different forms of PfEMP1 are encoded by different members of the multicopy var gene family. Expression of var genes is mutually exclusive, and by switching which gene is expressed, parasites alter both their antigenic and virulence phenotypes. Regulation of var gene expression involves gene activation, silencing, and cellular memory, and the details of the mechanisms that control this process are not understood. Here, we provide evidence that active transcription is required for the maintenance of the cellular memory that marks a specific var gene to be stably expressed through numerous cell cycles. Forcing transfected parasites to express increasing numbers of unregulated episomal var promoters led to a corresponding down-regulation of the active var gene in the parasite's genome, presumably by competing for the transcriptional machinery of the parasite and suggesting the existence of a limited nuclear factor that is required for var gene activation. This process allowed us to repress transcription of the active var gene without acting through the mechanism that controls mutually exclusive expression and, thus, to investigate the role of transcription itself in maintaining epigenetic memory. When the competing episomes were removed, the parasites did not return to their previous var gene expression pattern, but rather displayed random var gene activation, demonstrating that the epigenetic imprint that controls var gene expression had been completely erased and, thus, linking active transcription to the maintenance of cellular memory.

25 - *J Mol Med.* 2008 Jul 19.

A central role for free heme in the pathogenesis of severe malaria: the missing link?

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Malaria, the disease caused by *Plasmodium* infection, is endemic to poverty in so-called underdeveloped countries. *Plasmodium falciparum*, the main infectious *Plasmodium* species in sub-Saharan countries, can trigger the development of severe malaria, including cerebral malaria, a neurological syndrome that claims the lives of more than one million children (<5 years old) per year. Attempts to eradicate *Plasmodium* infection, and in particular its lethal outcomes, have so far been unsuccessful. Using well-established rodent models of malaria infection, we found that survival of a *Plasmodium*-infected host is strictly dependent on the host's ability to up-regulate the expression of heme oxygenase-1 (HO-1 encoded by the gene *Hmox1*). HO-1 is a stress-responsive enzyme that catabolizes free heme into biliverdin, via a reaction that releases Fe and generates the gas carbon monoxide (CO). Generation of CO through heme catabolism by HO-1 prevents the onset of cerebral malaria. The protective effect of CO is mediated via its binding to cell-free hemoglobin (Hb) released from infected red blood cells during the blood stage of *Plasmodium* infection. Binding of CO to cell-free Hb prevents heme release and thus generation of free heme, which we found to play a central role in the pathogenesis of cerebral malaria. We will address hereby how defense mechanisms that prevent the deleterious effects of free heme, including the expression of HO-1, impact on the pathologic outcome of *Plasmodium* infection and how these may be used therapeutically to suppress its lethal outcomes.

26 - *Lancet.* 2008 Jul 12

Effect of intermittent preventive treatment of malaria on health and education in schoolchildren: a cluster-randomised, double-blind, placebo-controlled trial.

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BACKGROUND: Malaria is a major cause of morbidity and mortality in early childhood, yet its consequences for health and education during the school-age years remain poorly understood. We examined the effect of intermittent preventive treatment (IPT) in reducing anaemia and improving classroom attention and educational achievement in semi-immune schoolchildren in an area of high perennial transmission. **METHODS:** A stratified, cluster-randomised, double-blind, placebo-controlled trial of IPT was done in 30 primary schools in western Kenya. Schools were randomly assigned to treatment (sulfadoxine-pyrimethamine in combination with amodiaquine or dual placebo) by use of a computer-generated list. Children aged 5-18 years received three treatments at 4-month intervals (IPT n=3535, placebo n=3223). The primary endpoint was the prevalence of anaemia, defined as a haemoglobin concentration below 110 g/L. This outcome was assessed through cross-sectional surveys 12 months post-intervention. Analysis was by both intention to treat, excluding children with missing data, and per protocol. This study is registered with ClinicalTrials.gov, number NCT00142246. **FINDINGS:** 2604 children in the IPT group and 2302 in the placebo group were included in the intention-to-treat analysis of the primary outcome; the main reason for exclusion was loss to follow-up. Prevalence of anaemia at 12 months averaged 6.3% in the IPT group and 12.6% in the placebo group (adjusted risk ratio 0.52, 95% CI 0.29-0.93; p=0.028). Significant improvements were also seen in two of the class-based tests of sustained attention, with a mean increase in code transmission test score of 6.05 (95% CI 2.83-9.27; p=0.0007) and counting sounds test score of 1.80 (0.19-3.41; p=0.03), compared with controls. No effect was shown for inattentive or hyperactive-compulsive behaviours or on educational achievement. The per-protocol analysis yielded similar results. 23 serious adverse events were reported within 28 days of any treatment (19 in the IPT group and four in the placebo group); the main side-effects were problems of balance, dizziness, feeling faint, nausea, and/or vomiting shortly after treatment. **INTERPRETATION:** IPT of malaria improves the health and cognitive ability of semi-immune schoolchildren. Effective malaria interventions could be a valuable addition to school health programmes.

27 - *Malar J.* 2008 Jul 31;7(1):146.

Malaria vector control practices in an irrigated rice agro-ecosystem in central Kenya and implications for malaria control.

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ABSTRACT: **BACKGROUND:** Malaria transmission in most agricultural ecosystems is complex and hence the need for developing a holistic malaria control strategy with adequate consideration of socio-economic factors driving transmission at community level. A cross-sectional household survey was conducted in an irrigated ecosystem with the aim of investigating vector control practices applied and factors affecting their application both at household and community level. **METHODS:** Four villages representing the socio-economic, demographic and geographical diversity within the study area were purposefully selected. A total of 400 households were randomly sampled from the four study villages. Both semi-structured questionnaires and focus group discussions were used to gather both qualitative and quantitative data. **RESULTS:** The results showed that malaria was perceived to be a major public health problem in the area and the role of the vector *Anopheles* mosquitoes in malaria transmission was generally recognized. More than 80% of respondents were aware of the major breeding sites of the vector. Reported personal protection methods applied to prevent mosquito bites

included; use of treated bed nets (57%), untreated bed nets (35%), insecticide coils (21%), traditional methods such as burning of cow dung (8%), insecticide sprays (6%), and use of skin repellents (2%). However, 39% of respondents could not apply some of the known vector control methods due to unaffordability (50.5%), side effects (19.9%), perceived lack of effectiveness (16%), and lack of time to apply (2.6%). Lack of time was the main reason (56.3%) reported for non-application of environmental management practices, such as draining of stagnant water (77%) and clearing of vegetations along water canals (67%). CONCLUSION: The study provides relevant information necessary for the management, prevention and control of malaria in irrigated agro-ecosystems, where vectors of malaria are abundant and disease transmission is stable.

Malar J. 2008 Jul 30;7(1):145.

Utilization of insecticide-treated nets by under-five children in Nigeria: Assessing progress towards the Abuja targets.

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ABSTRACT: BACKGROUND: The Abuja target of increasing the proportion of people sleeping under insecticide-treated nets (ITNs) to 60% by the year 2005, as one of the measures for malaria control in Africa, has generated an influx of resources for malaria control in several countries in the region. A national household survey conducted in 2005 by the Malaria Control Programme in Nigeria assessed the progress made with respect to ITN ownership and use among pregnant women and children under five years of age since 2000. The survey was the first nationally representative study of ITN use assessing progress towards the Abuja target amongst vulnerable groups. Population and Method A cross-sectional survey of a sample of 7,200 households, selected by a multistage stratified sampling technique from 12 randomly selected states from the six geopolitical zones of the country. Data collection was done during the malarious rainy season (October 2005) using a modified WHO Malaria Indicator Survey structured questionnaire about household ownership and utilization of mosquito nets (treated or untreated) from household heads. RESULTS: Household ownership of any net was 23.9% (95% CI, 22.8%-25.1%) and 10.1% for ITNs (95% CI, 9.2%-10.9%). Education, wealth index, presence of an under-five child in the household, family size, residence, and region by residence were predictive of ownership of any net. The presence of an under-five child in the household, family size, education, presence of health facility in the community, gender of household head, region by residence and wealth index by education predicted ITN ownership. Utilization of any net by children under-five was 11.5% (95% CI, 10.4%-12.6%) and 1.7% (95% CI, 1.3%-2.2%) for ITN. Predictors of use of any net among under-five children were fever in the previous two weeks, presence of health facility in the community, caregiver's education, residence, and wealth index by caregiver's education; while religion, presence of health facility and wealth index by caregiver's education predicted the use of ITN among this group. CONCLUSION: This study demonstrated that the substantial increase in ITN utilization among children under five years of age in Nigeria is still far from the Abuja targets.

Malar J. 2008 Jul 30;7(1):144.

Evaluation of the genetic polymorphism of Plasmodium falciparum P126 protein (SERA or SERP) and its influence on naturally acquired specific antibody responses in malaria-infected individuals living in the Brazilian Amazon.

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ABSTRACT: BACKGROUND: The Plasmodium falciparum P126 protein is an asexual blood-stage malaria vaccine candidate antigen. Antibodies against P126 are able

to inhibit parasite growth in vitro, and a major parasite-inhibitory epitope has been recently mapped to its 47 kDa N-terminal extremity (octamer repeat domain - OR domain). The OR domain basically consists of six octamer units, but variation in the sequence and number of repeat units may appear in different alleles. The aim of the present study was to investigate the polymorphism of P126 N-terminal region OR domain in *P. falciparum* isolates from two Brazilian malaria endemic areas and its impact on anti-OR naturally acquired antibodies. METHODS: The study was carried out in two villages, Candeias do Jamari (Rondonia state) and Peixoto de Azevedo (Mato Grosso state), both located in the south-western part of the Amazon region. The repetitive region of the gene encoding the P126 antigen was PCR amplified and sequenced with the di-deoxy chain termination procedure. The antibody response was evaluated by ELISA with the Nt47 synthetic peptide corresponding to the P126 OR-II domain. RESULTS: Only two types of OR fragments were identified in the studied areas, one of 175 bp (OR-I) and other of 199 bp (OR-II). A predominance of the OR-II fragment was observed in Candeias do Jamari whereas in Peixoto de Azevedo both fragments OR-I and OR-II were frequent as well as mixed infection (both fragments simultaneously) reported here for the first time. Comparing the DNA sequencing of OR-I and OR-II fragments, there was a high conservation among predicted amino acid sequences of the P126 N-terminal extremity. Data of immune response demonstrated that the OR domain is highly immunogenic in natural conditions of exposure and that the polymorphism of the OR domain does not apparently influence the specific immune response. CONCLUSIONS: These findings confirm a limited genetic polymorphism of the P126 OR domain in *P. falciparum* isolates and that this limited genetic polymorphism does not seem to influence the development of a specific humoral immune response to P126 and its immunogenicity in the studied population.

Malar J. 2008 Jul 29;7(1):143.

Early home treatment of childhood fevers with ineffective antimalarials is deleterious in the outcome of severe malaria.

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ABSTRACT: BACKGROUND: Early diagnosis and prompt treatment including appropriate home-based treatment of malaria is a major strategy for malaria control. A major determinant of clinical outcome in case management, is compliance and adherence to effective antimalarial regimen. Home-based malaria treatment with inappropriate medicines is ineffective and there is insufficient evidence on how this contributes to the outcome of severe malaria. This study evaluated the effects of pre-hospital antimalarial drugs use on the presentation and outcome of severe malaria in children in Ibadan, Nigeria. METHODS: Two hundred and sixty-eight children with a median age of 30 months comprising 114 children with cerebral malaria and 154 with severe malarial anaemia (as defined by WHO) were prospectively enrolled. Data on socio-demographic data, treatments given at home, clinical course and outcome of admission were collected and analysed. RESULTS: A total of 168 children had treatment with an antimalarial treatment at home before presenting at the hospital when there was no improvement. There were no significant differences in the haematocrit levels, parasite counts and nutritional status of the pre-hospital treated and untreated groups. The most commonly used antimalarial medicine was chloroquine. Treatment policy was revised to Artemisinin-based Combination Therapy (ACT) in 2005 as a response to unacceptable levels of therapeutic failures with chloroquine, however chloroquine use remains high. The risk of presenting as cerebral malaria was 1.63 times higher with pre-hospital use of chloroquine for treatment of malaria, with a four-fold increase in the risk of mortality. Controlling for other confounding factors including age and clinical severity, pre-hospital treatment with chloroquine was an independent predictor of mortality. CONCLUSION: This study showed that, home treatment with chloroquine significantly impacts on the outcome of severe malaria. This finding underscores the need for wide-scale monitoring to withdraw chloroquine from circulation in Nigeria and efforts intensified at

promoting prompt treatment with effective medicines in the community.

Malar J. 2008 Jul 29;7(1):142.

Cohort study of the association of antibody levels to AMA1, MSP119, MSP3 and GLURP with protection from clinical malaria in Ghanaian children.

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ABSTRACT: BACKGROUND: Antigen-specific antibody-mediated immune responses play an important role in natural protection against clinical malaria, but conflicting estimates of this association have emerged from immuno-epidemiological studies in different geographical settings. This study was aimed at assessing in a standardized manner the relationship between the antibody responses to four malaria vaccine candidate antigens and protection from clinical malaria, in a cohort of Ghanaian children. **METHODS:** Standardized ELISA protocols were used to measure isotype and IgG subclass levels to Apical Membrane Antigen 1 (AMA1), Merozoite Surface Protein 1-19 (MSP119), Merozoite Surface Protein 3 (MSP3) and Glutamate Rich Protein (GLURP) antigens in plasma samples from 352 Ghanaian children, aged three to 10 years with subsequent malaria surveillance for nine months. This is one of a series of studies in different epidemiological settings using the same standardized ELISA protocols to permit comparisons of results from different laboratories. **RESULTS:** The incidence rate of malaria was 0.35 episodes per child per year. Isotype and IgG subclasses for all antigens investigated increased with age, while the risk of malaria decreased with age. After adjusting for age, higher levels of IgG to GLURP, MSP119, MSP3 and IgM to MSP119, MSP3 and AMA1 were associated with decreased malaria incidence. Of the IgG subclasses, only IgG1 to MSP119 was associated with reduced incidence of clinical malaria. A previous study in the same location failed to find an association of antibodies to MSP119 with clinical malaria. The disagreement may be due to differences in reagents, ELISA and analytical procedures used in the two studies. When IgG, IgM and IgG subclass levels for all four antigens were included in a combined model, only IgG1 [(0.80 (0.67-0.97), p=0.018)] and IgM [(0.48 (0.32-0.72), p<0.001)] to MSP119 were independently associated with protection from malaria. **CONCLUSION:** Using standardized procedures, the study has confirmed the importance of antibodies to MSP119 in reducing the risk of clinical malaria in Ghanaian children, thus substantiating its potential as a malaria vaccine candidate.

Malar J. 2008 Jul 26;7(1):141.

A physiological time analysis of the duration of the gonotrophic cycle of Anopheles pseudopunctipennis and its implications for malaria transmission in Bolivia.

Lardeux FJ, Tejerina RH, Quispe V, Chavez TK.

ABSTRACT: BACKGROUND: The length of the gonotrophic cycle varies the vectorial capacity of a mosquito vector and therefore its exact estimation is important in epidemiological modelling. Because the gonotrophic cycle length depends on temperature, its estimation can be satisfactorily computed by means of physiological time analysis. **METHODS:** A model of physiological time was developed and calibrated for *Anopheles pseudopunctipennis*, one of the main malaria vectors in South America, using data from laboratory temperature controlled experiments. The model was validated under varying temperatures and could predict the time elapsed from blood engorgement to oviposition according to the temperature. **RESULTS:** In laboratory experiments, a batch of *An. pseudopunctipennis* fed at the same time may lay eggs during several consecutive nights (2-3 at high temperature and >10 at low temperature). The model took into account such pattern and was used to predict the range of the gonotrophic cycle duration of *An. pseudopunctipennis* in four characteristic sites of Bolivia. It showed that the

predicted cycle duration for *An. pseudopunctipennis* exhibited a seasonal pattern, with higher variances where climatic conditions were less stable. Predicted mean values of the (minimum) duration ranged from 3.3 days up to >10 days, depending on the season and the geographical location. The analysis of ovaries development stages of field collected biting mosquitoes indicated that the phase 1 of Beklemishev might be of significant duration for *An. pseudopunctipennis*. The gonotrophic cycle length of *An. pseudopunctipennis* correlates with malaria transmission patterns observed in Bolivia which depend on locations and seasons. CONCLUSIONS: A new presentation of cycle length results taking into account the number of ovipositing nights and the proportion of mosquitoes laying eggs is suggested. The present approach using physiological time analysis might serve as an outline to other similar studies and allows the inclusion of temperature effects on the gonotrophic cycle in transmission models. However, to better explore the effects of temperature on malaria transmission, the others parameters of the vectorial capacity should be included in the analysis and modelled accordingly.

Malar J. 2008 Jul 24;7(1):140.

Epochal changes in the association between malaria epidemics and El Nino in Sri Lanka.

Zubair L, Galappaththy GN, Yang H, Chandimala J, Yahiya Z, Amerasinghe P, Ward N, Connor SJ.

ABSTRACT: BACKGROUND: El Nino events were suggested as a potential predictor for malaria epidemics in Sri Lanka based on the coincidence of nine out of 16 epidemics with El Nino events from 1870 to 1945. Here the potential for the use of El Nino predictions to anticipate epidemics was examined using enhanced climatic and epidemiological data from 1870 to 2000. METHODS: The epidemics start years were identified by the National Malaria Control Programme and verified against epidemiological records for consistency. Monthly average rainfall climatologies were estimated for epidemic and non-epidemic years; as well El Nino, Neutral and La Nina climatic phases. The relationship between El Nino indices and epidemics was examined to identify 'epochs' of consistent association. The statistical significance of the association between El Nino and epidemics for different epochs was characterized. The changes in the rainfall-El Nino relationships over the decade were examined using running windowed correlations. The anomalies in rainfall climatology during El Nino events for different epochs were compared. RESULTS: The relationship between El Nino and epidemics from 1870 to 1927 was confirmed. The anomalies in monthly average rainfall during El Nino events resembled the anomalies in monthly average rainfall during epidemics during this period. However, the relationship between El Nino and epidemics broke down from 1928 to 1980. Of the three epidemics in these six decades, only one coincided with an El Nino. Not only did this relationship breakdown but epidemics were more likely to occur in periods with a La Nina tendency. After 1980, three of four epidemics coincided with El Nino. CONCLUSIONS: The breakdown of the association between El Nino and epidemics after 1928 is likely due to an epochal change in the El Nino-rainfall relationship in Sri Lanka around the 1930's. It is unlikely that this breakdown is due to the insecticide spraying programme that began in 1945 since the breakdown started in 1928. Nor does it explain the occurrence of epidemics during La Nina phase from 1928 to 1980. Although there has been renewed coincidence with El Nino after 1980, this record is too short for establishing a reliable relationship.

Malar J. 2008 Jul 23;7(1):138.

Efficacy of local neem extracts for sustainable malaria vector control in an African village.

Gianotti RL, Bomblies A, Dafalla M, Issa-Arzika I, Duchemin JB, Eltahir EA.

ABSTRACT: BACKGROUND: Larval control of malaria vectors has been historically successful in reducing malaria transmission, but largely fell out of favour with the introduction of synthetic insecticides and bed nets. However, an integrated approach to malaria control, including larval control methods, continues to be the best chance for success, in view of insecticide resistance, the behavioural adaptation of the vectors to changing environments and the difficulties of reaching the poorest populations most at risk. Laboratory studies investigating the effects of neem seed (*Azadirachta indica*) extracts on *Anopheles* larvae have shown high rates of larval mortality and reductions in adult longevity, as well as low potential for resistance development. **METHODS:** This paper describes a method whereby seeds of the neem tree can be used to reduce adult *Anopheles gambiae* s.l. abundance in a way that is low cost and can be implemented by residents of rural villages in western Niger. The study was conducted in Banizoumbou village, western Niger. Neem seeds were collected from around the village. Dried seeds were ground into a coarse powder, which was then sprinkled onto known *Anopheles* larvae breeding habitats twice weekly during the rainy season 2007. Adult mosquitoes were captured on a weekly basis in the village and captures compared to those from 2005 and 2006 over the same period. Adult mosquitoes were also captured in a nearby village, Zindarou, as a control data set and compared to those from Banizoumbou. **RESULTS:** It was found that twice-weekly applications of the powder to known breeding habitats of *Anopheles* larvae in 2007 resulted in 49% fewer adult female *Anopheles gambiae* s.l. mosquitoes in Banizoumbou, compared with previous captures under similar environmental conditions and with similar habitat characteristics in 2005 and 2006. The productivity of the system in 2007 was found to be suppressed compared to the mean behaviour of 2005 and 2006 in Banizoumbou, whereas no change was found in Zindarou. **CONCLUSIONS:** With a high abundance of neem plants in many villages in this area, the results of this study suggest that larval control using neem seed powder offers a sustainable additional tool for malaria vector control in the Sahel region of Niger.

Malar J. 2008 Jul 22;7(1):135.

Prospects, achievements, challenges and opportunities for scaling-up malaria chemoprevention in pregnancy in Tanzania: the perspective of national level officers.

Mubyazi GM, Bygbjerg IC, Magnussen P, Olsen O, Byskov J, Hansen KS, Bloch P.

ABSTRACT: Objectives To describe the prospects, achievements, challenges and opportunities for implementing intermittent preventive treatment for malaria in pregnancy (IPTp) in Tanzania in light of national antenatal care (ANC) guidelines and ability of service providers to comply with them. **METHODS:** In-depth interviews were made with national level malaria control officers in 2006 and 2007. Data was analysed manually using a qualitative content analysis approach. **RESULTS:** IPTp has been under implementation countrywide since 2001 and the 2005 evaluation report showed increased coverage of women taking two doses of IPTp from 29% to 65% between 2001 and 2007. This achievement was acknowledged, however, several challenges were noted including (i) the national antenatal care (ANC) guidelines emphasizing two IPTp doses during a woman's pregnancy, while other agencies operating at district level were recommending three doses, this confuses frontline health workers (HWs); (ii) focused ANC guidelines have been revised, but printing and distribution to districts has often been delayed; (iii) reports from district management teams demonstrate constraints related to women's late booking, understaffing, inadequate skills of most HWs and their poor motivation. Other problems were unreliable supply of free SP at private clinics, clean and safe water shortage at many government ANC clinics limiting direct observation treatment and occasionally pregnant women asked to pay for ANC services. Finally, supervision of peripheral health facilities has been inadequate and national guidelines on district budgeting for health services have

been inflexible. IPTp coverage is generally low partly because IPTp is not systematically enforced like programmes on immunization, tuberculosis, leprosy and other infectious diseases. Necessary concerted efforts towards fostering uptake and coverage of two IPTp doses were emphasized by the national level officers, who called for further action including operational health systems research to understand challenges and suggest ways forward for effective implementation and high coverage of IPTp. CONCLUSION: The benefit of IPTp is appreciated by national level officers who are encouraged by trends in the coverage of IPTp doses. However, their appeal for concerted efforts towards IPTp scaling-up through rectifying the systemic constraints and operational research is important and supported by suggestions by other authors.

Malar J. 2008 Jul 22;7(1):134.

The impact of HIV-1 on the malaria parasite biomass in adults in sub-Saharan Africa contributes to the emergence of antimalarial drug resistance.

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ABSTRACT: BACKGROUND: HIV-related immune-suppression increases the risk of malaria (infection, disease and treatment failure) and probably the circulating parasite biomass, favouring the emergence of drug-resistant parasites. METHODS: The additional malaria parasite biomass related to HIV-1 co-infection in sub-Saharan Africa was estimated by a mathematical model. Parasite biomass was computed as the incidence rate of clinical malaria episodes multiplied by the number of parasites circulating in the peripheral blood of patients at the time symptoms appear. A mathematical model estimated the influence of HIV-1 infection on parasite density in clinical malaria by country and by age group, malaria transmission intensity and urban/rural area. In a multivariate sensitivity analysis, 95% confidence intervals (CIs) were calculated using the Monte Carlo simulation. RESULTS: The model shows that in 2005 HIV-1 increased the overall malaria parasite biomass by 18.0 % (95%CI: 11.6-26.9). The largest relative increase (134.9-243.9%) was found in southern Africa where HIV-1 prevalence is the highest and malaria transmission unstable. The largest absolute increase was found in Zambia, Malawi, the Central African Republic and Mozambique, where both malaria and HIV are highly endemic. A univariate sensitivity analysis shows that estimates are sensitive to the magnitude of the impact of HIV-1 infection on the malaria incidence rates and associated parasite densities. CONCLUSIONS: The HIV-1 epidemic by increasing the malaria parasite biomass in sub-Saharan Africa may also increase the emergence of antimalarial drug resistance, potentially affecting the health of the whole population in countries endemic for both HIV-1 and malaria.

Malar J. 2008 Jul 21;7(1):133.

Malaria in pregnant women in an area with sustained high coverage of insecticide-treated bed nets.

Kabanywany AM, Macarthur JR, Stolk WA, Habbema JD, Mshinda H, Bloland PB, Abdulla S, Kachur SP.

ABSTRACT: BACKGROUND: Since 2000, the World Health Organization has recommended a package of interventions to prevent malaria during pregnancy and its sequelae that includes the promotion of insecticide-treated bed nets (ITNs), intermittent preventive treatment in pregnancy (IPTp), and effective case management of malarial illness. It is recommended that pregnant women in malaria-endemic areas receive at least two doses of sulphadoxine-pyrimethamine in the second and third trimesters of pregnancy. This study assessed the prevalence of placental malaria at delivery in women during 1st or 2nd pregnancy, who did not receive intermittent preventive treatment for malaria (IPTp) in a malaria-endemic area with high bed net coverage. METHODS: A hospital-based cross-sectional study was

done in Ifakara, Tanzania, where bed net coverage is high. Primi- and secundigravid women, who presented to the labour ward and who reported not using IPTp were included in the study. Self-report data were collected by questionnaire; whereas neonatal birth weight and placenta parasitaemia were measured directly at the time of delivery. RESULTS: Overall, 413 pregnant women were enrolled of which 91% reported to have slept under a bed net at home the previous night, 43% reported history of fever and 62% were primigravid. Malaria parasites were detected in 8% of the placenta samples; the geometric mean (95%CI) placental parasite density was 3,457 (1,060-1,1271) parasites/ul in primigravid women and 2,178 (881-5,383) parasites/ul in secundigravid women. Fifteen percent of newborns weighed <2,500g at delivery. Self-reported bed net use was statistically associated with lower risk for low birth weight [OR 0.34 (95% CI: 0.16 - 0.74) and OR 0.22 (95% CI: 0.08 - 0.59) for untreated and treated bed nets, respectively], but was not associated with placental parasitaemia [OR 0.74 (0.21 - 2.68) and OR 1.64 (0.44 - 6.19) for untreated and treated bed nets, respectively]. CONCLUSION: The observed incidence of LBW and prevalence of placental parasitaemia at delivery suggests that malaria remains a problem in pregnancy in this area with high bed net coverage when eligible women do not receive IPTp. Delivery of IPTp should be emphasized at all levels of implementation to achieve maximum community coverage.

Malar J. 2008 Jul 16;7:132.

Presumptive treatment of fever cases as malaria: help or hindrance for malaria control?

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BACKGROUND: Malaria incidence has been reported to be falling in several countries in sub-Saharan Africa in recent years. This fall appears to have started before the widespread introduction of insecticide-treated nets. In the new era of calls to eliminate and eradicate malaria in sub-Saharan Africa, exploring possible causes for this fall seem pertinent. PRESENTATION OF THE HYPOTHESIS: The authors explore an argument that presumptive treatment of fever cases as malaria may have played a role in reducing transmission of malaria by the prophylactic effect of antimalarials and their widespread use. This strategy, which is already in practice is termed Opportunistic Presumptive Treatment (OPT). TESTING THE HYPOTHESIS: Further comparison of epidemiological indicators between areas with OPT and more targeted treatment is required. If data suggest a benefit of OPT, combining long acting antimalarials that have an anti-gametocytocidal activity component plus using high levels of vector control measures may reduce transmission, prevent resistant strains spreading and be easily implemented. IMPLICATIONS OF THE HYPOTHESIS: OPT is practised widely by presumptive treatment of fever in health facilities and home management of fever. Improving diagnosis using rapid diagnostic tests and thus reducing the number of doses of antimalarials given may have counter intuitive effects on transmission in the context of elimination of malaria in high to moderate transmission settings.

Malar J. 2008 Jul 15;7(1):131.

Competency of *Anopheles stephensi mysorensis* strain for *Plasmodium vivax* and the role of inhibitory carbohydrates to block its sporogonic cycle.

Basseri HR, Doosti S, Akbarzadeh K, Nategpour M, Whitten MM, Ladoni H.

ABSTRACT: BACKGROUND: Despite the abundance of studies conducted on the role of mosquitoes in malaria transmission, the biology and interaction of *Plasmodium* with its insect host still holds many mysteries. This paper provides the first

study to follow the sporogonic cycle of Plasmodium vivax in a wild insecticide-resistant mysorensis strain of Anopheles stephensi, a major vector of vivax malaria in south-eastern Iran. The study subsequently demonstrates that host-parasite sugar binding interactions are critical to the development of this parasite in the salivary glands of its mosquito host. The identity of the receptors or sugars involved was revealed by a receptor "pre-saturation" strategy in which sugars fed to the mosquitoes inhibited normal host-parasite interactions. METHODS: Anopheles stephensi mysorensis mosquitoes were artificially infected with P. vivax by feeding on the blood of gametocytaemic volunteers reporting to local malaria clinics in the Sistan-Beluchistan province of south-eastern Iran. In order to determine the inhibitory effect of carbohydrates on sporogonic development, vector mosquitoes were allowed to ingest blood meals containing both gametocytes and added carbohydrates. The carbohydrates tested were GlcNAc, GalNAc, arabinose, fucose, mannose, lactose, glucose and galactose. Sporogonic development was assessed by survival of the parasite at both the oocyst and sporozoite stages. RESULTS: Oocyst development was observed among nearly 6% of the fed control mosquitoes but the overall number of mosquitoes exhibiting sporozoite invasion of the salivary glands was 47.5% lower than the number supporting oocysts in their midgut. Of the tested carbohydrates, only arabinose and fucose slightly perturbed the development of P. vivax oocysts at the basal side of the mosquito midgut, and the remaining sugars caused no reductions in oocyst development. Strikingly however, sporozoites were completely absent from the salivary glands of mosquitoes treated with mannose, GalNAc, and lactose. CONCLUSION: The study indicates that An. stephensi in southern Iran has the potential to survive long enough to be re-infected and transmit vivax malaria several times, based on the average adult female longevity (about 30 days) and its gonotrophic cycle (2-3 days) during the malaria transmission season. Certain sugar binding interactions are important for the development of P. vivax sporozoites, and this information may be instrumental for the development of transmission blocking strategies.

Malar J. 2008 Jul 15;7:130.

MalHaploFreq: a computer programme for estimating malaria haplotype frequencies from blood samples.

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BACKGROUND: Molecular markers, particularly those associated with drug resistance, are important surveillance tools that can inform policy choice. People infected with falciparum malaria often contain several genetically-distinct clones of the parasite; genotyping the patients' blood reveals whether or not the marker is present (i.e. its prevalence), but does not reveal its frequency. For example a person with four malaria clones may contain both mutant and wildtype forms of a marker but it is not possible to distinguish the relative frequencies of the mutant and wildtypes i.e. 1:3, 2:2 or 3:1. METHODS: An appropriate method for obtaining frequencies from prevalence data is by Maximum Likelihood analysis. A computer programme has been developed that allows the frequency of markers, and haplotypes defined by up to three codons, to be estimated from blood phenotype data. RESULTS: The programme has been fully documented [see Additional File 1] and provided with a user-friendly interface suitable for large scale analyses. It returns accurate frequencies and 95% confidence intervals from simulated dataset sets and has been extensively tested on field data sets. CONCLUSION: The programme is included [see Additional File 2] and/or may be freely downloaded from 1. It can then be used to extract molecular marker and haplotype frequencies from their prevalence in human blood samples. This should enhance the use of frequency data to inform antimalarial drug policy choice.

Malar J. 2008 Jul 14;7:129.

Evaluation of immunoglobulin purification methods and their impact on quality and yield of antigen-specific antibodies.

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BACKGROUND: Antibodies are the main effectors against malaria blood-stage parasites. Evaluation of functional activities in immune sera from Phase 2a/b vaccine trials may provide invaluable information in the search for immune correlates of protection. However, the presence of anti-malarial-drugs, improper collection/storage conditions or concomitant immune responses against other pathogens can contribute to non-specific anti-parasite activities when the sera/plasma are tested in vitro. Purification of immunoglobulin is a standard approach for reducing such non-specific background activities, but the purification method itself can alter the quality and yield of recovered Ag-specific antibodies. METHODS: To address this concern, various immunoglobulin (Ig) purification methods (protein G Sepharose, protein A/G Sepharose, polyethylene glycol and caprylic acid-ammonium sulphate precipitation) were evaluated for their impact on the quality, quantity and functional activity of purified rabbit and human Igs. The recovered Igs were analysed for yield and purity by SDS-PAGE, for quality by Ag-specific ELISAs (determining changes in titer, avidity and isotype distribution) and for functional activity by in vitro parasite growth inhibition assay (GIA). RESULTS: This comparison demonstrated that overall polyethylene glycol purification of human serum/plasma samples and protein G Sepharose purification of rabbit sera are optimal for recovering functional Ag-specific antibodies. CONCLUSION: Consequently, critical consideration of the purification method is required to avoid selecting non-representative populations of recovered Ig, which could influence interpretations of vaccine efficacy, or affect the search for immune correlates of protection.

Malar J. 2008 Jul 14;7:128.

Performance of self-diagnosis and standby treatment of malaria in international oilfield service employees in the field.

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ABSTRACT: BACKGROUND: Falciparum malaria remains a major occupational illness that accounts for several deaths per year and numerous lost working days among the expatriate population, working or living in high-risk malarious areas. Compliance to preventive strategies is poor in travellers, especially business travellers, expatriates and long-term travellers. METHODS: In this cross-sectional, web-based study the adherence to and outcome of a preventive malaria programme on knowledge, attitudes and practices, including the practice of self-diagnosis and standby treatment (curative malaria kit, CMK) was evaluated in 2,350 non-immune expatriates, who had been working in highly malaria endemic areas. RESULTS: One-third (N = 648) of these expatriates visited a doctor for malaria symptoms and almost half (29 of 68) of all hospitalizations were due to malaria. The mandatory malaria training for non-immunes was completed by 92% of those who visited or worked in a high risk malaria country; 70% of the respondents at risk also received the CMK. The malaria awareness training and CMK significantly increased malaria knowledge [relative risk (RR) of 1.5, 95%CI

1.2-2.1], attitudes and practices, including compliance to chemoprophylaxis [RR = 2.2, 95%CI 1.6-3.2]. Hospitalization for malaria tended to be reduced by the programme [RR = 0.4, 95%CI 0.1-1.1], albeit not significantly. Respondents who did not receive instructions on the rapid diagnostic test were two times [RR = 2.3, 95%CI 1.6-3.3] more likely to have difficulties. Those who did receive instructions adhered poorly to the timing of repeating the test. Moreover, 6% (31 of 513) of those with a negative test result were diagnosed with malaria by a local doctor. 77% (N = 393) of the respondents with a negative test result did not take curative medication. 57% (252 of 441) of the respondents who took the curative medication that was included in the kit did not have a positive self-test or clinical malaria diagnosis made by a doctor. CONCLUSION: This survey demonstrated that a comprehensive programme targeting malaria prevention in expatriates can be effectively implemented and that it significantly increased malaria awareness.

Malar J. 2008 Jul 11;7:127.

Amodiaquine-artesunate vs artemether-lumefantrine for uncomplicated malaria in Ghanaian children: a randomized efficacy and safety trial with one year follow-up.

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BACKGROUND: Artesunate-amodiaquine (AS+AQ) and artemether-lumefantrine (AM-L) are efficacious artemisinin combination therapy (ACT) regimens that have been widely adopted in sub-Saharan Africa. However, there is little information on the efficacy of these regimens on subsequent episodes beyond 28 days, or on the safety of repeated treatments. METHODS: Children aged six months to 14 years with uncomplicated malaria were randomly assigned to treatment with AS+AQ (n = 116), or AM-L (n = 111). Recruited subjects were followed-up, initially for 28 days, and then monthly for up to one year. All subsequent attacks of uncomplicated malaria after 28 days were treated with the same regimen as at randomization. Investigations aimed at determining efficacy and side effects were conducted. RESULTS: Adequate clinical and parasitological response in subjects with evaluable end-points were, 97.1% (100/103) and 98.2% (107/109) on day 14, and 94.2% (97/103) and 95.3% (102/107) on day 28 in the AM-L and AS+AQ groups, respectively. Similar results were obtained after PCR correction. The incidence of malaria attacks in the year following recruitment was similar between the two treatment groups (p = 0.93). There was a high incidence of potentially AQ-resistant parasites in the study area. The incidence of adverse events, such as pruritus, fatigue and neutropaenia were similar in the two treatment groups. No patient showed signs of hearing impairment, and no abnormal neurological signs were observed during one year of follow-up. Other adverse events were mild in intensity and overlapped with known malaria symptomatology. No adverse event exacerbation was observed in any of the subjects who received multiple treatment courses with these ACT regimens during one year follow-up. CONCLUSION: AS+AQ and AM-L were efficacious for treatment of children with uncomplicated malaria in Ghana and drug-related adverse events were rare in treated subjects during one year of follow-up. The high prevalence of potentially AQ resistant parasites raises questions about the utility of AQ as a partner drug for ACT in Ghana. The efficacy of AS+AQ in Ghana requires, therefore, continuous monitoring and evaluation. TRIAL REGISTRATION: NCT 00406146 <http://www.clinicaltrials.gov>.

Malar J. 2008 Jul 11;7:126.

The usefulness of a new rapid diagnostic test, the First Response Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India.

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BACKGROUND: Malaria presents a diagnostic challenge in tribal belt of central India where two Plasmodium species, Plasmodium falciparum and Plasmodium vivax, are prevalent. In these areas, rapid detection of the malaria parasites and early treatment of infection remain the most important goals of disease management. Therefore, the usefulness of a new rapid diagnostic (RDT), the First Response(R) Combo Malaria Ag (pLDH/HRP2) card test was assessed for differential diagnosis between P. falciparum with other Plasmodium species in remote villages of Jabalpur district. **METHODS:** A finger prick blood sample was collected to prepare blood smear and for testing with the RDT after taking informed consent. The figures for sensitivity, specificity, accuracy and predictive values were calculated using microscopy as gold standard. **RESULTS:** Analysis revealed that overall, the RDT was 93% sensitive, 85% specific with a positive predictive value (PPV) of 79%, and a negative predictive value (NPV) of 95%. The accuracy 88% and J-index was 0.74. For P. falciparum, the sensitivity and specificity of the test were 96% and 95% respectively, with a PPV of 85% and a NPV of 99%. The RDT accuracy 95% and J-index was 0.84. For non-falciparum malaria, the sensitivity, specificity and accuracy were 83%, 94% and 92% respectively with a PPV of 69% and a NPV of 97%. **CONCLUSION:** The RDTs are easy to use, reliable and simple to interpret. RDTs are more suited to health workers in situations where health services are deficient or absent. Therefore, the test can be used as an epidemiological tool for the rapid screening of malaria.

Malar J. 2008 Jul 9;7:125.

Reduction of transmission from malaria patients by artemisinin combination therapies: a pooled analysis of six randomized trials.

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BACKGROUND: Artemisinin combination therapies (ACT), which are increasingly being introduced for treatment of Plasmodium falciparum malaria, are more effective against sexual stage parasites (gametocytes) than previous first-line antimalarials and therefore have the potential to reduce parasite transmission. The size of this effect is estimated in symptomatic P. falciparum infections. **METHODS:** Data on 3,174 patients were pooled from six antimalarial trials conducted in The Gambia and Kenya. Multivariable regression was used to investigate the role of ACT versus non-artemisinin antimalarial treatment, treatment failure, presence of pre-treatment gametocytes and submicroscopic gametocytaemia on transmission to mosquitoes and the area under the curve (AUC) of gametocyte density during the 28 days of follow up. **RESULTS:** ACT treatment was associated with a significant reduction in the probability of being gametocytaemic on the day of transmission experiments (OR 0.20 95% CI 0.16-0.26), transmission to mosquitoes by slide-positive gametocyte carriers (OR mosquito infection 0.49 95% CI 0.33-0.73) and AUC of gametocyte density (ratio of means 0.35 95% CI 0.31-0.41). Parasitological treatment failure did not account for the difference between ACT and non-artemisinin impact. The presence of slide-positive gametocytaemia prior to treatment significantly reduced ACT impact on

gametocytaemia ($p < 0.001$). Taking account of submicroscopic gametocytaemia reduced estimates of ACT impact in a high transmission setting in Kenya, but not in a lower transmission setting in the Gambia. CONCLUSION: Treatment with ACT significantly reduces infectiousness of individual patients with uncomplicated falciparum malaria compared to previous first line treatments. Rapid treatment of cases before gametocytaemia is well developed may enhance the impact of ACT on transmission.

Malar J. 2008 Jul 8;7(1):124.

Antimalarial drug use in general populations of tropical Africa.

Gardella F, Assi S, Simon F, Bogreau H, Eggelte T, Ba F, Foumane V, Henry MC, Traore Kientaga P, Basco L, Trape JF, Lalou R, Martelloni M, Desbordes M, Baragatti M, Briolant S, Almeras L, Pradines B, Fusai T, Rogier C.

ABSTRACT: BACKGROUND: The burden of Plasmodium falciparum malaria has worsened because of the emergence of chloroquine resistance. Antimalarial drug use and drug pressure are critical factors contributing to the selection and spread of resistance. The present study explores the geographical, socio-economic and behavioural factors associated with the use of antimalarial drugs in Africa. METHODS: The presence of chloroquine (CQ), pyrimethamine (PYR) and other antimalarial drugs has been evaluated by immuno-capture and high-performance liquid chromatography in the urine samples of 3,052 children (2-9y), randomly drawn in 2003 from the general populations at 30 sites in Senegal (10), Burkina-Faso (10) and Cameroon (10). Questionnaires have been administered to the parents of sampled children and to a random sample of households in each site. The presence of CQ in urine was analysed as dependent variable according to individual and site characteristics using a random - effect logistic regression model to take into account the interdependency of observations made within the same site. RESULTS: According to the sites, the prevalence rates of CQ and PYR ranged from 9% to 91% and from 0% to 21%, respectively. In multivariate analysis, the presence of CQ in urine was significantly associated with a history of fever during the three days preceding urine sampling (OR=1.22, $p = 0.043$), socio-economic level of the population of the sites (OR=2.74, $p = 0.029$), age (2-5y = reference level; 6-9y OR = 0.76, $p = 0.002$), prevalence of anti-circumsporozoite protein (CSP) antibodies (low prevalence : reference level; intermediate level OR = 2.47, $p = 0.023$), proportion of inhabitants who lived in another site one year before (OR= 2.53, $p = 0.003$), and duration to reach the nearest tarmacked road (duration less than one hour = reference level, duration equal to or more than one hour OR= 0.49, $p = 0.019$). CONCLUSIONS: Antimalarial drug pressure varied considerably from one site to another. It was significantly higher in areas with intermediate malaria transmission level and in the most accessible sites. Thus, P. falciparum strains arriving in cross-road sites or in areas with intermediate malaria transmission are exposed to higher drug pressure, which could favour the selection and the spread of drug resistance.

Malar J. 2008 Jul 8;7(1):123.

Impact of intermittent preventive treatment with sulphadoxine-pyrimethamine targeting the transmission season on the incidence of clinical malaria in children in Mali.

Dicko A, Sagara I, Sissoko MS, Guindo O, Diallo AI, Kone M, Toure OB, Sacko M, Doumbo OK.

ABSTRACT: BACKGROUND: Recent studies have shown that intermittent preventive malaria treatment (IPT) in infants in areas of stable malaria transmission reduces malaria and severe anaemia incidence. However in most areas malaria morbidity and mortality remain high in older children. METHODS: To evaluate the effect of seasonal IPT with sulphadoxine pyrimethamine (SP) on incidence of

malaria disease in area of seasonal transmission, 262 children 6 months-10 years in Kambila, Mali were randomized to receive either IPT with SP twice at eight weeks interval or no IPT during the transmission season of 2002 and were followed up for 12 months. Subjects were also followed during the subsequent transmission season in 2003 to assess possible rebound effect. Clinical malaria cases were treated with SP and followed to assess the in vivo response during both periods. RESULTS: The incidence rate of malaria disease per 1,000 person-months during the first 12 months was 3.2 episodes in the treatment group vs. 5.8 episodes in the control group with age-adjusted Protective Efficacy (PE) of 42.5%; [95% CI 28.6%-53.8%]. When the first 16 weeks of follow up is considered age-adjusted PE was 67.5% [95% CI 55.3% - 76.6%]. During the subsequent transmission season, the incidence of clinical malaria per 1000 persons-days was similar between the two groups (23.0 vs 21.5 episodes, age-adjusted IRR = 1.07 [95% CI, 0.90 - 1.27]). No significant difference was detected in in vivo response between the groups during both periods. CONCLUSIONS: Two malaria intermittent treatments targeting the peak transmission season reduced the annual incidence rate of clinical malaria by 42.5% in an area with intense seasonal transmission. This simple strategy is likely to be one of the most effectives in reducing malaria burden in such areas. Trial Registration: Clinicaltrials.gov NCT00623155.

Malar J. 2008 Jul 8;7:122.

No PfATPase6 S769N mutation found in Plasmodium falciparum isolates from China.

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BACKGROUND: Artemisinin and its derivatives have been used for falciparum malaria treatment in China since late 1970s. Monotherapy and uncontrolled use of artemisinin drugs were common practices for a long period of time. In vitro tests showed that the susceptibility of Plasmodium falciparum to artemisinins was declining in China. A concern was raised about the resistance to artemisinins of falciparum malaria in the country. It has been reported that in vitro artemisinin resistance was associated with the S769N mutation in the PfATPase6 gene. The main purpose of this study was to investigate whether that mutation has occurred in field isolates from China. METHODS: Plasmodium falciparum field isolates were collected in 2006-2007 from Hainan and Yunnan provinces, China. A nested PCR-sequencing assay was developed to analyse the genotype of the PfATPase6 S769N polymorphism in the P. falciparum field isolates. RESULTS: The genotyping results of six samples could not be obtained due to failure of PCR amplification, but no S769N mutation was detected in any of the 95 samples successfully analysed. CONCLUSION: The results indicate that the S769N mutation in the PfATPase6 gene is not present in China, suggesting that artemisinin resistance has not yet developed, but the situation needs to be watched very attentively.

28 - Microbes Infect. 2008 Jul 10.

Immune interactions in malaria co-infections with other endemic infectious diseases: implications for the development of improved disease interventions.

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Today targeted research efforts are in progress with the goal to develop vaccines, microbicides, new drugs and alternative treatments for some of the neglected infectious diseases (NIDs). Until now the world is far from having effective cures and/or prophylactic vaccines in place. People living in endemic

areas generally are more skewed towards a TH2 profile (i.e. anti-inflammatory) that could greatly affect the induction of an inflammatory TH1 type response needed to combat many infectious microorganisms. Despite this, very little is today known about how co-infections with NID can affect the outcome of the different diseases and the possibilities for prophylactic vaccination and treatment. Thus, if we are to intervene successfully to eradicate infections or prevent immune pathology either by vaccination or other immune intervention therapies it will be crucial to understand how co-infections with different pathogens affect the adaptive immunity and the establishment of immunological memory. The aim of this paper is to review what is known about co-infection with malaria and certain other pathogens.

29 - Mol Biochem Parasitol. 2008 Aug;160(2):107-15.

N-terminal processing of proteins exported by malaria parasites.

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Malaria parasites utilize a short N-terminal amino acid motif termed the Plasmodium export element (PEXEL) to export an array of proteins to the host erythrocyte during blood stage infection. Using immunoaffinity chromatography and mass spectrometry, insight into this signal-mediated trafficking mechanism was gained by discovering that the PEXEL motif is cleaved and N-acetylated. PfHRP2 and PfEMP2 are two soluble proteins exported by Plasmodium falciparum that were demonstrated to undergo PEXEL cleavage and N-acetylation, thus indicating that this N-terminal processing may be general to many exported soluble proteins. It was established that PEXEL processing occurs upstream of the brefeldin A-sensitive trafficking step in the P. falciparum secretory pathway, therefore cleavage and N-acetylation of the PEXEL motif occurs in the endoplasmic reticulum (ER) of the parasite. Furthermore, it was shown that the recognition of the processed N-terminus of exported proteins within the parasitophorous vacuole may be crucial for protein transport to the host erythrocyte. It appears that the PEXEL may be defined as a novel ER peptidase cleavage site and a classical N-acetyltransferase substrate sequence.

Mol Biochem Parasitol. 2008 Aug;160(2):81-9.

The RhopH complex is transferred to the host cell cytoplasm following red blood cell invasion by Plasmodium falciparum.

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The high-molecular mass rhoptry protein complex (PfRhopH), which comprises three distinct gene products, RhopH1, RhopH2, and RhopH3, is known to be secreted and transferred to the parasitophorous vacuole membrane upon invasion of a red blood cell by the malaria parasite Plasmodium falciparum. Here we show that the merozoite-acquired RhopH complex is also transferred to defined domains of the red blood cell cytoplasm, and possibly transiently associated with Maurer's clefts. This is the first report of trafficking in the host cell cytoplasm for P. falciparum rhoptry proteins secreted upon red blood cell invasion. Based on its newly identified sub-cellular location and the phenotype of RhopH1 mutants, we propose that the RhopH complex participate in the assembly of the cytoadherence complex.

Mol Biochem Parasitol. 2008 Aug;160(2):138-42.

Susceptibility of Plasmodium falciparum cyclic AMP-dependent protein kinase and its mammalian homologue to the inhibitors.

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Cyclic AMP-dependent protein kinase (protein kinase A, PKA) is a key element in many cell signaling pathways. An essential role of Plasmodium falciparum PKA (PfPKA) activity was reported in the intraerythrocytic growth of the malaria parasite. However, molecular characterization of PfPKA using purified recombinant proteins has not yet been performed. Here, we report the first successful purification of the enzymatically active PKA catalytic subunit of P. falciparum (PfPKA-C) using a wheat germ cell-free expression system. Interestingly, parasite enzymatic activity was weakly inhibited as compared with the inhibition of mammalian PKA catalytic subunit (PKA-C) by the specific PKA inhibitor, H89. Furthermore, PfPKA-C was only slightly inhibited by protein kinase inhibitor (PKI). These results suggest that substrate sites of PfPKA-C may be different from those of mammalian PKA-Cs. In addition, potential PKI corresponding to malarial PKA-C would also be different from those of mammalian cells.

Mol Biochem Parasitol. 2008 Aug;160(2):148-51.

Sex- and stage-specific reporter gene expression in Plasmodium falciparum.

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For malaria transmission, Plasmodium parasites must successfully complete gametocytogenesis in the vertebrate host. Differentiation into mature male or female Plasmodium falciparum gametocytes takes 9-12 days as the parasites pass through five distinct morphologic stages (I-V). To evaluate the signals controlling the initiation of stage- and/or sex-specific expression, reporter constructs containing the 5'-flanking regions (FR) of seven genes with distinct expression patterns through gametogenesis were developed. The regulatory information present in the 5'-FR of each selected gene was found to be sufficient to drive appropriate sex- and stage-specific reporter gene expression. The transformed parasite lines also provide in vivo markers to identify gametocytes at specific stages, including a subpopulation of schizonts that express early gametocyte markers.

Mol Biochem Parasitol. 2008 Aug;160(2):143-7.

In vivo studies support the role of trafficking and cytoskeletal-binding motifs in the interaction of MESA with the membrane skeleton of Plasmodium falciparum-infected red blood cells.

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In red blood cells (RBCs) infected with the malaria parasite Plasmodium falciparum, a 19-residue region of the mature parasite-infected erythrocyte surface antigen (MESA) associates with RBC cytoskeleton protein 4.1R; an interaction essential for parasite survival. This region in MESA is adjacent to a host targeting motif found in other malaria parasite proteins exported to the

membrane skeleton. To demonstrate function of these motifs in vivo, regions of MESA fused to a reporter were expressed in malaria parasites. Immunochemical analyses confirmed the requirement for both motifs in the trafficking and interaction of MESA with the cytoskeleton and demonstrates their function in vivo.

30 - Parasitol Res. 2008 Aug;103(3):625-31.

Diagnosis of vivax malaria using an IgM capture ELISA is a sensitive method, even for low levels of parasitemia.

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Although diagnosis of Plasmodium vivax malaria has been difficult when it is present at a low parasite density, it was recently revealed that an antibody assay was a good method of screening for malaria in blood banks. However, the use of this method for the diagnosis of malaria is limited due to the persistence of specific immunoglobulin (Ig) G. Therefore, we evaluated specific IgM antibody responses against the C-terminal region of the merozoite surface protein 1 of P. vivax (PvMSP1c) in sera obtained from patients with vivax malaria using various assays. The IgM capture enzyme-linked immunosorbent assay showed good sensitivity (97.7%; 308/315) and specificity (99.1%, 446/450). In addition, the results of this assay were not related to parasite density, and a high reactivity was observed when there was a low level of parasitemia. Furthermore, we found that patients with cases of malaria that had relapsed still had the IgM titers against PvMSP1c. Therefore, the use of IgM ELISA for the detection of specific IgM that was not involved in memorial immune activity could be an alternative tool for the diagnosis of malaria and blood screening, even in areas in which malaria is endemic.

Parasitol Res. 2008 Aug;103(3):691-5.

Studies on effect of Acalypha indica L. (Euphorbiaceae) leaf extracts on the malarial vector, Anopheles stephensi Liston (Diptera:Culicidae).

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The leaf extract of Acalypha indica with different solvents viz, benzene, chloroform, ethyl acetate and methanol were tested for larvicidal, ovicidal activity and oviposition attractancy against Anopheles stephensi. The larval mortality was observed after 24 h exposure. The LC(50) values are 19.25, 27.76, 23.26 and 15.03 ppm, respectively. Mean percent hatchability of the ovicidal activity was observed 120 h after treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. The highest effective attractancy of 90.09%, 94.20%, 85.43% and 95.75% were observed at 100 ppm concentration viz, benzene, chloroform, ethyl acetate and methanol, respectively. The lowest effective attractancy of 47.17%, 61.94%, 49.28% and 68.12% were observed at 25 ppm concentration viz, benzene, chloroform, ethyl acetate and methanol, respectively. The results that the leaf extract of A. indica is promising as larvicidal and ovicidal activity and oviposition attractancy against malaria vector A. stephensi.

Parasitol Res. 2008 Aug;103(3):529-34.

Laboratory evaluation of traditional insect/mosquito repellent plants against Anopheles arabiensis, the predominant malaria vector in Ethiopia.

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Laboratory study was carried out to evaluate the repellent efficiency of most commonly known four traditional insect/mosquito repellent plants Wogert [vernacular name (local native language, Amharic); *Silene macroserene*], Kebercho [vernacular name (local native language, Amharic); *Echinops* sp.], Tinjut [vernacular name (local native language, Amharic); *Ostostegia integrifolia*], and Woira [vernacular name (local native language, Amharic); *Olea europaea*] against *Anopheles arabiensis* under the laboratory conditions. One hundred (4-5 days old) female *A. arabiensis* were introduced into the both 'control' and 'test' repellent chamber through the hole on top. Traditional charcoal stoves were used for direct burning. The experiment was conducted by applying the smoke into the repellent "test" mosquito cage by direct burning of 25 gm of dried plant materials (leaves and roots) until plant materials completely burned. The number of mosquitoes driving away from the "test" and "control" cage was recorded for every 5 min. In the present investigation, the results clearly revealed that the roots of *S. macroserene* has potent repellent efficiency (93.61%) and was the most effective. The leaves of *Echinops* sp. (92.47%), leaves of *O. integrifolia* (90.10%) and *O. europaea* (79.78%) were also effective. Roots of *S. macroserene* exhibited the highest repellent efficiency by direct burning. The present study identified these four traditional indigenous insect/mosquito repellent plant materials are very promising and can be used as safer alternative to modern synthetic chemical repellents against mosquito vectors of disease. Since people have been using these plants for some medicinal purposes, no side effects have been found.

31 - *PLoS Med.* 2008 Jul 22;5(7):e142.

International funding for malaria control in relation to populations at risk of stable *Plasmodium falciparum* transmission.

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BACKGROUND: The international financing of malaria control has increased significantly in the last ten years in parallel with calls to halve the malaria burden by the year 2015. The allocation of funds to countries should reflect the size of the populations at risk of infection, disease, and death. To examine this relationship, we compare an audit of international commitments with an objective assessment of national need: the population at risk of stable *Plasmodium falciparum* malaria transmission in 2007. **METHODS AND FINDINGS:** The national distributions of populations at risk of stable *P. falciparum* transmission were projected to the year 2007 for each of 87 *P. falciparum*-endemic countries. Systematic online- and literature-based searches were conducted to audit the international funding commitments made for malaria control by major donors between 2002 and 2007. These figures were used to generate annual malaria funding allocation (in US dollars) per capita population at risk of stable *P. falciparum* in 2007. Almost US\$1 billion are distributed each year to the 1.4 billion people exposed to stable *P. falciparum* malaria risk. This is less than US\$1 per person at risk per year. Forty percent of this total comes from the Global Fund to Fight AIDS, Tuberculosis and Malaria. Substantial regional and national variations in disbursements exist. While the distribution of funds is found to be broadly appropriate, specific high population density countries receive disproportionately less support to scale up malaria control. Additionally, an inadequacy of current financial commitments by the international community was

found: under-funding could be from 50% to 450%, depending on which global assessment of the cost required to scale up malaria control is adopted. CONCLUSIONS: Without further increases in funding and appropriate targeting of global malaria control investment it is unlikely that international goals to halve disease burdens by 2015 will be achieved. Moreover, the additional financing requirements to move from malaria control to malaria elimination have not yet been considered by the scientific or international community.

32 - *PLoS ONE*. 2008 Jul 30;3(7):e2830.

Protection induced by Plasmodium falciparum MSP1(42) is strain-specific, antigen and adjuvant dependent, and correlates with antibody responses.

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Vaccination with Plasmodium falciparum MSP1(42)/complete Freund's adjuvant (FA) followed by MSP1(42)/incomplete FA is the only known regimen that protects Aotus nancymae monkeys against infection by erythrocytic stage malaria parasites. The role of adjuvant is not defined; however complete FA cannot be used in humans. In rodent models, immunity is strain-specific. We vaccinated Aotus monkeys with the FVO or 3D7 alleles of MSP1(42) expressed in Escherichia coli or with the FVO allele expressed in baculovirus (bv) combined with complete and incomplete FA, Montanide ISA-720 (ISA-720) or AS02A. Challenge with FVO strain P. falciparum showed that suppression of cumulative day 11 parasitemia was strain-specific and could be induced by E. coli expressed MSP1(42) in combination with FA or ISA-720 but not with AS02A. The coli42-FVO antigen induced a stronger protective effect than the bv42-FVO antigen, and FA induced a stronger protective effect than ISA-720. ELISA antibody (Ab) responses at day of challenge (DOC) were strain-specific and correlated inversely with c-day 11 parasitemia ($r = -0.843$). ELISA Ab levels at DOC meeting a titer of at least 115,000 ELISA Ab units identified the vaccinees not requiring treatment (noTx) with a true positive rate of 83.3% and false positive rate of 14.3%. Correlation between functional growth inhibitory Ab levels (GIA) and cumulative day 11 parasitemia was weaker ($r = -0.511$), and was not as predictive for a response of noTx. The lowest false positive rate for GIA was 30% when requiring a true positive rate of 83.3%. These inhibition results along with those showing that antigen/FA combinations induced a stronger protective immunity than antigen/ISA-720 or antigen/AS02 combinations are consistent with protection as ascribed to MSP1-specific cytophilic antibodies. Development of an effective MSP1(42) vaccine against erythrocytic stage P. falciparum infection will depend not only on antigen quality, but also upon the selection of an optimal adjuvant component.

PLoS ONE. 2008 Jul 23;3(7):e2775.

Effect of transmission setting and mixed species infections on clinical measures of malaria in Malawi.

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BACKGROUND: In malaria endemic regions people are commonly infected with multiple species of malaria parasites but the clinical impact of these Plasmodium co-infections is unclear. Differences in transmission seasonality and transmission intensity between endemic regions have been suggested as important

factors in determining the effect of multiple species co-infections. **PRINCIPAL FINDINGS:** In order to investigate the impact of multiple-species infections on clinical measures of malaria we carried out a cross-sectional community survey in Malawi, in 2002. We collected clinical and parasitological data from 2918 participants aged >6 months, and applied a questionnaire to measure malaria morbidity. We examined the effect of transmission seasonality and intensity on fever, history of fever, haemoglobin concentration ([Hb]) and parasite density, by comparing three regions: perennial transmission (PT), high intensity seasonal transmission (HIST) and low intensity seasonal transmission (LIST). These regions were defined using multi-level modelling of PCR prevalence data and spatial and geo-climatic measures. The three Plasmodium species (*P. falciparum*, *P. malariae* and *P. ovale*) were randomly distributed amongst all children but not adults in the LIST and PT regions. Mean parasite density in children was lower in the HIST compared with the other two regions. Mixed species infections had lower mean parasite density compared with single species infections in the PT region. Fever rates were similar between transmission regions and were unaffected by mixed species infections. A history of fever was associated with single species infections but only in the HIST region. Reduced mean [Hb] and increased anaemia was associated with perennial transmission compared to seasonal transmission. Children with mixed species infections had higher [Hb] in the HIST region. **CONCLUSIONS:** Our study suggests that the interaction of Plasmodium co-infecting species can have protective effects against some clinical outcomes of malaria but that this is dependent on the seasonality and intensity of malaria transmission.

PLoS ONE. 2008 Jul 16;3(7):e2727.

Antimalarial therapy selection for quinolone resistance among *Escherichia coli* in the absence of quinolone exposure, in tropical South America.

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BACKGROUND: Bacterial resistance to antibiotics is thought to develop only in the presence of antibiotic pressure. Here we show evidence to suggest that fluoroquinolone resistance in *Escherichia coli* has developed in the absence of fluoroquinolone use. **METHODS:** Over 4 years, outreach clinic attendees in one moderately remote and five very remote villages in rural Guyana were surveyed for the presence of rectal carriage of ciprofloxacin-resistant gram-negative bacilli (GNB). Drinking water was tested for the presence of resistant GNB by culture, and the presence of antibacterial agents and chloroquine by HPLC. The development of ciprofloxacin resistance in *E. coli* was examined after serial exposure to chloroquine. Patient and laboratory isolates of *E. coli* resistant to ciprofloxacin were assessed by PCR-sequencing for quinolone-resistance-determining-region (QRDR) mutations. **RESULTS:** In the very remote villages, 4.8% of patients carried ciprofloxacin-resistant *E. coli* with QRDR mutations despite no local availability of quinolones. However, there had been extensive local use of chloroquine, with higher prevalence of resistance seen in the villages shortly after a *Plasmodium vivax* epidemic ($p < 0.01$). Antibacterial agents were not found in the drinking water, but chloroquine was demonstrated to be present. Chloroquine was found to inhibit the growth of *E. coli* in vitro. Replica plating demonstrated that 2-step QRDR mutations could be induced in *E. coli* in response to chloroquine. **CONCLUSIONS:** In these remote communities, the heavy use of chloroquine to treat malaria likely selected for ciprofloxacin resistance in *E. coli*. This may be an important public health problem in malarious areas.

Genistein-supplemented diet decreases malaria liver infection in mice and constitutes a potential prophylactic strategy.

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In tropical regions millions of people still live at risk of malaria infection. Indeed the emergence of resistance to chloroquine and other drugs in use in these areas reinforces the need to implement alternative prophylactic strategies. Genistein is a naturally occurring compound that is widely used as a food supplement and is thought to be effective in countering several pathologies. Results presented here show that genistein inhibits liver infection by the Plasmodium parasite, the causative agent of malaria. In vitro, genistein decreased the infection rates of both mouse and human hepatoma cells by inhibiting the early stages of the parasite's intracellular development. Oral or intraperitoneal administration of genistein decreased the liver parasite load of P. berghei-infected mice. Moreover, mice fed on a genistein-supplemented diet showed a significant reduction in Plasmodium liver infection as well as a reduced blood parasitemia and partial protection from severe disease. Since genistein is a safe, low-cost, natural compound that can be used permanently in a diet, we propose its use as a prophylactic agent against malaria for endemic populations and long-time travelers.

PLoS ONE. 2008 Jul 16;3(7):e2661.

Modelling the epidemiological impact of intermittent preventive treatment against malaria in infants.

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BACKGROUND: Trials of intermittent preventive treatment against malaria in infants (IPTi) using sulphadoxine-pyrimethamine (SP) have shown a positive, albeit variable, protective efficacy against clinical malaria episodes. The impact of IPTi in different epidemiological settings and over time is unknown and predictions are hampered by the lack of knowledge about how IPTi works. We investigated mechanisms proposed for the action of IPTi and made predictions of the likely impact on morbidity and mortality. **METHODS/PRINCIPAL FINDINGS:** We used a comprehensive, individual-based, stochastic model of malaria epidemiology to simulate recently published trials of IPTi using SP with site-specific characteristics as inputs. This baseline model was then modified to represent hypotheses concerning the duration of action of SP, the temporal pattern of fevers caused by individual infections, potential benefits of avoiding fevers on immunity and the effect of sub-therapeutic levels of SP on parasite dynamics. The baseline model reproduced the pattern of results reasonably well. None of the models based on alternative hypotheses improved the fit between the model predictions and observed data. Predictions suggest that IPTi would have a beneficial effect across a range of transmission intensities. IPTi was predicted to avert a greater number of episodes where IPTi coverage was higher, the health system treatment coverage lower, and for drugs which were more efficacious and had longer prophylactic periods. The predicted cumulative benefits were proportionately slightly greater for severe malaria episodes and malaria-attributable mortality than for acute episodes in the settings modelled.

Modest increased susceptibility was predicted between doses and following the last dose, but these were outweighed by the cumulative benefits. The impact on transmission intensity was negligible. CONCLUSIONS: The pattern of trial results can be accounted for by differences between the trial sites together with known features of malaria epidemiology and the action of SP. Predictions suggest that IPTi would have a beneficial impact across a variety of epidemiological settings.

PLoS ONE. 2008 Jul 16;3(7):e2659.

Genetic immunisation by liver stage antigen 3 protects chimpanzees against malaria despite low immune responses.

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BACKGROUND: The true interest of genetic immunisation might have been hastily underestimated based on overall immunogenicity data in humans and lack of parallelism with other, more classical immunisation methods. PRINCIPAL FINDINGS: Using malaria Liver Stage Antigen-3 (LSA-3), we report that genetic immunization induces in chimpanzees, the closest relative of humans, immune responses which are as scarce as those reported using other DNA vaccines in humans, but which nonetheless confer strong, sterile and reproducible protection. The pattern was consistent in 3/4 immunized apes against two high dose sporozoite challenges performed as late as 98 and 238 days post-immunization and by a heterologous strain. CONCLUSIONS: These results should, in our opinion, lead to a revisiting of the value of this unusual means of immunisation, using as a model a disease, malaria, in which virulent challenges of volunteers are ethically acceptable.

PLoS ONE. 2008 Jul 9;3(7):e2636.

Phase 1 trial of malaria transmission blocking vaccine candidates Pfs25 and Pvs25 formulated with montanide ISA 51.

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BACKGROUND: Pfs25 and Pvs25, surface proteins of mosquito stage of the malaria parasites *P. falciparum* and *P. vivax*, respectively, are leading candidates for vaccines preventing malaria transmission by mosquitoes. This single blinded, dose escalating, controlled Phase 1 study assessed the safety and immunogenicity of recombinant Pfs25 and Pvs25 formulated with Montanide ISA 51, a water-in-oil emulsion. METHODOLOGY/PRINCIPAL FINDINGS: The trial was conducted at The Johns Hopkins Center for Immunization Research, Washington DC, USA, between May 16, 2005-April 30, 2007. The trial was designed to enroll 72 healthy male and non-pregnant female volunteers into 1 group to receive adjuvant control and 6 groups to receive escalating doses of the vaccines. Due to unexpected reactogenicity, the vaccination was halted and only 36 volunteers were enrolled into 4 groups: 3 groups of 10 volunteers each were immunized with 5 microg of Pfs25/ISA 51, 5 microg of Pvs25/ISA 51, or 20 microg of Pvs25/ISA 51, respectively. A fourth group of 6 volunteers received adjuvant control (PBS/ISA 51). Frequent local reactogenicity was observed. Systemic adverse events included two cases of erythema nodosum considered to be probably related to the combination of the antigen and the adjuvant. Significant antibody responses were detected in volunteers who completed the lowest scheduled doses of Pfs25/ISA 51. Serum anti-Pfs25 levels correlated with transmission blocking activity.

CONCLUSION/SIGNIFICANCE: It is feasible to induce transmission blocking immunity in humans using the Pfs25/ISA 51 vaccine, but these vaccines are unexpectedly reactogenic for further development. This is the first report that the formulation is associated with systemic adverse events including erythema nodosum. TRIAL REGISTRATION: ClinicalTrials.gov NCT00295581.

PLoS ONE. 2008 Jul 9;3(7):e2608.

Both functional LTbeta receptor and TNF receptor 2 are required for the development of experimental cerebral malaria.

Togbe D, de Sousa PL, Fauconnier M, Boissay V, Fick L, Scheu S, Pfeffer K, Menard R, Grau GE, Doan BT, Beloeil JC, Renia L, Hansen AM, Ball HJ, Hunt NH, Ryffel B, Quesniaux VF.

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BACKGROUND: TNF-related lymphotoxin alpha (LTalpha) is essential for the development of Plasmodium berghei ANKA (PbA)-induced experimental cerebral malaria (ECM). The pathway involved has been attributed to TNFR2. Here we show a second arm of LTalpha-signaling essential for ECM development through LTbeta-R, receptor of LTalphabeta2 heterotrimer. METHODOLOGY/PRINCIPAL FINDINGS: LTbetaR deficient mice did not develop the neurological signs seen in PbA induced ECM but died at three weeks with high parasitaemia and severe anemia like LTalphabeta deficient mice. Resistance of LTalphabeta or LTbetaR deficient mice correlated with unaltered cerebral microcirculation and absence of ischemia, as documented by magnetic resonance imaging and angiography, associated with lack of microvascular obstruction, while wild-type mice developed distinct microvascular pathology. Recruitment and activation of perforin(+) CD8(+) T cells, and their ICAM-1 expression were clearly attenuated in the brain of resistant mice. An essential contribution of LIGHT, another LTbetaR ligand, could be excluded, as LIGHT deficient mice rapidly succumbed to ECM. CONCLUSIONS/SIGNIFICANCE: LTbetaR expressed on radioresistant resident stromal, probably endothelial cells, rather than hematopoietic cells, are essential for the development of ECM, as assessed by hematopoietic reconstitution experiment. Therefore, the data suggest that both functional LTbetaR and TNFR2 signaling are required and non-redundant for the development of microvascular pathology resulting in fatal ECM.

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Antibodies targeting the PfRH1 binding domain inhibit invasion of Plasmodium falciparum merozoites.

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Invasion by the malaria merozoite depends on recognition of specific erythrocyte surface receptors by parasite ligands. Plasmodium falciparum uses multiple ligands, including at least two gene families, reticulocyte binding protein homologues (RBLs) and erythrocyte binding proteins/ligands (EBLs). The combination of different RBLs and EBLs expressed in a merozoite defines the invasion pathway utilized and could also play a role in parasite virulence. The binding regions of EBLs lie in a conserved cysteine-rich domain while the binding domain of RBL is still not well characterized. Here, we identify the erythrocyte binding region of the P. falciparum reticulocyte binding protein homologue 1 (PfRH1) and show that antibodies raised against the functional binding region efficiently inhibit invasion. In addition, we directly demonstrate that changes

in the expression of RBLs can constitute an immune evasion mechanism of the malaria merozoite.

34 - *Soc Sci Med.* 2008 Aug;67(4):487-96.

Determinants of provider choice for malaria treatment: Experiences from The Gambia.

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Malaria is responsible for an estimated one million deaths per year, the vast majority in sub-Saharan Africa. Many of these deaths are attributed to delays in seeking treatment and poor adherence to drug regimes. While there are a growing number of studies describing the factors influencing treatment seeking for malaria, far less is known about the relative weight given to these factors in different settings. This study estimates two models of demand for malaria treatment in the Farafenni region of The Gambia. The first examines the determinants of seeking malaria treatment outside the home versus no treatment or self-care while the second identifies the determinants of provider choice conditional on having decided to seek malaria treatment outside the home. Providers included hospital; health centre; and 'other' which included pharmacies, kiosks; petty traders; neighbours; and traditional healers. Results show that older people were more likely to opt for self-care, or no treatment. The longer the time spent ill or the more severe the fever, the more likely a treatment was sought outside the home. Time of the year and availability of community infrastructure played a key role in both models. Poorer households and those from the Fula ethnic group were much more likely to visit an 'other' provider than a hospital. The policy and methodological implications of these findings are discussed.

35 - *Trans R Soc Trop Med Hyg.* 2008 Aug;102(8):751-8.

Adherence and effectiveness of drug combination in curative treatment among children suffering uncomplicated malaria in rural Senegal.

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Increased *Plasmodium falciparum* resistance to chloroquine has prompted national malaria programs to develop new policies in several African countries. Less than a year after the introduction of amodiaquine/sulfadoxine-pyrimethamine (AQ/SP) as first-line treatment in Senegal, we examined adherence rates to therapy and its efficacy among children. The study was conducted in five dispensaries in rural Senegal. Children aged 2-10 years with a presumptive diagnosis of malaria were prescribed AQ/SP. Thick blood film analyses were carried out on days 0, 3, 7, 14 and 28. Blood and urine samples were collected on day 3 for drug level measurements. The principal caregivers were questioned on treatment adherence. Among the 289 recruited children, 144 had a parasitemia >2500/μl. The results demonstrated markedly good efficacy for the treatment, as no detectable parasitemia was observed on day 28 for 97.9% of the children. However, we noticed that 35.3% of children did not comply with the recommended doses and 62.3% did not exactly adhere to the drug schedule. Despite the good efficacy of the drugs, adherence to the therapeutic scheme was poor. Strategies to promote patient adherence would improve drug performance and thus might help to prevent the rapid emergence of drug resistance.

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Monitoring malaria control in Khammouane province, Laos: an active case detection survey of Plasmodium falciparum malaria using the Paracheck rapid diagnostic test.

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In Khammouane province, Laos, over 5000 slide-positive malaria cases were reported at local health facilities in 1997. To combat the spread of malaria, insecticide-treated nets (ITNs) and community health education were provided to the people in this province by the Lao Ministry of Health and the Japan International Cooperation Agency. In order to document the current malaria situation, an active case detection (ACD) survey using rapid diagnostic tests was conducted at 23 sites from June to July, the rainy season, in 2005. A total of 1711 villagers from 403 households participated in the survey. The proportion of positive cases was 0.7% (12/1711) with a range in each village of 0-8.2%. The low infection rate observed in this ACD survey was consistent with the decrease in the number of slide-positive malaria cases at local health facilities (from over 5000 cases to 536 cases), indicating the substantial progress made in malaria control. Although the reduction of malaria cases can be attributed to multiple factors, continued promotion of the proper use of ITNs as well as community-based testing and treatment services, especially in remote areas, may lead to a further reduction of malaria cases in the province.

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Community factors affecting long-lasting impregnated mosquito net use for malaria control in Sri Lanka.

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The Anti Malaria Campaign distributed approximately 300000 long-lasting impregnated nets (LLINs) to malaria-endemic areas in Sri Lanka during the years 2005 to 2007. We conducted a community-based cross-sectional survey among 2467 households distributed among the three major ethnic groups of Sri Lanka to study the perceptions and practices with regard to the use of LLINs in order to improve their use. In a majority of households the number of LLINs available was not sufficient for the number of people, although there was a small percentage of households that had excess nets. The information and advice given at the time of distribution regarding use of the nets differed amongst the three groups and was not consistent. Dissemination of this knowledge within the family was not observed. A relationship between knowledge regarding LLINs and reported practices on washing and drying of LLINs was found. It was noted that net shape may influence net use, with cone shaped nets being more popular. Efforts to increase knowledge on LLINs using behaviour change communication techniques would have more effectively contributed to achieve planned outcomes. Proper use of LLINs will undoubtedly contribute to further reduction of malaria in Sri Lanka.

Plasmodium falciparum population dynamics: only snapshots in time?

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Infections caused by the malaria parasite *Plasmodium falciparum* often comprise multiple genetically distinct clones. Individuals in endemic areas can have different clones detected in their peripheral blood over a few days or even hours. This reveals interesting within-host dynamics of multiclonal infections, which seem to differ in asymptomatic and symptomatic infections. As well as being an intriguing biological phenomenon that merits further understanding, the extensive dynamics of *P. falciparum* infections have practical implications on the design and interpretation of malaria studies. Most assessments will, indeed, only provide snapshots of the parasite population dynamics.

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Flipping the paradigm on malaria transmission-blocking vaccines.

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The idea of malaria transmission-blocking vaccines (TBVs) surfaced more than two decades ago. Since then, the research paradigm focused on developing TBVs that target surface antigens of parasite sexual stages. Only recently has an effort emerged that flipped this paradigm, targeting antigens of the parasite's obligate invertebrate vector, the *Anopheles* mosquito. Here, we review the current state of knowledge of mosquito-based TBVs and discuss the utility of this approach for future vaccine development.