

Malaria Bulletin: A Compendium of Current Literature

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Abstracts

1: *Am J Trop Med Hyg.* 2008 Jan;78(1):106-13.

Patterns of malaria: cause-specific and all-cause mortality in a malaria-endemic area of west Africa.

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Information on cause-specific mortality is sparse in sub-Saharan Africa. We present seasonal patterns of malaria and all-cause mortality from a longitudinal study with 60,000 individuals in rural northwestern Burkina Faso. The study is based on a demographic surveillance system and covers the period 1999-2003. Overall, 3,492 deaths were observed. Cause of death was ascertained by verbal autopsy. Age-specific death rates by cause and month of death were calculated. Seasonal and temporal trends were modeled with parametric Poisson regression. Infant and children less than 5 years of age mortality was 60.6 (95% CI, 56.2-65.3) and 31.9 (95% CI, 30.4-33.5) per 1,000 for all causes and 23.4 (95% CI, 20.7-26.4) and 13.3 (95% CI, 12.3-14.3) for malaria, respectively. Mortality was significantly higher in the rainy season. It is well described parametrically with a sinusoidal function. In adults, the highest all-cause mortality rates were observed in the dry season. Here, HIV/AIDS has become a leading cause of mortality.

2: *Am J Trop Med Hyg.* 2008 Jan;78(1):2-6.

Habitat-based larval interventions: a new perspective for malaria control.

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Interest in environmental management of mosquito larval habitats has been rekindled due to deterioration of malaria in tropical Africa. Environmental management programs were typically implemented as "all-out" campaigns by treating all potential breeding habitats. In contrast, targeted environmental management is based on a sound understanding of the heterogeneity in mosquito productivity. However, deficiencies in field methodology for measuring productivity hamper our progress in understanding of mosquito productivity. To address these issues, we develop a framework of habitat-based interventions by adoption of a landscape approach to elucidate mechanisms underlying mosquito productivity. The importance of vigorously quantitative estimation of the productivity is highlighted. Spatial models are proposed to examine the interrelationship between mosquito productivity and oviposition of gravid mosquitoes. In our view, environmental management approaches must take into account variability in productivity, in efforts to improve feasibility, cost-effectiveness, and sustainability of such approaches, particularly when implemented along with other malaria control measures.

3: *Am J Trop Med Hyg.* 2007 Dec;77(6 Suppl):193-7.

A major transition in malaria treatment: the adoption and deployment of artemisinin-based combination therapies.

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Parasite resistance to conventional antimalarial medicines has led, in recent years, to a dramatic shift in malaria treatment. Sixty-seven countries with endemic *Plasmodium falciparum* malaria, 41 of them in Africa, have recently adopted the highly effective artemisinin-based combination therapies (ACTs). In 2005, 31.3 million ACT treatment courses were procured globally for public sector use, 25.5 million of them in Africa. However, in the 39 countries, and in particular the 21 African countries in which ACTs are being deployed, access to these medicines is still unacceptably low. After a period of market instability, the global manufacturing capacity for ACTs is now sufficient to meet the demand. However, increased and sustained financing will be necessary to extend the current levels of ACT coverage. Artemisinins as monotherapies are widely available in the private sector of 47 endemic countries, and their consumption will, if unabated, promote resistance to artemisinins and compromise the effectiveness of ACTs.

4: *Am J Trop Med Hyg.* 2007 Dec;77(6 Suppl):181-92.

Artemisinin-based combination treatment of falciparum malaria.

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Artemisinin-based combination treatments (ACTs) are now generally accepted as the best treatments for uncomplicated falciparum malaria. They are rapidly and reliably effective. Efficacy is determined by the drug partnering the artemisinin derivative and, for artesunate-mefloquine, artemether-lumefantrine, and dihydroartemisinin-piperaquine, this usually exceeds 95%. Artesunate-sulfadoxine-pyrimethamine and artesunate-amodiaquine are effective in some areas, but in other areas resistance to the partner precludes their use. There is still uncertainty over the safety of artemisinin derivatives in the first trimester of pregnancy, when they should not be used unless there are no effective alternatives. Otherwise, except for occasional hypersensitivity reactions, the artemisinin derivatives are safe and remarkably well tolerated. The adverse effect profiles of the artemisinin-based combination treatments are determined by the partner drug. Most malaria endemic countries have now adopted artemisinin-based combination treatments as first-line treatment of falciparum malaria, but in most of these only a minority of the patients that need artemisinin-based combination treatments actually receive them.

5: *Am J Trop Med Hyg.* 2007 Dec;77(6 Suppl):170-80.

Intensity of malaria transmission and the spread of *Plasmodium falciparum* resistant malaria: a review of epidemiologic field evidence.

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Malaria transmission intensity has been proposed, based on theoretical models, as an important factor for the spread of falciparum-resistant malaria, but the

predictions obtained vary according to the assumptions inherent in the model used. We summarized the available field data on transmission intensity and the prevalence of malaria drug resistance. Resistance to chloroquine and sulphadoxine-pyrimethamine monotherapy was invariably higher where transmission was intense. Vector control interventions were associated with a better chloroquine and sulfadoxine-pyrimethamine efficacy. However, high resistance to chloroquine and also to combination therapy (chloroquine plus sulphadoxine-pyrimethamine and amodiaquine plus sulfadoxine-pyrimethamine) was also observed in very low transmission areas. Reducing transmission intensity is likely to slow the spread of drug resistance. Nevertheless, where transmission is extremely low, to limit the unnecessary use of antimalarials and a consequent paradoxical acceleration of the spread of resistance, patients should be treated only after laboratory confirmation of malaria.

6: *Ann Trop Med Parasitol.* 2008 Jan;102(1):39-44.

The antischistosomal efficacies of artesunate-sulfamethoxypyrazine-pyrimethamine and artemether-lumefantrine administered as treatment for uncomplicated, *Plasmodium falciparum* malaria.

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Although artemisinin and its derivatives are widely used for the treatment of malaria, they also have antischistosomal activity. In a small study in eastern Sudan, the effects of the treatment of uncomplicated, *Plasmodium falciparum* malaria with artesunate-sulfamethoxypyrazine-pyrimethamine (AS-SMP) and artemether-lumefantrine (AT-LU) on co-infections with *Schistosoma mansoni* were therefore investigated. Faecal samples from 14 of the 306 patients screened on presentation, at the start of a clinical trial of antimalarial treatment, were found to contain *Schistosoma mansoni* eggs. For the treatment of their malaria, the 14 egg-positive cases, who were aged 6-40 years (mean = 13.7 years), were each subsequently treated with three tablets of a fixed combination of AS-SMP, with a 12-h (six patients) or 24-h interval (five patients) between each tablet, or with six doses of AT-LU given over 3 days. When checked 28 and 29 days after the initiation of treatment, all 14 patients were found stool-negative for schistosome eggs. These results indicate that AS-SMP and AT-LU are currently very effective treatments not only for uncomplicated, *P. falciparum* malaria but also for *S. mansoni* infections.

7: *Ann Trop Med Parasitol.* 2008 Jan;102(1):3-9.

Comparative study of interactions between chloroquine and chlorpheniramine or promethazine in healthy volunteers: a potential combination-therapy phenomenon for resuscitating chloroquine for malaria treatment in Africa.

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Although, in in-vitro and limited in-vivo studies, chlorpheniramine (CP) and promethazine (PR) have each been shown to reverse chloroquine (CQ) resistance, the pharmacokinetic basis of this reversal has not been fully elucidated. In the present study, 15 healthy volunteers were randomly allotted to receive standard doses of CQ alone or in combination with CP or PR. Blood samples were collected from each volunteer at 21 time-points, from immediately before to 168 h after the initial dose. These samples were used to follow the changes in the plasma and

erythrocytic concentrations of CQ. The ratio between the mean maximum CQ concentration in the erythrocytes and that in the plasma was 4.2 for the volunteers given CQ alone, 7.3 in those given CQ-CP, and 3.2 in those given CQ-PR. CP significantly enhanced the erythrocytic accumulation of CQ, increasing the maximum CQ concentration observed in the erythrocytes by 24% ($P = 0.02$). The bio-availability of CQ was also significantly increased in the presence of CP, with the mean value for the area under the curve, of erythrocytic concentration v. time, increasing from 99,921 to 214,516 ng/ml.h ($P=0.001$). The mean half-life of CQ in the erythrocytes also increased when CP was used, from 51 to 100 h, but this change was not statistically significant ($P=0.83$). In contrast to CP, PR had no statistically significant effect on the disposition of CQ. As CP clearly enhances disposition of CQ, a combination of CQ with CP may be useful in the management of CQ-resistant infections. Detailed toxicological studies are required to understand the full clinical implications of CP's elevation of erythrocytic CQ concentrations.

8: *Antimicrob Agents Chemother.* 2008 Jan 14

Drug-regulated gene expression of Plasmodium falciparum P-glycoprotein homologue 1: a putative role for nuclear receptors.

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Acquired resistance to therapeutic agents is a major clinical concern in the prevention/treatment of malaria. The parasite has developed resistance to specific drugs through two mechanisms: mutations in target proteins such as dihydrofolate reductase and bcl complex for antifolates and nathoquinones respectively and alterations in parasite predicted transporter molecules such as p-glycoprotein homologue 1 (Pgh1) and PfCRT. Alterations in the expression of Pgh1 have been associated with modified susceptibility to a range of unrelated drugs. The molecular mechanism(s) that are responsible for this phenotype are unknown. We have shown previously (21) that the anticonvulsant phenobarbitone (PB) can induce reduced susceptibility to chloroquine (CQ) in Plasmodium falciparum, and in the current study we provide the first evidence for a molecular mechanism underlying this phenomenon. We demonstrate that pre-treatment with PB can elicit decreased susceptibility to CQ in both CQ-resistant and CQ-sensitive parasite lines, and that this is associated with increased expression of the drug transporter Pgh1, but not PfCRT. Furthermore we have investigated the proximal promoter regions from both pfmdr1 and pfcr1, and identified a number of putative binding sites for nuclear receptors with sequence similarities to regions known to be activated by phenobarbitone in mammals. Whole genome analysis has revealed a putative nuclear receptor gene, providing the first evidence that nuclear-receptor mediated responses to drug exposure may be a mechanism of gene regulation in Plasmodium falciparum.

9: *Antimicrob Agents Chemother.* 2008 Jan 7

Population pharmacokinetics of piperazine after two different treatment regimens of dihydroartemisinin-piperazine in patients with Plasmodium falciparum malaria in Thailand.

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The population pharmacokinetics of piperazine in adults and children with uncomplicated Plasmodium falciparum malaria treated with two different dosage regimens of dihydroartemisinin-piperazine were characterized. Piperazine pharmacokinetics in 98 Burmese and Karen patients aged 3-55 years were described by a two-compartment disposition model with first-order absorption and inter-individual random variability on all parameters, and were similar with the three and four dose regimens. Children had a lower body-weight normalized oral clearance than adults, resulting in longer terminal elimination half-lives and higher total exposure of piperazine (AUCday 0-63). But children had lower plasma concentrations in the therapeutically relevant post-treatment prophylactic period (AUCday 3-20) because of smaller body-weight normalized central volumes of distribution and shorter distribution half-lives. Our data lend further support of a simplified once daily treatment regimen to improve treatment adherence and efficacy, and indicate that weight adjusted piperazine doses in children may need to be higher than in adults.

10: *BMJ*. 2008 Jan 26;336(7637):202-5.

The impact of response to the results of diagnostic tests for malaria: cost-benefit analysis.

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OBJECTIVE: Rapid diagnostic tests for malaria seem cost effective in standard analyses, but these do not take account of clinicians' response to test results. This study tested the impact of clinicians' response to rapid diagnostic test or microscopy results on the costs and benefits of testing at different levels of malaria transmission and in different age groups. **DESIGN:** Cost-benefit analysis using a decision tree model and clinical data on the effectiveness of diagnostic tests for malaria, their costs, and clinicians' response to test results. **SETTING:** Tanzania. **METHODS:** Data were obtained from a clinical trial of 2425 patients carried out in three settings of varying transmission. **RESULTS:** At moderate and low levels of malaria transmission, rapid diagnostic tests were more cost beneficial than microscopy, and both more so than presumptive treatment, but only where response was consistent with test results. At the levels of prescription of antimalarial drugs to patients with negative tests that have been found in observational studies and trials, neither test methods likely to be cost beneficial, incurring costs 10-250% higher, depending on transmission rate, than would have been the case with fully consistent responses to all test results. Microscopy becomes more cost beneficial than rapid diagnostic tests when its sensitivity under operational conditions approaches that of rapid diagnostic tests. **CONCLUSIONS:** Improving diagnostic methods, including rapid diagnostic tests, can reduce costs and enhance the benefits of effective antimalarial drugs, but only if the consistency of response to test results is also improved. Investing in methods to improve rational response to tests is essential. Economic evaluations of diagnostic tests should take into account whether clinicians' response is consistent with test results.

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

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

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

12: *Clin Microbiol Rev.* 2008 Jan;21(1):97-110.

Update on rapid diagnostic testing for malaria.

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To help mitigate the expanding global impact of malaria, with its associated increasing drug resistance, implementation of prompt and accurate diagnosis is needed. Malaria is diagnosed predominantly by using clinical criteria, with microscopy as the current gold standard for detecting parasitemia, even though it is clearly inadequate in many health care settings. Rapid diagnostic tests (RDTs) have been recognized as an ideal method for diagnosing infectious diseases, including malaria, in recent years. There have been a number of RDTs developed and evaluated widely for malaria diagnosis, but a number of issues related to these products have arisen. This review highlights RDTs, including challenges in assessing their performance, internationally available RDTs, their effectiveness in various health care settings, and the selection of RDTs for different health care systems.

The impact of HIV infection in pregnant women on variant specific immunity to malaria.

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HIV increases susceptibility to malaria infection, and this has been most clearly demonstrated in pregnant women. Variant surface antigens on the surface of erythrocytes infected with *Plasmodium falciparum* are major targets of protective immunity. We studied the impact of HIV infection on pregnant women's humoral immunity to variant surface antigens expressed by placental and pediatric isolates of *P. falciparum*. By flow cytometry, sera from HIV-infected women more frequently lacked antibodies to these antigens than did sera from HIV-uninfected women. This difference was similar in magnitude for pediatric (unadjusted OR = 6.36; 95% CI = 1.14, 35.32, $p < 0.05$) and placental isolates (unadjusted OR = 6.47; 95% CI = 0.75, 55.64 $p < 0.10$). We divided women into high and low responders, based on antibody levels. After adjusting for CD4 count, maternal age and gravidity, we found that HIV infected women more frequently had low responses to both pediatric (OR = 5.34; 95% CI: 1.23, 23.16; $p = 0.025$) and placental isolates (OR = 4.14; 95% CI: 1.71, 10.02; $p = 0.002$). Relative quantity of antibodies to both pediatric ($p = 0.035$) and placental isolates ($p = 0.005$) was lower in HIV infected women than uninfected women. HIV infection has a broad impact on variant specific immunity, which may explain the susceptibility of infected individuals to clinical malaria episodes.

14: *Curr Med Chem.* 2008;15(2):161-71.

The fight against drug-resistant malaria: novel plasmodial targets and antimalarial drugs.

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Malaria, one of the major reemerging parasitic diseases, is caused by protozoal parasites belonging to the genus plasmodia. Antimalarial drugs have played a mainstream role in controlling the spread of malaria through the treatment of patients infected with the plasmodial parasites and controlling its transmissibility. The current line of therapy against malaria is faced with the hurdles of a low or total lack of efficacy due to the evolution of drug-resistant strains of the malarial parasites. Preventive vaccination against malaria is an ideal solution to this problem but is not expected to arrive for at least a decade. Development of antimalarial drugs involving novel mechanisms of action is therefore of imminent importance. Several novel drug candidates of synthetic and natural products origin as well as their combination therapies are currently being evaluated for their efficacy against the drug-resistant strains of the parasites. Various plasmodial targets/pathways, such as the Purine salvage pathway, Pyrimidine biosynthesis pathway as well as the processes in the apicoplast, have been identified and are being utilized for the discovery and development of novel antimalarial therapies. This review provides an overview of

the latest developments in terms of drugs, combination therapies and novel plasmodial targets being carried out to counter the menace of drug-resistant malaria.

15: *Curr Med Chem.* 2007;14(30):3243-59.

Chemical instability determines the biological action of the artemisinins.

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Artemisinin is a sesquiterpene compound of plant origin. It has a low molecular weight, and it contains five oxygen atoms, two in a lactone function, one is part of a seven membered ring system and two forms a peroxide function bridging over the seven-membered ring. It is a highly energetic molecule prone to lose its activity if circumstances permit. Reduction of its lactone function into dihydroartemisinin makes derivatization easy. Esterification and ether formation contribute to stability. Dihydroartemisinin exists preferably in a beta epimeric format but flip-flops with the alpha epimer. Solvation effects play a role. In doing so, open forms are created and they contribute to the instability, both of the peroxide and of the seven-membered ring. Artemisinins constitute a remarkable class of compounds which display instability both biologically and chemically due to the presence of various functional groups. Activity ranges from a wonderful action against a series of parasites, in particular malaria and schistosomiasis, to bacteria, fungi and selected viruses. The latest developments indicate a potential use in adjuvant cancer chemotherapy. The built-in chemical instability, necessary for biological action, causes serious pharmaceutical problems and only a restricted number of derivatives are useful. Problems are accelerated under tropical conditions and the basic active drug dihydroartemisinin cannot be used as such since it is prone to accelerated breakdown into a series of inactive products. The pitfalls of chemical instability and pharmaceutical stability are discussed in relation to the current uses of the drugs.

16: *Endocr Metab Immune Disord Drug Targets.* 2007 Dec;7(4):259-65.

Protein structure based strategies for antigen discovery and vaccine development against malaria and other pathogens.

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The review surveys potential "structural antigens" which represent small protein domains that can be chemically synthesized and, isolated from the context of the whole protein, can fold in the same native structure. They include natively unfolded protein regions, small globular domains, alpha-helical coiled coils and regions with tandem repeats forming structures ranging from the collagen triple helices to solenoid-like arrangements. We also describe and compare new strategies for development of vaccine that use the concept of structural epitopes. One type of approach is based on engineering artificial mini-proteins able to mimic structural epitopes of natural proteins. The review compares the "engineering" methodologies with "bioinformatics" approaches that became possible recently, after the sequencing of the genomes of many pathogens, and involve genome-wide bioinformatics searches for "structural antigens". In particular, based on the known *P. falciparum* genome, we identified putative alpha-helical coiled coil regions, 30-40 amino acids long, in proteins presented in asexual malaria blood stages. Peptides of such regions frequently fold into the "native" structure. A hundred such peptides were synthesized and all of them were

recognized at various degrees (5-80%) by a panel of sera from donors living in malaria-endemic areas. The results obtained demonstrate that a bioinformatics/chemical synthesis strategy can rapidly lead to the identification of new proteins that can be targets of potential vaccines and/or drugs against malaria and other infectious organisms.

17: *Gene*. 2008 Jan 15

Positive selection on the Plasmodium falciparum sporozoite threonine-asparagine-rich protein: Analysis of isolates mainly from low endemic areas.

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The sporozoite threonine-asparagine-rich protein (STARP) of Plasmodium falciparum is an attractive target for a pre-erythrocytic stage malaria vaccine because both naturally acquired and experimentally induced anti-STARP antibodies can block sporozoite invasion of hepatocytes. To explore the extent of sequence variation, we surveyed nucleotide polymorphism across the entire gene, encompassing 2 exons and an intron, of 124 P. falciparum-infected blood samples from Thailand and 10 from 4 other endemic areas. In total 24 haplotypes were identified despite low-level nucleotide diversity at this locus. The mean number of nonsynonymous substitutions per nonsynonymous site (d(N)) significantly exceeded that of synonymous substitutions per synonymous site (d(S)), suggesting that the STARP gene has evolved under positive selection, probably from host immune pressure. The preponderance of conservative amino acid exchanges and a strongly biased T-nucleotide toward the third position of codons in repeat arrays have reflected simultaneous constraints on this molecule, probably from its respective unknown function and nucleotide composition. Sequence conservation in the STARP locus among clinical isolates from different disease endemic areas would not compromise vaccine incorporation.

18: *Haematologica*. 2008 Jan;93(1):143-4.

Assessment of malaria in pregnancy using rapid diagnostic tests and its association with HIV infection and hematologic parameters in South-Eastern Nigeria.

Uneke CJ, Iyare FE, Oke P, Duhlińska DD.

P. falciparum malaria in pregnancy was evaluated using histidine-rich proteins-2 RDT and related to HIV infection and hematologic parameters. Prevalence of malaria, HIV and anemia were 19.7%, 3.1% and 17.2% respectively. Primigravidae were significantly more infected with malaria. Malaria was not significantly associated with anemia, blood group, genotype and HIV infection.

19: *Infect Genet Evol*. 2007 Nov 29

Bionomics, taxonomy, and distribution of the major malaria vector taxa of Anopheles subgenus Cellia in Southeast Asia: An updated review.

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There is high diversity of Anopheles mosquitoes in Southeast Asia and the main

vectors of malaria belong to complexes or groups of species that are difficult or impossible to distinguish due to overlapping morphological characteristics. Recent advances in molecular systematics have provided simple and reliable methods for unambiguous species identification. This review summarizes the latest information on the seven taxonomic groups that include principal malaria vectors in Southeast Asia, i.e. the *Minimus*, *Fluviatilis*, *Culicifacies*, *Dirus*, *Leucosphyrus*, and *Sundaicus* Complexes, and the *Maculatus* Group. Main issues still to be resolved are highlighted. The growing knowledge on malaria vectors in Southeast Asia has implications for vector control programs, the success of which is highly dependant on precise information about the biology and behavior of the vector species. Acquisition of this information, and consequently the application of appropriate, sustainable control measures, depends on our ability to accurately identify the specific vectors.

20: *Infect Immun.* 2008 Jan 22

Duration of naturally acquired antibody responses to blood stage *Plasmodium falciparum* is age dependent and antigen specific.

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Naturally acquired antibody responses provide partial protection from clinical malaria, and blood stage vaccines under development aim to prime such responses. To investigate the determinants of antibody response longevity, serum IgG to several blood stage vaccine candidate antigens was examined in two cohorts of children aged up to 6 years during the dry seasons of 2003 and 2004 in The Gambia. The first cohort showed that most antibodies were lost within less than four months of the first sampling, if a persistent infection was not present, so the second year cohort involved sampling of individuals every 2 weeks over a three month period. Antibody responses in this second cohort were also influenced by persistent malaria infection, so analysis focused particularly on children who did not have parasites detected after the first time point. Antibodies to most antigens declined more slowly in the oldest age group of children (> 5 years old), and more rapidly in the youngest (< 3 years old). However, antibodies to merozoite surface protein 2 (MSP2) were shorter-lived and were not more persistent in older children. The age-specific and antigen-specific differences were not explained by different IgG subclass response profiles, indicating the probable importance of differential longevity of plasma cell populations rather than antibody molecules. It is likely that young children mostly have short-lived plasma cells and thus experience rapid decline in antibody levels, while older children have longer lasting antibody responses that depend on long-lived plasma cells.

21: *Infect Immun.* 2008 Jan 22

Fetal responses during placental malaria modify the risk of low birth weight.

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Inflammation during placental malaria (PM) is associated with low birth weight

(LBW), especially during first pregnancy, but the relative contribution of maternal or fetal factors that mediate this effect remains unclear, and the role of IFN-gamma has been controversial. We examined the relationship of maternal and cord plasma levels of IFN-gamma, TNF-alpha, IL-10, ferritin and leptin to birth weight among Tanzanian women delivering in an area of high malaria transmission. Placental levels of inflammatory cytokines including IFN-gamma increased significantly during PM of primigravid and multigravid women but not secundigravid women. PM also increased maternal peripheral levels of all inflammatory markers except IFN-gamma, but had strikingly little effect on cord levels of these proteins. In multivariate analysis, placental IFN-gamma was negatively associated ($P = 0.01$) and cord ferritin was positively associated ($P < 0.0001$) with birth weight in infected (PM+) first-time mothers. This relationship was absent in other mothers, consistent with the epidemiology of PM and disease. Cord leptin had a strong positive relationship with birth weight in offspring of PM- women ($P = 0.02$ to < 0.0001) but not PM+ women (all $P = NS$) from the three gravidity groups. The results confirm that placental IFN-gamma is related to LBW due to PM during first pregnancies, and suggest that fetal ferritin plays a protective role. Because fetal cells are a source of placental IFN-gamma and cord ferritin, the fetal response to PM may modify the risk of LBW.

22: *J Biol Chem.* 2008 Jan 4

Biochemical and genetic analysis of the phosphoethanolamine methyltransferase of the human malaria parasite plasmodium falciparum.

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The PfPMT enzyme of *P. falciparum*, the agent of severe human malaria, is a member of a large family of known and predicted phosphoethanolamine methyl-transferases (PMTs) recently identified in plants, worms, insects and protozoa. Functional studies in *P. falciparum* revealed that PfPMT plays a critical role in the synthesis of phosphatidylcholine via a plant-like pathway involving serine decarboxylation and phosphoethanolamine methylation. Despite their important biological functions, PMT structures have not yet been solved and nothing is known about which amino acids in these enzymes are critical for catalysis and binding to *s*-adenosyl-methionine (SAM) and phosphoethanolamine substrates. Here we have performed a mutational analysis of PfPMT focused on 24 residues within and outside the predicted catalytic motif. The ability of PfPMT to complement the choline auxotrophy of a yeast mutant defective in phospholipid methylation enabled us to characterize the activity of the PfPMT mutants. Mutations in residues Asp61, Gly83 and Asp128 dramatically altered PfPMT activity, and its complementation of the yeast mutant. Our analyses identify the importance of these residues in PfPMT activity and set the stage for advanced structural understanding of this class of enzymes.

23: *J Infect Dis.* 2008 Jan 1;197(1):85-93.

Spatial Variation of Malaria Incidence in Young Children from a Geographically Homogeneous Area with High Endemicity.

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Background. In sub-Saharan Africa, malaria is a leading cause of morbidity and mortality among young children. Detailed knowledge of spatial variation of malaria epidemiology and associated risk factors is important for planning and evaluating malaria-control measures. Methods. The spatial variation of malaria incidences and socioeconomic factors were assessed over 21 months, from January 2003 to September 2005, in 535 children from 9 villages of a small rural area with high *Plasmodium falciparum* transmission in Ghana. Household positions were mapped by use of a global positioning system, and the spatial effects on malaria rates were assessed by means of ecological analyses and bivariate Poisson regression controlling for possible confounding factors. Results. Malaria incidence was surprisingly heterogeneous between villages, and ecological analyses showed strong correlations with village area ($[Formula: see text]$; $[Formula: see text]$) and population size ($[Formula: see text]$; $[Formula: see text]$). Malaria risk was affected by a number of socioeconomic factors. Poisson regression showed an independent linear rate reduction with increasing distance between children's households and the fringe of the forest. Conclusions. The exact location of households in villages is an independent and important factor for the variation of malaria incidence in children from high-transmission areas. This fact should be considered in the planning of intervention trials and in spatial targeting of malaria interventions at a local level.

24: *J Infect Dis.* 2008 Jan 1;197(1):72-78.

Platelet-Induced Clumping of *Plasmodium falciparum*-Infected Erythrocytes from Malawian Patients with Cerebral Malaria-Possible Modulation In Vivo by Thrombocytopenia.

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Platelets may play a role in the pathogenesis of human cerebral malaria (CM), and they have been shown to induce clumping of *Plasmodium falciparum*-parasitized red blood cells (PRBCs) in vitro. Both thrombocytopenia and platelet-induced PRBC clumping are associated with severe malaria and, especially, with CM. In the present study, we investigated the occurrence of the clumping phenomenon in patients with CM by isolating and coincubating their plasma and PRBCs ex vivo. Malawian children with CM all had low platelet counts, with the degree of thrombocytopenia directly proportional to the density of parasitemia. Plasma samples obtained from these patients subsequently induced weak PRBC clumping. When the assays were repeated, with the plasma platelet concentrations adjusted to within the physiological range considered to be normal, massive clumping occurred. The results of this study suggest that thrombocytopenia may, through reduction of platelet-mediated clumping of PRBCs, provide a protective mechanism for the host during CM.

25: *J Infect Dis.* 2007 Dec 15;196(12):1827-35.

Efficient development of plasmodium liver stage-specific memory CD8+ T cells during the course of blood-stage malarial infection.

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Immunity to Plasmodium liver stages in individuals in malaria-endemic areas is inextricably linked to concomitant blood-stage parasitemia. Although Plasmodium sporozoite infection induces measurable CD8+ T cell responses, the development of memory T cells during active erythrocytic infection remains uncharacterized. Using transgenic T cells, we assessed antigen-specific effector CD8+ T cell responses induced by normal (NorSpz) and radiation-attenuated (IrrSpz) Plasmodium yoelii sporozoites. The magnitude, phenotypic activation, and differentiation pathway of CD8+ T cells were similarly induced by NorSpz and IrrSpz. Moreover, in normal mice, memory T cells elicited after priming with NorSpz and IrrSpz generated identical recall responses after a heterologous boost strategy. Furthermore, these recall responses exhibited comparable in vivo antiparasite activity. Our results indicate that sporozoites that retain their infective capacity induce memory CD8+ T cells that are robustly recalled by secondary immunization. Thus, erythrocytic infection does not preclude the establishment of memory CD8+ T cell responses to malarial liver stages.

26: *J Trop Pediatr.* 2008 Jan 22

In-Hospital Risk Estimation in Children with Malaria--Early Predictors of Morbidity and Mortality.

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Background: Rapid diagnosis and adequate therapy are crucial to prevent development of severe disease and death in children suffering from malaria. A reliable but easy system for disease severity assessment would help to fast track seriously ill children and provide suitable therapies for different patient groups. Objectives: To examine risk factors and appropriate scoring systems in children suffering from malaria for outcome in terms of morbidity and mortality. Methods: A prospective, consecutive study in children admitted to the Muhimbili Medical Centre in Dar es Salaam was conducted to evaluate risk factors and test appropriate scoring systems. The simplified Multi-Organ Dysfunction Score (sMODS), a severity of disease classification consisting mainly of clinical data, was applied. Chosen outcome parameters were morbidity and mortality. Results were compared to those obtained from the World Health Organisation (WHO) classification of severe malaria, the Blantyre Coma Scale (BCS) and selected single clinical parameters. Results: Seventy-five children were recruited into the study. Mean age was 28 months ranging from 6 months to 8 years. 'Severe Malaria', according to WHO criteria was evident in 57 patients (76%). Mean sMODS on admission was 15.6 +/- 2. Seven patients (9%) died. Among single symptoms, impaired consciousness and respiratory distress predicted both, fatal outcome and morbidity. In terms of scoring systems, the sMODS correlated with both outcome parameters. In comparison, the WHO criteria did not correlate with any of the two parameters, the BCS correlated with mortality only. Conclusion: In our study, sMODS has been shown to represent a useful quantitative approach towards disease severity classification in resource poor settings and can be used for risk estimation in children suffering from malaria in terms of both morbidity and mortality.

27: *Malar J.* 2008 Jan 29;7(1):24

Assessment of a treatment guideline to improve home management of malaria in children in rural south-west Nigeria.

Ajayi IO, Falade CO, Bamgboye AE, Oduola AM, Kale OO.

ABSTRACT: **BACKGROUND:** Many Nigerian children with malaria are treated at home. Treatments are mostly incorrect, due to caregivers' poor knowledge of appropriate and correct dose of drugs. A comparative study was carried out in two rural health districts in southwest Nigeria to determine the effectiveness of a guideline targeted at caregivers, in the treatment of febrile children using chloroquine. **METHODS:** Baseline and post intervention knowledge, attitude and practice household surveys were conducted. The intervention strategy consisted of training a core group of mothers (another trainers) in selected communities on the correct treatment of malaria and distributing a newly developed treatment guideline to each household. "Mother trainers" disseminated the educational messages about malaria and the use of the guideline to their communities. **RESULTS:** Knowledge of cause, prevention and treatment of malaria increased with the one-year intervention. Many, (70.4%) of the respondents stated that they used the guideline each time a child was treated for malaria. There was a significant increase in the correct use of chloroquine from 2.6% at baseline to 52.3% after intervention among those who treated children at home in the intervention arm compared with 4.2% to 12.7% in the control arm. The correctness of use was significantly associated with use of the guideline. The timeliness of commencing treatment was significantly earlier in those who treated febrile children at home using chloroquine than those who took their children to the chemist or health facility ($p < 0.005$). Mothers considered the guideline to be explicit and useful. Mother trainers were also considered to be effective and acceptable. **CONCLUSIONS:** The use of the guideline with adequate training significantly improved correctness of malaria treatment with chloroquine at home. Adoption of this mode of intervention is recommended to improve compliance with drug use at home. The applicability for deploying artemisinin-based combination therapy at the community level need to be investigated.

28: *Malar J.* 2008 Jan 28;7(1):23

In vitro atovaquone/proguanil susceptibility and characterization of the cytochrome b gene of Plasmodium falciparum from different endemic regions of Thailand.

Khositnithikul R, Tan-Ariya P, Mungthin M.

ABSTRACT: **BACKGROUND:** The emergence of Plasmodium falciparum resistant to most currently used antimalarial drugs is the major problem in malaria control along the Thai-Myanmar and Thai-Cambodia borders. Although artemisinin-based combination therapy has been recommended for the treatment of multidrug-resistant falciparum malaria, these combinations are not available for some people, such as travelers from North America. A fixed-dose combination of atovaquone and proguanil (Malarone) has been proved to be effective for the treatment and prophylaxis of malaria which is already approved by countries in North America and Europe. Determination of the phenotypes and genotypes related to atovaquone/proguanil response in Thai isolates of P. falciparum will be useful for rationale drug use. The main purpose of this study was to explore the in vitro atovaquone/proguanil susceptibility of recently adapted Thai isolates of P. falciparum. Genotypic characterization of the cytb gene of these isolates was also determined since it has been reported that point mutations, particularly codon 268 in the cytochrome b gene (cytb) have been linked to atovaquone/proguanil treatment failure. **METHODS:** Eighty three P. falciparum isolates collected during 1998 to 2005 from four different multidrug resistance areas of Thailand were determined for the in vitro atovaquone/proguanil

susceptibilities using radioisotopic assay. Mutations in the cytb gene were determined by PCR-RFLP and sequence analysis. RESULTS: The mean atovaquone and proguanil IC50 was 3.4 nM and 36.5 μ M, respectively. All 83 Thai isolates were atovaquone sensitive. None of the 83 isolates contained the mutations at codon 268 of the cytb gene. DNA sequencing of the cytb gene of 20 parasite isolates showed no other mutations. CONCLUSION: In agreement with a recent efficacy study of atovaquone/proguanil, the present information indicates that atovaquone/proguanil can be one of the drugs of choice for the treatment and prophylaxis of multidrug-resistant falciparum malaria in Thailand.

29: *Malar J.* 2008 Jan 28;7(1):22

Pilot assessment of the sensitivity of the malaria thin film.

Ohrt C, Prudhomme O'Meara W, Remich S, McEvoy P, Ogutu B, Mtalib R, Sande Odera J.

ABSTRACT: BACKGROUND: Malaria microscopy remains the reference standard for malaria diagnosis in clinical trials (drug and vaccine), new diagnostic evaluation, as well as in clinical care in much of the world today. It is known that microscopy is an imperfect gold standard, and that very low false positive rates can dramatically lower protective efficacy estimates in malaria prevention trials. Although new methods are now available, including malaria rapid diagnostic tests and PCR, neither is as yet validated in the clinical trial setting and both have limitations. Surprisingly, the sensitivity of thin smears is not well established and thin smears are not commonly used in the developing world. METHODS: Malaria thick and thin films were collected in the lowlands of Western Kenya. All had density determined by four readings with two methods, as well as species identified. Thirty-six with low density parasitaemia had the thin smear read by five independent microscopists, two were expert and three were qualified. Microscopists read the entire thin film. For the first 10 parasites seen, they reported the species, appearance, time, field number, and red blood cells in the field. Total parasites, total fields and total time to examine the smear were also recorded. RESULTS: Median parasitaemia was 201 parasites/ul, mean 1,090 +/-2,195, range 6-11,124 parasites/ul for the 36 smears evaluated. The data revealed a density dependent increase in sensitivity, with 100% sensitivity achieved at >200 parasites/ul for experts and >500 parasites/ul for qualified readers. Thin film readings confirmed parasitaemia 74% of the time by experts, and 65% of the time for qualified microscopists. The 95th percentile for time to detect parasitaemia was 15 minutes for experts, 17 minutes for qualified microscopists. This decreased to 4-10 minutes for experts at densities of > 200 parasites/ul. Additionally, substantial discordance for species identification was observed. CONCLUSIONS: The thin film is sensitive enough to be a useful tool to confirm malaria diagnosis in study subjects in some settings. Specificity of the thin film and its utility for confirming thick film or other diagnostic test results should be assessed further.

30: *Malar J.* 2008 Jan 28;7(1):21

An interactive model for the assessment of the economic costs and benefits of different rapid diagnostic tests for malaria.

Lubell Y, Hopkins H, Whitty CJ, Staedke SG, Mills A.

ABSTRACT: BACKGROUND: Rapid diagnostic tests (RDTs) for malaria are increasingly being considered for routine use in Africa. However, many RDTs are available and selecting the ideal test for a particular setting is challenging. The appropriateness of RDT choice depends in part on patient population and epidemiological setting, and on decision makers' priorities. The model presented (available online) can be used by decision makers to evaluate alternative RDTs and assess the circumstances under which their use is justified on economic

grounds. METHODS: An interactive model based on a decision-tree structure and a cost-benefit framework was designed to compare different diagnostic strategies. Variables included in the model can be modified by users, including RDT and treatment costs, test accuracies (sensitivity and specificity), probabilities for developing severe illness, case-fatality rates, and clinician response to negative test results. To illustrate how the model can be used, a comparison is made of presumptive treatment with two available RDTs, one detecting histidine-rich protein-2 (HRP2) and one detecting Plasmodium lactate dehydrogenase (pLDH). Data inputs were obtained from a study comparing the RDTs at seven sites in Uganda. RESULTS: Applying the model in the illustrative Ugandan context demonstrates if only direct expenditures are considered, the pLDH test is the preferred option for adult patients except in high transmission settings, while young children are best treated presumptively in all settings. When health outcomes are considered, the HRP2 test gains an advantage in almost all settings and for all age groups. Introducing possible adverse consequences of using an antimalarial into the analysis, such as adverse drug reactions, or the development of resistance, considerably strengthens the case for using RDTs. When the model is adjusted to account for less than complete adherence to test results, the efficiency of using RDTs drops sharply. CONCLUSIONS: Model output demonstrates that which test is preferable varies by location, depending on factors such as malaria transmission intensity and the costs and accuracies of the RDTs under consideration. Despite the uncertainties and complexities involved, adaptable models such as the one presented here can serve as a practical tool to assist policy makers in efficient deployment of new technologies.

31: *Malar J.* 2008 Jan 25;7(1):20

A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania.

Fillinger U, Kannady K, William G, Vanek MJ, Dongus S, Nyika D, Geissbuehler Y, Chaki PP, Govella NJ, Mathenge EM, Singer BH, Mshinda H, Lindsay SW, Tanner M, Mtasiwa D, de Castro MC, Killeen GF.

ABSTRACT: BACKGROUND: As the population of Africa rapidly urbanizes, large populations could be protected from malaria by controlling aquatic stages of mosquitoes if cost-effective and scalable implementation systems can be designed. METHODS: A recently initiated Urban Malaria Control Programme in Dar es Salaam delegates responsibility for routine mosquito control and surveillance to modestly-paid community members, known as Community-Owned Resource Persons (CORPs). New vector surveillance, larviciding and management systems were designed and evaluated in 15 city wards to allow timely collection, interpretation and reaction to entomologic monitoring data using practical procedures that rely on minimal technology. After one year of baseline data collection, operational larviciding with *Bacillus thuringiensis* var. *israelensis* commenced in March 2006 in three selected wards. RESULTS: The procedures and staff management systems described greatly improved standards of larval surveillance relative to that reported at the outset of this programme. In the first year of the programme, over 65,000 potential *Anopheles* habitats were surveyed by 90 CORPs on a weekly basis. Reaction times to vector surveillance at observations were one day, week and month at ward, municipal and city levels, respectively. One year of community-based larviciding reduced transmission by the primary malaria vector, *Anopheles gambiae* s.l., by 31% (95% C.I.=21.6-37.6%; $p=0.04$). CONCLUSION: This novel management, monitoring and evaluation system for implementing routine larviciding of malaria vectors in African cities has shown considerable potential for sustained, rapidly responsive, data-driven and affordable application. Nevertheless, the true programmatic value of larviciding in urban Africa can only be established through longer-term programmes which are stably financed and allow the operational teams and management infrastructures to mature by learning from experience.

32: *Malar J.* 2008 Jan 23;7(1):18

The effects of a partitioned var gene repertoire of *Plasmodium falciparum* on antigenic diversity and the acquisition of clinical immunity.

Recker M, Arinaminpathy N, Buckee CO.

ABSTRACT: **BACKGROUND:** The human malaria parasite *Plasmodium falciparum* exploits antigenic diversity and within-host antigenic variation to evade the host's immune system. Of particular importance is the highly polymorphic var gene family that encodes the cell surface antigens PfEMP1 (*Plasmodium falciparum* Erythrocyte Membrane Protein 1). It has recently been shown that in spite of their extreme diversity, however, these genes fall into distinct groups according to chromosomal location or sequence similarity, and that recombination may be confined within these groups. **METHODS:** This study presents a mathematical analysis of how recombination hierarchies affect diversity, and, by using simple stochastic simulations, investigates how intra- and inter-genic diversity influence the rate at which individuals acquire clinical immunity. **RESULTS:** The analysis demonstrates that the partitioning of the var gene repertoire has a limiting effect on the total diversity attainable through recombination and that the limiting effect is strongly influenced by the respective sizes of each of the partitions. Furthermore, by associating expression of one of the groups with severe malaria it is demonstrated how a small number of infections can be sufficient to protect against disease despite a seemingly limitless number of possible non-identical repertoires. **CONCLUSION:** Recombination hierarchies within the var gene repertoire of *P.falciparum* have a severe effect on strain diversity and the process of acquired immunity against malaria. Future studies will show how the existence of these recombining groups can offer an evolutionary advantage in spite of their restriction on diversity.

33: *Malar J.* 2008 Jan 23;7(1):17

Multiplicity of *Plasmodium falciparum* infection in asymptomatic children in Senegal: relation to transmission, age and erythrocyte variants.

Vafa M, Troye-Blomberg M, Anchang J, Garcia A, Migot-Nabias F.

ABSTRACT: **BACKGROUND:** Individuals living in malaria endemic areas generally harbour multiple parasite strains. Multiplicity of infection (MOI) can be an indicator of immune status. However, whether this is good or bad for the development of immunity to malaria, is still a matter of debate. This study aimed to examine the MOI in asymptomatic children between two and ten years of age and to relate it to erythrocyte variants, clinical attacks, transmission levels and other parasitological indexes. **METHODS:** Study took place in Niakhar area in Senegal, where malaria is mesoendemic and seasonal. Three hundred and seventy two asymptomatic children were included. Sickle-cell trait, G6PD deficiency (A- and Santamaria) and alpha-thalassaemia were determined using PCR. Multiplicity of *Plasmodium falciparum* infection, i.e. number of concurrent clones, was defined by PCR-based genotyping of the merozoite surface protein-2 (*mSP2*), before and at the end of the malaria transmission season. The Chi Square test, ANOVA, multivariate linear regression and logistic regression statistical tests were used for data analysis. **RESULTS:** MOI was significantly higher at the end of transmission season. The majority of PCR positive subjects had multiple infections at both time points (64% before and 87% after the transmission season). MOI did not increase in alpha-thalassaemic and G6PD mutated children. The ABO system and HbAS did not affect MOI at any time points. No association between MOI and clinical attack was observed. MOI did not vary over age at any time points. There was a significant correlation between MOI and parasite density, as the higher parasite counts increases the probability of having multiple infections. **CONCLUSIONS:** Taken together our data revealed that alpha-thalassaemia may have a role in

protection against certain parasite strains. The protection against the increase in MOI after the transmission season conferred by G6PD deficiency is probably due to clearance of the malaria parasite at early stages of infection. The ABO system and HbAS are involved in the severity of the disease but do not affect asymptomatic infections. MOI was not age-dependent, in the range of two to ten years, but was correlated with parasite density. However some of these observations need to be confirmed including larger sample size with broader age range and using other msp2 genotyping method.

34: *Malar J.* 2008 Jan 19;7(1):16

Randomized, comparative study of the efficacy and safety of artesunate plus amodiaquine, administered as a single daily intake versus two daily intakes in the treatment of uncomplicated falciparum malaria.

Ndiaye JL, Faye B, Diouf AM, Kuete T, Cisse M, Seck PA, Brasseur P, Same-Ekobo A, Lameyre V, Gaye O.

ABSTRACT: **BACKGROUND:** Artesunate plus amodiaquine is a coblistered ACT, given as a single daily intake. It has been suggested that, in view of the number of tablets to be taken (particularly in adults), it may be possible to improve compliance by allowing patients to divide the daily dose. The objectives of this randomized, comparative, open-label, multicentre study, conducted in Senegal and in Cameroon in 2005, was to demonstrate the non-inferiority and to compare the safety of artesunate plus amodiaquine, as a single daily intake versus two daily intakes. **METHODS:** A three-day treatment period and 14-day follow-up period was performed in any subject weighting more than 10 kg, presenting with a malaria paroxysm confirmed by parasitaemia $\geq 1,000/\mu\text{l}$, after informed consent. Patients were randomly allocated into one of the two regimens, with dosage according to bodyweight range. All products were administered by an authorized person, blinded to both the investigating physician and the biologist. The primary endpoint was an adequate response to treatment on D14 (WHO definition). The two-sided 90% confidence interval of the difference was calculated on intent to treat (ITT) population; the acceptance limit for non-inferiority was 3%. The safety was evaluated by incidence of adverse events. **RESULTS:** Three-hundred and sixteen patients were included in the study. The two patient groups were strictly comparable on D0. The adequate responses to treatment were similar for the two treatment regimens on D14, PCR-corrected (99,4% in the one-daily intake group versus 99,3% in the comparative group). The statistical analyses demonstrated the non-inferiority of administering artesunate/amodiaquine as two intakes. The drug was well tolerated. The main adverse events were gastrointestinal disorders (2.5%) and pruritus (2.5%); safety profiles were similar in the two groups. **CONCLUSION:** This pilot study confirms the efficacy and good tolerability of artesunate plus amodiaquine, administered either in one or in two daily intakes.

35: *Malar J.* 2008 Jan 18;7(1):15

Comparison of all-cause and malaria-specific mortality from two West African countries with different malaria transmission patterns.

Ndugwa RP, Ramroth H, Mueller O, Jasseh M, Sie A, Kouyate B, Greenwood B, Becher H.

ABSTRACT: **BACKGROUND:** Malaria is a leading cause of death in children below five years of age in sub-Saharan Africa. All-cause and malaria-specific mortality rates for children under-five years old in a mesoendemic malaria area (The Gambia) were compared with those from a hyper/holoendemic area (Burkina Faso). **METHODS:** Information on observed person-years (PY), deaths and cause of death was extracted from online search, using key words: "Africa, The Gambia, Burkina Faso, malaria, Plasmodium falciparum, mortality, child survival, morbidity". Missing person-years were estimated and all-cause and malaria-specific mortality were

calculated as rates per 1,000 PY. Studies were classified as longitudinal/clinical studies or surveys/censuses. Linear regression was used to investigate mortality trends. RESULTS: Overall, 39 and 18 longitudinal/clinical studies plus 10 and 15 surveys and censuses were identified for The Gambia and Burkina Faso respectively (1960-2004). Model-based estimates for under-five all-cause mortality rates show a decline from 1960 to 2000 in both countries (Burkina Faso: from 71.8 to 39.0), but more markedly in The Gambia (from 104.5 to 28.4). The weighted-average malaria-specific mortality rate per 1,000 person-years for Burkina Faso (15.4, 95% CI: 13.0-18.3) was higher than that in The Gambia (9.5, 95% CI: 9.1-10.1). Malaria mortality rates did not decline over time in either country. CONCLUSIONS: Child mortality in both countries declined significantly in the period 1960 to 2004, possibly due to socio-economic development, improved health services and specific intervention projects. However, there was little decline in malaria mortality suggesting that there had been no major impact of malaria control programmes during this period. The difference in malaria mortality rates across countries points to significant differences in national disease control policies and/or disease transmission patterns.

36: *Malar J.* 2008 Jan 14;7(1):13

Polymorphisms of TNF-enhancer and gene for FcγRIIIa correlate with the severity of falciparum malaria in the ethnically diverse Indian population.

Sinha S, Mishra SK, Sharma S, Patibandla PK, Mallick PK, Sharma SK, Mohanty S, Pati SS, Mishra SK, Ramteke BK, Bhatt RM, Joshi H, Dash AP, Ahuja RC, Awasthi S, Consortium IG, Venkatesh V, Habib S.

ABSTRACT: BACKGROUND: Susceptibility/resistance to Plasmodium falciparum malaria has been correlated with polymorphisms in more than 30 human genes with most association analyses having been carried out on patients from Africa and south-east Asia. The aim of this study was to examine the possible contribution of genetic variants in the TNF and FCGR2A genes in determining severity/resistance to P. falciparum malaria in Indian subjects. METHODS: Allelic frequency distribution in populations across India was first determined by typing genetic variants of the TNF enhancer and the FCGR2A G/A SNP in 1,871 individuals from 55 populations. Genotyping was carried out by DNA sequencing, single base extension (SNaPshot), and DNA mass array (Sequenom). Plasma TNF was determined by ELISA. Comparison of datasets was carried out by Kruskal-Wallis and Mann-Whitney tests. Haplotypes and LD plots were generated by PHASE and Haploview, respectively. Odds ratio for risk assessment was calculated using EpiInfo™ version 3.4. RESULTS: A novel single nucleotide polymorphism (SNP) at position -76 was identified in the TNF enhancer along with other reported variants. Five TNF enhancer SNPs and one FCGR2A R131H (G/A) SNP were analysed for association with severity of P. falciparum malaria in a malaria-endemic and a non-endemic region of India in a case-control study with ethnically-matched controls enrolled from both regions. TNF -1031C and -863A alleles as well as homozygotes for the TNF enhancer haplotype CACGG (-1031T>C, -863C>A, -857C>T, -308G>A, -238G>A) correlated with enhanced plasma TNF levels in both patients and controls. Significantly higher TNF levels were observed in patients with severe malaria. Minor alleles of -1031 and -863 SNPs were associated with increased susceptibility to severe malaria. The high-affinity IgG2 binding FcγRIIIa AA (131H) genotype was significantly associated with protection from disease manifestation, with stronger association observed in the malaria non-endemic region. These results represent the first genetic analysis of the two immune regulatory molecules in the context of P. falciparum severity/resistance in the Indian population. CONCLUSIONS: Association of specific TNF and FCGR2A SNPs with cytokine levels and disease severity/resistance was indicated in patients from areas with differential disease endemicity. The data emphasizes the need for addressing the contribution of human genetic factors in malaria in the context of disease epidemiology and population genetic substructure within India.

Estimation of heterogeneity in malaria transmission by stochastic modelling of apparent deviations from mass action kinetics.

Smith TA.

ABSTRACT: **BACKGROUND:** Quantifying heterogeneity in malaria transmission is a prerequisite for accurate predictive mathematical models, but the variance in field measurements of exposure overestimates true micro-heterogeneity because it is inflated to an uncertain extent by sampling variation. Descriptions of field data also suggest that the rate of *Plasmodium falciparum* infection is not proportional to the intensity of challenge by infectious vectors. This appears to violate the principle of mass action that is implied by malaria biology. Micro-heterogeneity may be the reason for this anomaly. It is proposed that the level of micro-heterogeneity can be estimated from statistical models that estimate the amount of variation in transmission most compatible with a mass-action model for the relationship of infection to exposure. **METHODS:** The relationship between the entomological inoculation rate (EIR) for *falciparum* malaria and infection risk was reanalysed using published data for cohorts of children in Saradidi (western Kenya). Infection risk was treated as binomially distributed, and measurement-error (Poisson and negative binomial) models were considered for the EIR. Models were fitted using Bayesian Markov chain Monte Carlo algorithms and model fit compared for models that assume either mass-action kinetics, facilitation, competition or saturation of the infection process with increasing EIR. **RESULTS:** The proportion of inocula that resulted in infection in Saradidi was inversely related to the measured intensity of challenge. Models of facilitation showed, therefore, a poor fit to the data. When sampling error in the EIR was neglected, either competition or saturation needed to be incorporated in the model in order to give a good fit. Negative binomial models for the error in exposure could achieve a comparable fit while incorporating the more parsimonious and biologically plausible mass action assumption. Models that assume negative binomial micro-heterogeneity predict lower incidence of infection at a given average exposure than do those assuming exposure to be uniform. The negative binomial model moreover provides an estimate of the variance of the within-cohort distribution of the EIR and hence of within cohort heterogeneity in exposure. **CONCLUSIONS:** Apparent deviations from mass action kinetics in parasite transmission can arise from spatial and temporal heterogeneity in the inoculation rate, and from imprecision in its measurement. For parasites like *P. falciparum*, where there is no plausible biological rationale for deviations from mass action, this provides a strategy for estimating true levels of heterogeneity, since if mass-action is assumed, the within-population variance in exposure becomes identifiable in cohort studies relating infection to transmission intensity. Statistical analyses relating infection to exposure thus provide a valid general approach for estimating heterogeneity in transmission but only when they incorporate mass action kinetics and shrinkage estimates of exposure. Such analyses make it possible to include realistic levels of heterogeneity in dynamic models that predict the impact of control measures on transmission intensity.

Distribution of *Anopheles* in Vietnam, with particular attention to malaria vectors of the *Anopheles minimus* complex.

Garros C, Nguyen CV, Trung HD, Van Bortel W, Coosemans M, Manguin S.

ABSTRACT: **BACKGROUND:** The distribution of anopheline mosquitoes in Vietnam was examined, with a particular interest for the two sibling species of the *Anopheles minimus* complex (*Cellia*: *Myzomyia*), *An. minimus* and *Anopheles harrisoni*, respectively former species A and C. Because the morphological identification of both sibling species is difficult and may lead to misidentifications, accurate

data on their respective distribution are missing. This is of fundamental importance since the two species seem to exhibit differential vectorial capacities for malaria transmission. METHODS: Large entomological surveys based on cattle collections and molecular identifications of *An. minimus* s.l. were carried out in 23 sites throughout northern, central and south-eastern regions of Vietnam. RESULTS: Based on previous molecular works and our data, the distribution of anopheline species and the relative densities of *An. minimus* and *An. harrisoni* were mapped. It is noteworthy that there was a high specific biodiversity at each study site. *Anopheles minimus* s.l. and *Anopheles sinensis* were the main anopheline species in the northern region, whereas *Anopheles aconitus* and *Anopheles vagus* were the most frequent ones in the central region. The southern limit of *An. harrisoni* was increased to the latitude of 11 degrees N. Sympatry between both sibling species has been extended to new provinces. CONCLUSIONS: Malaria transmission is still high in central Vietnam and along bordering countries. Therefore, it is important to know and map the precise distribution of the main and secondary malaria vectors in Vietnam for applying efficient vector control programmes. Moreover, these maps should be regularly updated and linked to environmental characteristics relative to disease epidemiology, and environmental and climatic changes occurring in southeast Asia.

39: *Malar J.* 2008 Jan 11;7(1):10

Differential evolution of anti-*VAR2CSA* IgG3 in primigravidae and multigravidae pregnant women infected by *Plasmodium falciparum*.

Guitard J, Cottrell G, Mouloupo Magnouha N, Salanti A, Li T, Sow S, Deloron P, Tuikue Ndam N.

ABSTRACT: BACKGROUND: Pregnant women develop protective anti-VSA IgG1 and IgG3 when infected by *Plasmodium falciparum*. The major target of IgG from serum of infected pregnant women is VAR2CSA. METHODS: In this study, ELISA was used to compare the level of VAR2CSA DBL5e- specific IgG subclasses at enrolment and at delivery in a cohort of pregnant women in Senegal. All antibody measures were analysed in relation to placental infection according to parity. RESULTS: The results show an interaction between immune response to placental malaria and parity. A higher level of anti- DBL5e- IgG3 at enrolment and a higher increase between enrolment and delivery were found in primigravidae who presented with uninfected placenta at delivery in comparison to those who presented with an infection of the placenta. However, high antibody level at delivery was associated with the infection of the placenta in multigravidae. CONCLUSION: This high level of IgG3 in uninfected primigravidae suggests a protective role of these antibodies in this susceptible group, highlighting the importance of VAR2CSA in general and of some of its variants still to be defined, in the induction of protective immunity to pregnancy malaria.

40: *Malar J.* 2008 Jan 11;7(1):9

How antimalarial drug resistance affects post treatment prophylaxis.

White NJ.

ABSTRACT: Slowly eliminated antimalarial drugs suppress malaria reinfections for a period of time determined by the dose, the pharmacokinetic properties of the drug, and the susceptibility of the infecting parasites. This effect is called post-treatment prophylaxis (PTP). The clinical benefits of preventing recrudescence (reflecting treatment efficacy) compared with preventing reinfection (reflecting PTP) need further assessment. Antimalarial drug resistance shortens PTP. While blood concentrations are in the terminal elimination phase, the degree of shortening may be estimated from measurements of in-vitro susceptibility and the terminal elimination half-life. More information is needed on PTP following intermittent preventive treatments, and on the

relationship between the duration of PTP and immunity, so that policy recommendations can have a firmer evidence base.

41: *Malar J.* 2008 Jan 9;7(1):8

Correction: Monitoring the operational impact of insecticide usage for malaria control on *Anopheles funestus* from Mozambique.

Casimiro SL, Hemingway J, Sharp BL, Coleman M.

ABSTRACT: Since publication of our article [*Malaria J* 2007, 6:142], we have been made aware of several errors.

42: *Malar J.* 2008 Jan 9;7(1):7

Malaria risk and access to prevention and treatment in the paddies of the Kilombero Valley, Tanzania.

Hetzel MW, Alba S, Fankhauser M, Mayumana I, Lengeler C, Obrist B, Nathan R, Makemba AM, Mshana C, Schulze A, Mshinda H.

ABSTRACT: BACKGROUND: The Kilombero Valley is a highly malaria-endemic agricultural area in south-eastern Tanzania. Seasonal flooding of the valley is favourable to malaria transmission. During the farming season, many households move to distant field sites (shamba in Swahili) in the fertile river floodplain for the cultivation of rice. In the shamba, people live for several months in temporary shelters, far from the nearest health services. This study assessed the impact of seasonal movements to remote fields on malaria risk and treatment-seeking behaviour. METHODS: A longitudinal study followed approximately 100 randomly selected farming households over six months. Every household was visited monthly and whereabouts of household members, activities in the fields, fever cases and treatment seeking for recent fever episodes were recorded. RESULTS: Fever incidence rates were lower in the shamba compared to the villages and moving to the shamba did not increase the risk of having a fever episode. Children aged 1-4 years, who usually spend a considerable amount of time in the shamba with their caretakers, were more likely to have a fever than adults (odds ratio=4.47, 95% confidence interval 2.35-8.51). Protection with mosquito nets in the fields was extremely good (98% usage) but home-stocking of antimalarials was uncommon. Despite the long distances to health services, 55.8% (37.9-72.8) of the fever episodes were treated at a health facility, while home-management was less common (37%, 17.4-50.5). CONCLUSIONS: Living in the shamba does not appear to result in a higher fever-risk. Mosquito nets usage and treatment of fever in health facilities reflect awareness of malaria. Inability to obtain drugs in the fields may contribute to less irrational use of drugs but may pose an additional burden on poor farming households. A comprehensive approach is needed to improve access to treatment while at the same time assuring rational use of medicines and protecting fragile livelihoods.

43: *Malar J.* 2008 Jan 8;7(1):6

Feasibility and acceptability of artemisinin-based combination therapy for the home management of malaria in four African sites.

Ajayi IO, Browne EN, Garshong B, Bateganya F, Yusuf B, Agyei-Baffour P, Doamekpor L, Balyeku A, Munguti K, Cousens S, Pagnoni F.

ABSTRACT: BACKGROUND: The Home Management of Malaria (HMM) strategy was developed using chloroquine, a now obsolete drug, which has been replaced by artemisinin-based combination therapy (ACT) in health facility settings. Incorporation of ACT in HMM would greatly expand access to effective antimalarial therapy by the populations living in underserved areas in malaria endemic

countries. The feasibility and acceptability of incorporating ACT in HMM needs to be evaluated. METHODS: A multi-country study was performed in four district-size sites in Ghana (two sites), Nigeria and Uganda, with populations ranging between 38,000 and 60,000. Community medicine distributors (CMDs) were trained in each village to dispense pre-packaged ACT to febrile children aged 6-59 months, after exclusion of danger signs. A community mobilization campaign accompanied the programme. Artesunate-amodiaquine (AA) was used in Ghana and artemether-lumefantrine (AL) in Nigeria and Uganda. Harmonized qualitative and quantitative data collection methods were used to evaluate CMD performance, caregiver adherence and treatment coverage of febrile children with ACTs obtained from CMDs. RESULTS: Some 20,000 fever episodes in young children were treated with ACT by CMDs across the four study sites. Cross-sectional surveys identified 2,190 children with fever in the two preceding weeks, of whom 1,289 (59%) were reported to have received ACT from a CMD. Coverage varied from 52% in Nigeria to 75% in Ho District, Ghana. Coverage rates did not appear to vary greatly with the age of the child or with the educational level of the caregiver. A very high proportion of children were reported to have received the first dose on the day of onset or the next day in all four sites (range 86-97%, average 90%). The proportion of children correctly treated in terms of dose and duration was also high (range 74-97%, average 85%). Overall, the proportion of febrile children who received prompt treatment and the correct dose for the assigned duration of treatment ranged from 71% to 87% (average 77%). Almost all caregivers perceived ACT to be effective, and no severe adverse events were reported. CONCLUSION: ACTs can be successfully integrated into the HMM strategy.

44: *Malar J.* 2008 Jan 8;7(1):5

Improving equity in malaria treatment: Relationship of socio-economic status with health-seeking as well as with perceptions of ease of using the services of different providers for the treatment of malaria in Nigeria.

Onwujekwe O, Uzochukwu B, Eze S, Obikeze E, Okoli C, Ochonma O.

ABSTRACT: BACKGROUND: Equitable improvement of treatment-seeking for malaria will depend partly on how different socio-economic groups perceive the ease of accessing and utilizing malaria treatment services from different healthcare providers. Hence, it was important to investigate the link between socioeconomic status (SES) with differences in perceptions of ease of accessing and receiving treatment as well as with actual health-seeking for treatment of malaria from different providers. METHODS: Structured questionnaires were used to collect data from 1,351 health providers in four malaria-endemic communities in Enugu state, southeast Nigeria. Data was collected on the people's perceptions of ease of accessibility and utilization of different providers of malaria treatment using a pre-tested questionnaire. A SES index was used to examine inequities in perceptions and health-seeking. RESULTS: Patent medicine dealers (vendors) were the most perceived easily accessible providers, followed by private hospitals/clinics in two communities with full complement of healthcare providers: public hospital in the community with such a health provider and traditional healers in a community that is devoid of public healthcare facilities. There were inequities in perception of accessibility and use of different providers. There were also inequity in treatment-seeking for malaria and the poor spend proportionally more to treat the disease. CONCLUSION: Inequities exist in how different SES groups perceive the levels of ease of accessibility and utilization of different providers for malaria treatment. The differentials in perceptions of ease of access and use as well as health seeking for different malaria treatment providers among SES groups could be decreased by reducing barriers such as the cost of treatment by making health services accessible, available and at reduced cost for all groups.

45: *Malar J.* 2008 Jan 7;7(1):4

The costs of introducing artemisinin-based combination therapy: evidence from district- wide implementation in rural Tanzania.

Njau JD, Goodman CA, Kachur SP, Mulligan J, Munkondya JS, McHomvu N, Abdulla S, Bloland P, Mills A.

ABSTRACT: **BACKGROUND:** The development of antimalarial drug resistance has led to increasing calls for the introduction of artemisinin-based combination therapy (ACT). However, little evidence is available on the full costs associated with changing national malaria treatment policy. This paper presents findings on the actual drug and non-drug costs associated with deploying ACT in one district in Tanzania, and uses these data to estimate the nationwide costs of implementation in a setting where identification of malaria cases is primarily dependant on clinical diagnosis. **METHODS:** Detailed data were collected over a three year period on the financial costs of providing ACT in Rufiji District as part of a large scale effectiveness evaluation, including costs of drugs, distribution, training, treatment guidelines and other information, education and communication (IEC) materials and publicity. The district-level costs were scaled up to estimate the costs of nationwide implementation, using four scenarios to extrapolate variable costs. **RESULTS:** The total district costs of implementing ACT over the three year period were slightly over one million USD, with drug purchases accounting for 72.8% of this total. The composite (best) estimate of nationwide costs for the first three years of ACT implementation was 48.3 million USD (1.29 USD per capita), which varied between 21 and 67.1 million USD in the sensitivity analysis (2003 USD). In all estimates drug costs constituted the majority of total costs. However, non-drug costs such as IEC materials, drug distribution, communication, and health worker training were also substantial, accounting for 31.4% of overall ACT implementation costs in the best estimate scenario. Annual implementation costs are equivalent to 9.5% of Tanzania's recurrent health sector budget, and 28.7% of annual expenditure on medical supplies, implying a 6-fold increase in the national budget for malaria treatment. **CONCLUSION:** The costs of implementing ACT are substantial. Although drug purchases constituted a majority of total costs, non-drug costs were also considerable. It is clear that substantial external resources will be required to facilitate and sustain effective ACT delivery across Tanzania and other malaria-endemic countries.

46: *Malar J.* 2008 Jan 7;7(1):3

Recombinant human erythropoietin increases survival and reduces neuronal apoptosis in a murine model of cerebral malaria.

Wiese L, Hempel C, Penkowa M, Kirkby N, Kurtzhals JA.

ABSTRACT: **BACKGROUND:** Cerebral malaria (CM) is an acute encephalopathy with increased pro-inflammatory cytokines, sequestration of parasitized erythrocytes and localized ischaemia. In children CM induces cognitive impairment in about 10% of the survivors. Erythropoietin (Epo) has - besides of its well known haematopoietic properties - significant anti-inflammatory, antioxidant and anti-apoptotic effects in various brain disorders. The neurobiological responses to exogenously injected Epo during murine CM were examined. **METHODS:** Female C57BL/6j mice (4-6 weeks), infected with *Plasmodium berghei* ANKA, were treated with recombinant human Epo (rhEpo; 50-5000U/kg/OD, i.p.) at different time points. The effect on survival was measured. Brain pathology was investigated by TUNEL (Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-digoxigenin nick end labelling), as a marker of apoptosis. Gene expression in brain tissue was measured by real time PCR. **RESULTS:** Treatment with rhEpo increased survival in mice with CM in a dose- and time-dependent manner and reduced apoptotic cell death of neurons as well as the expression of

pro-inflammatory cytokines in the brain. This neuroprotective effect appeared to be independent of the haematopoietic effect. CONCLUSIONS: These results and its excellent safety profile in humans makes rhEpo a potential candidate for adjunct treatment of CM.

47: *Malar J.* 2008 Jan 7;7(1):2

Risk factors for house-entry by malaria vectors in a rural town and satellite villages in The Gambia.

Kirby MJ, Green C, Milligan PM, Sismanidis C, Jasseh M, Conway DJ, Lindsay SW.

ABSTRACT: BACKGROUND: In the pre-intervention year of a randomized controlled trial investigating the protective effects of house screening against malaria-transmitting vectors, a multi-factorial risk factor analysis study was used to identify factors that influence mosquito house entry. METHODS: Mosquitoes were sampled using CDC light traps in 976 houses, each on one night, in Farafenni town and surrounding villages during the malaria-transmission season in The Gambia. Catches from individual houses were both (a) left unadjusted and (b) adjusted relative to the number of mosquitoes caught in four sentinel houses that were operated nightly throughout the period, to allow for night-to-night variation. Houses were characterized by location, architecture, human occupancy and their mosquito control activities, and the number and type of domestic animals within the compound. RESULTS: 106,536 mosquitoes were caught, of which 55% were *Anopheles gambiae sensu lato*, the major malaria vectors in the region. There were seven fold higher numbers of *An. gambiae s.l.* in the villages (geometric mean per trap night = 43.7, 95% confidence intervals, CIs = 39.5-48.4) than in Farafenni town (6.3, 5.7-7.2) and significant variation between residential blocks ($p < 0.001$). A negative binomial multivariate model performed equally well using unadjusted or adjusted trap data. Using the unadjusted data the presence of nuisance mosquitoes was reduced if the house was located in the town (odds ratio, OR = 0.11, 95% CIs = 0.09-0.13), the eaves were closed (OR = 0.71, 0.60-0.85), a horse was tethered near the house (OR = 0.77, 0.73-0.82), and churai, a local incense, was burned in the room at night (OR = 0.56, 0.47-0.66). Mosquito numbers increased per additional person in the house (OR = 1.04, 1.02-1.06) or trapping room (OR = 1.19, 1.13-1.25) and when the walls were made of mud blocks compared with concrete (OR = 1.44, 1.10-1.87). CONCLUSIONS: This study demonstrates that the risk of malaria transmission is greatest in rural areas, where large numbers of people sleep in houses made of mud blocks, where the eaves are open, horses are not tethered nearby and where churai is not burnt at night. These factors need to be considered in the design and analysis of intervention studies designed to reduce malaria transmission in The Gambia and other parts of sub-Saharan Africa.

48: *Med Trop (Mars)*. 2007 Oct;67(5):477-80.

Randomized clinical trial of two malaria prophylaxis regimens for pregnant women in Faladie, Mali [Article in French]

Diallo M, Dabo CA, Saye R, Yattara O, Diarra MA, Kayentao K, Ongoiba A, Sangho H, Doumbo O.

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From June 2003 to May 2004 we carried out a comparative study of two malaria prophylaxis regimens for pregnant women. The purpose was to compare the efficacy of two regimens using chloroquine (CQ) or sulfadoxine-pyrimethamine (SP) during pregnancy and delivery in a village located in an endemic area of Mali. The study was carried out in Faladié (District of Kati) located 80 km from Bamako. Prophylaxis was administered during the second and third trimesters of pregnancy

(except the 9th month for SP). A total of 301 pregnant women were enrolled including 150 in the CQ group and 151 in the SP group. At the onset of the study, the two groups were comparable with regard to socio-demographic and malaria factors. At the time of delivery, malaria infection was reduced by 43.3% in the CQ group ($P < 10^{-6}$), and by 79.1% in the SP group ($p < 10^{-6}$). The anemia rate was reduced by 57.5% in the CQ group (Ch2 of McNemar = 0.017), and by 74.8% in the SP group (Ch2 of McNemar = 0.025). The incidence of placental infection was 20.6 % in the CQ group versus 8.3 % in the SP group ($p = 4.10^{-3}$). Overall 16.7% of newborns presented low birth weight at delivery including 70.4% in the CQ group. The findings of this study suggest that intermittent presumptive treatment using SP is more effective than intermittent presumptive treatment using CQ in protecting both the mother and newborn against intra-uterine malaria transmission and its consequences.

49: *Mem Inst Oswaldo Cruz*. 2007 Dec;102(8):953-8.

Population structure of the malaria vector *Anopheles darlingi* in Rondônia, Brazilian Amazon, based on mitochondrial DNA.

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Anopheles darlingi is the most important Brazilian malaria vector, with a widespread distribution in the Amazon forest. Effective strategies for vector control could be better developed through knowledge of its genetic structure and gene flow among populations, to assess the vector diversity and competence in transmitting Plasmodium. The aim of this study was to assess the genetic diversity of *An. darlingi* collected at four locations in Porto Velho, by sequencing a fragment of the ND4 mitochondrial gene. From 218 individual mosquitoes, we obtained 20 different haplotypes with a diversity index of 0.756, equivalent to that found in other neotropical anophelines. The analysis did not demonstrate significant population structure. However, haplotype diversity within some populations seems to be over-represented, suggesting the presence of sub-populations, but the presence of highly represented haplotypes complicates this analysis. There was no clear correlation among genetic and geographical distance and there were differences in relation to seasonality, which is important for malarial epidemiology.

50: *Mem Inst Oswaldo Cruz*. 2007 Dec;102(8):943-51.

Naturally acquired antibodies to merozoite surface protein (MSP)-1(19) and cumulative exposure to *Plasmodium falciparum* and *Plasmodium vivax* in remote populations of the Amazon Basin of Brazil.

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To infer recent patterns of malaria transmission, we measured naturally acquired IgG antibodies to the conserved 19-kDa C-terminal region of the merozoite surface protein (MSP)-1 of both *Plasmodium vivax* (PvMSP-1(19)) and *Plasmodium falciparum* (PfMSP-1(19)) in remote malaria-exposed populations of the Amazon Basin. Community-based cross-sectional surveys were carried out between 2002 and 2003 in subjects of all age groups living along the margins of the Unini and Jaú rivers, Northwestern Brazil. We found high prevalence rates of IgG antibodies to PvMSP-1(19) (64.0 - 69.6%) and PfMSP-1(19) (51.6 - 52.0%), with significant differences in the proportion of subjects with antibodies to PvMSP-1(19)

according to age, place of residence and habitual involvement in high-risk activities, defining some groups of highly exposed people who might be preferential targets of malaria control measures. In contrast, no risk factor other than age was significantly associated with seropositivity to PfMSP-1(19). Only 14.1% and 19.3% of the subjects tested for antibodies to PvMSP-1(19) and PfMSP-1(19) in consecutive surveys (142 - 203 days apart) seroconverted or had a three fold or higher increase in the levels of antibodies to these antigens. We discuss the extent to which serological data correlated with the classical malariometric indices and morbidity indicators measured in the studied population at the time of the seroprevalence surveys and highlight some limitations of serological data for epidemiological inference.

51: *Mol Ecol.* 2007 Dec 20

Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in *Anopheles arabiensis* sampled from an area of cotton fields in Northern Cameroon.

Müller P, Chouaïbou M, Pignatelli P, Etang J, Walker ED, Donnelly MJ, Simard F, Ranson H.

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Spraying of agricultural crops with insecticides can select for resistance in nontarget insects and this may compromise the use of insecticides for the control of vector-borne diseases. The tolerance of the malaria vector, *Anopheles arabiensis* to deltamethrin was determined in a field population from a cotton-growing region of Northern Cameroon both prior to and midway through the 4-month period of insecticide application to the cotton crop. A 1.6-fold increase in the median knockdown time was observed. To determine whether this increased tolerance was associated with constitutively elevated levels of genes commonly associated with insecticide resistance, RNA was extracted from F(1) progeny from family lines of field-caught mosquitoes and hybridized to the *Anopheles gambiae* detox chip. The experimental design avoided the confounding effects of colonization, and this study is the first to measure gene expression in the progeny of gravid, wild-caught mosquitoes. Several genes with antioxidant roles, including superoxide dismutases, a glutathione S-transferase and a thioredoxin-dependent peroxidase, and a cytochrome P450 showed elevated expression in mosquito families collected during the insecticide-spraying programme. These genes may constitute an important general defence mechanism against insecticides. Intriguingly, the levels of expression of these genes were strongly correlated suggesting a common regulatory mechanism.

52: *Parasite Immunol.* 2007 Dec 19

A decrease of plasma macrophage migration inhibitory factor concentration is associated with lower numbers of circulating lymphocytes in experimental *Plasmodium falciparum* malaria.

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Macrophage migration inhibitory factor (MIF) has recently been implicated in the pathogenesis of malarial anaemia. However, field studies have reported contradictory results on circulating MIF concentrations in patients with clinically overt *Plasmodium falciparum* malaria. We determined plasma MIF levels over time in 10 healthy volunteers during experimental *P. falciparum* infection.

Under fully controlled conditions, MIF levels decreased significantly during early blood-stage infection and reached a nadir at day 8 post-infection. A decrease in the number of circulating lymphocytes, which are an important source of MIF production, paralleled the decrease in MIF levels. Monocyte/macrophage counts remained unchanged. At MIF nadir, the anti-inflammatory cytokine interleukin (IL)-10, which is an inhibitor of T-cell MIF production, was detectable in only 2 of 10 volunteers. Plasma concentrations of the pro-inflammatory cytokines IL-8 and IL-1beta were only marginally elevated. We conclude that circulating MIF levels decrease early in blood-stage malaria as a result of the decline in circulating lymphocytes.

53: *Parasitol Res.* 2008 Jan 5

Inhibition of glutathione-S-transferase from Plasmodium yoelii by protoporphyrin IX, cibacron blue and menadione: implications and therapeutic benefits.

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The rapidly developing resistance to drugs used for prophylaxis and treatment of malaria makes the identification of novel drug targets necessary. Glutathione-S-transferase (GST, E.C. 2.5.1.18), an important enzyme of the glutathione (GSH) cycle, is considered to be an essential detoxification enzyme in malarial parasites. Selective inhibition of this enzyme from malarial parasites by various classes of inhibitors may be viewed as a potential chemotherapeutic strategy to combat malaria. Purified GST from *Plasmodium yoelii* was inhibited by compounds like protoporphyrin IX, cibacron blue, as well as by the GSH depletor menadione. Cytosolic GST was inhibited to varying degrees by each compound. A characteristic inhibitor constant (K_i) was obtained for each inhibitor. The possible consequences of selective inhibition of parasitic GST to that of the host are discussed in relation to the chemotherapy of malaria.

54: *Parasitology.* 2008 Jan 24;:1-8

Using evolutionary costs to enhance the efficacy of malaria control via genetically manipulated mosquitoes.

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SUMMARYAn earlier mathematical model exploring the use of genetically manipulated mosquitoes for malaria control suggested that the prevalence of malaria is reduced significantly only if almost all mosquitoes become completely resistant to malaria. Central to the model was the 'cost of resistance': the reduction of a resistant mosquito's evolutionary fitness in comparison with a sensitive one's. Here, we consider the possibility of obtaining more optimistic outcomes by taking into account the epidemiological (in addition to the evolutionary) consequences of a cost of resistance that decreases the life-span of adult mosquitoes (the most relevant parameter for the parasite's epidemiology). There are two main results. First, if despite its cost, resistance is fixed in the population, increasing the cost of resistance decreases the intensity of transmission. However, this epidemiological effect is weak if resistance is effective enough to be considered relevant for control. Second, if the cost of resistance prevents its fixation, increasing it intensifies transmission. Thus, the epidemiological effect of the cost of resistance cannot compensate for the lower frequency of resistant mosquitoes in the population. Overall, our conclusion remains pessimistic: so that genetic manipulation can become a promising method of malaria control, we need techniques that enable almost all mosquitoes to be almost completely resistant to infection.

55: *Pathol Biol (Paris)*. 2008 Jan 3

Tolerance and efficacy of mefloquine as the first line treatment of uncomplicated *P. falciparum* malaria in children. [Article in French]

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INTRODUCTION: Given the national therapeutic guidelines in France, halofantrine represents the first line treatment of uncomplicated *Plasmodium falciparum* (*P. falciparum*) malaria in children. But several disadvantages exist using halofantrine in paediatrics. **OBJECTIVES:** The primary objective of this study is to evaluate the tolerance and the efficacy of mefloquine as the first line treatment of uncomplicated *P. falciparum* malaria in a paediatric emergency department. The secondary objective of the study is to evaluate whether symptomatic measures may improve the gastrointestinal tolerance of mefloquine. **PATIENTS AND METHODS:** This retrospective observational cohort study includes all the patients who have been treated for acute uncomplicated *P. falciparum* malaria in the paediatric emergency department of the Hospital Trousseau (Paris, France) in 2003. **RESULTS:** First line treatment was mefloquine in 35 children. Early vomiting occurred in 22 (63%) cases. All children responded to mefloquine therapy except two children who had persistent vomiting early after mefloquine therapy and required intravenous quinine. Those two children had initial vomiting. Light meal and metopimazine prophylaxis did not precede mefloquine intake in those two children. **CONCLUSION:** This study suggests that mefloquine treatment of uncomplicated *P. falciparum* malaria is effective and well tolerated in children. Furthermore, a light meal and metopimazine prophylaxis preceding mefloquine intake may improve its gastrointestinal tolerance.

56: *Pediatr Infect Dis J*. 2008 Feb;27(2):130-135.

Life-Threatening Malaria in African Children: A Prospective Study in a Mesoendemic Urban Setting.

Ranque S, Poudiougou B, Traoré A, Keita M, Oumar AA, Safeukui I, Marquet S, Cabantous S, Diakitè M, Mintha D, Cissé MB, Keita MM, Dessein AJ, Doumbo OK.

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BACKGROUND:: The population exposed to malaria within African cities has steadily increased. However, comprehensive data on life-threatening malaria features and risk factors in children from urban areas with seasonal malaria transmission, such as in Bamako (Mali), are lacking. **METHODS::** Children admitted to the Gabriel Touré Hospital in Bamako with severe malarial anemia (SMA) and/or cerebral malaria (CM) were prospectively included in the study. Indicators of either SMA or CM were analyzed using logistic regression; and death hazard ratios (HRs) were estimated through survival analysis. **RESULTS::** The study included 455 children: 66% presented with CM, 34% with SMA, 3% with hypoglycemia (HG); 5% with dehydration; 17% with respiratory distress (RD); 25% with splenomegaly; and 92% with hepatomegaly. The children with CM were older than those with SMA. CM was more often associated with dehydration, HG, and RD, whereas SMA was more often associated with splenomegaly. The overall case fatality rate was 16%, and 94% of the children who died had CM. HG [HR: 2.37; 95% confidence interval (CI): 1.04-5.39; P = 0.040], RD (HR: 4.23; 95% CI: 2.46-7.30; P < 10) and a deep coma with a Blantyre score of less than 3 (HR: 6.78, 95% CI: 2.43-18.91; P < 10), were

all independent predictors of death. CONCLUSIONS:: These findings delineate the patterns of severe malaria in children in a West African mesoendemic urban setting. They validate practicable prognostic indicators of life-threatening malaria for use in the limited facilities available in African health centers and provide a frame of reference for further research addressing life-threatening malaria in this setting.

57: *Phytochem Anal.* 2008 Jan 15

Quantification of artemisinin in *Artemisia annua* extracts by (1)H-NMR.

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Artemisinin is a polycyclic sesquiterpene lactone that is highly effective against multidrug-resistant strains of *Plasmodium falciparum*, the etiological agent of the most severe form of malaria. Determination of artemisinin in the source plant, *Artemisia annua*, is a challenging problem since the compound is present in very low concentrations, is thermolabile and unstable, and lacks chromophoric or fluorophoric groups. The aim of this study was to develop a simple protocol for the quantification of artemisinin in a plant extract using an (1)H-NMR method. Samples were prepared by extraction of leaf material with acetone, treatment with activated charcoal to remove chlorophylls and removal of solvent. (1)H-NMR spectra were measured on samples dissolved in deuteriochloroform with tert-butanol as internal standard. Quantification was carried out using the δ 5.864 signal of artemisinin and the δ 1.276 signal of tert-butanol. The method was optimised and fully validated against a reference standard of artemisinin. The results were compared with those obtained from the same samples quantified using an HPLC-refractive index (RI) method. The (1)H-NMR method gave a linear response for artemisinin within the range 9.85-97.99 μ g (r² = 0.9968). Using the described method, yields of artemisinin in the range 0.77-1.06% were obtained from leaves of the *A. annua* hybrid CPQBA x POP, and these values were in agreement with those obtained using an HPLC-RI. Copyright (c) 2008 John Wiley & Sons, Ltd.

58: *PLoS Comput Biol.* 2007 Dec;3(12):e255.

Determination of the processes driving the acquisition of immunity to malaria using a mathematical transmission model.

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Acquisition of partially protective immunity is a dominant feature of the epidemiology of malaria among exposed individuals. The processes that determine the acquisition of immunity to clinical disease and to asymptomatic carriage of malaria parasites are poorly understood, in part because of a lack of validated immunological markers of protection. Using mathematical models, we seek to better understand the processes that determine observed epidemiological patterns. We have developed an age-structured mathematical model of malaria transmission in which acquired immunity can act in three ways ("immunity functions"): reducing the probability of clinical disease, speeding the clearance of parasites, and increasing tolerance to subpatent infections. Each immunity function was allowed to vary in efficacy depending on both age and malaria transmission intensity. The results were compared to age patterns of parasite prevalence and clinical disease in endemic settings in northeastern Tanzania and The Gambia. Two types of immune function were required to reproduce the epidemiological age-prevalence curves

seen in the empirical data; a form of clinical immunity that reduces susceptibility to clinical disease and develops with age and exposure (with half-life of the order of five years or more) and a form of anti-parasite immunity which results in more rapid clearance of parasitaemia, is acquired later in life and is longer lasting (half-life of >20 y). The development of anti-parasite immunity better reproduced observed epidemiological patterns if it was dominated by age-dependent physiological processes rather than by the magnitude of exposure (provided some exposure occurs). Tolerance to subpatent infections was not required to explain the empirical data. The model comprising immunity to clinical disease which develops early in life and is exposure-dependent, and anti-parasite immunity which develops later in life and is not dependent on the magnitude of exposure, appears to best reproduce the pattern of parasite prevalence and clinical disease by age in different malaria transmission settings. Understanding the effector mechanisms underlying these two immune functions will assist in the design of transmission-reducing interventions against malaria.

59: *PLoS ONE*. 2008 Jan 23;3(1):e1471.

A trial of the efficacy, safety and impact on drug resistance of four drug regimens for seasonal intermittent preventive treatment for malaria in senegalese children.

Sokhna C, Cissé B, Bâ el H, Milligan P, Hallett R, Sutherland C, Gaye O, Boulanger D, Simondon K, Simondon F, Targett G, Lines J, Greenwood B, Trape JF.

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SUMMARY: In the Sahel, most malaria deaths occur among children 1-4 years old during a short transmission season. A trial of seasonal intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP) and a single dose of artesunate (AS) showed an 86% reduction in the incidence of malaria in Senegal but this may not be the optimum regimen. We compared this regimen with three alternatives. **METHODS:** 2102 children aged 6-59 months received either one dose of SP plus one dose of AS (SP+1AS) (the previous regimen), one dose of SP plus 3 daily doses of AS (SP+3AS), one dose of SP plus three daily doses of amodiaquine (AQ) (SP+3AQ) or 3 daily doses of AQ and AS (3AQ+3AS). Treatments were given once a month on three occasions during the malaria transmission season. The primary end point was incidence of clinical malaria. Secondary end-points were incidence of adverse events, mean haemoglobin concentration and prevalence of parasites carrying markers of resistance to SP. **FINDINGS:** The incidence of malaria, and the prevalence of parasitaemia at the end of the transmission season, were lowest in the group that received SP+3AQ: 10% of children in the group that received SP+1AS had malaria, compared to 9% in the SP+3AS group (hazard ratio HR 0.90, 95%CI 0.60, 1.36); 11% in the 3AQ+3AS group, HR 1.1 (0.76-1.7); and 5% in the SP+3AQ group, HR 0.50 (0.30-0.81). Mutations associated with resistance to SP were present in almost all parasites detected at the end of the transmission season, but the prevalence of *Plasmodium falciparum* was very low in the SP+3AQ group. **CONCLUSIONS:** Monthly treatment with SP+3AQ is a highly effective regimen for seasonal IPT. Choice of this regimen would minimise the spread of drug resistance and allow artemisinins to be reserved for the treatment of acute clinical malaria. **TRIAL REGISTRATION:** Clinicaltrials.gov NCT00132548.

60: *PLoS ONE*. 2008 Jan 23;3(1):e1465.

Safety and Immunogenicity of an AMA-1 Malaria Vaccine in Malian Adults: Results of a Phase 1 Randomized Controlled Trial.

Thera MA, Doumbo OK, Coulibaly D, Diallo DA, Kone AK, Guindo AB, Traore K, Dicko A, Sagara I, Sissoko MS, Baby M, Sissoko M, Diarra I, Niangaly A, Dolo A, Daou M, Diawara SI, Heppner DG, Stewart VA, Angov E, Bergmann-Leitner ES, Lanar DE, Dutta

S, Soisson L, Diggs CL, Leach A, Owusu A, Dubois MC, Cohen J, Nixon JN, Gregson A, Takala SL, Lyke KE, Plowe CV.

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BACKGROUND: The objective was to evaluate the safety, reactogenicity and immunogenicity of the AMA-1-based blood-stage malaria vaccine FMP2.1/AS02A in adults exposed to seasonal malaria. **METHODOLOGY/PRINCIPAL FINDINGS:** A phase 1 double blind randomized controlled dose escalation trial was conducted in Bandiagara, Mali, West Africa, a rural town with intense seasonal transmission of *Plasmodium falciparum* malaria. The malaria vaccine FMP2.1/AS02A is a recombinant protein (FMP2.1) based on apical membrane antigen-1 (AMA-1) from the 3D7 clone of *P. falciparum*, adjuvanted with AS02A. The comparator vaccine was a cell-culture rabies virus vaccine (RabAvert). Sixty healthy, malaria-experienced adults aged 18-55 y were recruited into 2 cohorts and randomized to receive either a half dose or full dose of the malaria vaccine (FMP2.1 25 microg/AS02A 0.25 mL or FMP2.1 50 microg/AS02A 0.5 mL) or rabies vaccine given in 3 doses at 0, 1 and 2 mo, and were followed for 1 y. Solicited symptoms were assessed for 7 d and unsolicited symptoms for 30 d after each vaccination. Serious adverse events were assessed throughout the study. Titers of anti-AMA-1 antibodies were measured by ELISA and *P. falciparum* growth inhibition assays were performed on sera collected at pre- and post-vaccination time points. Transient local pain and swelling were common and more frequent in both malaria vaccine dosage groups than in the comparator group. Anti-AMA-1 antibodies increased significantly in both malaria vaccine groups, peaking at nearly 5-fold and more than 6-fold higher than baseline in the half-dose and full-dose groups, respectively. **CONCLUSION/SIGNIFICANCE:** The FMP2.1/AS02A vaccine had a good safety profile, was well-tolerated, and was highly immunogenic in malaria-exposed adults. This malaria vaccine is being evaluated in Phase 1 and 2 trials in children at this site. **TRIAL REGISTRATION:** ClinicalTrials.gov NCT00308061.

61: *PLoS Pathog.* 2007 Dec 28;3(12):e195.

Progression of *Plasmodium berghei* through *Anopheles stephensi* is density-dependent.

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It is well documented that the density of *Plasmodium* in its vertebrate host modulates the physiological response induced; this in turn regulates parasite survival and transmission. It is less clear that parasite density in the mosquito regulates survival and transmission of this important pathogen. Numerous studies have described conversion rates of *Plasmodium* from one life stage to the next within the mosquito, yet few have considered that these rates might vary with parasite density. Here we establish infections with defined numbers of the rodent malaria parasite *Plasmodium berghei* to examine how parasite density at each stage of development (gametocytes; ookinetes; oocysts and sporozoites) influences development to the ensuing stage in *Anopheles stephensi*, and thus the delivery of infectious sporozoites to the vertebrate host. We show that every developmental transition exhibits strong density dependence, with numbers of the ensuing stages saturating at high density. We further show that when fed ookinetes at very low densities, oocyst development is facilitated by increasing ookinete number (i.e., the efficiency of ookinete-oocyst transformation follows a sigmoid relationship). We discuss how observations on this model system generate important hypotheses for the understanding of malaria biology, and how these might guide the rational analysis of interventions against the transmission of the malaria parasites of humans by their diverse vector species.

62: *Proc Natl Acad Sci U S A.* 2008 Jan 29;105(4):1321-6.

Protection against cerebral malaria by the low-molecular-weight thiol pantethine.

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We report that administration of the low-molecular-weight thiol pantethine prevented the cerebral syndrome in *Plasmodium berghei* ANKA-infected mice. The protection was associated with an impairment of the host response to the infection, with in particular a decrease of circulating microparticles and preservation of the blood-brain barrier integrity. Parasite development was unaffected. Pantethine modulated one of the early steps of the inflammation-coagulation cascade, i.e., the transbilayer translocation of phosphatidylserine at the cell surface that we demonstrated on red blood cells and platelets. In this, pantethine mimicked the inactivation of the ATP-binding-cassette transporter A1 (ABCA1), which also prevents the cerebral syndrome in this malaria model. However, pantethine acts through a different pathway, because ABCA1 activity was unaffected by the treatment. The mechanisms of pantethine action were investigated, using the intact molecule and its constituents. The disulfide group (oxidized form) is necessary to lower the platelet response to activation by thrombin and collagen. Thio-sensitive mechanisms are also involved in the impairment of microparticle release by TNF-activated endothelial cells. In isolated cells, the effects were obtained by cystamine that lacks the pantothenic moiety of the molecule; however, the complete molecule is necessary to protect against cerebral malaria. Pantethine is well tolerated, and it has already been administered in other contexts to man with limited side effects. Therefore, trials of pantethine treatment in adjunctive therapy for severe malaria are warranted.

63: *Proc Natl Acad Sci U S A.* 2008 Jan 22;105(3):991-6.

Impaired cytoadherence of *Plasmodium falciparum*-infected erythrocytes containing sickle hemoglobin.

Cholera R, Brittain NJ, Gillrie MR, Lopera-Mesa TM, Diakitè SA, Arie T, Krause MA, Guindo A, Tubman A, Fujioka H, Diallo DA, Doumbo OK, Ho M, Wellem TE, Fairhurst RM.

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Sickle trait, the heterozygous state of normal hemoglobin A (HbA) and sickle hemoglobin S (HbS), confers protection against malaria in Africa. AS children infected with *Plasmodium falciparum* are less likely than AA children to suffer the symptoms or severe manifestations of malaria, and they often carry lower parasite densities than AA children. The mechanisms by which sickle trait might confer such malaria protection remain unclear. We have compared the cytoadherence properties of parasitized AS and AA erythrocytes, because it is by these properties that parasitized erythrocytes can sequester in postcapillary microvessels of critical tissues such as the brain and cause the life-threatening complications of malaria. Our results show that the binding of parasitized AS erythrocytes to microvascular endothelial cells and blood monocytes is significantly reduced relative to the binding of parasitized AA erythrocytes. Reduced binding correlates with the altered display of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), the parasite's major cytoadherence ligand and virulence factor on the erythrocyte surface. These findings identify a mechanism of protection for HbS that has features in common with that of hemoglobin C

(HbC). Coinherited hemoglobin polymorphisms and naturally acquired antibodies to PfEMP-1 may influence the degree of malaria protection in AS children by further weakening cytoadherence interactions.

64: *Proc Natl Acad Sci U S A.* 2008 Jan 8;105(1):305-10.

A combined transcriptome and proteome survey of malaria parasite liver stages.

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For 50 years since their discovery, the malaria parasite liver stages (LS) have been difficult to analyze, impeding their utilization as a critical target for anti-infection vaccines and drugs. We have undertaken a comprehensive transcriptome analysis in combination with a proteomic survey of LS. Green fluorescent protein-tagged *Plasmodium yoelii* (PyGFP) was used to efficiently isolate LS-infected hepatocytes from the rodent host. Genome-wide LS gene expression was profiled and compared with other parasite life cycle stages. The analysis revealed approximately 2,000 genes active during LS development, and proteomic analysis identified 816 proteins. A subset of proteins appeared to be expressed in LS only. The data revealed exported parasite proteins and LS metabolic pathways including expression of FASII pathway enzymes. The FASII inhibitor hexachlorophene and the antibiotics, tetracycline and rifampicin, that target the apicoplast inhibited LS development, identifying FASII and other pathways localized in the apicoplast as potential drug targets to prevent malaria infection.

65: *Recent Patents Anti-Infect Drug Disc.* 2007 Nov;2(3):222-32.

Recent patent reviews on small molecule-based antimalarial drugs.

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Malaria is the number one disease in the world responsible for 1-3 million deaths each year. The world wide number of malaria patients is estimated at 400 to 900 million. Approximately one third of the world's population lives in malaria-endemic areas, including Central and South America, Asia, and Africa. *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* are malaria parasites responsible for infecting humans. Mosquitoes that carry malaria parasites have become resistant to insecticides, and the deadliest parasites have become resistant to previously effective antimalarial drugs such as chloroquine, quinine and other clinically used agents. Because of the widespread incidence of malaria in certain parts of the world and because of the increasing parasite resistance to standard anti-malarial agents, there is an urgent need for introducing new effective drugs. This review presents the recent patents that reveal development of novel antimalarial drugs.

66: *Reprod Toxicol.* 2007 Nov 17

Toxicity of artemisinin [*Artemisia annua* L.] in two different periods of pregnancy in Wistar rats.

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Artemisinin compounds are important for treating multidrug-resistant malaria;

however, the possible resorption and abnormalities observed in animal reproduction studies may contraindicate artemisinin use during the first trimester. To evaluate whether artemisinin interferes with developmental outcomes at different periods of pregnancy, Wistar rats were treated by gavage with increasing doses of 7, 35 and 70mg/kg/day from gestational day [GD] 7 to 13 or 14 to 20. Viable embryos and post-implantation losses, and progesterone and testosterone levels, were monitored in the former treatment group and pregnancy and outcomes data, post-implantation losses and male and female developmental endpoints of the offspring were evaluated in the latter treatment group. Results indicate toxicity for both periods of treatment, with lower sensitivity at later stages of pregnancy. The results showed that dosing with 35 or 75mg/kg of artemisinin caused high percentages of post-implantation losses that correlated with a trend to lower maternal progesterone and a significant maternal testosterone decrease. These findings demonstrate that oral administration of artemisinin can adversely affect post-implantation development and pregnancy in the rat.

67: *Ther Drug Monit.* 2008 Feb;30(1):10-15.

Polymorphism of Antimalaria Drug Metabolizing, Nuclear Receptor, and Drug Transport Genes among Malaria Patients in Zanzibar, East Africa.

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Artemisinin-based combination therapy is a main strategy for malaria control in Africa. Zanzibar introduced this new treatment policy in 2003. The authors have studied the prevalence of a number of functional single nucleotide polymorphisms (SNPs) in genes associated with the elimination of the artemisinin-based combination therapy compounds in use in Zanzibar to investigate the frequencies of subgroups potentially at higher drug exposure and therefore possible higher risk of toxicity. One hundred three unrelated children with uncomplicated malaria from the Unguja and Pemba islands of Zanzibar were enrolled. With use of polymerase chain reaction (PCR)-restriction fragment length polymorphism and real-time PCR-based allele discrimination methods, the CYP2B6 (G15631T), CYP3A4 (A-392G), CYP3A5 (A6986G, G14690A, 27131-132 insT, C3699T) SNPs and MDR1 SNPs C3435T, G2677T/A, and T-129C were analyzed. PCR product sequencing was applied to regulatory regions of MDR1, the CYP3A4 proximal promoter, and to exons 2 and 5 of PXR, a gene coding for a nuclear factor activated by artemisinin antimalarials and associated with the transcription induction of most of the studied genes. Homozygous subjects for alleles coding for low activity proteins were found at the following frequencies: 1) MDR1: 2.9%; 2) CYP2B6: 9.7%; 3) CYP3A5: 14.1%; and 4) CYP3A4: 49.5%. No functionally relevant allele was found in the analyzed regions of PXR. A new MDR1 SNP was found (T-158C), located in a putative antigen recognition element. Ten (10.1%) subjects were predicted to be low metabolizers simultaneously for CYP3A4 and CYP3A5. This fraction of the population is suggested to be under higher exposure to certain antimalarials, including lumefantrine and quinine.

Is chemical genetics the new frontier for malaria biology?

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Malaria is a global disease, causing at least 500 million clinical cases and more than one million deaths each year. Moreover, drug-resistant *Plasmodium falciparum*, the organism that causes most malaria-associated deaths, has become a major problem. Therefore, discovery and investigation of novel targets for anti-malarial drug design is essential to combat this disease. The malarial genome has been sequenced, revealing approximately 5500 genes. The current post-genomic challenge is functionally to evaluate the essential genes and validate them for therapeutic design. Unfortunately, standard genetics techniques are limited in scope because of low transfection efficiency and a lack of knockdown techniques, thereby rendering the analysis of essential genes difficult.