

Malaria Bulletin: A Compendium of Current Literature

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ABSTRACTS

1: *Acta Trop.* 2008 Apr;106(1):39-43.

Alteration of platelet counts and lipid profiles after treatment of acute Plasmodium vivax.

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During malaria infections, thrombocytopenia and low cholesterol levels are frequently observed changes. We compared these changes in patients admitted with fevers and infected with *Plasmodium vivax*, patients admitted with fevers with respiratory/urinary infections and afebrile normal (control) non-infected volunteers. Changes in the platelet count and lipid parameters are reported for malaria patients after treatment with hydroxychloroquine and primaquine for acute *P. vivax* malaria. Of a total 141 participants, 55 patients were diagnosed with malaria (positive blood smear) prior to treatment. Compared to the normal (n=52) and non-malaria fever groups (n=34), there was a significant decrease in five hematologic indices (white blood cell, red blood cell, hemoglobin, hematocrit and platelet) and three lipid parameters (total cholesterol, HDL-c and LDL-c) in the vivax malaria group at day 0 (pre-treatment). Following treatment, the platelet counts returned to normal limits ($P < 0.05$) from 91,058/microL on day 0 to 246,833/microL by day 17 after treatment. However, changes in the lipid parameters of malaria patients showed a slow recovery to normal limits compared to the platelet counts. The HDL-c and LDL-c remained low for 1 month after treatment but increased at 3 and 6 months post-treatment. At 12 months after treatment, the levels of two lipid parameters had fully recovered to the normal limits. Thus, special attention should be applied when interpreting laboratory blood profiles of malaria patients, especially platelet and lipid based tests, until full recovery after treatment.

2: *Acta Trop.* 2008 Apr;106(1):75-9.

Molecular and phylogenetic analysis of a novel salivary defensin cDNA from malaria vector *Anopheles stephensi*.

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Manipulating the endogenous immune responses of the mosquito such as temporal and spatial expression of antimicrobial peptides may help in the development of a refractory mosquito, unable to transmit malaria. In mosquito several small antimicrobial peptides are activated locally in the midgut and salivary glands upon *Plasmodium* infection. *Anopheles stephensi*, the major urban malaria vector in India, has been considered as an important insect model to study vector-parasite interactions; however, so far no reports are available on the antimicrobial peptides from this mosquito species. In the present study, we report identification and molecular characterization of a novel cDNA encoding defensin like peptide, isolated from the salivary gland subtractive hybridization cDNA library of mosquito *A. stephensi*. Defensin cDNA is 396 base pair long, bearing an open reading frame of 96 amino acids. Deduced amino acid sequence of *A. stephensi* defensin (Astp_def) contains a signal peptide sequence of 24 amino acids followed by 32-amino acids long putative propeptide domain and a 40-amino acid mature

peptide domain carrying 23-amino acid long consensus sequence signature of insect defensin. Mature peptide of *Astp_def* carries six conserved cysteine residues, with a predicted molecular weight of 4.20kDa, and isoelectric point of 8.30, characteristic features of cationic defensins. Amino acid sequence similarity and phylogenetic analysis indicated a higher variation in the pre-propeptide region, as compared to the mature defensin peptide, assuring the presence of finely tuned immune responses to counter pathogens.

3: *Acta Trop.* 2008 Apr;106(1):9-15.

Detection of Plasmodium falciparum derived macrophage migration inhibitory factor homologue in the sera of malaria patients.

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Macrophage migration inhibitory factor homologues have been identified from several genera of parasites, including *Plasmodium*, and have shown some functional similarities to the host molecule. It was hypothesized that MIF molecules can act as a regulator in host-parasite interaction in favor of parasites survival during malaria infection. Although there has been some progress in recent studies, the biological function of the malaria parasite-derived MIF is still far from clear. In this study, cDNA of *Pfmif* was synthesized from mRNA of *Plasmodium falciparum* 3D7 strain and the recombinant protein was generated and analyzed for both enzymatic and chemotactic activities. The *Plasmodium*-derived MIF homologue molecules are conservative both inter-strain and interspecies. And all the sequences of them have typical structure of CC chemokine family: CC-C-C. PfMIF was proved to have chemotactic activity on human monocytes, which was similar to human-derived MIF, but at lower concentration than the latter. Meanwhile, the proline at position 2 was confirmed to be important for its tautomerase activity. With specific monoclonal and polyclonal antibodies, we demonstrated the release of PfMIF from cultured parasite-infected erythrocytes and the secretion of it from transfected eukaryotic cells in vitro, and more importantly, we found the existence of parasite derived MIF homologue in the sera of the patients infected by *P. falciparum*. These results will contribute to the understanding of the parasite-derived MIFs role during malaria infection.

4: *Am J Trop Med Hyg.* 2008 Apr;78(4):641-2.

Pharmacokinetics of dihydroartemisinin in a murine malaria model.

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Pharmacokinetic properties of dihydroartemisinin (DHA) were determined in mice given 100 mg/kg intraperitoneal DHA. Half-life, CL/F, and V/F were 25 min, 61.3 L/hr/kg, and 36.3 L/kg in malaria-infected mice and 19 min, 50.9 L/hr/kg, and 23.0 L/kg in controls. These data are valuable for pharmacokinetic-pharmacodynamic evaluations of DHA in murine models.

5: *Am J Trop Med Hyg.* 2008 Apr;78(4):633-640.

Association of pfCRT But Not pfmdr1 Alleles with Chloroquine Resistance in Iranian Isolates of Plasmodium falciparum.

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This study was designed to analyze the Plasmodium falciparum chloroquine resistance transporter (pfCRT) and P. falciparum multidrug resistance 1 (pfmdr1) mutations as markers of chloroquine (CQ) resistance in 200 blood samples collected from malaria patients in south-eastern Iran during 2002-2005. Among these, 25 (post-treatment) fulfilled the 28-day follow-up study. A high number of Iranian P. falciparum (97%) strains harbored quadruple mutations at codons 76T, 220S, 326D, and 356L. All post-treatment isolates harbored the mutant allele 76T, but low rates of the mutant allele 86Y (44%) of the pfmdr1 gene were detected. No wild haplotype of pfCRT (72-CVMNKAQNIR-371) was found in post-treatment samples; however, 56% of clinical "failure" samples carried the wild type of pfmdr1 (NYSND). The present results suggest a strong association between pfCRT 76T, but not pfmdr1 86Y mutation and in vivo CQ resistance. Furthermore, we found the CQ resistance-associated SVMNT haplotype, which previously had been seen in South American isolates. Although Iran is located more proximally to Southeast Asia than to South America, no CQ resistance-associated CVIET haplotype has been observed in this region. Therefore, these results were not consistent with the earlier presumed spread of CQR parasites from Southeast Asia to Africa via the Indian subcontinent. In conclusion, P. falciparum mutations associated with resistance to CQ are abundant in south-eastern Iran and this finding strongly supports that CQ as the first line drug is inadequate for treatment of uncomplicated falciparum malaria in Iran.

6: *Am J Trop Med Hyg.* 2008 Apr;78(4):552-555.

Impaired Clinical Response in a Patient with Uncomplicated Falciparum Malaria Who Received Poor-Quality and Underdosed Intramuscular Artemether.

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We describe an adult with uncomplicated Plasmodium falciparum malaria who did not improve clinically despite 5 days of intramuscular artemether therapy. He was prescribed a lower dose (kg body weight) than that recommended, and a vial from the packet contained only 74% of the artemether dose as stated by the manufacturer. The combination of underdosing, poor-quality drug, and the intrinsic low bioavailability of artemether may have contributed to his poor clinical response. Analysis of the packaging and chemical "fingerprinting" of the artemether suggested that the drug was genuine but was either substandard or had deteriorated after manufacture.

7: *Am J Trop Med Hyg.* 2008 Apr;78(4):543-545.

Dihydroartemisinin--Piperaquine Rescue Treatment of Multidrug-resistant Plasmodium falciparum Malaria in Pregnancy: A Preliminary Report.

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Dihydroartemisinin-piperaquine (DHA-PPQ) is a promising new artemisinin combination treatment. There are no published data on the intentional use of the drug in pregnancy. Between June 2006 and January 2007, 50 Karen pregnant women with recurrent *P. falciparum* infections, despite 7-day treatments with quinine or artesunate (+/-clindamycin) or both, were treated with DHA-PPQ. This rescue treatment was effective and well tolerated and there was no evidence of toxicity for the mothers or the fetus. The PCR adjusted cure rate by Kaplan Meier analysis at day 63 was 92.2% (95% CI: 76.9-97.4).

8: *Antimicrob Agents Chemother.* 2008 Apr 14

Discordant patterns of genetic variation at two chloroquine-resistant loci in worldwide populations of malaria parasite Plasmodium falciparum.

Mehlotra RK, Mattera G, Bockarie MJ, Maguire JD, Baird JK, Sharma YD, Alifrangis M, Dorsey G, Rosenthal PJ, Fryauff DJ, Kazura JW, Stoneking M, Zimmerman PA.

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Mutations in the chloroquine resistance transporter gene of *Plasmodium falciparum* (*pfcr*, chromosome 7) play a key role in chloroquine resistance (CQR), while mutations in the multidrug resistance gene (*pfmdr1*, chromosome 5) play a significant role in the parasite's resistance to a variety of antimalarials, and also modulate CQR. To compare patterns of genetic variation at *pfcr* and *pfmdr1* loci, we investigated 460 *P. falciparum*-infected blood samples from four Asian, three African, and three South American countries, analyzing microsatellite (MS) loci flanking *pfcr* (five loci, approximately 40 kb) and *pfmdr1* (either two loci [approximately 5 kb] or four loci [approximately 10 kb]). CQR *pfmdr1* allele-associated MS haplotypes showed considerably higher genetic diversity and higher subdivision than CQR *pfcr* allele-associated MS haplotypes in both Asian and African parasite populations. However, both *pfcr* and *pfmdr1* MS haplotypes showed similar levels of low diversity in South American parasite populations. Median-joining network analyses showed that *pfcr* MS haplotypes correlated well with geography and CQR *pfcr* alleles, whereas there was no distinct *pfmdr1* MS haplotype that correlated with geography and/or CQR *pfmdr1* alleles. Furthermore, multiple independent origins of CQR *pfmdr1* alleles in Asia and Africa were inferred. These results suggest that variation at *pfcr* and *pfmdr1* loci in both Asian and African parasite populations is generated and/or maintained via substantially different mechanisms. Since *pfmdr1* mutations may be associated with resistance to artemisinin combination therapies that are replacing CQ,

particularly in Africa, it is important to determine if, and how, the genetic characteristics of this locus change over time.

9: *Antimicrob Agents Chemother.* 2008 Apr 14

First Case of Emergence of Atovaquone-Proguanil (Malarone™) Resistance in Plasmodium falciparum During Treatment in Traveller from Comoros.

Savini H, Bogreau H, Bertaux L, Bouchiba H, Kraemer P, Parzy D, Garnotel E, Rogier C, Simon F, Pradines B.

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Malarone(TM) (GlaxoSmithKline) is now commonly used for the treatment and prophylaxis of falciparum malaria in France....

10: *Antimicrob Agents Chemother.* 2008 Apr;52(4):1493-5.

Malaria treatment with atovaquone-proguanil in malaria-immune adults: implications for malaria intervention trials and for pre-exposure prophylaxis of malaria.

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

11: *Antimicrob Agents Chemother.* 2008 Apr;52(4):1215-20.

New Active Drugs against Liver Stages of Plasmodium Predicted by Molecular Topology.

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We conducted a quantitative structure-activity relationship (QSAR) study based on a database of 127 compounds previously tested against the liver stage of Plasmodium yoelii in order to develop a model capable of predicting the in vitro antimalarial activities of new compounds. Topological indices were used as structural descriptors, and their relation to antimalarial activity was determined by using linear discriminant analysis. A topological model consisting of two discriminant functions was created. The first function discriminated between active and inactive compounds, and the second identified the most active among the active compounds. The model was then applied sequentially to a large

database of compounds with unknown activity against liver stages of Plasmodium. Seventeen drugs that were predicted to be active or inactive were selected for testing against the hepatic stage of *P. yoelii* in vitro. Antiretroviral, antifungal, and cardiotoxic drugs were found to be highly active (nanomolar 50% inhibitory concentration values), and two ionophores completely inhibited parasite development. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed on hepatocyte cultures for all compounds, and none of these compounds were toxic in vitro. For both ionophores, the same in vitro assay as those for *P. yoelii* has confirmed their in vitro activities on *Plasmodium falciparum*. A similar topological model was used to estimate the octanol/water partition of each compound. These results demonstrate the utility of the QSAR and molecular topology approaches for identifying new drugs that are active against the hepatic stage of malaria parasites. We also show the remarkable efficacy of some drugs that were not previously reported to have antiparasitic activity.

12: *Antimicrob Agents Chemother.* 2008 Apr;52(4):1454-61.

Potent antimalarial activity of histone deacetylase inhibitor analogues.

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The malaria parasite *Plasmodium falciparum* has at least five putative histone deacetylase (HDAC) enzymes, which have been proposed as new antimalarial drug targets and may play roles in regulating gene transcription, like the better-known and more intensively studied human HDACs (hHDACs). Fourteen new compounds derived from L-cysteine or 2-aminosuberonic acid were designed to inhibit *P. falciparum* HDAC-1 (PfHDAC-1) based on homology modeling with human class I and class II HDAC enzymes. The compounds displayed highly potent antiproliferative activity against drug-resistant (Dd2) or drug sensitive (3D7) strains of *P. falciparum* in vitro (50% inhibitory concentration of 13 to 334 nM). Unlike known hHDAC inhibitors, some of these new compounds were significantly more toxic to *P. falciparum* parasites than to mammalian cells. The compounds inhibited *P. falciparum* growth in erythrocytes at both the early and late stages of the parasite's life cycle and caused altered histone acetylation patterns (hyperacetylation), which is a marker of HDAC inhibition in mammalian cells. These results support PfHDAC enzymes as being promising targets for new antimalarial drugs.

13: *Antimicrob Agents Chemother.* 2008 Apr;52(4):1438-45.

Drug-Regulated 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 the bcl complex for antifolates and naphoquinones, respectively, and alterations in predicted parasite transporter molecules such as P-glycoprotein homologue 1 (Pgh1) and *Plasmodium falciparum* CRT (PfCRT).

Alterations in the expression of Pgh1 have been associated with modified susceptibility to a range of unrelated drugs. The molecular mechanism(s) that is responsible for this phenotype is unknown. We have shown previously (A. M. Ndifor, R. E. Howells, P. G. Bray, J. L. Ngu, and S. A. Ward, *Antimicrob. Agents Chemother.* 37:1318-1323, 2003) that the anticonvulsant phenobarbitone (PB) can induce reduced susceptibility to chloroquine (CQ) in *P. falciparum*, and in the current study, we provide the first evidence for a molecular mechanism underlying this phenomenon. We demonstrate that pretreatment with PB can elicit decreased susceptibility to CQ in both CQ-resistant and CQ-sensitive parasite lines and that this is associated with the increased expression of the drug transporter Pgh1 but not PfCRT. Furthermore, we have investigated the proximal promoter regions from both *pfmdr1* and *pfCRT* and identified a number of putative binding sites for nuclear receptors with sequence similarities to regions known to be activated by PB 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 *P. falciparum*.

14: *Antimicrob Agents Chemother.* 2008 Mar 31

Antiparasitic Activity and Toxicity of Individual Enantiomers of the 8-Aminoquinoline, 8-[(4-Amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline Succinate.

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8-Aminoquinolines are an important class of antiparasitic agents, with broad utility and excellent efficacy, but also limitations due to hematological toxicities, primarily methemoglobinemia and hemolysis. One representative from this class, (+/-)-8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline succinate (NPC1161C) proved extremely efficacious in animal models of malaria and pneumocystis pneumonia. This racemic mixture was separated into its component enantiomers by chemical and chromatographic means. The enantiomers were evaluated for blood schizonticidal activity and duration of protection in murine models of *Plasmodium berghei*, *Pneumocystis carinii*, and *Leishmania donovani* infection, as well as the propensity to elicit hematotoxicity in dogs. The (-)-enantiomer NPC1161B was found to be more active (by several-fold, depending on the dosing regimen) than the (+)-enantiomer NPC1161A in all of these murine models. In addition, the (-)-enantiomer showed markedly reduced general toxicity in mice and reduced hematotoxicity in the dog model of methemoglobinemia. It is concluded that the configuration at the asymmetric center in the 8-amino side chain differentially affects efficacy and toxicity profiles, and thus may be an important determinant of the "therapeutic window" for compounds in this class.

15: *Biol Pharm Bull.* 2008 Apr;31(4):703-8.

Detection of malaria parasites in mosquitoes from the malaria-endemic area of Chakaria, Bangladesh.

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Malaria is one of the major public health problems of Bangladesh. We investigated the mosquito populations infected with malaria parasites in a malaria-endemic area Chakaria, Bangladesh, where *Anopheles dirus* and *Anopheles minimus* are the principal vectors. *Anopheles* mosquitoes were collected with a CDC miniature light trap from inside households in June 2007. A total of 868 mosquitoes were collected, among which females numbered 669 (77.1%). The species of female *Anopheles* mosquitoes were identified morphologically, and 651 were *A. minimus* and the remaining 18 were other *Anopheles* species. Malaria parasite DNA from individual female mosquitoes was extracted and distinguished using the microtiter plate hybridization (MPH) technique targeting the 18S rRNA of human malaria parasites. Nineteen mosquitoes were malaria parasite positive: 12 for *Plasmodium falciparum*, 1 for *Plasmodium vivax*, and 6 for both *P. falciparum* and *P. vivax*. This is the first time that the MPH technique was used for distinguishing malaria parasites in mosquitoes and the first report from Chakaria. Our results may contribute to planning and assessing malaria control strategies in Chakaria.

16: *Biotechnol Lett.* 2008 Apr;30(4):581-92.

Production of artemisinin by genetically-modified microbes.

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Artemisinin, an endoperoxidized sesquiterpene originally extracted from the medicinal plant *Artemisia annua* L., is a potent malaria-killing agent. Due to the urgent demand and short supply of this new antimalarial drug, engineering enhanced production of artemisinin by genetically-modified or transgenic microbes is currently being explored. Cloning and expression of the artemisinin biosynthetic genes in *Saccharomyces cerevisiae* and *Escherichia coli* have led to large-scale microbial production of the artemisinin precursors such as amorpho-4,11-diene and artemisinic acid. Although reconstruction of the complete biosynthetic pathway toward artemisinin in transgenic yeast and bacteria has not been achieved, artemisinic acid available from these transgenic microbes facilitates the subsequent partial synthesis of artemisinin by either chemical or biotransformational process, thereby providing an attractive strategy alternative to the direct extraction of artemisinin from *A. annua* L. In this review, we update the current trends and summarize the future prospects on genetic engineering of the microorganisms capable of accumulating artemisinin precursors through heterologous and functional expression of the artemisinin biosynthetic genes.

17: *BMC Bioinformatics.* 2008 Apr 16;9(1):201

Identification of Proteins Secreted by Malaria Parasite into Erythrocyte using SVM and PSSM profiles.

Verma R, Tiwari A, Kaur S, Varshney GC, Raghava GP.

ABSTRACT: BACKGROUND: Malaria parasite secretes various proteins in infected RBC for its growth and survival. Thus identification of these secretory proteins is

important for developing vaccine/drug against malaria. The existing motif-based methods have got limited success due to lack of universal motif in all secretory proteins of malaria parasite. RESULTS: In this study a systematic attempt has been made to develop a general method for predicting secretory proteins of malaria parasite. All models were trained and tested on a non-redundant dataset of 252 secretory and 252 non-secretory proteins. We developed SVM models and achieved maximum MCC 0.72 with 85.65% accuracy and MCC 0.74 with 86.45% accuracy using amino acid and dipeptide composition respectively. SVM models were developed using split-amino acid and split-dipeptide composition and achieved maximum MCC 0.74 with 86.40% accuracy and MCC 0.77 with accuracy 88.22% respectively. In this study, for the first time PSSM profiles obtained from PSI-BLAST, have been used for predicting secretory proteins. We achieved maximum MCC 0.86 with 92.66% accuracy using PSSM based SVM model. All models developed in this study were evaluated using 5-fold cross-validation technique. CONCLUSION: This study demonstrates that secretory proteins have different residue composition than non-secretory proteins. Thus, it is possible to predict secretory proteins from its residue composition-using machine learning technique. The multiple sequence alignment provides more information than sequence itself. Thus performance of method based on PSSM profile is more accurate than method based on sequence composition. A web server PSEApred has been developed for predicting secretory proteins of malaria parasites (<http://www.imtech.res.in/raghava/pseapred/>).

18: *Br J Clin Pharmacol*. 2008 Apr;65(4):493-501.

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

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT: * Both chloroquine (CQ) and sulfadoxine/ pyrimethamine (SDx/PYR) remain important drugs in the control of malaria. * The available data on CQ, SDx and PYR are summary pharmacokinetic parameters based on classical/traditional methods, mostly in adults. * No study has described the population pharmacokinetics of a fixed-dose CQ + SDx/PYR combination in children with falciparum malaria. WHAT THIS STUDY ADDS: * This study presents population pharmacokinetic data on CQ and SDx in children with uncomplicated falciparum malaria. * The study demonstrates that in age-based fixed-dose regimens with CQ and SDx, drug exposures and outcomes may be correctly predicted, although correlation with body weight is poor. * The study proposes dose modification to improve response with the CQ + SDx/PYR combination. AIMS: To describe the pharmacokinetics of chloroquine (CQ) and sulfadoxine (SDx), and to identify predictors of treatment response in children with malaria given the CQ + SDx and pyrimethamine (PYR) combination. METHODS: Eighty-six Ugandan children with uncomplicated falciparum malaria, 6 months to 5 years old, were randomly treated with prepacked fixed-dose CQ + SDx/PYR. The youngest children (<24 months) received half strength and the older (>24 months) full strength treatment. The reported day 14 failure rates were 48% and 18%, respectively. Capillary blood (100 microl) applied on to filter paper was collected on eight occasions during 28 days of follow up. Concentrations of CQ and SDx were determined. A population approach was used for the pharmacokinetic analysis. RESULTS: A two-compartment model adequately described the data for both CQ and SDx. For CQ, the typical apparent clearance (CL/F) and volume of distribution (V(C)/F) values were estimated to be 2.84 l h(-1) and 230 l. The typical CL/F for SDx was 0.023 l h(-1), while the factor relating its V(C)/F to normalized body weight was 1.6 l kg(-1). Post hoc parameter estimates for both drugs showed lower maximum concentrations (C(max)) and concentration-time curve areas (AUC(0,336 h)) in younger children. The AUC(0,336 h) for SDx and CQ were independently

significant factors for prediction of cure. Simulations suggest that giving the higher dose to the youngest children would result in higher CQ and SDx concentrations and improved outcome. CONCLUSIONS: The study results suggest that full-strength combination to all children would improve the cure rate.

19: *Clin Vaccine Immunol.* 2008 Apr;15(4):650-8.

Similar cytokine responses and degrees of anemia in patients with Plasmodium falciparum and Plasmodium vivax infections in the Brazilian Amazon region.

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The mechanisms of malarial anemia induction are poorly understood, but cytokines and autoantibodies are considered to play important roles. This work aimed at evaluating the degree of anemia and the plasmatic profile of the cytokines tumor necrosis factor alpha (TNF-alpha), gamma interferon (IFN-gamma), interleukin-12 (IL-12), migration inhibitory factor (MIF), and IL-10 and the monocyte chemotactic protein-1 (MCP-1) chemokine, as well as evaluating the presence of antibodies directed to components of the normal erythrocyte membrane and to cardiolipin in individuals with malaria from the Brazilian Amazon. No difference was observed in the frequency of anemia between patients infected by Plasmodium vivax and those infected by Plasmodium falciparum, and there was no relationship between the levels of parasitemia and the manifestations of anemia in P. vivax and P. falciparum patients. Significant increases in the concentrations of TNF-alpha, IFN-gamma, MIF, and MCP-1 were observed in patients with P. falciparum and P. vivax malaria, whereas the concentrations of IL-10 was increased only in patients with P. vivax infection. Higher concentrations of IL-12 and IL-10 were observed in the P. falciparum anemic patients, while for TNF-alpha this profile was observed in the nonanemic ones. P. vivax-infected and P. falciparum-infected patients with positive immunoglobulin M (IgM) or IgM and IgG responses, respectively, against blood-stage forms of the parasites had significantly lower hemoglobin levels than did those with negative responses. There was no correlation between the presence of anti-erythrocyte and anti-cardiolipin antibodies and the presence or intensity of the anemia. Our data suggest that in areas of low endemicity and unstable transmission of malaria, P. vivax and P. falciparum infections present similar characteristics in terms of the induction of anemia and cytokine responses.

20: *Clin Vaccine Immunol.* 2008 Apr;15(4):617-21.

Impact of human immunodeficiency virus infection in pregnant women on variant-specific immunity to malaria.

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Human immunodeficiency virus (HIV) increases susceptibility to Plasmodium falciparum infection, and this has most clearly been demonstrated in pregnant women. Variant surface antigens on the surfaces of erythrocytes infected with P. 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 sera from HIV-uninfected women. This difference was similar

in magnitude for pediatric isolates (unadjusted odds ratio [OR] = 6.36; 95% confidence interval [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 on the basis of their antibody levels. After adjustment for CD4 count, maternal age, and gravidity, we found that HIV-infected women more frequently had low responses to both pediatric isolates (OR = 5.34; 95% CI = 1.23, 23.16; $P = 0.025$) and placental isolates (OR = 4.14; 95% CI = 1.71, 10.02; $P = 0.002$). The relative quantity of antibodies to both pediatric isolates ($P = 0.035$) and placental isolates ($P = 0.005$) was lower in HIV-infected women than in HIV-uninfected women. HIV infection has a broad impact on variant-specific immunity, which may explain the susceptibility of infected individuals to clinical malaria episodes.

21: *Cochrane Database Syst Rev.* 2008 Apr 16;(2):CD003756.

Chemoprophylaxis and intermittent treatment for preventing malaria in children.

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BACKGROUND: Malaria causes repeated illness in children living in endemic areas. Policies of giving antimalarial drugs at regular intervals (prophylaxis or intermittent treatment) are being considered for preschool children. **OBJECTIVES:** To evaluate prophylaxis and intermittent treatment with antimalarial drugs to prevent malaria in young children living in malaria-endemic areas. **SEARCH STRATEGY:** We searched the Cochrane Infectious Diseases Group Specialized Register (August 2007), CENTRAL (The Cochrane Library 2007, Issue 3), MEDLINE (1966 to August 2007), EMBASE (1974 to August 2007), LILACS (1982 to August 2007), mRCT (February 2007), and reference lists of identified trials. We also contacted researchers. **SELECTION CRITERIA:** Individually randomized and cluster-randomized controlled trials comparing antimalarial drugs given at regular intervals (prophylaxis or intermittent treatment) with placebo or no drug in children aged one month to six years or less living in a malaria-endemic area. **DATA COLLECTION AND ANALYSIS:** Two authors independently extracted data and assessed methodological quality. We used relative risk (RR) or weighted mean difference with 95% confidence intervals (CI) for meta-analyses. Where we detected heterogeneity and considered it appropriate to combine the trials, we used the random-effects model (REM). **MAIN RESULTS:** Twenty-one trials (19,394 participants), including six cluster-randomized trials, met the inclusion criteria. Prophylaxis or intermittent treatment with antimalarial drugs resulted in fewer clinical malaria episodes (RR 0.53, 95% CI 0.38 to 0.74, REM; 7037 participants, 10 trials), less severe anaemia (RR 0.70, 95% CI 0.52 to 0.94, REM; 5445 participants, 9 trials), and fewer hospital admissions for any cause (RR 0.64, 95% CI 0.49 to 0.82; 3722 participants, 5 trials). We did not detect a difference in the number of deaths from any cause (RR 0.90, 95% CI 0.65 to 1.23; 7369 participants, 10 trials), but the CI do not exclude a potentially important difference. One trial reported three serious adverse events with no statistically significant difference between study groups (1070 participants). Eight trials measured morbidity and mortality six months to two years after stopping regular antimalarial drugs; overall, there was no statistically significant difference, but participant numbers were small. **AUTHORS' CONCLUSIONS:** Prophylaxis and intermittent treatment with antimalarial drugs reduce clinical malaria and severe anaemia in preschool children.

22: *Curr Biol.* 2008 Apr 22;18(8):607-13.

Male Fertility of Malaria Parasites Is Determined by GCS1, a Plant-Type Reproduction Factor.

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Malaria, which is caused by Plasmodium parasites, is transmitted by anopheline mosquitoes. When gametocytes, the precursor cells of Plasmodium gametes, are transferred to a mosquito, they fertilize and proliferate, which render the mosquito infectious to the next vertebrate host [1]. Although the fertilization of malaria parasites has been considered as a rational target for transmission-blocking vaccines [2], the underlying mechanism is poorly understood. Here, we show that the rodent malaria parasite gene Plasmodium berghei GENERATIVE CELL SPECIFIC 1 (PbGCS1) plays a central role in its gametic interaction. PbGCS1 knockout parasites show male sterility, resulting in unsuccessful fertilization. Because such a male-specific function of GCS1 has been observed in angiosperms [3, 4], this indicates, for the first time, that parasite sexual reproduction is controlled by a machinery common to flowering plants. Our present findings provide a new viewpoint for understanding the parasitic fertilization system and important clues for novel strategies to attack life-threatening parasites.

23: *Eur J Clin Pharmacol.* 2008 Apr 16

Pharmacokinetics and tolerability of artesunate and amodiaquine alone and in combination in healthy volunteers.

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OBJECTIVES: The WHO recommends artemisinin-based combination therapies for treatment of uncomplicated falciparum malaria. At least 15 African countries have adopted artesunate plus amodiaquine as treatment policy. As no pharmacokinetic data on this combination have been published to date, we investigated its pharmacokinetic interactions and tolerability in healthy volunteers in Africa. **METHODS:** In a randomized, three-phase, cross-over study, amodiaquine (10 mg/kg) and artesunate (4 mg/kg) were given as single oral doses to 15 healthy volunteers. Artesunate was given to all volunteers on day 0. On day 7 they received either amodiaquine or amodiaquine plus artesunate and the alternative regimen on day 28. The pharmacokinetics of artesunate and amodiaquine and their main active metabolites dihydroartemisinin and desethylamodiaquine were compared following monotherapy and combination therapy using analysis of variance. **RESULTS:** Thirteen volunteers completed the study, and pharmacokinetic parameters could be determined for twelve volunteers. When given in combination, the mean AUC was lower for dihydroartemisinin [ratio 67% (95% CI 51-88%); P = 0.008] and desethylamodiaquine [ratio 65% (95% CI 46-90%); P = 0.015] when compared with monotherapy. Adverse events of concern occurred in four volunteers (27%): grade 3 transaminitis (n = 1), neutropaenia (n = 2), and hypersensitivity (n = 1). **CONCLUSION:** The total drug exposure to both drugs was reduced significantly when they were given in combination. The clinical significance of these interactions is unclear and must be studied in malaria patients. The frequency and nature of adverse events among the healthy volunteers were of concern, and suggest laboratory monitoring would be needed in malaria patients treated with artesunate plus amodiaquine.

24: *Expert Opin Biol Ther.* 2008 Apr;8(4):441-8.

Malaria vaccines: the case for a whole-organism approach.

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BACKGROUND: Malaria is a significant health problem causing morbidity and mortality worldwide. Vaccine development has been an imperative for decades. However, the intricacy of the parasite's lifecycle coupled with the lack of evidence for robust infection-induced immunity has made vaccine development exceptionally difficult. OBJECTIVE: To review some of the key advances in the field and discuss potential ways forward for a whole-organism vaccine. METHODS: The authors searched PubMed using the words 'malaria and vaccine'. We searched for manuscripts detailing antigen characterisation and vaccine strategies with emphasis on subunit versus whole-parasite approaches. Abstracts were selected and relevant articles are discussed. The searches were not restricted by language or date. CONCLUSIONS: The early cloning of malaria antigens has fuelled rapid development of subunit vaccines. However, the disappointing results of clinical trials have resulted in reappraisal of current strategies. Whole-parasite approaches have re-emerged as an alternative strategy. Immunization using radiation or genetically attenuated sporozoites has been shown to result in sterile immunity and immunization with blood-stage parasites curtailed by antimalarials has demonstrated delayed parasitemia in rodent models as well as in human malaria.

25: *Infect Immun.* 2008 Apr;76(4):1702-8.

Wheat germ cell-free system-based production of malaria proteins for discovery of novel vaccine candidates.

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

protocol for the discovery of malaria vaccine candidates will be discussed.

26: *Infect Immun.* 2008 Apr;76(4):1791-800.

Evidence for globally shared, cross-reacting polymorphic epitopes in the pregnancy-associated malaria vaccine candidate VAR2CSA.

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Pregnancy-associated malaria (PAM) is characterized by the placental sequestration of *Plasmodium falciparum*-infected erythrocytes (IEs) with the ability to bind to chondroitin sulfate A (CSA). VAR2CSA is a leading candidate for a pregnancy malaria vaccine, but its large size (approximately 350 kDa) and extensive polymorphism may pose a challenge to vaccine development. In this study, rabbits were immunized with individual VAR2CSA Duffy binding-like (DBL) domains expressed in *Pichia pastoris* or var2csa plasmid DNA and sera were screened on different CSA-binding parasite lines. Rabbit antibodies to three recombinant proteins (DBL1, DBL3, and DBL6) and four plasmid DNAs (DBL1, DBL3, DBL5, and DBL6) reacted with homologous FCR3-CSA IEs. By comparison, antibodies to the DBL4 domain were unable to react with native VAR2CSA protein unless it was first partially proteolyzed with trypsin or chymotrypsin. To investigate the antigenic relationship of geographically diverse CSA-binding isolates, rabbit immune sera were screened on four heterologous CSA-binding lines from different continental origins. Antibodies did not target conserved epitopes exposed in all VAR2CSA alleles; however, antisera to several DBL domains cross-reacted on parasite isolates that had polymorphic loops in common with the homologous immunogen. This study demonstrates that VAR2CSA contains common polymorphic epitopes that are shared between geographically diverse CSA-binding lines.

27: *Infect Immun.* 2008 Apr;76(4):1678-85.

Elevated gamma interferon-producing NK cells, CD45RO memory-like T cells, and CD4 T cells are associated with protection against malaria infection in pregnancy.

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Previous studies have shown that gamma interferon (IFN-gamma) production in the placenta is associated with protection against placental malaria. However, it remains unknown which IFN-gamma-producing cell subpopulations are involved in this protection and whether the cellular immune components of protection are the same in the peripheral and the placental blood compartments. We investigated cell subpopulations for CD4, CD8, and CD45RO memory-like T cells and CD56+/CD3- natural killer (NK) cells and for IFN-gamma production by these cells in maternal peripheral and placental intervillous blood in relation to the status of malaria infection in pregnancy. Of 52 human immunodeficiency virus-negative enrolled pregnant women residing in Western Kenya, 20 had placental parasitemia. We found that the percentages of CD45RO memory-like and CD4 T cells were significantly higher in the periphery than in the placenta, while the CD56/CD3- NK-cell percentage was higher in the placenta than in the periphery, suggesting differences in immune cell profiles between the two blood compartments. Furthermore, the percentages of peripheral CD45RO memory-like and CD4 T cells were significantly elevated in aparasitemic women compared to levels in the parasitemic group, with aparasitemic multigravid women having the highest percentages of CD45RO memory-like and CD4 T cells. In contrast, at the placental

level, IFN-gamma production by innate NK cells was significantly increased in aparasitemic women compared to parasitemic women, regardless of gravidity. These results suggest that the elevated IFN-gamma-producing NK cells in the placenta and CD45RO memory-like and CD4 T cells in peripheral blood may be involved in protection against malaria infection in pregnancy.

28: *Infect Immun.* 2008 Apr;76(4):1748-55.

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 parasite vaccines under development aim to prime such responses. To investigate the determinants of antibody response longevity, immunoglobulin G (IgG) antibodies to several blood-stage vaccine candidate antigens in the sera of two cohorts of children of up to 6 years of age during the dry seasons of 2003 and 2004 in The Gambia were examined. The first cohort showed that most antibodies were lost within less than 4 months of the first sampling if a persistent infection was not present, so the study of the second-year cohort involved collecting samples from individuals every 2 weeks over a 3-month period. Antibody responses in the second cohort were also influenced by persistent malaria infection, so analysis focused particularly on children in whom parasites were not detected after the first time point. Antibodies to most antigens declined more slowly in children in the oldest age group (>5 years old) and more rapidly in children in the youngest group (<3 years old). However, antibodies to merozoite surface protein 2 were shorter lived than antibodies to other antigens 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 longevities of plasma cell populations rather than antibody molecules. It is likely that young children mostly have short-lived plasma cells and thus experience rapid declines in antibody levels but that older children have longer-lasting antibody responses that depend on long-lived plasma cells.

29: *Infect Immun.* 2008 Apr;76(4):1527-34.

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 the first pregnancy, but the relative contribution of maternal or fetal factors that mediate this effect remains unclear and the role of gamma interferon (IFN-gamma) has been controversial. We examined the relationship of maternal and cord plasma levels of IFN-gamma, tumor necrosis factor alpha, interleukin-10, ferritin, and leptin to birth weight for Tanzanian women delivering in an area where there is a high rate of malaria transmission. The placental levels of inflammatory cytokines, including IFN-gamma, increased significantly during PM in primigravid and multigravid women but not in 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 a 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-positive [PM+]) first-time mothers. This relationship was not observed 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-negative women ($P = 0.02$ to $P < 0.0001$) but not in offspring of PM+ women (all differences were not significant) in the three gravidity groups. The results confirmed 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.

30: *Infect Immun.* 2008 Apr;76(4):1709-18.

Impact of recombinant adenovirus serotype 35 priming versus boosting of a Plasmodium falciparum protein: characterization of T- and B-cell responses to liver-stage antigen 1.

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Prime-boost vaccination regimens with heterologous antigen delivery systems have indicated that redirection of the immune response is feasible. We showed earlier that T-cell responses to circumsporozoite (CS) protein improved significantly when the protein is primed with recombinant adenovirus serotype 35 coding for CS (rAd35.CS). The current study was designed to answer the question whether such an effect can be extended to liver-stage antigens (LSA) of Plasmodium falciparum such as LSA-1. Studies with mice have demonstrated that the LSA-1 protein induces strong antibody response but a weak T-cell immunity. We first identified T-cell epitopes in LSA-1 by use of intracellular gamma interferon (IFN-gamma) staining and confirmed these epitopes by means of enzyme-linked immunospot assay and pentamer staining. We show that a single immunization with rAd35.LSA-1 induced a strong antigen-specific IFN-gamma CD8(+) T-cell response but no measurable antibody response. In contrast, vaccinations with the adjuvanted recombinant LSA-1 protein induced remarkably low cellular responses but strong antibody responses. Finally, both priming and boosting of the adjuvanted protein by rAd35 resulted in enhanced T-cell responses without impairing the level of antibody responses induced by the protein immunizations alone. Furthermore, the incorporation of rAd35 in the vaccination schedule led to a skewing of LSA-1-specific antibody responses toward a Th1-type immune response. Our results show the ability of rAd35 to induce potent T-cell immunity in combination with protein in a prime-boost schedule without impairing the B-cell response.

31: *Infect Immun.* 2008 Mar 31

A diversity-covering approach to immunisation with Plasmodium falciparum AMA1 induces broader allelic recognition and growth inhibition responses in rabbits.

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Plasmodium falciparum AMA1 (PfAMA1), a candidate malaria vaccine, is polymorphic. This polymorphism is believed to be generated predominantly under immune selection pressure, and as a result may compromise attempts at vaccination. Alignment of 355 PfAMA1 sequences shows that around 10% of the 622 amino acid residues can vary between alleles and that linkages between polymorphic residues occur. Using this analysis we have designed three Diversity-Covering (DiCo) PfAMA1 sequences that take account of these linkages and, when taken together, on average incorporate 97% of amino acid variability observed. For each of the three DiCo sequences a synthetic gene was constructed and used to transform the

methylothrophic yeast *Pichia pastoris*, allowing recombinant expression. All three DiCo proteins were reactive with the reduction sensitive monoclonal antibody 4G2, suggesting the DiCo's had a similar conformation to naturally occurring PfAMA1. Rabbits were immunised with FVO strain PfAMA1 or with the DiCo's either individually or as a mixture. Antibody titers and the ability to inhibit parasite growth in vitro were determined. Animals immunised with the DiCo mix performed similarly to animals immunised with FVO AMA1 when measured against FCR3 strain parasites, but outperformed animals immunised with FVO AMA1 when assessed against other strains. The levels of growth inhibition (70%) induced by the mix of three DiCo's were comparable for FVO, 3D7 and HB3, suggesting a considerable degree of diversity in AMA1 is adequately covered. This suggests that vaccines based upon the DiCo mix approach provide a broader functional immunity than immunisation with a single allele.

32: *Insect Mol Biol.* 2008 Apr;17(2):103-12.

The malaria vector mosquito *Anopheles gambiae* expresses a suite of larval-specific defensin genes.

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cdNAs of *Anopheles gambiae* Defensin 2 (AgDef2), Defensin 3 (AgDef3) and Defensin 4 (AgDef4), identified in the genome sequence, have been characterized and their expression profiles investigated. In contrast to both typical defensins and insect antimicrobial peptides generally, the newly identified defensins were not upregulated with acute-phase kinetics following immune challenge in insects or cell culture. However, mRNA abundance of AgDef2, AgDef3 and AgDef4 increased significantly during the larval stages. Promoter analysis of all three genes failed to identify putative immune response elements previously identified in other mosquito defensin genes. As previous studies failed to identify these larval-specific defensins, it seems likely that further antimicrobial peptide genes with nontypical expression profiles will be identified as more genome sequences become available.

33: *Int J Gynaecol Obstet.* 2008 Apr;101(1):62-6.

Anemia and iron deficiency in pregnant Ghanaian women from urban areas.

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OBJECTIVES: To determine the prevalence and identify risk factors for iron deficiency and anemia in pregnant Ghanaian women from urban areas. **METHODS:** A cross-sectional study of 452 healthy pregnant women receiving prenatal care in Accra, Ghana, was conducted. A sociodemographic health questionnaire was performed and hematologic parameters were measured. Logistic regression methods were used to identify risk factors for anemia and iron status. **RESULTS:** Complete data were available for 428 women. Anemia (hemoglobin <11 g/dL) was present in 144 (34%), iron deficiency (ferritin \leq 16 μ g/L) in 69 (16%), and iron deficiency anemia in 32 (7.5%) women. The adjusted odds ratio (OR) for anemia was 3.4 and 9.8 if iron deficiency and malaria parasitemia were present, respectively; the OR was 0.6 if women were at \geq 36 weeks of pregnancy. The adjusted OR for iron deficiency was 2.7 if women were at \geq 36 weeks of pregnancy and 0.12 if they had sickle trait. **CONCLUSION:** Although anemia and iron deficiency remain substantial problems in pregnant Ghanaian women from urban areas, their prevalence is less than previously reported.

34: *Int J Pharm.* 2008 Apr 16;354(1-2):28-33.

Anticancer properties of artemisinin derivatives and their targeted delivery by transferrin conjugation.

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Artemisinin and its derivatives are well known antimalaria drugs and particularly useful for the treatment of infection of *Plasmodium falciparum* malaria parasites resistant to traditional antimalarials. Artemisinin has an endoperoxide bridge that is activated by intraparasitic heme-iron to form free radicals, which kill malaria parasites by alkylating biomolecules. In recent years, there are many reports of anticancer activities of artemisinins both in vitro and in vivo. Artemisinins have inhibitory effects on cancer cell growth, including many drug- and radiation-resistant cancer cell lines. The cytotoxic effect of artemisinin is specific to cancer cells because most cancer cells express a high concentration of transferrin receptors on cell surface and have higher iron ion influx than normal cells via transferrin mechanism. In addition, some artemisinin analogs have been shown to have antiangiogenesis activity. Artemisinin tagged to transferrin via carbohydrate chain has also been shown to have high potency and specificity against cancer cells. The conjugation enables targeted delivery of artemisinin into cancer cells. In this review, we discuss the anticancer activities and mechanisms of action of artemisinins and the transferrin-conjugate.

35: *Int J Pharm.* 2008 Apr 2;353(1-2):1-7.

In vitro release and stability of an artesunate rectal gel suitable for pediatric use.

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The rectal route is indicated to treat patients with rapidly evolving malaria who cannot take oral medication to prevent progression to severe forms of the disease. Improvement can be made in terms of rectal bioavailability and stability of current formulations. We studied a new two-compartment, muco-adhesive gel formulation of artesunate which is adapted for use in children and storage in tropical climates. The formulation contains 50mg of artesunate per gram of gel. Because of its instability in aqueous solutions, artesunate is in the dry component of the gel with Carbopol((R)) and separate from the liquid phase until reconstitution. Artesunate is stable in the dry blend for 6 months at 45 degrees C and 60% RH. The gel should be used between 1 and 72h after being reconstituted. Artesunate release was measured by with a rapid, simple and reliable HPLC-UV which allowed the analysis of artesunate and dihydroartemisinin with an analysis time at 3min. The amount of artesunate released over 6h was 56+/-0.97%. Compared to the reference suspension, total release and dissolution efficiency were lower and rate of release was slower (time to 50% dissolution 271+/-21min), probably because of the higher viscosity of the gel, but the drug release profiles were similar. The calculated in vitro release exponent (n) value suggested that artesunate is released from the gel by non-Fickian transport.

36: *J Acquir Immune Defic Syndr.* 2008 Apr 1;47(4):472-6.

Association of HIV and malaria with mother-to-child transmission, birth outcomes, and child mortality.

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OBJECTIVE: To assess the impact of HIV and malaria coinfection on mother-to-child HIV transmission (MTCT) and adverse birth outcomes. **METHODS:** One hundred nine HIV-positive mother-infant pairs with a malaria diagnosis were identified in a community cohort and followed up postpartum. Maternal malaria was diagnosed by a rapid immunochromatographic test (ICT) on sera and histopathologic examination of placenta. Infant HIV was diagnosed within 6 weeks of birth using polymerase chain reaction (PCR) to capture in-utero and intrapartum HIV transmission. Log binomial models were used to assess the relative risk of MTCT, low birth weight, and preterm birth associated with malaria. **RESULTS:** Approximately 17.4% of infants were HIV positive at or around birth, and the prevalence of serologic and placental malaria were 31% and 32%, respectively. HIV-positive mothers with serological ICT malaria were significantly more likely to have low-birth-weight infants, and low-birth-weight infants had significantly higher risk of MTCT compared with infants of normal birth weight. Although placental and serologic ICT malaria were significantly associated with MTCT, after adjusting for maternal HIV viral load, the risk of MTCT was significantly increased only for mothers coinfecting with placental malaria (relative risk [RR] = 7.9, P = 0.025). **CONCLUSIONS:** Placental malaria increases the risk of MTCT after adjustment for viral load. Programs should focus on enhanced malaria prevention during pregnancy to decrease the risk of adverse birth outcomes and MTCT.

37: *J Exp Med.* 2008 Apr 21

C5 deficiency and C5a or C5aR blockade protects against cerebral malaria.

Patel SN, Berghout J, Lovegrove FE, Ayi K, Conroy A, Serghides L, Min-Oo G, Gowda DC, Sarma JV, Rittirsch D, Ward PA, Liles WC, Gros P, Kain KC.

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Experimental infection of mice with *Plasmodium berghei* ANKA (PbA) provides a powerful model to define genetic determinants that regulate the development of cerebral malaria (CM). Based on the hypothesis that excessive activation of the complement system may confer susceptibility to CM, we investigated the role of C5/C5a in the development of CM. We show a spectrum of susceptibility to PbA in a panel of inbred mice; all CM-susceptible mice examined were found to be C5 sufficient, whereas all C5-deficient strains were resistant to CM. Transfer of the C5-defective allele from an A/J (CM resistant) onto a C57BL/6 (CM-susceptible) genetic background in a congenic strain conferred increased resistance to CM; conversely, transfer of the C5-sufficient allele from the C57BL/6 onto the A/J background recapitulated the CM-susceptible phenotype. The role of C5 was further explored in B10.D2 mice, which are identical for all loci other than C5. C5-deficient B10.D2 mice were protected from CM, whereas C5-sufficient B10.D2 mice were susceptible. Antibody blockade of C5a or C5a receptor (C5aR) rescued susceptible mice from CM. In vitro studies showed that C5a-potentiated cytokine secretion induced by the malaria product *P. falciparum* glycosylphosphatidylinositol and C5aR blockade abrogated these amplified responses. These data provide evidence implicating C5/C5a in the pathogenesis of CM.

Molecular Markers of Resistance to Sulfadoxine-Pyrimethamine during Intermittent Preventive Treatment for Malaria in Mozambican Infants.

Mayor A, Serra-Casas E, Sanz S, Aponte JJ, Macete E, Mandomando I, Puyol L, Berzosa P, Dobaño C, Aide P, Sacarlal J, Benito A, Alonso P, Menéndez C.

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Background. Intermittent preventive treatment in infants (IPTi) with sulfadoxine-pyrimethamine (SP) is a potential malaria control strategy. There is concern about the impact that increasing in vivo resistance to SP has on the efficacy of IPTi, as well as about the potential contribution of IPTi to increases in resistance. **Methods.** We compared the frequency of clinical episodes of malaria caused by *P. falciparum* parasites with mutations in dhfr and dhps among sick children who received SP or placebo in the context of a randomized, double-blind, placebo-controlled IPTi trial in Mozambique. **Results.** Half of the children who received placebo harbored quintuple-pure mutant parasites. Nevertheless, the protective efficacy of IPTi within the 35 days after the third dose was 70.8% (95% confidence interval [CI], 40.7%-85.6%). Between month 2 after the third IPTi dose and the end of the follow-up period, children receiving SP harbored more dhps codon 437 mixed infections (odds ratio [OR], 10.56 [95% CI, 1.30-86.14]) and fewer dhps double-pure mutant parasites (OR, 0.43 [95% CI, 0.22-0.84]) than did placebo recipients. **Conclusions.** IPTi appears to be associated with some changes in the prevalence of genotypes involved in SP resistance. In the face of a high prevalence of quintuple-mutant parasites, SP exhibited a high level of efficacy in the prevention of new episodes of malaria in infants. **Trial registration.** ClinicalTrials.gov identifier: NCT00209794 .

Cognitive Dysfunction in Mice Infected with Plasmodium berghei Strain ANKA.

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Cerebral malaria complicated by cognitive sequelae is a major cause of morbidity in humans infected with *Plasmodium falciparum*. To model cognitive function after malaria, we created a rodent model of cerebral malaria by infecting C57BL/6 mice with *Plasmodium berghei* strain ANKA. After 7 days, an object-recognition test of working memory revealed a significant impairment in the visual memory of infected mice. This impairment was observed in the absence of confounding effects of infection. The cognitive dysfunction correlated with hemorrhage and inflammation. Furthermore, microglial activity and morphological changes detected throughout the brains of infected mice were absent from the brains of control mice, and this correlated with the measured cognitive defects. Similar testing methods in human studies could help identify subjects at risk for an adverse cognitive outcome.

This murine model should facilitate the study of adjunctive methods to ameliorate adverse neurological outcomes in cerebral malaria.

40: *J Infect Dis.* 2008 Apr 1;197(7):1062-73.

Rho kinase inhibition in severe malaria: thwarting parasite-induced collateral damage to endothelia.

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Acute clinical manifestations of falciparum malaria, such as multiorgan failure and cerebral malaria, occur unpredictably and lead to coma and death within hours if left untreated. Despite the emergency administration of effective antimalarial drugs, 15%-20% of patients die. Other therapeutic approaches are therefore urgently needed. There is increasing evidence that endothelial changes play a key role in the pathogenesis of severe malaria. We therefore used coculture models to study interactions between infected erythrocytes and endothelium. We found that adhesion of Plasmodium falciparum to endothelial cells in vitro activated the Rho kinase signaling pathway, which is strongly involved in various vascular diseases. When added concomitantly with parasites, the Rho kinase inhibitor fasudil (HA-1077), a drug already in clinical use, decreased both NF-kappaB activation and endothelial cell apoptosis. Fasudil also helped to restore endothelial barrier integrity after P. falciparum adhesion. Rho kinase inhibition thus appears to be a promising adjunctive therapeutic approach to the management of severe human malaria.

41: *J Trop Pediatr.* 2008 Apr;54(2):94-101.

Malaria Mortality in Venezuela: Focus on Deaths due to Plasmodium vivax in Children.

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Morbidity and mortality burden of malaria in the childhood represents a public health threat not only in countries with high levels of transmission, but also in those, such as Venezuela and others in Latin America, with moderate to low transmission. Usually its mortality has been attributed just to Plasmodium falciparum malaria, but the changing patterns of increase in Plasmodium vivax malaria morbidity and mortality are now causing concern. We studied malaria mortality by analyzing different epidemiological variables during a 10-year period in Venezuela, finding mortality rates ranging 0.10-0.36 deaths/100 000 population, with almost a third of deaths in children (<10 years old), corresponding 270 deaths to P. falciparum cases and 30 to P. vivax; but along the period with a decrease trend for P. falciparum and an increase trend for P. vivax.

42: *J Virol.* 2008 Apr;82(8):3822-33.

Single-dose protection against Plasmodium berghei by a simian adenovirus vector using a human cytomegalovirus promoter containing intron A.

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Human adenovirus serotype 5 (AdH5) vector vaccines elicit strong immune responses to the encoded antigen and have been used in various disease models. We designed AdH5 vectors expressing antigen under the control of a human cytomegalovirus (HCMV) immediate-early promoter containing its intron A sequence. The transcriptional levels of antigen and immune responses to antigen for vectors with the HCMV promoter with the intron A sequence (LP) were greater than those for AdH5 vectors using the HCMV promoter sequence without intron A (SP). We compared an E1E3-deleted AdH5 adenoviral vector, which affords more space for insertion of foreign sequences, and showed it to be as immunogenic as an E1-deleted AdH5 vector. Neutralizing antibodies to AdH5 limit the efficacy of vaccines based on the AdH5 serotype, and simian adenoviral vectors offer an attractive option to overcome this problem. We constructed E1E3-deleted human and simian adenoviral vectors encoding the pre-erythrocytic-stage malarial antigen *Plasmodium berghei* circumsporozoite protein. We compared the immunogenicity and efficacy of AdC6, a recombinant simian adenovirus serotype 6 vector, in a murine malaria model to those of AdH5 and the poxviral vectors MVA and FP9. AdC6 induced sterile protection from a single dose in 90% of mice, in contrast to AdH5 (25%) and poxviral vectors MVA and FP9 (0%). Adenoviral vectors maintained potent CD8(+) T-cell responses for a longer period after immunization than did poxviral vectors and mainly induced an effector memory phenotype of cells. Significantly, AdC6 was able to maintain protection in the presence of preexisting immunity to AdH5.

43: *Lancet Infect Dis.* 2008 Apr 1

Measuring malaria endemicity from intense to interrupted transmission.

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The quantification of malaria transmission for the classification of malaria risk has long been a concern for epidemiologists. During the era of the Global Malaria Eradication Programme, measurements of malaria endemicity were institutionalised by their incorporation into rules outlining defined action points for malaria control programmes. We review the historical development of these indices and their contemporary relevance. This is at a time when many malaria-endemic countries are scaling-up their malaria control activities and reconsidering their prospects for elimination. These considerations are also important to an international community that has recently been challenged to reevaluate the prospects for malaria eradication.

44: *Malar J.* 2008 Apr 27;7(1):69

Evaluation of an operational malaria outbreak identification and response system in Mpumalanga Province, South Africa.

Coleman M, Coleman M, Mabuza AM, Kok G, Coetzee M, Durrheim DN.

ABSTRACT: Background and objective To evaluate the performance of a novel malaria outbreak identification system in the epidemic prone rural area of Mpumalanga Province, South Africa, for timely identification of malaria outbreaks and guiding integrated public health responses. **METHODS:** Using five years of historical notification data, two binomial thresholds were determined for each primary health care facility in the highest malaria risk area of Mpumalanga province. Whenever the thresholds were exceeded at health facility level (tier

1), primary health care staff notified the malaria control programme, which then confirmed adequate stocks of malaria treatment to manage potential increased cases. The cases were followed up at household level to verify the likely source of infection. The binomial thresholds were reviewed at village/town level (tier 2) to determine whether additional response measures were required. In addition, an automated electronic outbreak identification system at town/village level (tier 2) was integrated into the case notification database (tier 3) to ensure that unexpected increases in case notification were not missed. The performance of these binomial outbreak thresholds was evaluated against other currently recommended thresholds using retrospective data. The acceptability of the system at primary health care level was evaluated through structured interviews with health facility staff. RESULTS: Eighty four percent of health facilities reported outbreaks within 24 hours (n=95), 92% (n=104) within 48 hours and 100% (n=113) within 72 hours. Appropriate response to all malaria outbreaks (n=113, tier 1, n=46, tier 2) were achieved within 24 hours. The system was positively viewed by all health facility staff. When compared to other epidemiological systems for a specified 12 month outbreak season (June 2003 to July 2004) the binomial exact thresholds produced one false weekly outbreak, the C-sum 12 weekly outbreaks and the mean + 2 SD nine false weekly outbreaks. Exceeding the binomial level 1 threshold triggered an alert four weeks prior to an outbreak, but exceeding the binomial level 2 threshold identified an outbreak as it occurred. CONCLUSION: The malaria outbreak surveillance system using binomial thresholds achieved its primary goal of identifying outbreaks early facilitating appropriate local public health responses aimed at averting a possible large-scale epidemic in a low, and unstable, malaria transmission setting.

45: *Malar J.* 2008 Apr 25;7(1):68

The severity of malarial anaemia in *Plasmodium chabaudi* infections of BALB/c mice is determined independently of the number of circulating parasites.

Lamb TJ, Langhorne J.

ABSTRACT: Background Severe malarial anaemia is a major complication of malaria infection and is multi-factorial resulting from loss of circulating red blood cells (RBCs) from parasite replication, as well as immune-mediated mechanisms. An understanding of the causes of severe malarial anaemia is necessary to develop and implement new therapeutic strategies to tackle this syndrome of malaria infection. METHODS: Using analysis of variance, this work investigated whether parasite-destruction of RBCs always accounts for the severity of malarial anaemia during infections of the rodent malaria model *Plasmodium chabaudi* in mice of a BALB/c background. Differences in anaemia between two different clones of *P. chabaudi* were also examined. RESULTS: Circulating parasite numbers were not correlated with the severity of anaemia in either BALB/c mice or under more severe conditions of anaemia in BALB/c RAG2 deficient mice (lacking T and B cells). Mice infected with *P. chabaudi* clone CB suffered more severe anaemia than mice infected with clone AS, but this was not correlated with the number of parasites in the circulation. Instead, the peak percentage of parasitized RBCs was higher in CB-infected animals than in AS-infected animals, and was correlated with the severity of anaemia, suggesting that the availability of uninfected RBCs was impaired in CB-infected animals. CONCLUSIONS: This work shows that parasite numbers are a more relevant measure of parasite levels in *P. chabaudi* infection than % parasitaemia, a measure that does not take anaemia into account. The lack of correlation between parasite numbers and the drop in circulating RBCs in this experimental model of malaria support a role for the host response in the impairment or destruction of uninfected RBC in *P. chabaudi* infections, and thus development of acute anaemia in this malaria model.

Clinically immune hosts as a refuge for drug-sensitive malaria parasites.

Klein EY, Smith DL, Boni MF, Laxminarayan R.

ABSTRACT: BACKGROUND: Mutations in *Plasmodium falciparum* that confer resistance to first-line antimalarial drugs have spread throughout the world from a few independent foci, all located in areas that were likely characterized by low or unstable malaria transmission. One of the striking differences between areas of low or unstable malaria transmission and hyperendemic areas is the difference in the size of the population of immune individuals. However, epidemiological models of malaria transmission have generally ignored the role of immune individuals in transmission, assuming that they do not affect the fitness of the parasite. This model reconsiders the role of immunity in the dynamics of malaria transmission and its impact on the evolution of antimalarial drug resistance under the assumption that immune individuals are infectious. METHODS: The model is constructed as a two-stage susceptible-infected-susceptible (SIS) model of malaria transmission that assumes that individuals build up clinical immunity over a period of years. This immunity reduces the frequency and severity of clinical symptoms, and thus their use of drugs. It also reduces an individual's level of infectiousness, but does not impact the likelihood of becoming infected. RESULTS: Simulations found that with the introduction of resistance into a population, clinical immunity can significantly alter the fitness of the resistant parasite, and thereby impact the ability of the resistant parasite to spread from an initial host by reducing the effective reproductive number of the resistant parasite as transmission intensity increases. At high transmission levels, despite a higher basic reproductive number, R_0 , the effective reproductive number of the resistant parasite may fall below the reproductive number of the sensitive parasite. CONCLUSION: These results suggest that high-levels of clinical immunity create a natural ecological refuge for drug-sensitive parasites. This provides an epidemiological rationale for historical patterns of resistance emergence and suggests that future outbreaks of resistance are more likely to occur in low- or unstable-transmission settings. This finding has implications for the design of drug policies and the formulation of malaria control strategies, especially those that lower malaria transmission intensity.

Enhanced detection of gametocytes by magnetic deposition microscopy predicts higher potential for *Plasmodium falciparum* transmission.

Karl S, David M, Moore L, Grimberg BT, Michon P, Mueller I, Zborowski M, Zimmerman PA.

ABSTRACT: BACKGROUND: Aggregated haemozoin crystals within malaria-infected erythrocytes confer susceptibility of parasitized cells to a magnetic field. Here the utility of this method for diagnosis of human malaria is evaluated in a malaria-endemic region of Papua New Guinea (PNG). Methods and findings Individuals with *Plasmodium falciparum* malaria symptoms (n=55) provided samples for conventional blood smear (CBS) and magnetic deposition microscopy (MDM) diagnosis. Standard Giemsa staining and light microscopy was performed to evaluate all preparations. *Plasmodium falciparum* parasitaemia observed on MDM slides was consistently higher than parasitaemia observed by (CBS) for ring (CBS = 2.6 vs. MDM = 3.4%; t-test P-value = 0.13), trophozoite (CBS = 0.5 vs. MDM = 1.6%; t-test P-value = 0.01), schizont (CBS = 0.003 vs. MDM = 0.1%; t-test P-value = 0.08) and gametocyte (CBS = 0.001 vs. MDM = 0.4%; t-test P-value = 0.0002) parasitaemias. Gametocyte prevalence determined by CBS compared to MDM increased from 7.3 % to 45 %, respectively. CONCLUSIONS: MDM increased detection sensitivity of *P. falciparum*-infected, haemozoin-containing erythrocytes from

infected humans while maintaining detection of ring-stage parasites. Gametocyte prevalence five-fold higher than observed by CBS suggests higher malaria transmission potential in PNG endemic sites compared to previous estimates.

48: *Malar J.* 2008 Apr 25;7(1):65

Towards a sterile insect technique field release of *Anopheles arabiensis* mosquitoes in Sudan: Irradiation, transportation, and field cage experimentation.

Helinski ME, Hassan MM, El-Motasim WM, Malcolm CA, Knols BG, El-Sayed B.

ABSTRACT: **BACKGROUND:** The work described in this article forms part of a study to suppress a population of the malaria vector *Anopheles arabiensis* in Northern State, Sudan, with the Sterile Insect Technique. No data have previously been collected on the irradiation and transportation of anopheline mosquitoes in Africa, and the first series of attempts to do this in Sudan are reported here. In addition, experiments in a large field cage under near-natural conditions are described. **METHODS:** Mosquitoes were irradiated in Khartoum and transported as adults by air to the field site earmarked for future releases (400 km from the laboratory). The field cage was prepared for experiments by creating resting sites with favourable conditions. The mating and survival of (irradiated) laboratory males and field-collected males was studied in the field cage, and two small-scale competition experiments were performed. **RESULTS:** Minor problems were experienced with the irradiation of insects, mostly associated with the absence of a rearing facility in close proximity to the irradiation source. The small-scale transportation of adult mosquitoes to the release site resulted in minimal mortality (< 6%). Experiments in the field cage showed that mating occurred in high frequencies (i.e. an average of 60% insemination of females after one or two nights of mating), and laboratory reared males (i.e. sixty generations) were able to inseminate wild females at rates comparable to wild males. Based on wing length data, there was no size preference of males for mates. Survival of mosquitoes from the cage, based on recapture after mating, was satisfactory and approximately 60% of the insects were recaptured after one night. Only limited information on male competitiveness was obtained due to problems associated with individual egg laying of small numbers of wild females. **CONCLUSIONS:** It is concluded that although conditions are challenging, there are no major obstacles associated with the small-scale irradiation and transportation of insects in the current setting. The field cage is suitable for experiments and studies to test the competitiveness of irradiated males can be pursued. The scaling up of procedures to accommodate much larger numbers of insects needed for a release is the next challenge and recommendations to further implementation of this genetic control strategy are presented.

49: *Malar J.* 2008 Apr 22;7(1):64

Natural relapses in vivax malaria induced by *Anopheles* mosquitoes.

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ABSTRACT: **BACKGROUND:** Monthly malaria cases in Finland during 1750-1850 revealed regionally different peaks. The main peak was in late spring in the whole country, but additional peaks occurred in August and December in some regions of Finland. Both primary infections and relapses caused deaths from malaria. The cause and timing of relapses are analysed. **METHODS:** Monthly data of deaths from malaria in 1750-1850 were successively correlated with mean temperatures of June and July of five years in succession forwards from the current year and through 10 years in succession backwards to identify timing of relapses in *Plasmodium vivax*. **RESULTS:** Malaria cases showed an increasing correlation with June-July temperatures, with peaks in late summer, midwinter and late spring and then dropped gradually during two to nine years from the first summer depending on the region. The longest incubation time identified was eight years and seven months.

CONCLUSION: High correlations of June-July temperatures with deaths from malaria in August to September in the same year indicate a close connection to the new generation of hatching Anopheles mosquitoes. Because rapid sporogony before October is impossible in Finland, the most plausible explanation is an early induction of relapses of vivax malaria by uninfected anophelines. Malaria cases during the winter and the following spring are caused by both primary infections and induced relapses. All subsequent cases represent relapses. It is proposed that the basic relapse patterns in vivax malaria are regulated by anophelines. It is also proposed that the Plasmodium is enhancing blood sucking of Anopheles messeae, which so far has been considered a bad vector.

50: *Malar J.* 2008 Apr 21;7(1):62

Investigation of a novel approach to scoring Giemsa-stained malaria-infected thin blood films.

Proudfoot O, Drew N, Scholzen A, Xiang S, Plebanski M.

ABSTRACT: Daily assessment of the percentage of erythrocytes that are infected ('percent-parasitaemia') across a time-course is a necessary step in many experimental studies of malaria, but represents a time-consuming and unpopular task among researchers. The most common method is extensive microscopic examination of Giemsa-stained thin blood-films. This study explored a method for the assessment of percent-parasitaemia that does not require extended periods of microscopy and results in a descriptive and permanent record of parasitaemia data that is highly amenable to subsequent 'data-mining'. Digital photography was utilized in conjunction with a basic purpose-written computer programme to test the viability of the concept. Partial automation of the determination of percent parasitaemia was then explored, resulting in the successful customization of commercially available broad-spectrum image analysis software towards this aim. Lastly, automated discrimination between infected and uninfected RBCs based on analysis of digital parameters of individual cell images was explored in an effort to completely automate the calculation of an accurate percent-parasitaemia.

51: *Malar J.* 2008 Apr 19;7(1):61

The usefulness of twenty-four molecular markers in predicting treatment outcome with combination therapy of amodiaquine plus sulphadoxine-pyrimethamine against falciparum malaria in Papua New Guinea.

Marfurt J, Muller I, Sie A, Oa O, Reeder JC, Smith TA, Beck HP, Genton B.

ABSTRACT: BACKGROUND: In Papua New Guinea (PNG), combination therapy with amodiaquine (AQ) or chloroquine (CQ) plus sulphadoxine-pyrimethamine (SP) was introduced as first-line treatment against uncomplicated malaria in 2000. METHODS: In vivo treatment failure rates with AQ+SP were assessed in two different areas in PNG and characterized twenty-four molecular drug resistance markers of Plasmodium falciparum in pre-treatment samples. The aim of the study was to investigate the association between infecting genotype and treatment response in order to identify useful predictors of treatment failure with AQ+SP. RESULTS: In 2004, Day-28 treatment failure rates for AQ+SP were 29% in the Karimui and 19% in the South Wosera area, respectively. The strongest independent predictors for treatment failure with AQ+SP were pfmdr1 N86Y (OR=7.87, p<0.01) and pfdhps A437G (OR=3.44, p<0.01). Mutations found in CQ/AQ related markers pfcrt K76T, A220S, N326D, and I356L did not help to increase the predictive value, the most likely reason being that these mutations reached almost fixed levels. Though mutations in SP related markers pfdhfr S108N and C59R were not associated with treatment failure, they increased the predictive value of pfdhps A437G. The difference in treatment failure rate in the two sites was reflected in the corresponding genetic profile of the parasite populations, with significant

differences seen in the allele frequencies of mutant pfmdr1 N86Y, pfmdr1 Y184F, pfcrt A220S, and pfdhps A437G. CONCLUSIONS: The study provides evidence for high levels of resistance to the combination regimen of AQ+SP in PNG and indicates which of the many molecular markers analysed are useful for the monitoring of parasite resistance to combinations with AQ+SP.

52: *Malar J.* 2008 Apr 18;7(1):60

Socio-economic status is inversely related to bed net use in Gabon.

Goesch JN, Schwarz NG, Decker ML, Oyakhirome S, Borchert LB, Kombila UD, Poetschke M, Lell B, Issifou S, Kremsner PG, Grobusch MP.

ABSTRACT: BACKGROUND: Insecticide-treated bed nets (ITNs) range among the most effective measures of malaria prophylaxis, yet their implementation level in sub-Saharan Africa is still low. The goal of this study was to investigate the influence of socio-economic factors on the use of bed nets by mothers in Gabon. METHODS: A cross-sectional study was conducted completing pre-tested, interviewer-administered questionnaires exploring socioeconomic proxy measures with 397 mothers or guardians of young children. Respondents were grouped according to their socio-economic situation, using scores. The condition of the bed nets was evaluated during a home visit. RESULTS: Socio-economic factors of wellbeing were negatively associated with bed net use, such as living in a stone house (OR 0.26, 95% CI 0.14-0.48), running water in the house (OR 0.44, 95% CI 0.21-0.92), shower/flush toilet in the house (OR 0.39/0.34, 95% CI 0.21-0.75/0.16-0.73), ownership of a freezer (OR 0.50, 95% CI 0.26-0.96) and belonging to the highest group in the economic score (OR 0.32, 95% CI 0.15-0.67). In contrast, similar factors were positively associated with a good maintenance condition of the bed nets: higher monthly income (OR 5.64, 95% CI 2.41-13.19) and belonging to the highest group in the economic score (OR 2.55, 95% CI 1.19 - 5.45). CONCLUSIONS: Among the poorest families in Lambarene the coverage with untreated nets (UTNs) is the highest, but the condition of these UTNs is the worst. To achieve a broad implementation of ITNs in Lambarene, there is an urgent need for educational programmes as well as need-tailored marketing strategies for ITNs.

53: *Malar J.* 2008 Apr 18;7(1):59

Mosquito abundance, bed net coverage and other factors associated with variations in sporozoite infectivity rates in four villages of rural Tanzania.

Kweka EJ, Nkya WM, Mahande AM, Assenga C, Mosha FW, Lyatuu EE, Massenga CP, Nyale EM, Mwakalinga SB, Lowassa A.

ABSTRACT: BACKGROUND: Entomological surveys are of great importance in decision-making processes regarding malaria control strategies because they help to identify associations between vector abundance both species-specific ecology and disease intervention factors associated with malaria transmission. Sporozoite infectivity rates, mosquito host blood meal source, bed net coverage and mosquito abundance were assessed in this study. Methodology: A longitudinal survey was conducted in four villages in two regions of Tanzania. Malaria vectors were sampled using the CDC light trap and pyrethrum spray catch methods. In each village, ten paired houses were selected for mosquitoes sampling. Sampling was done in fortnight case and study was undertaken for six months in both Kilimanjaro (Northern Tanzania) and Dodoma (Central Tanzania) regions. RESULTS: A total of 6,883 mosquitoes were collected including: 5,628 (81.8%) *Anopheles arabiensis*, 1,100 (15.9%) *Culex quinquefasciatus*, 89 (1.4%) *Anopheles funestus*, and 66 (0.9%) *Anopheles gambiae* s.s. Of the total mosquitoes collected 3,861 were captured by CDC light trap and 3,022 by the pyrethrum spray catch method. The overall light trap: spray catch ratio was 1.3:1. Mosquito densities per room were 96.5 and 75.5 for light trap and pyrethrum spray catch, respectively. Mosquito

infectivity rates between villages that have high proportion of bed net owners and those without bed nets was significant ($P < 0.001$) and there was a significant difference in sporozoite rates between households with and without bed nets in these four villages ($P < 0.001$). CONCLUSION: Malaria remains a major problem in the study areas characterized as low transmission sites. Further studies are required to establish the annual entomological inoculation rates and to observe the annual parasitaemia dynamics in these communities. Outdoor mosquitoes collection should also be considered.

54: *Malar J.* 2008 Apr 17;7(1):58

Efficacy of amodiaquine in the treatment of uncomplicated falciparum malaria in young children of rural north-western Burkina Faso.

Mandi G, Mockenhaupt FP, Coulibaly B, Meissner P, Mueller O.

ABSTRACT: BACKGROUND: Combination therapy has become a new paradigm in malaria treatment. Amodiaquine is a common partner drug in different malaria combination therapies used or investigated in sub-Saharan Africa, but data on its efficacy as a single drug are scarce. METHODS: The objective of the study was to determine the efficacy of amodiaquine against falciparum malaria in neighbouring rural and urban areas of north-western Burkina Faso. The study was designed as an uncontrolled trial in children aged 6-59 months with uncomplicated falciparum malaria in the Nouna Health District. RESULTS: During the rainy season 2005, 117 children were enrolled, 62 from the rural and 55 from the urban study area. The crude adequate clinical and parasitological response (ACPR) rate was 103/117 (88%) by day 14 but decreased to 28/117 (24%) by day 28. After PCR correction for reinfections, ACPR rates were 108/117 (92%) and 71/117 (61%) by day 14 and day 28, respectively. There were no significant differences in efficacy between urban and rural areas. The Plasmodium falciparum crt K76T mutation did not predict AQ failure, but was selected in parasites re-appearing following treatment. No serious adverse events occurred and only 16 other adverse events were recorded. CONCLUSIONS: Compared to chloroquine, amodiaquine is more effective against uncomplicated falciparum malaria in Burkina Faso. However, a considerable degree of amodiaquine resistance already exists and it is currently unclear how this resistance will develop when amodiaquine in combination with other drugs is used on a large scale. Trial registration: Current Controlled Trials ISRCTN73824458.

55: *Malar J.* 2008 Apr 11;7(1):57

Clinical uncomplicated Plasmodium falciparum malaria with high schizontaemia: A case report.

Lwin KM, Ashley EA, Proux S, Silamut K, Nosten F, McGready R.

ABSTRACT: BACKGROUND: The treatment options for acute Plasmodium falciparum malaria are based on the clinician classifying the patient as uncomplicated or severe according to the clinical and parasitological findings. This process is not always straightforward. CASE PRESENTATION: An adult male presented to a clinic on the western border of Thailand with a physical examination and P. falciparum trophozoite count (1.2% of infected red blood cells, IRBC) from malaria blood smear, consistent with a diagnosis of uncomplicated P. falciparum infection. However, the physician on duty treated the patient for severe malaria based on the reported P. falciparum schizont count, which was very high (0.3% IRBC), noticeably in relation to the trophozoite count and schizont:trophozoite ratio 0.25:1. On intravenous artesunate, the patient deteriorated clinically in the first 24 hours. The trophozoite count increased from 1.2% RBC at baseline to 20.5% RBC 18 hours following the start of treatment. By day three, the patient recovered and was discharged on day seven having completed a seven-day treatment with artesunate and mefloquine. CONCLUSIONS: The malaria blood smear provides only a guide to the overall parasite biomass in the body, due to the ability of

P. falciparum to sequester in the microvasculature. In severe malaria, high schizont counts are associated with worse prognosis. In low transmission areas or in non-immune travelers the presence of schizonts in the peripheral circulation is an indication for close patient supervision. In this case, an unusually high schizont count in a clinically uncomplicated patient was indicative of potential deterioration. Prompt treatment with intravenous artesunate is likely to have been responsible for the good clinical outcome in this case.

56: *Malar J.* 2008 Apr 8;7(1):56

Imported malaria in a cosmopolitan European city: a mirror image of the world epidemiological situation.

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ABSTRACT: **BACKGROUND:** International travel and migration have been related with an increase of imported malaria cases. There has been considerable immigration to Barcelona from low-income countries (LIC) in recent years. The objective is to describe the epidemiology and to determine the trends of the disease in Barcelona. **METHODS:** Analysis of the cases notified among city residents between 1989 and 2005. Patients were classified as: tourists, voluntary workers, resident immigrants (visiting friends and relatives, VFR) and recently arrived immigrants. An analysis was conducted using the chi² test and comparison of means. As a measure of association we calculated the Relative Risk (RR) and Odds Ratio (OR) with a Confidence Interval of 95% (CI) and carried out a trends analysis. **RESULTS:** Of the total of 1,579 imported cases notified, 997 (63.1%) lived in Barcelona city, and 55.1% were male. The mean age of patients was 32.7 years. The incidence increased from 2.4 cases/100,000 in 1989 to 3.5 cases/100,000 in 2005 (RR 1.46 CI:1.36-1.55). This increase was not statistically significant (trends analysis, p= 0.36). In terms of reason for travelling, 40.7% were VFR, 33.6% tourists, 12.1% voluntary workers and 13.6% were recently arrived immigrants. The most frequent species found was *Plasmodium falciparum* (71.3%), mainly in visitors to Africa (OR=2.3,CI=1.7-3.2). The vast majority (82.2%) had had some contact with Africa (35.9% with Equatorial Guinea, a Spanish ex-colony) and 96.6% had not completed chemoprophylaxis. Six deaths were observed, all tourists who had travelled to Africa and not taken chemoprophylaxis (3.9% fatality rate). Lack of compliance and the association between Africa and *P. falciparum* are very clear in the imported cases. **CONCLUSIONS:** Over the period studied there is an increase in malaria incidence, however the trend is not statistically significant. There is evidence of an association between *P. falciparum* and Africa. Most of the patients with malaria did not take chemoprophylaxis.

57: *Malar J.* 2008 Apr 4;7:55.

Assessment of the efficacy of antimalarial drugs recommended by the National Malaria Control Programme in Madagascar: up-dated baseline data from randomized and multi-site clinical trials.

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BACKGROUND: In order to improve the monitoring of the antimalarial drug resistance in Madagascar, a new national network based on eight sentinel sites was set up. In 2006/2007, a multi-site randomized clinical trial was designed to assess the therapeutic efficacy of chloroquine (CQ), sulphadoxine-pyrimethamine (SP), amodiaquine (AQ) and artesunate plus amodiaquine combination (ASAQ), the

antimalarial therapies recommended by the National Malaria Control Programme (NMCP). METHODS: Children between six months and 15 years of age, with uncomplicated falciparum malaria, were enrolled. Primary endpoints were the day-14 and day-28 risks of parasitological failure, either unadjusted or adjusted by genotyping. Risks of clinical and parasitological treatment failure after adjustment by genotyping were estimated using Kaplan-Meier survival analysis. Secondary outcomes included fever clearance, parasite clearance, change in haemoglobin levels between Day 0 and the last day of follow-up, and the incidence of adverse events. RESULTS: A total of 1,347 of 1,434 patients (93.9%) completed treatment and follow-up to day 28. All treatment regimens, except for the chloroquine (CQ) treatment group, resulted in clinical cure rates above 97.6% by day-14 and 96.7% by day-28 (adjusted by genotyping). Parasite and fever clearance was more rapid with artesunate plus amodiaquine, but the extent of haematological recovery on day-28 did not differ significantly between the four groups. No severe side-effects were observed during the follow-up period. CONCLUSION: These findings (i) constitute an up-dated baseline data on the efficacy of antimalarial drugs recommended by the NMCP, (ii) show that antimalarial drug resistance remains low in Madagascar, except for CQ, compared to the bordering countries in the Indian Ocean region such as the Comoros Archipelago and (iii) support the current policy of ASAQ as the first-line treatment in uncomplicated falciparum malaria.

58: *Malar J.* 2008 Apr 3;7:54.

Can changes in malaria transmission intensity explain prolonged protection and contribute to high protective efficacy of intermittent preventive treatment for malaria in infants?

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BACKGROUND: Intermittent preventive (or presumptive) treatment of infants (IPTi), the administration of a curative anti-malarial dose to infants whether or not they are known to be infected, is being considered as a new strategy for malaria control. Five of the six trials using sulphadoxine-pyrimethamine (SP) for IPTi showed protective efficacies (PEs) against clinical malaria ranging from 20.1 - 33.3% whilst one, the Ifakara study, showed a protective efficacy of 58.6%. MATERIALS AND METHODS: The possible mechanisms that could explain the differences in the reported PE of IPTi were examined by comparing output from a mathematical model to data from the six published IPTi trials. RESULTS: Under stable transmission, the PE of IPTi predicted by the model was comparable with the observed PEs in all but the Ifakara study (ratio of the mean predicted PE to that observed was 1.02, range 0.39 - 1.59). When a reduction in the incidence of infection during the study was included in the model, the predicted PE of IPTi increased and extended into the second year of life, as observed in the Ifakara study. CONCLUSION: A decrease in malaria transmission during the study period may explain part of the difference in observed PEs of IPTi between sites and the extended period of protection into the second year of life observed in the Ifakara study. This finding of continued benefit of interventions in settings of decreasing transmission may explain why rebound of clinical malaria was absent in the large scale trials of insecticide-treated bed nets.

59: *Malar J.* 2008 Apr 2;7:53.

Guidelines and mindlines: why do clinical staff over-diagnose malaria in Tanzania? A qualitative study.

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BACKGROUND: Malaria over-diagnosis in Africa is widespread and costly both financially and in terms of morbidity and mortality from missed diagnoses. An understanding of the reasons behind malaria over-diagnosis is urgently needed to inform strategies for better targeting of antimalarials. **METHODS:** In an ethnographic study of clinical practice in two hospitals in Tanzania, 2,082 patient consultations with 34 clinicians were observed over a period of three months at each hospital. All clinicians were also interviewed individually as well as being observed during routine working activities with colleagues. Interviews with five tutors and 10 clinical officer students at a nearby clinical officer training college were subsequently conducted. **RESULTS:** Four, primarily social, spheres of influence on malaria over-diagnosis were identified. Firstly, the influence of initial training within a context where the importance of malaria is strongly promoted. Secondly, the influence of peers, conforming to perceived expectations from colleagues. Thirdly, pressure to conform with perceived patient preferences. Lastly, quality of diagnostic support, involving resource management, motivation and supervision. Rather than following national guidelines for the diagnosis of febrile illness, clinician behaviour appeared to follow 'mindlines': shared rationales constructed from these different spheres of influence. Three mindlines were identified in this setting: malaria is easier to diagnose than alternative diseases; malaria is a more acceptable diagnosis; and missing malaria is indefensible. These mindlines were apparent during the training stages as well as throughout clinical careers. **CONCLUSION:** Clinicians were found to follow mindlines as well as or rather than guidelines, which incorporated multiple social influences operating in the immediate and the wider context of decision making. Interventions to move mindlines closer to guidelines need to take the variety of social influences into account.

60: *Malar J.* 2008 Mar 31;7:52.

Bionomics of *Anopheles latens* in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite *Plasmodium knowlesi*.

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BACKGROUND: A large focus of human infections with *Plasmodium knowlesi*, a simian parasite naturally found in long-tailed and pig-tailed macaques was discovered in the Kapit Division of Sarawak, Malaysian Borneo. A study was initiated to identify the vectors of malaria, to elucidate where transmission is taking place and to understand the bionomics of the vectors in Kapit. **METHODS:** Three different ecological sites in the forest, farm and longhouse in the Kapit district were selected for the study. Mosquitoes were collected by human landing collection at all sites and at the forest also by monkey-baited-traps situated on three different levels. All mosquitoes were identified and salivary glands and midguts of anopheline mosquitoes were dissected to determine the presence of malaria parasites. **RESULTS AND DISCUSSIONS:** Over an 11-month period, a total of 2,504 *Anopheles* mosquitoes comprising 12 species were caught; 1,035 at the farm, 774 at the forest and 425 at the longhouse. *Anopheles latens* (62.3%) and *Anopheles watsonii* (30.6%) were the predominant species caught in the forested ecotypes,

while in the farm *Anopheles donaldi* (49.9%) and *An. latens* (35.6%) predominated. In the long house, *An. latens* (29.6%) and *An. donaldi* (22.8%) were the major Anopheline species. However, *An. latens* was the only mosquito positive for sporozoites and it was found to be attracted to both human and monkey hosts. In monkey-baited net traps, it preferred to bite monkeys at the canopy level than at ground level. *An. latens* was found biting early as 18.00 hours. CONCLUSION: *Anopheles latens* is the main vector for *P. knowlesi* malaria parasites in the Kapit District of Sarawak, Malaysian Borneo. The study underscores the relationship between ecology, abundance and bionomics of anopheline fauna. The simio-anthropophagic and acrodendrophilic behaviour of *An. latens* makes it an efficient vector for the transmission of *P. knowlesi* parasites to both human and monkey hosts.

61: *Med Princ Pract.* 2008;17(3):197-201.

Enhanced efficacy of amodiaquine and chlorpheniramine combination over amodiaquine alone in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in children.

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OBJECTIVE: To evaluate the comparative efficacy of amodiaquine (AMQ) alone and the combination of AMQ and chlorpheniramine (CP) in the treatment of acute uncomplicated malaria in children. SUBJECTS: Of the 110 children enrolled in the study, 103 with acute uncomplicated malaria, aged 6 months to 12 years, were evaluated using the 14-day modification of the WHO field test. The patients were randomized to 2 groups. Group 1 received supervised treatment with AMQ alone (10 mg AMQ base/kg daily for 3 days), while group 2 received supervised treatment with AMQ (same dose as group 1) plus CP (AMQCP) for 7 days. RESULTS: Both treatment regimens were well tolerated and no patient was withdrawn as a result of recurrent vomiting or drug-related adverse events. There was no significant difference in mean fever and parasite clearance times. The cure rates at day 7 were 90.2 versus 100% ($\rho = 0.027$) for AMQ versus AMQCP, while the day 14 cure rates were 85.9 versus 98.1% for AMQ versus AMQCP, respectively ($\rho = 0.016$). CONCLUSION: The combination of AMQ plus CP proved significantly more effective than AMQ alone in the treatment of acute uncomplicated *falciparum* malaria, most probably due to the enhancement of the antimalarial effect of AMQ by CP. The combination of AMQCP could be a better alternative to AMQ alone as a companion drug in artemisinin-based combination therapies. (c) 2008 S. Karger AG, Basel

62: *Mol Biochem Parasitol.* 2008 Apr;158(2):208-12.

Effect of protease inhibitors on exflagellation in *Plasmodium falciparum*.

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Enzymes involved in sexual differentiation and fertilization of the human malaria parasite *Plasmodium falciparum* represent potential targets for transmission blocking strategies. Parasite proteases are putatively involved in several steps during fertilization, but the types of proteases, their targets and modes of action remain hitherto unknown. We investigated the involvement of proteases in gametogenesis via exflagellation and immunofluorescence assays, using a variety of commercially available as well as newly designed protease inhibitors. The assays revealed a blockade of microgamete formation by the cysteine/serine protease inhibitors TLCK and TPCK. The serine protease inhibitor PMSF, the

falcipain-targeting inhibitor RV112D, and the aspartic protease inhibitor EPNP also significantly decreased formation of microgametes. The metalloprotease inhibitor 1,10-phenanthroline, on the other hand, inhibited exflagellation by interfering with microgamete motility. Furthermore, EPNP reduced the activation of male and female gametocytes. Our data point to a major involvement of serine proteases and a non-thermolysin-like zinc metalloprotease in microgametocyte exflagellation.

63: *Mol Biochem Parasitol.* 2008 Apr;158(2):213-6.

An efficient strategy for gene targeting and phenotypic assessment in the Plasmodium yoelii rodent malaria model.

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In this report, we describe a cloning procedure for gene replacement by double homologous recombination in *Plasmodium yoelii*, which requires only one digestion and ligation step. This significantly shortens the time required to complete the production of the targeting vector. Furthermore, for more efficient phenotypic evaluation of the gene knockout parasites, we have also introduced a fluorescent protein cassette into the targeting vector. This allows for a more rapid assessment of parasite growth in all of its developmental stages. In addition, the introduction of the fluorescent marker via the replacement strategy confers the stable integration of the marker.

64: *Mol Biochem Parasitol.* 2008 Apr;158(2):139-51.

Biochemical characterization of Plasmodium falciparum Sir2, a NAD⁺-dependent deacetylase.

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In *Plasmodium falciparum*, the causative agent of cerebral malaria, silent information regulator 2 (Sir2) has been implicated in pathogenesis through its role in var gene silencing. *P. falciparum* Sir2 (PfSir2) in addition to the catalytic core, has a 13 residue N-terminal and 4 residue C-terminal extension over the shorter *Archaeoglobus fulgidus* Sir2. In this paper, we highlight our studies aimed at understanding the kinetic mechanism of PfSir2 and the role of N- and C-terminal extensions in protein function and oligomerization. Bisubstrate kinetic analysis showed that PfSir2 exhibits a rapid equilibrium ordered sequential mechanism, with peptide binding preceding NAD(+). This study also reports on surfactin as a novel Sir2 inhibitor exhibiting competitive inhibition with respect to NAD(+) and uncompetitive inhibition with acetylated peptide. This inhibition pattern with surfactin provides further support for ordered binding of substrates. Surfactin was also found to be a potent inhibitor of intra-erythrocytic growth of *P. falciparum* with 50% inhibitory concentration in the low micromolar range. PfSir2, like the yeast homologs (yHst2 and Sir2p), is a trimer in solution. However, dissociation of trimer to monomers in the presence of NAD(+) is characteristic of the parasite enzyme. Oligomerization studies on N- and/or C-terminal deletion constructs of PfSir2 highlight the role of C-terminus of the protein in mediating homotrimerization. N-terminal deletion resulted in reduced catalytic efficiency although substrate affinity was not altered in the constructs. Interestingly, deletion of both the ends relaxed NAD(+) specificity.

65: *Mol Biosyst.* 2008 Apr;4(4):328-40.

Intrinsic disorder in pathogenic and non-pathogenic microbes: discovering and analyzing the unfoldomes of early-branching eukaryotes.

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Parasitic protozoal infections have long been known to cause profound degrees of sickness and death in humans as well as animal populations. Despite the increase in the number of annotated genomes available for a large variety of protozoa, a great deal more has yet to be learned about them, from their fundamental physiology to mechanisms invoked during host-pathogen interactions. Most of these genomes share a common feature, namely a high prevalence of low complexity regions in their predicted proteins, which is believed to contribute to the uniqueness of the individual species within this diverse group of early-branching eukaryotes. In the case of *Plasmodium* species, which cause malaria, such regions have also been reported to hamper the identification of homologues, thus making functional genomics exceptionally challenging. One of the better accepted theories accounting for the high number of low complexity regions is the presence of intrinsic disorder in these microbes. In this study we compare the degree of disordered proteins that are predicted to be expressed in many such ancient eukaryotic cells. Our findings indicate an unusual bias in the amino acids comprising protozoal proteomes, and show that intrinsic disorder is remarkably abundant among their predicted proteins. Additionally, the intrinsically disordered regions tend to be considerably longer in the early-branching eukaryotes. An analysis of a *Plasmodium falciparum* interactome indicates that protein-protein interactions may be at least one function of the intrinsic disorder. This study provides a bioinformatics basis for the discovery and analysis of unfoldomes (the complement of intrinsically disordered proteins in a given proteome) of early-branching eukaryotes. It also provides new insights into the evolution of intrinsic disorder in the context of adapting to a parasitic lifestyle and lays the foundation for further work on the subject.

66: *Mol Immunol.* 2008 Apr 25

Altered cord blood gammadelta T cell repertoire in Nigeria: Possible impacts of environmental factors on neonatal immunity.

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Infectious diseases during pregnancy can impact the development of fetal immunity, leading to reduced neonatal resistance to infection and decreased responses to pediatric vaccines. *Plasmodium falciparum* causes placental infection in low parity pregnant women and is among the pathogens that affect fetal immunity. Recognizing the relationship between malaria and gammadelta T lymphocytes in adults, we asked whether neonatal gammadelta T cells would be altered in malaria-endemic regions as a marker for changes in fetal immunity. Our initial studies compared cord blood gammadelta T cells from deliveries to HIV-mothers in Jos (Nigeria) where malaria is endemic, or in Rome (Italy). We noted substantial differences in the Vgamma2 repertoire for cord blood collected in Jos or Rome; differences were consistent with a negative selection mechanism operating on the fetal Vgamma2 chain repertoire in neonates from Jos. A specific disruption affected the fraction of gammadelta T cells that we expect will respond to Bacille Calmette-Guerin (BCG). Fetal gammadelta T cell depletion might

be a mechanism for impaired neonatal immunity and lowered responses to pediatric vaccines.

67: *Mol Microbiol.* 2008 Apr 11

Plasmodium falciparum Sec24 Marks Transitional ER that Exports a Model Cargo via a Diacidic Motif.

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Exit from the endoplasmic reticulum (ER) often occurs at distinct sites of vesicle formation known as transitional ER (tER) that are enriched for COPII vesicle coat proteins. We have characterized the organization of ER export in the malaria parasite, *Plasmodium falciparum*, by examining the localization of two components of the COPII machinery, PfSec12 and PfSec24a. PfSec12 was found throughout the ER, whereas the COPII cargo adaptor, PfSec24a, was concentrated at distinct foci that likely correspond to tER sites. These foci were closely opposed to cis-Golgi sites marked by PfGRASP-GFP, and upon treatment with brefeldin A they accumulated a model cargo protein via a process dependent on the presence of an intact diacidic export motif. Our data suggest that the cargo-binding function of PfSec24a is conserved and that accumulation of cargo in discrete tER sites depends upon positive sorting signals. Furthermore, the number and position of tER sites with respect to the cis-Golgi suggests a coordinated biogenesis of these domains.

68: *Mol Microbiol.* 2008 Apr;68(1):124-38.

MSP1(19) miniproteins can serve as targets for invasion inhibitory antibodies in *Plasmodium falciparum* provided they contain the correct domains for cell surface trafficking.

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Antibodies from malaria-exposed individuals can agglutinate merozoites released from *Plasmodium* schizonts, thereby preventing them from invading new erythrocytes. Merozoite coat proteins attached to the plasma membrane are major targets for host antibodies and are therefore considered important malaria vaccine candidates. Prominent among these is the abundant glycosylphosphatidylinositol (GPI)-anchored merozoite surface protein 1 (MSP1) and particularly its C-terminal fragment (MSP1(19)) comprised of two epidermal growth factor (EGF)-like modules. In this paper, we revisit the role of agglutination and immunity using transgenic fluorescent marker proteins. We describe expression of heterologous MSP1(19)'miniproteins' on the surface of *Plasmodium falciparum* merozoites. To correctly express these proteins, we determined that GPI-anchoring and the presence of a signal sequence do not allow default export of proteins from the endoplasmic reticulum to merozoite surface and that extra sequence elements are required. The EGFs are insufficient for correct trafficking unless they are fused to additional residues that normally reside upstream of this fragment. Antibodies specifically targeting the surface-expressed miniprotein can inhibit erythrocyte invasion in vitro despite the presence of endogenous MSP1. Using a line expressing a green fluorescent protein-MSP1 fusion protein, we demonstrate that one mode of inhibition by antibodies targeting the MSP1(19) domain is the rapid agglutinating of merozoites prior to erythrocyte attachment.

69: *Mol Phylogenet Evol.* 2008 Apr;47(1):45-53.

Evolution and phylogeny of the heterogeneous cytosolic SSU rRNA genes in the genus *Plasmodium*.

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Unlike other eukaryotes, malaria parasites in the genus *Plasmodium* have structurally and functionally different paralogous copies of the cytosolic (cyto-) SSU rRNA (18S rRNA) gene that are expressed at different developmental stages. In *P. falciparum*, *P. vivax*, and *P. berghei*, A-type cyto-SSU rRNA is expressed in asexual stage, while S-type in sporozoite stage. A third type (O-type) has been described in *P. vivax*. It is expressed only in oocyst stage in the mosquito. Recently, it has been shown that the maintenance of heterogeneous cyto-SSU rRNAs in *Plasmodium* can be modeled as a birth-and-death process under strong purifying selection [Rooney, A.P., 2004. Mechanisms underlying the evolution and maintenance of functionally heterogeneous 18S rRNA genes in Apicomplexans. *Mol. Biol. Evol.* 21, 1704-1711]. In this study, we performed detailed phylogenetic analyses of *Plasmodium* cyto-SSU rRNAs with special emphasis on the evolution of multi-copy genes in simian *Plasmodium* species. We sequenced paralogous copies of the cyto-SSU rRNA genes from an African simian *Plasmodium* species, *P. gonderi*, and Asian simian *Plasmodium* species, *P. fragile*, *P. coatneyi*, *P. inui*, *P. hylobati*, *P. fieldi*, *P. simiovale*, and *P. cynomolgi*. Interestingly, all Asian simian *Plasmodium* species have a single S-type-like gene and several A-type-like genes. Alignment analysis demonstrated for the first time that an approximately 50-residue insertion in the V7 variable region near the stem 43 is shared exclusively by the S-type-like sequences of the Asian simian *Plasmodium* species and the S- and O-type sequences of *P. vivax*. We comprehensively analyzed all cyto-SSU rRNA sequences of the genus *Plasmodium* currently available in the database. Phylogenetic analyses of all publicly available cyto-SSU rRNA sequences for the genus *Plasmodium* clearly demonstrated that gene duplication events giving rise to A- and S-type-like sequences took place independently at least three times in the *Plasmodium* evolution, supporting the hypothesis that these genes evolve according to a birth-and-death model.

70: *Parasit Vectors.* 2008 Apr 10;1(1):9

A stable isotope dual-labelling approach to detect multiple insemination in un-irradiated and irradiated *Anopheles arabiensis* mosquitoes.

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ABSTRACT: BACKGROUND: In the context of a Sterile Insect Technique programme, the occurrence of multiple insemination in the malaria mosquito *Anopheles arabiensis* Patton was studied using a novel labelling system with the stable isotopes ¹⁵N and ¹³C. The incidence of multiple insemination in the absence of radiation, and when males were irradiated in the pupal stage and competed against un-irradiated males were assessed. Males used in the experiments were labelled with either ¹⁵N or ¹³C and the label was applied to the larval rearing water. Males with either label and virgin females were caged at a 1:1:1 ratio. Males used in the radiation treatments were irradiated in the pupal stage with a partially or fully-sterilizing dose of 70 or 120 Gy, respectively. After mating, females were dissected and inseminated spermathecae analysed using mass spectrometry. RESULTS: The data indicate that about 25% of inseminated females had been inseminated multiply. The presence of irradiated males in the experiments did not affect the incidence of multiple insemination. In line with previous research, irradiated

males were generally less competitive than un-irradiated males. CONCLUSIONS: The implications of these findings for the Sterile Insect Technique are discussed, and further experiments recommended. The dual-labelling system used to determine paternity gave good results for ¹³C, however, for ¹⁵N it is recommended to increase the amount of label in future studies.

71: *Parasite Immunol.* 2008 Apr 23

Cellular immune responses to recombinant *Plasmodium vivax* tryptophan-rich antigen (PvTRAg) among individuals exposed to vivax malaria.

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Plasmodium vivax, the most widespread species of human malaria parasite responsible for 70-80 million cases each year requires a vaccine. In recent years, many potential vaccine candidate antigens have been identified from *P. vivax* including PvTRAg. We describe here cellular immune response to recombinant PvTRAg expressed in *Escherichia coli*. The in vitro stimulation of PBMCs derived from *P. vivax*-exposed individuals (n = 16) showed strong proliferative response (SI > 2.2) to PvTRAg as compared to PBMCs from normal healthy controls (n = 8). Although both Th1 (IFN-gamma, TNF-alpha and IL-12) and Th2 (IL-4 and IL-10) cytokines were secreted by the PBMCs of the *P. vivax*-exposed individuals in response to PvTRAg, the overall response was more inclined towards Th2. In conclusion, recombinant PvTRAg was found to elicit strong cellular immune response among the *P. vivax*-exposed individuals.

72: *Parasite Immunol.* 2008 Apr 14

Effect of GPI anchor moiety on the immunogenicity of DNA plasmids encoding the 19-kDa C-terminal portion of *Plasmodium falciparum* MSP-1.

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The glycosylphosphatidylinositol (GPI)-anchored *Plasmodium falciparum* merozoite surface protein 1 (MSP-1) is a widely studied malaria vaccine candidate. The C-terminal 19-kDa portion of MSP-1 (MSP-1(19)) is of particular interest because this polypeptide moiety remains bound to the parasite even after erythrocyte invasion, while the remainder of MSP-1 is shed during invasion. Studies have shown that antibodies against MSP-1(19) inhibit merozoite invasion of erythrocytes efficiently, and that MSP-1(19) produces protective immunity in mice and monkeys. To investigate the efficacy of MSP-1(19) DNA vaccine and role of GPI anchor moiety in the immunogenicity of MSP-1(19), we constructed expression vectors that produce MSP-1(19) as either secretory or GPI-anchored polypeptide. Both constructs efficiently expressed MSP-1(19) in transfected HEK-293 cells. While the recombinant plasmid lacking GPI anchor signal sequence expressed MSP-1(19) mainly as secreted polypeptide, that containing GPI anchor signal sequence produced GPI-anchored MSP-1(19) on cell surface. In immunized mice, both constructs produced substantial levels of MSP-1(19)-specific IgG1, IgG2a, IgG2b, IgG3, IgA and IgM antibodies. In both cases, the IgG1 level was significantly higher than other isotypes. Interestingly, the plasmid containing GPI anchor signal sequence produced significantly higher levels of IgG2a and IgG2b than the plasmid that lacks GPI signal sequence.

73: *Parasite Immunol.* 2008 Apr;30(4):223-33.

What can transgenic parasites tell us about the development of Plasmodium-specific immune responses?

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Malaria infects 500 million people and kills an estimated 2.7 million annually, representing one of the most significant diseases in the world. However, efforts to develop effective vaccines have met with limited success. One reason is our lack of basic knowledge of how and where the immune system responds to parasite antigens. This is important as the early events during induction of an immune response influence the acquisition of effector function and development of memory responses. Our knowledge of the interactions of Plasmodia with the host immune system has largely been derived through in vitro study. This is a significant issue as the component parts of the immune system do not work in isolation and their interactions occur in distinct and specialized micro- and macro-anatomical locations that can only be assessed in the physiological context, in vivo. In this context, the availability of transgenic malaria parasites over the last 10 years has greatly enhanced our ability to understand and evaluate factors involved in host-parasite interactions in vivo. In this article, we review the current status of this area and speculate on what parasite transgenesis approaches will tell us about the development of Plasmodium-specific immune responses in the future.

74: *Parasitology.* 2008 Apr 1;:1-8

Worms and malaria: blind men feeling the elephant?

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SUMMARYFor thousands of years the deadliest human parasite, Plasmodium falciparum, has been evolving in populations also infected by the most prevalent parasites, worms. This is likely to have shaped the genome of all 3 protagonists - man, worms and malaria. Observational studies in Thailand have shown that although P. falciparum malaria incidence increased two-fold in helminth-infected patients, there was a 64% reduction of cerebral malaria and an 84% reduction of acute renal failure in helminth-infected patients relative to those without helminths. In addition, it was suggested that mixed infections, anaemia and gametocyte carriage were more frequent in helminth-infected patients. On the contrary, fever was lower in helminth-infected patients. The present hypotheses, their implications and the limitations of the results described and of those from studies in Africa are discussed.

75: *Pediatr Infect Dis J.* 2008 Apr;27(4):319-324.

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

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BACKGROUND: Inpatient treatment for malaria without microscopic confirmation of the diagnosis occurs commonly in sub-Saharan Africa. Differences in mortality in children who are tested by microscopy for *Plasmodium falciparum* infection as compared with those not tested are not well characterized. **METHODS:** A retrospective chart review was conducted of all children up to 15 years of age admitted to Mulago Hospital, Kampala, Uganda from January 2002 to July 2005, with a diagnosis of malaria and analyzed according to microscopy testing for *P. falciparum*. **RESULTS:** A total of 23,342 children were treated for malaria during the study period, 991 (4.2%) of whom died. Severe malarial anemia in 7827 (33.5%) and cerebral malaria in 1912 (8.2%) were the 2 common causes of malaria-related admissions. Children who did not receive microscopy testing had a higher case fatality rate than those with a positive blood smear (7.5% versus 3.2%, $P < 0.001$). After adjustment for age, malaria complications, and comorbid conditions, children who did not have microscopy performed or had a negative blood smear had a higher risk of death than those with a positive blood smear [odds ratio (OR): 3.49, 95% confidence interval (CI): 2.88-4.22, $P < 0.001$; and OR: 1.59, 95% CI: 1.29-1.96, $P < 0.001$, respectively]. **CONCLUSIONS:** Diagnosis of malaria in the absence of microscopic confirmation is associated with significantly increased mortality in hospitalized Ugandan children. Inpatient diagnosis of malaria should be supported by microscopic or rapid diagnostic test confirmation.

76: *PLoS ONE*. 2008 Apr 23;3(4):e2000.

Genetic determination and linkage mapping of *Plasmodium falciparum* malaria related traits in Senegal.

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Plasmodium falciparum malaria episodes may vary considerably in their severity and clinical manifestations. There is good evidence that host genetic factors contribute to this variability. To date, most genetic studies aiming at the identification of these genes have used a case/control study design for severe malaria, exploring specific candidate genes. Here, we performed a family-based genetic study of *falciparum* malaria related phenotypes in two independent longitudinal survey cohorts, as a first step towards the identification of genes and mechanisms involved in the outcome of infection. We studied two Senegalese villages, Dielmo and Ndiop that differ in ethnicity, malaria transmission and endemicity. We performed genome-scan linkage analysis of several malaria-related phenotypes both during clinical attacks and asymptomatic infection. We show evidence for a strong genetic contribution to both the number of clinical *falciparum* malaria attacks and the asymptomatic parasite density. The asymptomatic parasite density showed linkage to chromosome 5q31 (LOD = 2.26, empirical $p = 0.0014$, Dielmo), confirming previous findings in other studies. Suggestive linkage values were also obtained at three additional chromosome regions: the number of clinical malaria attacks on chromosome 5p15 (LOD = 2.57, empirical $p = 0.001$, Dielmo) and 13q13 (LOD = 2.37, empirical $p = 0.0014$ Dielmo), and the maximum parasite density during asymptomatic infection on chromosome 12q21 (LOD = 3.1, empirical $p < 10^{-4}$, Ndiop). While regions of linkage show little overlap with genes known to be involved in severe malaria, the four regions appear to overlap with regions linked to asthma or atopy related traits, suggesting that common immune related pathways may be involved.

77: *PLoS ONE*. 2008 Apr 23;3(4):e1982.

Default Pathway of var2csa switching and translational repression in Plasmodium falciparum.

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Antigenic variation is a subtle process of fundamental importance to the survival of a microbial pathogen. In *Plasmodium falciparum* malaria, PfEMP1 is the major variable antigen and adhesin expressed at the surface of the infected erythrocyte, which is encoded for by members of a family of 60 var-genes. Peri-nuclear repositioning and epigenetic mechanisms control their mono-allelic expression. The switching of PfEMP1 depends in part on variable transition rates and short-lived immune responses to shared minor epitopes. Here we show var-genes to switch to a common gene that is highly transcribed, but sparsely translated into PfEMP1 and not expressed at the erythrocyte surface. Highly clonal and adhesive *P. falciparum*, which expressed distinct var-genes and the corresponding PfEMP1s at onset, were propagated without enrichment or panning. The parasites successively and spontaneously switched to transcribe a shared var-gene (var2csa) matched by the loss of PfEMP1 surface expression and host cell-binding. The var2csa gene repositioned in the peri-nuclear area upon activation, away from the telomeric clusters and heterochromatin to transcribe spliced, full-length RNA. Despite abundant transcripts, the level of intracellular PfEMP1 was low suggesting post-transcriptional mechanisms to partake in protein expression. In vivo, off-switching and translational repression may constitute one pathway, among others, coordinating PfEMP1 expression.

78: *PLoS ONE*. 2008 Apr 16;3(4):e1968.

High genetic differentiation between the M and S molecular forms of Anopheles gambiae in Africa.

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BACKGROUND: *Anopheles gambiae*, a major vector of malaria, is widely distributed throughout sub-Saharan Africa. In an attempt to eliminate infective mosquitoes, researchers are trying to develop transgenic strains that are refractory to the *Plasmodium* parasite. Before any release of transgenic mosquitoes can be envisaged, we need an accurate picture of the differentiation between the two molecular forms of *An. gambiae*, termed M and S, which are of uncertain taxonomic status. **METHODOLOGY/PRINCIPAL FINDINGS:** Insertion patterns of three transposable elements (TEs) were determined in populations from Benin, Burkina Faso, Cameroon, Ghana, Ivory Coast, Madagascar, Mali, Mozambique, Niger, and Tanzania, using Transposon Display, a TE-anchored strategy based on Amplified Fragment Length Polymorphism. The results reveal a clear differentiation between the M and S forms, whatever their geographical origin, suggesting an incipient speciation process. **CONCLUSIONS/SIGNIFICANCE:** Any attempt to control the transmission of malaria by *An. gambiae* using either conventional or novel technologies must take the M/S genetic differentiation into account. In addition, we localized three TE insertion sites that were present either in every individual or at a high frequency in the M molecular form. These sites were found to be located outside the chromosomal regions that are suspected of involvement in the speciation event between the two forms. This suggests that these chromosomal regions are either larger than previously thought, or there are additional differentiated genomic

regions interspersed with undifferentiated regions.

79: *PLoS ONE*. 2008 Apr 9;3(4):e1952.

Safety and immunogenicity of a malaria vaccine, Plasmodium falciparum AMA-1/MSP-1 chimeric protein formulated in montanide ISA 720 in healthy adults.

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BACKGROUND: The *P. falciparum* chimeric protein 2.9 (PfCP-2.9) consisting of the sequences of MSP1-19 and AMA-1 (III) is a malaria vaccine candidate that was found to induce inhibitory antibodies in rabbits and monkeys. This was a phase I randomized, single-blind, placebo-controlled, dose-escalation study to evaluate the safety and immunogenicity of the PfCP-2.9 formulated with a novel adjuvant Montanide ISA720. Fifty-two subjects were randomly assigned to 4 dose groups of 10 participants, each receiving the test vaccine of 20, 50, 100, or 200 microg respectively, and 1 placebo group of 12 participants receiving the adjuvant only. **METHODS AND FINDINGS:** The vaccine formulation was shown to be safe and well-tolerated, and none of the participants withdrew. The total incidence of local adverse events (AEs) was 75%, distributed among 58% of the placebo group and 80% of those vaccinated. Among the vaccinated, 65% had events that were mild and 15% experienced moderate AEs. Almost all systemic adverse reactions observed in this study were graded as mild and required no therapy. The participants receiving the test vaccine developed detectable antibody responses which were boosted by the repeated vaccinations. Sixty percent of the vaccinated participants had high ELISA titers (>1:10,000) of antigen-specific antibodies which could also recognize native parasite proteins in an immunofluorescence assay (IFA). **CONCLUSION:** This study is the first clinical trial for this candidate and builds on previous investigations supporting PfCP-2.9/ISA720 as a promising blood-stage malaria vaccine. Results demonstrate safety, tolerability (particularly at the lower doses tested) and immunogenicity of the formulation. Further clinical development is ongoing to explore optimizing the dose and schedule of the formulation to decrease reactogenicity without compromising immunogenicity. **TRIAL REGISTRATION:** Chinese State Food and Drug Administration (SFDA) 2002SL0046; Controlled-Trials.com ISRCTN66850051 [66850051].

80: *PLoS Pathog*. 2008 Apr 25;4(4):e1000053.

HDP-a novel heme detoxification protein from the malaria parasite.

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When malaria parasites infect host red blood cells (RBC) and proteolyze hemoglobin, a unique, albeit poorly understood parasite-specific mechanism, detoxifies released heme into hemozoin (Hz). Here, we report the identification and characterization of a novel Plasmodium Heme Detoxification Protein (HDP) that is extremely potent in converting heme into Hz. HDP is functionally conserved across Plasmodium genus and its gene locus could not be disrupted. Once expressed, the parasite utilizes a circuitous "Outbound-Inbound" trafficking route by initially secreting HDP into the cytosol of infected RBC. A subsequent endocytosis of host cytosol (and hemoglobin) delivers HDP to the food vacuole (FV), the site of Hz formation. As Hz formation is critical for survival, involvement of HDP in this process suggests that it could be a malaria drug target.

81: *Proc Natl Acad Sci U S A.* 2008 Apr 23

Critical role of a K⁺ channel in *Plasmodium berghei* transmission revealed by targeted gene disruption.

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Regulated K⁽⁺⁾ transport across the plasma membrane is of vital importance for the survival of most cells. Two K⁽⁺⁾ channels have been identified in the *Plasmodium falciparum* genome; however, their functional significance during parasite life cycle in the vertebrate host and during transmission through the mosquito vector remains unknown. We hypothesize that these two K⁽⁺⁾ channels mediate the transport of K⁽⁺⁾ in the parasites, and thus are important for parasite survival. To test this hypothesis, we identified the orthologue of one of the *P. falciparum* K⁽⁺⁾ channels, PfKch1, in the rodent malaria parasite *P. berghei* (PbKch1) and examined the biological role by performing a targeted disruption of the gene encoding PbKch1. The deduced amino acid sequence of the six transmembrane domains of PfKch1 and PbKch1 share 82% identity, and in particular the pore regions are completely identical. The PbKch1-null parasites were viable despite a marked reduction in the uptake of the K⁽⁺⁾ congener (⁸⁶Rb⁽⁺⁾), and mice infected with PbKch1-null parasites survived slightly longer than mice infected with WT parasites. However, the most striking feature of the phenotype was the virtually complete inhibition of the development of PbKch1-null parasites in *Anopheles stephensi* mosquitoes. In conclusion, these studies demonstrate that PbKch1 contributes to the transport of K⁽⁺⁾ in *P. berghei* parasites and supports the growth of the parasites, in particular the development of oocysts in the mosquito midgut. K⁽⁺⁾ channels therefore may constitute a potential antimalarial drug target.

82: *Proc Natl Acad Sci U S A.* 2008 Apr 21

The molecular and cellular basis of olfactory-driven behavior in *Anopheles gambiae* larvae.

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The mosquito *Anopheles gambiae* is the principal Afrotropical vector for human malaria. A central component of its vectorial capacity is the ability to maintain sufficient populations of adults. During both adult and preadult (larval) stages, the mosquitoes depend on the ability to recognize and respond to chemical cues that mediate feeding and survival. In this study, we used a behavioral assay to identify a range of odorant-specific responses of *An. gambiae* larvae that are dependent on the integrity of the larval antennae. Parallel molecular analyses have identified a subset of the *An. gambiae* odorant receptors (AgOrs) that are localized to discrete neurons within the larval antennae and facilitate odor-evoked responses in *Xenopus* oocytes that are consistent with the larval behavioral spectrum. These studies shed light on chemosensory-driven behaviors and represent molecular and cellular characterization of olfactory processes in mosquito larvae. These advances may ultimately enhance the development of vector control strategies, targeting olfactory pathways in larval-stage mosquitoes to reduce the catastrophic effects of malaria and other diseases.

83: *Science*. 2008 Apr 18;320(5874):330-4.

Qinghaosu (artemisinin): the price of success.

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Artemisinin and its derivatives have become essential components of antimalarial treatment. These plant-derived peroxides are unique among antimalarial drugs in killing the young intraerythrocytic malaria parasites, thereby preventing their development to more pathological mature stages. This results in rapid clinical and parasitological responses to treatment and life-saving benefit in severe malaria. Artemisinin combination treatments (ACTs) are now first-line drugs for uncomplicated falciparum malaria, but access to ACTs is still limited in most malaria-endemic countries. Improved agricultural practices, selection of high-yielding hybrids, microbial production, and the development of synthetic peroxides will lower prices. A global subsidy would make these drugs more affordable and available. ACTs are central to current malaria elimination initiatives, but there are concerns that tolerance to artemisinins may be emerging in Cambodia.

84: *Stat Methods Med Res*. 2008 Apr;17(2):191-206.

Efficacy studies of malaria treatments in Africa: efficient estimation with missing indicators of failure.

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The effect of missing data in causal inference problems is widely recognized. In malaria drug efficacy studies, it is often difficult to distinguish between new and old infections after treatment, resulting in indeterminate outcomes. Methods that adjust for possible bias from missing data include a variety of imputation procedures (extreme case analysis, hot-deck, single and multiple imputation), weighting methods, and likelihood based methods (data augmentation, EM procedures and their extensions). In this article, we focus our discussion on multiple imputation and two weighting procedures (the inverse probability weighted and the doubly robust (DR) extension), comparing the methods' applicability to the efficient estimation of malaria treatment effects. Simulation studies indicate that DR estimators are generally preferable because they offer protection to misspecification of either the outcome model or the missingness model. We apply the methods to analyze malaria efficacy studies from Uganda.

85: *Trans R Soc Trop Med Hyg*. 2008 Apr 9

Characterisation of DDT, pyrethroid and carbamate resistance in *Anopheles funestus* from Obuasi, Ghana.

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Indoor-resting anopheline mosquitoes were collected from Obuasi, Ghana, and were identified morphologically and by PCR as *Anopheles funestus* Giles. Wild-caught females were induced to lay eggs. Samples of F1 progeny from each family were divided into cohorts and were either exposed to DDT and permethrin or were stored

for biochemical analysis. Bioassay data by family show evidence of DDT and pyrethroid resistance in the parent *A. funestus* population. The sodium channel gene of DDT survivors and DDT-susceptible individuals was PCR amplified and sequenced to determine whether any *kdr*-type mutations were present. Molecular analysis of the IIS5-IIS6 segment of the sodium channel gene gave no indication of any *kdr*-type mutations associated with resistance phenotypes. Biochemical analysis suggests that DDT and pyrethroid resistance may be metabolically mediated, although there were no clear correlations between enzyme levels/activities and insecticide resistance across families. Furthermore, an altered acetylcholinesterase conferring carbamate resistance was evident. These results can be used to plan an effective malaria control strategy in the Obuasi region.

86: *Trans R Soc Trop Med Hyg.* 2008 Apr 9

Anaemia and malaria in Yanomami communities with differing access to healthcare.

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Inequitable access to healthcare has a profound impact on the health of marginalised groups that typically suffer an excess burden of infectious disease morbidity and mortality. The Yanomami are traditionally semi-nomadic people living in widely dispersed communities in Amazonian Venezuela and Brazil. Only communities living in the vicinity of a health post have relatively constant access to healthcare. To monitor the improvement in the development of Yanomami healthcare a cross-sectional survey of 183 individuals was conducted to investigate malaria and anaemia prevalence in communities with constant and intermittent access to healthcare. Demographic and clinical data were collected. Malaria was diagnosed by microscopy and haemoglobin concentration by HemoCue. Prevalence of malaria, anaemia, splenomegaly, fever and diarrhoea were all significantly higher in communities with intermittent access to healthcare (anaemia 80.8% vs. 53.6%, $P < 0.001$; malaria 18.2% vs. 6.0%, $P = 0.013$; splenomegaly 85.4% vs. 12.5%, $P < 0.001$; fever 50.5% vs. 28.6%, $P = 0.003$; diarrhoea 30.3% vs. 10.7% $P = 0.001$). Haemoglobin level (10.0g/dl vs. 11.5g/dl) was significantly associated with access to healthcare when controlling for age, sex, malaria and splenomegaly ($P = 0.01$). These findings indicate a heavy burden of anaemia in both areas and the need for interventions against anaemia and malaria, along with more frequent medical visits to remote areas.

87: *Trans R Soc Trop Med Hyg.* 2008 Apr 5

Combating the "other diseases" of MDG 6: changing the paradigm to achieve equity and poverty reduction?

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This paper suggests that the 'other diseases' of Millennium Development Goal 6 (MDG 6) are ignored by policy-makers and politicians who overfocus on unachievable objectives and targets around the 'big three' diseases of HIV, tuberculosis (TB) and malaria, which if the planet was viewed by aliens would be seen as the only diseases that existed on the planet. The diseases of the majority of the poor represent 'low hanging fruit' for control and elimination and opportunities are ignored despite the availability of cheap or donated drugs and ample evidence that such interventions are effective and reduce incidence, as

well as mortality and morbidity. The time frame available to achieve the MDGs of some 7-8 years requires a re-evaluation of what can be done with the tools available now and which can address the problems faced by the majority of poor people afflicted by disabling conditions which together represent a global burden greater than malaria or TB. The author considers also the volume of research relevant to the MDGs and their achievement is distorted by the focus on high tech end research which cannot be delivered by 2015 and that in terms of the 90:10 gap in research relevant to the problems of the poorest the real gap is 99:1. The concepts of distortion of donor funding for diseases of MDG 6 for implementation of largely curative interventions which do not reduce incidence as well as research which addresses problems that cannot reach poor people in the time frame to 2015 is emphasised. New paradigms are required if any impact on MDG 6 is to be achieved recognising the needs of the majority via an equitable distribution of funding.

88: Trends Parasitol. 2008 Apr 16

Ecological immunology of mosquito-malaria interactions.

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More than a century after the discovery of the complex life cycle of its causative agent, malaria remains a major health problem. Understanding mosquito-malaria interactions could lead to breakthroughs in malaria control. Novel strategies, such as the design of transgenic mosquitoes refractory to Plasmodium, or design of human vaccines emulating mosquito resistance to the parasite, require extensive knowledge of processes involved in immune responses and of microevolutionary mechanisms that create and maintain variation in immune responses in wild vector populations. The recent realization of how intimately and specifically mosquitoes and Plasmodium co-evolve in Nature is driving vector molecular biologists and evolutionary ecologists to move closer to the natural setting under the common umbrella of 'Ecological immunology'.

89: Trends Parasitol. 2008 Apr 9

Parental guidance? Trans-generational influences on offspring life history in mosquitoes.

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Parental effects are important factors that might influence evolutionary and ecological aspects of parasite vectors and the parasites they transmit. A recent study demonstrated the importance of parental rearing conditions on the malaria vector *Anopheles stephensi*. When parents are reared in a food-limited environment their offspring have increased bloodmeal sizes and larger clutches. The study highlights that ecological studies are vital for understanding vectors of disease and ultimately for developing effective control strategies.

90: Trends Parasitol. 2008 Apr 9

New insight into the role of dendritic cells in malaria immune pathogenesis.

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The mechanism by which the host develops protective immunity to malaria remains poorly understood. Dendritic cells (DCs) are central to the initiation and regulation of the adaptive immune response. Modulation of DC function might enable Plasmodium to evade the immune system. Millington et al. propose one mechanism by which malaria inhibits DC-T-cell interactions without interfering directly with T-cell receptor engagement. The consequence is a decrease in the co-stimulation required to develop an effective immune response.

91: *Trends Parasitol.* 2008 Apr 8

Plasmodium vivax in India.

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Four Plasmodium species cause malaria in humans: Plasmodium vivax is the most widespread and results in pronounced morbidity. India (population >1 billion) is a major contributor to the burden of vivax malaria. With a resurgence in interest concerning the neglected burden of vivax malaria and the completion of the P. vivax genome, it is timely to review what is known concerning P. vivax in India. The P. vivax population is highly diverse in terms of relapse patterns, drug response and clinical profiles, and highly genetically variable according to studies of antigen genes, isoenzyme markers and microsatellites. The unique epidemiology of malaria in India, where P. vivax predominates over Plasmodium falciparum, renders this location ideal for studying the dynamics of co-infection.

92: *Trends Parasitol.* 2008 Apr;24(4):159-63.

Simplified antimalarial therapeutic monitoring: using the day-7 drug level?

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The blood concentration profiles of most antimalarial drugs vary considerably between patients. The interpretation of antimalarial drug trials evaluating efficacy and effectiveness would be improved considerably if the exposure of the infecting parasite population to the antimalarial drug treatment could be measured. Artemisinin combination treatments are now recommended as first-line drugs for the treatment of falciparum malaria. Measurement of the blood, serum or plasma concentration of the slowly eliminated partner antimalarial drug on day 7 of follow-up is simpler and might be a better determinant of therapeutic response than the area under the concentration-time curve. Measurement of the day-7 drug level should be considered as a routine part of antimalarial drug trials.

93: *Trends Parasitol.* 2008 Apr;24(4):168-75.

Sex in Plasmodium: a sign of commitment.

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The gametocyte, or sexual blood-stage, of the malaria parasite represents the

only stage of the parasite that can be transmitted to the mosquito vector following sexual development within the infected bloodmeal. Little is known about the processes leading to this cellular differentiation and specialization. The recent completion of the Plasmodium genome, and subsequent transcriptome and proteome analyses have revealed for the first time a molecular map of the genes that are differentially regulated at the onset of and during gametocytogenesis. In this review, we outline the underlying mechanisms involved in this process, focusing on the transition between the asexual and the sexual blood-stages of the parasite.

94: *Trop Med Int Health*. 2008 May;13(5):626-34.

Very young children with uncomplicated falciparum malaria have higher risk of hypoglycaemia: a study from Suriname.

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OBJECTIVE: To measure glucose kinetics and the influence of age, nutritional status and fasting duration in children with uncomplicated falciparum malaria (UFM) under the age of 5 years. **METHODS:** Plasma glucose concentration, endogenous glucose production (EGP) and gluconeogenesis (GNG) were measured using [6,6-(2)H(2)]glucose and (2)H(2)O in 17 very young (<3 years) and 7 older (3-5 years) Surinamese children with UFM admitted to the Distrikt Hospital Stoelmanseiland and Diakonessen Hospital Paramaribo over 17 months. **RESULTS:** Plasma glucose concentration was lower in the group of very young children than in the older children (P = 0.028). There were no differences in EGP and GNG between the groups. Overall GNG contributed 56% (median, range 17-87%) to EGP, with no differences between the groups (P = 0.240). Glucose clearance was lower in the older children (P = 0.026). Glucose concentration did not differ between children with weight for length/height less than -1.3 SD and children with weight for length/height greater than -1.3 SD (P = 0.266). Plasma glucose concentration was not predicted by fasting duration (P = 0.762). **CONCLUSIONS:** Our data suggest a higher risk of hypoglycaemia in very young children with uncomplicated malaria as plasma glucose concentration was lower in this study group. Since this could not be attributed to an impaired EGP, and because glucose clearance was lower in the older children, we presume that older children were better capable of reducing glucose utilization during fasting. Studies on glucose kinetics are feasible in very young children with malaria and give more insight in the pathophysiology of hypoglycaemia.

95: *Trop Med Int Health*. 2008 Apr 10

Who develops severe malaria? Impact of access to healthcare, socio-economic and environmental factors on children in Yemen: a case-control study.

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Objective To investigate the impact of socio-economic and environmental factors on developing severe malaria in comparison with mild malaria in Yemen. **Method** Case-control study comparing 343 children aged 6 months to 10 years diagnosed with WHO-defined severe malaria (cases) at the main children's hospital in Taiz and 445 children with mild malaria (controls) diagnosed in the health centres, which serve the areas where the cases came from. **Results** In univariate analysis, age <1 year, distance from health centre, delay to treatment and driving time to health centre were associated with progression from mild to severe malaria. In

multivariate analysis, distance to nearest health centre >2 km was significantly associated with progression to severe disease. Environmental and vector control factors associated with protection from acquiring malaria (such as sleeping under bednets) were not associated with protection from moving from mild to severe disease. Conclusions Innovative ways to improve access to antimalarial treatment for those living more than 2 km away from health centres such as home management of malaria, especially for infants and young children, should be explored in malaria-endemic areas of Yemen.

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Dynamics of insecticide resistance in the malaria vector *Anopheles gambiae* s.l. from an area of extensive cotton cultivation in Northern Cameroon.

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OBJECTIVE: To explore temporal variation in insecticide susceptibility of *Anopheles gambiae* s.l. populations to the four chemical groups of insecticides used in public health and agriculture, in close match with the large-scale cotton spraying programme implemented in the cotton-growing area of North Cameroon. **METHODS:** Mosquito larvae were collected in 2005 before (mid June), during (mid August) and at the end (early October) of the cotton spraying programme. Larvae were sampled in breeding sites located within the cotton fields in Gaschiga and Pitoa, and in Garoua, an urban cotton-free area that served as a control. Insecticide susceptibility tests were carried out with 4% DDT (organochlorine), 0.4% chlorpyrifos methyl (organophosphate), 0.1% propoxur (carbamate), 0.05% deltamethrin and 0.75% permethrin (pyrethroids). **RESULTS:** Throughout the survey, *An. gambiae* s.l. populations were completely susceptible to carbamate and organophosphate, whereas a significant decrease of susceptibility to organochlorine and pyrethroids was observed during spraying in cotton-growing areas. Tolerance to these insecticides was associated with a slight increase of knockdown times compared to the reference strain. Among survivor mosquitoes, the East and West African Kdr mutations were detected only in two specimens of *An. gambiae* s.s. (n = 45) and not in *Anopheles arabiensis* (n = 150), suggesting metabolic-based resistance mechanisms. **CONCLUSIONS:** Environmental disturbance due to the use of insecticides in agriculture may provide local mosquito populations with the enzymatic arsenal selecting tolerance to insecticides.

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Phase 2a trial of 0, 1, and 3 month and 0, 7, and 28 day immunization schedules of malaria vaccine RTS,S/AS02 in malaria-naïve adults at the Walter Reed Army Institute of Research.

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BACKGROUND: Immunization with RTS,S/AS02 consistently protects some vaccinees against malaria infection in experimental challenges and in field trials. A brief immunization schedule against falciparum malaria would be compatible with the Expanded Programme on Immunization, or in combination with other prevention measures, interrupt epidemic malaria or protect individuals upon sudden travel to an endemic area. **METHODS:** We conducted an open label, Phase 2a trial of two

different full dose schedules of RTS,S/AS02 in 40 healthy malaria-naïve adults. Cohort 1 (n=20) was immunized on a 0, 1, and 3 month schedule and Cohort 2 (n=20) on a 0, 7, and 28 day schedule. Three weeks later, 38 vaccinees and 12 unimmunized infectivity controls underwent malaria challenge. RESULTS: Both regimens had a good safety and tolerability profile. Peak GMCs of antibody to the circumsporozoite protein (CSP) were similar in Cohort 1 (78mug/mL; 95% CI: 45-134) and Cohort 2 (65mug/mL; 95% CI: 40-104). Vaccine efficacy for Cohort 1 was 45% (95% CI: 18-62%) and for Cohort 2, 39% (95% CI: 11-56%). Protected volunteers had a higher GMC of anti-CSP antibody (114mug/mL) than did volunteers with a 2-day delay (70mug/mL) or no delay (30mug/mL) in the time to onset of parasitemia (Kruskal-Wallis, p=0.019). A trend was seen for higher CSP-specific IFN-gamma responses in PBMC from protected volunteers only in Cohort 1, but not in Cohort 2, for ex vivo and for cultured ELISPOT assays. CONCLUSION: In malaria-naïve adults, the efficacy of three-dose RTS,S/AS02 regimens on either a 0, 1, and 3 month schedule or an abbreviated 0, 7, and 28 day schedule was not discernibly different from two previously reported trials of two-dose regimens given at 0, 1 month that conferred 47% (95% CI: -19 to 76%) protection and in another trial 42% (95% CI: 5-63%). A strong association of CSP-specific antibody with protection against malaria challenge is observed and confirms similar observations made in other studies. Subsequent trials of adjuvanted RTS,S in African children and infants on a 0, 1, and 2 month schedule have demonstrated a favorable safety and efficacy profile.

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Longitudinal analyses of immune responses to Plasmodium falciparum derived peptides corresponding to novel blood stage antigens in coastal Kenya.

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We have recently described 95 predicted alpha-helical coiled-coil peptides derived from putative Plasmodium falciparum erythrocytic stage proteins. Seventy peptides recognized with the highest level of prevalence by sera from three endemic areas were selected for further studies. In this study, we sequentially examined antibody responses to these synthetic peptides in two cohorts of children at risk of clinical malaria in Kilifi district in coastal Kenya, in order to characterize the level of peptide recognition by age, and the role of anti-peptide antibodies in protection from clinical malaria. Antibody levels from 268 children in the first cohort (Chonyi) were assayed against 70 peptides. Thirty-nine peptides were selected for further study in a second cohort (Junju). The rationale for the second cohort was to confirm those peptides identified as protective in the first cohort. The Junju cohort comprised of children aged 1-6 years old (inclusive). Children were actively followed up to identify episodes of febrile malaria in both cohorts. Of the 70 peptides examined, 32 showed significantly (p<0.05) increased antibody recognition in older children and 40 showed significantly increased antibody recognition in parasitaemic children. Ten peptides were associated with a significantly reduced odds ratio (OR) for an episode of clinical malaria in the first cohort of children and two of these peptides (LR146 and AS202.11) were associated with a significantly reduced OR in both cohorts. LR146 is derived from hypothetical protein PFB0145c in PlasmoDB. Previous work has identified this protein as a target of antibodies effective in antibody dependent cellular inhibition (ADCI). The current study substantiates further the potential of protein PFB0145c and also identifies protein PF11_0424 as another likely target of protective antibodies against P. falciparum malaria.