

Journal/Title Index

- 1: *Acta Trop.* [Abandoning small-scale fish farming in western Kenya leads to higher malaria vector abundance.](#)
- 2: *Acta Trop.* [Seasonal patterns of Plasmodium falciparum gametocyte prevalence and density in a rural population of Burkina Faso.](#)
- 3: *Acta Trop.* [Marked differences in the prevalence of chloroquine resistance between urban and rural communities in Burkina Faso.](#)
- 4: *Am J Obstet Gynecol.* [Maternal infection and risk of preeclampsia: Systematic review and metaanalysis.](#)
- 5: *Antimicrob Agents Chemother.* [Pharmacokinetics and efficacy of piperazine and chloroquine in Melanesian children with uncomplicated malaria.](#)
- 6: *BMC Genomics.* [Gene expression analysis reveals early changes in several molecular pathways in cerebral malaria-susceptible mice versus cerebral malaria-resistant mice.](#)
- 7: *BMC Genomics.* [Continuous exposure to Plasmodium results in decreased susceptibility and transcriptomic divergence of the Anopheles gambiae immune system.](#)
- 8: *Genome Res.* [Genome-wide discovery and verification of novel structured RNAs in Plasmodium falciparum.](#)
- 9: *Haematologica.* [Assessment of malaria in pregnancy using rapid diagnostic tests and its association with HIV infection and hematologic parameters in South-Eastern Nigeria.](#)
- 10: *Hum Mol Genet.* [Haemoglobin S and haemoglobin C: 'quick but costly' versus 'slow but gratis' genetic adaptations to Plasmodium falciparum malaria.](#)
- 11: *Infect Immun.* [Preclinical evaluation of the safety and immunogenicity of a vaccine consisting of Plasmodium falciparum liver-stage antigen 1 with adjuvant AS01B...](#)
- 12: *Infect Immun.* [Humoral responses to P. falciparum blood stage antigens \(MSP3, MSP1-19, GLURP and AMA1\) and their association with the incidence of clinical malaria...](#)
- 13: *Int J Infect Dis.* [Intermittent preventive treatment of malaria in pregnancy: a community-based delivery system and its effect on parasitemia, anemia and low birth weight in Uganda.](#)
- 14: *Int J Parasitol.* [Ethnopharmacology and malaria: New hypothetical leads or old efficient antimalarials?](#)
- 15: *J Acquir Immune Defic Syndr.* [HIV-1 Infection in Patients Referred for Malaria Blood Smears at Government Health Clinics in Uganda.](#)
- 16: *J Biol Chem.* [Nuclear non-coding RNAs are transcribed from the centromeres of Plasmodium falciparum and are associated with centromeric chromatin.](#)
- 17: *J Biol Chem.* [Role of Ca²⁺/CaM-PfPKB signaling pathway in erythrocyte invasion by plasmodium falciparum.](#)
- 18: *J Biosoc Sci.* [Malaria-related health-seeking behaviour and challenges for care providers in rural ethiopia: implications for control.](#)
- 19: *J Cell Mol Med.* [Strategies for developing multi-epitope, subunit-based, chemically-synthesized antimalarial vaccines.](#)
- 20: *J Chromatogr B Analyt Technol Biomed Life Sci.* [High-performance liquid chromatographic assay for the determination of sulfadoxine and N-acetyl sulfadoxine...](#)
- 21: *J Clin Invest.* [Variation in use of erythrocyte invasion pathways by Plasmodium falciparum mediates evasion of human inhibitory antibodies.](#)
- 22: *J Clin Microbiol.* [Rapid Diagnosis of Vivax Malaria by SD BIOLINE Malaria Antigen test and Thrombocytopenia.](#)
- 23: *J Ethnopharmacol.* [Evaluation of Senegalese plants used in malaria treatment: Focus on Chrozophora senegalensis.](#)
- 24: *Malar J.* [Adding artesunate to sulphadoxine-pyrimethamine greatly improves the treatment efficacy in children with uncomplicated falciparum malaria...](#)
- 25: *Malar J.* [Estimated financial and human resources requirements for the treatment of malaria in Malawi.](#)
- 26: *Malar J.* [Duffy blood group gene polymorphisms among malaria vivax patients in four areas of the Brazilian Amazon region.](#)
- 27: *Malar J.* [Haemoglobin and haematocrit: the threefold conversion is also non valid for assessing anaemia in Plasmodium vivax malaria-endemic settings.](#)
- 28: *Malar J.* [Complement activation in Ghanaian children with severe Plasmodium falciparum malaria.](#)
- 29: *Malar J.* [Sequence analysis of Plasmodium falciparum cytochrome b in multiple geographic sites.](#)
- 30: *Malar J.* [Malaria incidence and efficacy of intermittent preventive treatment in infants \(IPTi\).](#)
- 31: *Malar J.* [Climate prediction of El Nino malaria epidemics in north-west Tanzania.](#)
- 32: *Malar J.* [How is childhood development of immunity to Plasmodium falciparum enhanced by certain antimalarial interventions?](#)
- 33: *Malar J.* [Intermittent preventive treatment for the prevention of malaria during pregnancy in high transmission areas](#)
- 34: *Med Mal Infect.* [Assessing the application of Rwanda's national protocol for uncomplicated malaria treatment in healthcare institutions in Kigali city, Rwanda.](#)

35: *Mol Med*. [Reduced immune complex binding capacity and increased complement susceptibility of red cells from children with severe malaria-associated anemia.](#)

36: *Mol Microbiol*. [Mapping a common interaction site used by Plasmodium falciparum Duffy binding-like domains to bind diverse host receptors.](#)

37: *Mol Microbiol*. [An atypical orthologue of 6-pyruvoyltetrahydropterin synthase can provide the missing link in the folate biosynthesis pathway of malaria parasites.](#)

38: *Mol Microbiol*. [The role of osmiophilic bodies and Pfg377 expression in female gametocyte emergence and mosquito infectivity in the human malaria parasite Plasmodium...](#)

39: *Nature*. [Distinct physiological states of Plasmodium falciparum in malaria-infected patients.](#)

40: *Parasitol Res*. [Are coinfections of malaria and filariasis of any epidemiological significance?](#)

41: *Parasitol Res*. [Blood coagulation in falciparum malaria-a review.](#)

42: *PLoS Comput Biol*. [Determination of the Processes Driving the Acquisition of Immunity to Malaria Using a Mathematical Transmission Model.](#)

43: *PLoS Genet*. [Localization of Candidate Regions Maintaining a Common Polymorphic Inversion \(2La\) in Anopheles gambiae.](#)

44: *PLoS ONE*. [Sterile Protection against Malaria Is Independent of Immune Responses to the Circumsporozoite Protein.](#)

45: *PLoS ONE*. [A Randomized Open-Label Trial of Artesunate- Sulfadoxine-Pyrimethamine with or without Primaquine for Elimination of Sub-Microscopic P. falciparum](#)

46: *PLoS ONE*. [A virosomal malaria Peptide vaccine elicits a long-lasting sporozoite-inhibitory antibody response in a phase 1a clinical trial.](#)

47: *PLoS Pathog*. [Progression of Plasmodium berghei through Anopheles stephensi Is Density-Dependent.](#)

48: *PLoS Pathog*. [Hemolytic C-Type Lectin CEL-III from Sea Cucumber Expressed in Transgenic Mosquitoes Impairs Malaria Parasite Development.](#)

49: *Prog Drug Res*. [Drug discovery and development with plant-derived compounds.](#)

50: *Prog Lipid Res*. [New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents.](#)

51: *Scand J Immunol*. [Naturally Acquired Immunity and Reduced Susceptibility to falciparum Malaria in Two Subpopulations of Endemic Eastern India.](#)

52: *Trans R Soc Trop Med Hyg*. [Assessment of three new parasite lactate dehydrogenase \(pan-pLDH\) tests for diagnosis of uncomplicated malaria.](#)

53: *Trans R Soc Trop Med Hyg*. [Malaria diagnosis under field conditions in the Venezuelan Amazon.](#)

54: *Trans R Soc Trop Med Hyg*. [Malaria drug and vaccine trials in Africa: obstacles and opportunities.](#)

55: *Trans R Soc Trop Med Hyg*. [DEET microencapsulation: a slow-release formulation enhancing the residual efficacy of bed nets against malaria vectors.](#)

56: *Trans R Soc Trop Med Hyg*. [Antimalarial resistance and DHFR/DHPS genotypes of Plasmodium falciparum three years after introduction of sulfadoxine-pyrimethamine and amodiaquine in rural Tanzania.](#)

57: *Vaccine*. [Expression and purification of a Plasmodium vivax antigen - PvTARAg55 tryptophan- and alanine-rich antigen and its immunological responses in human subjects.](#)

58: *Vaccine*. [The requirement of CD80, CD86, and ICAM-1 on the ability of adjuvant formulations to potentiate antibody responses to a Plasmodium falciparum blood-stage vaccine.](#)

Abstracts

1: *Acta Trop.* 2008 Jan;105(1):67-73.

Abandoning small-scale fish farming in western Kenya leads to higher malaria vector abundance.

Howard AF, Omlin FX. Human Health Department, International Centre of Insect Physiology and Ecology (ICIPE) P.O. Box 30772, 00100 Nairobi, Kenya.

Fishponds become abandoned due to lack of access to both young fish and technical support and faster economic returns from other activities. Certain conditions found in abandoned fishponds, such as absence of fish and presence of aquatic vegetation, are conducive to the presence of malaria vectors. We conducted a district-wide fishpond census to determine the maintenance status and mosquito populations of fishponds in Kisii Central District in western Kenya. Two hundred and sixty one fishponds were found, 186 active (fish present) and 75 abandoned (fish absent). Vegetation was not significantly associated with the distribution of *Anopheles gambiae* s.l., *Anopheles funestus* or culicines (Diptera: Culicidae) in active or abandoned ponds. The presence of fish, however, correlated significantly with the distribution of all mosquito species, with significantly higher mosquito densities in abandoned fishponds. *An. gambiae* s.l. was the most abundant mosquito species found in both active and abandoned ponds, being proportionally more abundant in the abandoned ponds. The proportion of *An. funestus* increased with altitude. Following the census the demand for fish to re-stock abandoned ponds rose by 67% when compared to the same time period in the previous year. This study highlights the potential public health problems associated with the abandonment of small-scale fish farming in the highlands of western Kenya.

2: *Acta Trop.* 2008 Jan;105(1):28-34.

Seasonal patterns of *Plasmodium falciparum* gametocyte prevalence and density in a rural population of Burkina Faso.

Ouédraogo AL, de Vlas SJ, Nébié I, Ilboudo-Sanogo E, Bousema JT, Ouattara AS, Verhave JP, Cuzin-Ouattara N, Sauerwein RW.

Centre National de Recherche et de Formation sur le Paludisme, 01BP 2208, Ouagadougou 01, Burkina Faso; Department of Medical Microbiology, Radboud University Medical Centre, Nijmegen, The Netherlands; Departement de Biochimie Microbiologie, Université de Ouagadougou, 03BP 7131, Ouagadougou 03, Burkina Faso.

Gametocytes are the malaria parasite stages that secure the transmission from the human host to the mosquito. The identification of natural parameters that influence gametocyte carriage can contribute to a better understanding of the dynamics of the sexual stage parasites for transmission reducing strategies. A total of 3400 blood slide readings were done during four cross-sectional surveys (2002-2003) including all age groups to determine the effect of season on *Plasmodium falciparum* gametocytes in a seasonal malaria transmission area of Burkina Faso. Entomological data were collected to determine the malaria transmission intensity in relation to seasons. Transmission intensity was estimated by monthly EIRs, averaging 28 and 32 infective bites/person/month in the wet seasons of 2002 and 2003, respectively. The EIR in the dry seasons was below one infective bite/person/month. The gametocyte prevalence was significantly higher at the start and peak of the wet season compared to the dry season when corrected for asexual parasite density and age. Gametocyte density significantly increased during the wet season after correction for asexual parasite density and age. In this study, season appears to be an independent

parameter that determines gametocyte prevalence and density and should be considered to be included in epidemiological studies on malaria transmission.

3: *Acta Trop.* 2008 Jan;105(1):81-6.

Marked differences in the prevalence of chloroquine resistance between urban and rural communities in Burkina Faso.

Meissner PE, Mandi G, Mockenhaupt FP, Witte S, Coulibaly B, Mansmann U, Frey C, Merkle H, Burhenne J, Walter-Sack I, Müller O.

Department of Tropical Hygiene and Public Health, Ruprecht-Karls-University, Heidelberg, Germany; Department of Paediatrics IV Neonatology, Ruprecht-Karls-University, Heidelberg, Germany.

BACKGROUND: Chloroquine (CQ) resistance has reached high levels in Africa in recent years. Little is known about variations of resistance between urban and rural areas. **OBJECTIVES:** To compare the rates of in vivo resistance to CQ and the prevalences of the main molecular marker for CQ resistance among young children from urban and rural areas in Burkina Faso. **METHODS:** The current analysis used the frame of a randomized controlled trial (ISRCTN27290841) on the combination CQ-methylene blue (MB) (n=177) compared to CQ alone (n=45) in young children with uncomplicated malaria. We examined clinical and parasitological failure rates as well as the prevalence of the Plasmodium falciparum chloroquine resistance transporter gene (pfcr1) T76 mutation. **RESULTS:** Clinical and parasitological failure rates of CQ-MB differed significantly between urban (70%) and rural areas (29%, p<0.0001). Likewise, CQ failure rates were higher in the urban setting. Matching this pattern, pfcr1 T76 was more frequently seen among parasite strains from urban areas (81%) when compared to rural ones (64%, p=0.01). In the presence of parasites exhibiting pfcr1 T76, the odds of overall clinical failure were increased to 2.6-fold ([1.33, 5.16], p(LR)=0.005). CQ was detected at baseline in 21% and 2% of children from the urban and the rural study area, respectively (p(Chi)=0.002). **CONCLUSION:** Even within circumscribed geographical areas, CQ efficacy can vary dramatically. The differences in the prevalence of pfcr1 T76 and in CQ failure rates are probably explained by a higher drug pressure in the urban area compared to the rural study area. This finding has important implications for national malaria policies.

4: *Am J Obstet Gynecol.* 2008 Jan;198(1):7-22.

Maternal infection and risk of preeclampsia: Systematic review and metaanalysis.

Conde-Agudelo A, Villar J, Lindheimer M.

Perinatology Research Branch, Intramural Division, NICHD/NIH/DHHS, Baltimore, MD, and Detroit, MI.

There are lingering questions regarding the association between maternal infection and preeclampsia. Systematic review and metaanalysis was conducted of observational studies that examined the relationship between maternal infection and preeclampsia. Forty-nine studies met the inclusion criteria. The risk of preeclampsia was increased in pregnant women with urinary tract infection (pooled odds ratio, 1.57; 95% CI, 1.45-1.70) and periodontal disease (pooled odds ratio, 1.76; 95% CI, 1.43-2.18). There were no associations between preeclampsia and presence of antibodies to Chlamydia pneumoniae, Helicobacter pylori, and cytomegalovirus, treated and nontreated HIV infection, and malaria. Individual studies did not find a relationship between herpes simplex virus type 2, bacterial vaginosis, and Mycoplasma hominis and preeclampsia. Urinary tract infection and periodontal disease during pregnancy are associated with an increased risk of preeclampsia. More studies are required to verify this as well as to explore whether or not such relationships are causal and, if so, the

mechanisms involved.

5: *Antimicrob Agents Chemother.* 2008 Jan;52(1):237-43.

Pharmacokinetics and efficacy of piperavaquine and chloroquine in melanesian children with uncomplicated malaria.

Karunajeewa HA, Ilett KF, Mueller I, Siba P, Law I, Page-Sharp M, Lin E, Lammeey J, Batty KT, Davis TM.

Department of Medicine, Fremantle Hospital, P.O. Box 480, Fremantle 6959, Western Australia, Australia. tdavis@cyllene.uwa.edu.au

The disposition of chloroquine (CQ) and the related 4-aminoquinoline, piperavaquine (PQ), were compared in Papua New Guinean children with uncomplicated malaria. Twenty-two children were randomized to 3 days of PQ phosphate at 20 mg/kg/day (12 mg of PQ base/kg/day) coformulated with dihydroartemisinin (DHA-PQ), and twenty children were randomized to 3 days of CQ at 10 mg base/kg/day with a single dose of sulfadoxine-pyrimethamine (CQ-SP). After a 42-day intensive sampling protocol, PQ, CQ, and its active metabolite monodesethyl-chloroquine (DECQ) were assayed in plasma by using high-performance liquid chromatography. A two-compartment model with first-order absorption was fitted to the PQ and CQ data. There were no significant differences in age, gender, body weight, or admission parasitemia between the two groups. The PCR-corrected 42-day adequate clinical and parasitological responses were 100% for DHA-PQ and 94% for CQ-SP, but *P. falciparum* reinfections during follow-up were common (33 and 18%, respectively). For PQ, the median volume of distribution at steady state, allowing for bioavailability ($V(ss)/F$), was 431 liters/kg (interquartile range [IQR], 283 to 588 liters/kg), the median clearance (CL/F) was 0.85 liters/h/kg (IQR, 0.67 to 1.06 liters/h/kg), the median distribution half-life ($t(1/2)(\alpha)$) was 0.12 h (IQR, 0.05 to 0.66 h), and the median elimination half-life ($t(1/2)(\beta)$) was 413 h (IQR, 318 to 516 h). For CQ, the median $V(ss)/F$ was 154 liters/kg (IQR, 101 to 210 liters/kg), the median CL/F was 0.80 liters/h/kg (IQR, 0.52 to 0.96 liters/h/kg), the median $t(1/2)(\alpha)$ was 0.43 h (IQR, 0.05 to 1.82 h), and the median $t(1/2)(\beta)$ was 233 h (IQR, 206 to 298 h). The noncompartmentally derived median DECQ $t(1/2)(\beta)$ was 290 h (IQR, 236 to 368 h). Combined molar concentrations of DECQ and CQ were higher than those of PQ during the elimination phase. Although PQ has a longer $t(1/2)(\beta)$ than CQ, its prompt distribution and lack of active metabolite may limit its posttreatment malaria-suppressive properties.

6: *BMC Genomics.* 2007 Dec 6;8(1):452

Gene expression analysis reveals early changes in several molecular pathways in cerebral malaria-susceptible mice versus cerebral malaria-resistant mice.

Delahaye NF, Coltel N, Puthier D, Barbier M, Benech P, Joly F, Iraqi FA, Grau GE, Nguyen C, Rihet P.

ABSTRACT: BACKGROUND: : Microarray analyses allow the identification and assessment of molecular signatures in whole tissues undergoing pathological processes. To better understand cerebral malaria pathogenesis, we investigated intra-cerebral gene-expression profiles in well-defined genetically cerebral malaria-resistant (CM-R) and CM-susceptible (CM-S) mice, upon infection by *Plasmodium berghei* ANKA (PbA). We investigated mouse transcriptional responses at early and late stages of infection by use of cDNA microarrays. RESULTS: : Through a rigorous statistical approach with multiple testing corrections, we showed that PbA significantly altered brain gene expression in CM-R (BALB/c), and in CM-S (CBA/J and C57BL/6) mice, and that 327 genes discriminated between early and late infection stages, between mouse strains, and between CM-R and CM-S mice. We further identified 104, 56, 84 genes with significant differential expression

between CM-R and CM-S mice on days 2, 5, and 7 respectively. The analysis of their functional annotation indicates that genes involved in metabolic energy pathways, the inflammatory response, and the neuroprotection/neurotoxicity balance play a major role in cerebral malaria pathogenesis. In addition, our data suggest that cerebral malaria and Alzheimer's disease may share some common mechanisms of pathogenesis, as illustrated by the accumulation of beta-amyloid proteins in brains of CM-S mice, but not of CM-R mice. CONCLUSION: Our microarray analysis highlighted marked changes in several molecular pathways in CM-S compared to CM-R mice, particularly at early stages of infection. This study revealed some promising areas for exploration that may both provide new insight into the knowledge of CM pathogenesis and the development of novel therapeutic strategies.

7: *BMC Genomics*. 2007 Dec 5;8(1):451

Continuous exposure to Plasmodium results in decreased susceptibility and transcriptomic divergence of the Anopheles gambiae immune system.

Aguilar R, Das S, Dong Y, Dimopoulos G.

BACKGROUND: Plasmodium infection has been shown to compromise the fitness of the mosquito vector, reducing its fecundity and longevity. However, from an evolutionary perspective, the impact of Plasmodium infection as a selective pressure on the mosquito is largely unknown. RESULTS: In the present study we have addressed the effect of a continuous Plasmodium berghei infection on the resistance to infection and global gene expression in Anopheles gambiae. Exposure of A. gambiae to P. berghei-infected blood and infection for 16 generations resulted in a decreased susceptibility to infection, altered constitutive expression levels for approximately 2.4% of the mosquito's total transcriptome and a lower basal level of immune genes expression, including several anti-Plasmodium factors. The infection-responsiveness for several anti-Plasmodium defense genes was elevated in the P. berghei exposed mosquito colonies. CONCLUSION: Our study establishes the existence of a selective pressure exerted by the parasite P. berghei on the malaria vector A. gambiae, which results in a decreased permissiveness to infection and changes in the mosquito transcriptome regulation that suggest a decreased constitutive immune gene activity but a more potent immune response upon Plasmodium challenge.

8: *Genome Res*. 2007 Dec 20

Genome-wide discovery and verification of novel structured RNAs in Plasmodium falciparum.

Mourier T, Carret C, Kyes S, Christodoulou Z, Gardner PP, Jeffares DC, Pinches R, Barrell B, Berriman M, Griffiths-Jones S, Ivens A, Newbold C, Pain A.

Ancient DNA and Evolution Group, Department of Biology, University of Copenhagen, Copenhagen DK-2100, Denmark;

We undertook a genome-wide search for novel noncoding RNAs (ncRNA) in the malaria parasite Plasmodium falciparum. We used the RNAz program to predict structures in the noncoding regions of the P. falciparum 3D7 genome that were conserved with at least one of seven other Plasmodium spp. genome sequences. By using Northern blot analysis for 76 high-scoring predictions and microarray analysis for the majority of candidates, we have verified the expression of 33 novel ncRNA transcripts including four members of a ncRNA family in the asexual blood stage. These transcripts represent novel structured ncRNAs in P. falciparum and are not represented in any RNA databases. We provide supporting evidence for purifying selection acting on the experimentally verified ncRNAs by comparing the nucleotide substitutions in the predicted ncRNA candidate structures in P. falciparum with the closely related chimp malaria parasite P. reichenowi. The

high confirmation rate within a single parasite life cycle stage suggests that many more of the predictions may be expressed in other stages of the organism's life cycle.

9: *Haematologica*. 2008 Jan;93(1):143-144.

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

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

Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria.
unekecj@yahoo.com

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

10: *Hum Mol Genet*. 2007 Dec 6

Haemoglobin S and haemoglobin C: 'quick but costly' versus 'slow but gratis' genetic adaptations to Plasmodium falciparum malaria.

Modiano D, Bancone G, Ciminelli BM, Pompei F, Blot I, Simporé J, Modiano G.

Dipartimento di Scienze di Sanità Pubblica, University of Rome "La Sapienza", Italy.

Haemoglobin S (HbS; beta6Glu-->Val) and HbC (beta6Glu-->Lys) strongly protect against clinical Plasmodium falciparum malaria. HbS, which is lethal in homozygosity, has a multi-foci origin and a widespread geographic distribution in sub-Saharan Africa and Asia whereas HbC, which has no obvious CC segregational load, occurs only in a small area of central West-Africa. To address this apparent paradox we adopted two partially independent haplotypic approaches in the Mossi population of Burkina Faso where both the local S (S(Benin)) and the C alleles are common (0.05 and 0.13). Here we show that: both C and S(Benin) are monophyletic; C has accumulated a fourfold higher recombinational and DNA slippage haplotypic variability than the S(Benin) allele (P = 0.003) implying higher antiquity; for a long initial lag period the C alleles did apparently remain very few. These results, consistently with epidemiological evidences, imply that the C allele has been accumulated mainly through a recessive rather than a semidominant mechanism of selection. This evidence explains the apparent paradox of the uni-epicentric geographic distribution of HbC, representing a 'slow but gratis' genetic adaptation to malaria through a transient polymorphism, compared to the polycentric 'quick but costly' adaptation through balanced polymorphism of HbS.

11: *Infect Immun*. 2008 Jan;76(1):229-38.

Preclinical evaluation of the safety and immunogenicity of a vaccine consisting of Plasmodium falciparum liver-stage antigen 1 with adjuvant AS01B administered alone or concurrently with the RTS,S/AS01B vaccine in rhesus primates.

Pichyangkul S, Kum-Arb U, Yongvanitchit K, Limsalakpetch A, Gettayacamin M, Lanar DE, Ware LA, Stewart VA, Heppner DG, Mettens P, Cohen JD, Ballou WR, Fukuda MM.

Department of Immunology and Medicine, USAMC-AFRIMS, 315/6 Rajvithi Rd., Bangkok

10400, Thailand. Sathitp@afirms.org

Several lines of evidence suggest that targeting pre-erythrocytic-stage parasites for malaria vaccine development can provide sterile immunity. The objectives of this study were (i) to evaluate preclinically the safety and immunogenicity of a new recombinant pre-erythrocytic-stage antigen, liver-stage antigen 1 (LSA1), in nonhuman primates; and (ii) to investigate the potential for immune interference between LSA1 and the leading malaria vaccine candidate, RTS,S, by comparing the immune responses after single-antigen vaccination to responses after simultaneous administration of both antigens at separate sites. Using a rhesus monkey model, we found that LSA1 formulated with the GlaxoSmithKline proprietary adjuvant system AS01B (LSA1/AS01B) was safe and immunogenic, inducing high titers of antigen-specific antibody and CD4+ T-cell responses, as monitored by the production of interleukin-2 and gamma interferon, using intracellular cytokine staining. RTS,S/AS01B vaccination was well tolerated and demonstrated robust antibody and moderate CD4+ T-cell responses to circumsporozoite protein (CSP) and HBsAg. Positive CD8+ T-cell responses to HBsAg were detected, whereas the responses to CSP and LSA1 were negligible. For both LSA1/AS01B and RTS,S/AS01B, no statistically significant differences were observed between individual and concurrent administration in the magnitude or duration of antibody and T-cell responses. Our results revealed that both pre-erythrocytic-stage antigens were safe and immunogenic, administered either separately or simultaneously to rhesus monkeys, and that no significant immune cross interference occurred with concurrent separate-site administration. The comparison of the profiles of immune responses induced by separate-site and single-site vaccinations with LSA1 and RTS,S warrants further investigation.

12: Infect Immun. 2007 Dec 10

Humoral responses to *P. falciparum* blood stage antigens (MSP3, MSP1-19, GLURP and AMA1) and their association with the incidence of clinical malaria in children living in a seasonal malaria transmission area of Burkina Faso (West Africa).

Nebie I, Diarra A, Ouedraogo A, Soulama I, Bougouma EC, Tiono AB, Konate AT, Chilengi R, Theisen M, Doodoo D, Remarque E, Bosomprah S, Milligan P, Sirima SB.

Centre National de Recherche et de Formation sur le paludisme, Ouagadougou, Burkina Faso; The African Malaria Network Trust, Tanzania Commission for Science and Technology Building, P.O. Box 33207, Dar es Salaam, Tanzania; Department of Clinical Biochemistry Statens Serum, Copenhagen, Denmark; Noguchi Memorial Institute for Medical Research, University of Ghana; Department of Parasitology Biomedical Primate Research Centre Lange Kleiweg 139 2288 GJ Rijswijk, The Netherlands; Ministry of Health, Ghana, P.O. Box M44 Accra Ghana; Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK.

There is longstanding evidence that immunoglobulins IgG have a role in protection against clinical malaria, and human antibodies of the cytophilic subclasses are thought to be particularly critical in this respect. In this cohort study, 286 Burkinabè children aged 6 months to 15 years were kept under malaria surveillance in order to assess the protective role of antibody responses against four antigens which are currently being evaluated as vaccine candidates (AMA1, MSP1-19, MSP3, and GLURP). Total IgG, IgM, and IgG subclass responses were measured just before the malaria transmission season. The incidence of malaria was 2.4 episodes per child year at risk. After adjusting for the confounding effects of age, the level of total IgG to GLURP was strongly associated with reduced malaria incidence (rate ratio associated with a doubling of total IgG IRR=0.79, 95%CI:0.66-0.94, P =0.009.); there was a borderline statistical significance association between the level of total IgG to MSP3 and malaria incidence, and no evidence of an association for total IgG to AMA1 and to MSP1-19. Of the IgG subclass responses studied only IgG3 and IgG4 against GLURP

and IgG1 against AMA1 were associated with reduced risk of clinical malaria. There was no evidence of an interaction between responses to AMA1 and baseline parasitaemia in their effects on malaria incidence. Currently included in malaria vaccine formulations for clinical trials in human, these blood stage antigens (AMA1 and GLURP) offer good prospects for malaria vaccine development.

13: *Int J Infect Dis.* 2008 Jan;12(1):22-9.

Intermittent preventive treatment of malaria in pregnancy: a community-based delivery system and its effect on parasitemia, anemia and low birth weight in Uganda.

Mbonye AK, Bygbjerg I, Magnussen P. Reproductive Health Division, Department of Community Health, Ministry of Health, PO Box 7272, Kampala, Uganda.

OBJECTIVE: The main objective of the study was to assess the impact of a community-based delivery system of intermittent preventive treatment (IPT) for malaria in pregnancy with sulfadoxine-pyrimethamine (SP) on access, parasitemia, anemia and low birth weight as primary outcome measures. **METHODS:** A study was designed to test the community-based delivery system of IPT through traditional birth attendants (TBAs), drug-shop vendors (DSVs), community reproductive health workers (CRHWs) and adolescent peer mobilizers (APMs), and to compare these with IPT at health units in an area of high malaria transmission - Mukono District, Uganda. **RESULTS:** Two thousand seven hundred and eighty-five pregnant women participated in the study. The majority of the women (92.4%) at the community-based approaches received their first dose of IPT during their second trimester compared to 76.1% at health units ($p < 0.0001$). At both health units and the community-based approaches, IPT increased mean hemoglobin by 6.7% ($p < 0.0001$) for all parities and by 10.2% among primigravidae. IPT reduced the prevalence of severe anemia from 5.7% to 3.1% ($p < 0.04$). The prevalence of parasitemia was reduced from 24.5% to 16.1% ($p < 0.001$), and parasite density reduced significantly ($p < 0.02$) after the first dose and remained stable with the second dose. Overall the proportion of low birth weight was 6.3% (8.3% at health units versus 6.0% at the community-based approaches, $p < 0.03$) highlighting the importance of access and adherence to IPT. This intervention was acceptable to 89.6% of the women at the community-based approaches intending to use IPT in the future, while 48.1% of them had recommended it to other women. **CONCLUSIONS:** The community-based approaches increased access and adherence to IPT with an effect on anemia, severe anemia, parasitemia and low birth weight. However the reduced effect of IPT on parasitemia points to drug resistance with SP and this requires further evaluation; research into the identification of other more efficacious drugs for malaria prevention in pregnancy is also required.

14: *Int J Parasitol.* 2008 Jan;38(1):33-41.

Ethnopharmacology and malaria: New hypothetical leads or old efficient antimalarials?

Bourdy G, Willcox ML, Ginsburg H, Rasoanaivo P, Graz B, Deharo E.

Laboratoire de Pharmacochimie des Substances Naturelles et Pharmacophores Redox, UMR-152 (IRD - Université Paul Sabatier, Université de Toulouse 3), Mission IRD, Casilla 18-1209, Lima 18, Peru.

New treatments are urgently needed to curb and eradicate malaria in developing countries. As most people living in malarial endemic areas use traditional medicine to fight this disease, why have new treatments not emerged recently from ethnopharmacology-oriented research? The rationale and limitations of the ethnopharmacological approach are discussed in this paper, focusing on ethnopharmacology methodologies and techniques used for assessing botanical samples for their antimalarial properties. Discrepancies often observed between

strong ethnopharmacological reputation and laboratory results are discussed, as well as new research perspectives.

15: *J Acquir Immune Defic Syndr.* 2007 Dec 15;46(5):624-630.

HIV-1 Infection in Patients Referred for Malaria Blood Smears at Government Health Clinics in Uganda.

Bebell LM, Gasasira A, Kiggundu M, Dokomajilar C, Kanya MR, Charlebois ED, Havlir D, Rosenthal PJ, Dorsey G.

From *Columbia University College of Physicians and Surgeons, New York, NY; †Makerere University Medical School, Kampala, Uganda; and the ‡Department of Medicine, University of California at San Francisco, San Francisco, CA.

BACKGROUND:: HIV is associated with an increased incidence of malaria in adult African populations. In children, the relationship between HIV and malaria is less clear. We investigated the relationship between malaria and HIV-1 infection among adults and children referred for malaria blood smears at government health clinics in Uganda. **METHODS::** This was a cross-sectional study in which 1000 consecutive patients referred for malaria blood smears over the course of 1 to 2 months at each of 7 government clinics (N = 7000) were tested for HIV-1 from dried blood spots using enzyme-linked immunosorbent assay (ELISA) screening and nucleic acid-based confirmatory testing. Risk factors for HIV-1 infection were identified using multivariate logistic regression. **RESULTS::** Among 4467 children aged 16 years or younger, 77 (1.7%) were HIV-1 infected. Of 2533 adults, 270 (10.7%) were HIV-1 infected. In children, having a negative malaria blood smear was associated with higher odds of HIV-1 infection (odds ratio [OR] = 1.90, 95% confidence interval [CI]: 1.18 to 3.06) after controlling for age and gender. In adults, having a positive malaria blood smear was moderately associated with higher odds of HIV-1 infection (OR = 1.41, 95% CI: 1.01 to 1.97) after controlling for age and gender. **CONCLUSIONS::** In Ugandans evaluated for suspected malaria, associations between malaria smear results and HIV infection differed between children and adults. Although further operations research is needed, our results suggest that counseling and testing for HIV may be of particular importance in children suspected of malaria but with negative malaria smears and in adults with positive malaria smears.

16: *J Biol Chem.* 2007 Dec 28

Nuclear non-coding RNAs are transcribed from the centromeres of Plasmodium falciparum and are associated with centromeric chromatin.

Li F, Sonbuchner L, Kyes SA, Epp C, Deitsch KW. Microbiology and Immunology, Weill Cornell Medical College, New York, NY 10021.

Non-coding RNAs (ncRNAs) play an important role in a variety of nuclear processes, including genetic imprinting, RNA interference (RNAi) mediated transcriptional repression and dosage compensation. These transcripts are thought to influence chromosome organization, and in some cases gene expression, by directing the assembly of specific chromatin modifications to targeted regions of the genome. In the malaria parasite *Plasmodium falciparum*, little is known about the regulation of nuclear organization or gene expression, although a notable scarcity of identifiable transcription factors encoded in its genome has led to the speculation that this organism may be unusually reliant on chromatin modifications as a mechanism for regulating gene expression. To study the mechanisms that regulate chromatin structure in malaria parasites, we examined the role of ncRNAs in the assembly of chromatin at the centromeres of *P. falciparum*. We show that centromeric regions within the *Plasmodium* genome contain bi-directional promoter activity driving the expression of short ncRNAs that are localized within the nucleus and appear to associate with the centromeres

themselves, strongly suggesting that they are central characters in maintenance and function of centromeric chromatin. These observations support the hypothesis that ncRNAs play an important role in the proper organizational assembly of chromatin in *P. falciparum*, perhaps compensating for a lack of both regulatory transcription factors and RNAi machinery.

17: *J Biol Chem.* 2007 Dec 28

Role of Ca²⁺/CaM-PfPKB signaling pathway in erythrocyte invasion by plasmodium falciparum.

Vaid A, Thomas DC, Vaid A. Eukaryotic Gene Expression Laboratory, National Institute of Immunology, New Delhi, Delhi 110067.

Molecular mechanisms via which signaling pathways operate in malaria parasite and control its development are promiscuous. Recently, we reported the identification of a signaling pathway in *P. falciparum*, which involves activation of protein kinase PfPKB1 by calcium/calmodulin (1). Studies carried out to elucidate the function of this pathway suggested that it may be important for erythrocyte invasion. Blocking the function of the upstream activators of this pathway, Calmodulin and Phospholipase C, resulted in impaired invasion. To evaluate if this signaling cascade controls invasion by regulating PfPKB, inhibitors against this kinase were developed. PfPKB inhibitors dramatically reduced the ability of the parasite to invade erythrocytes. Furthermore, we demonstrate that PfPKB associates with actin-myosin motor and phosphorylates Glideosome Associated Protein 45 (PfGAP45), one of the important components of the motor complex, which may help explain its role in erythrocyte invasion.

18: *J Biosoc Sci.* 2008 Jan;40(1):115-35.

Malaria-related health-seeking behaviour and challenges for care providers in rural ethiopia: implications for control.

Deressa W, Ali A, Hailemariam D. School of Public Health, Addis Ababa University, Addis Ababa, Ethiopia.

SummaryA range of activities are currently underway to improve access to malaria prevention and control interventions. As disease control strategies change over time, it is crucial to understand the health-seeking behaviour and the local socio-cultural context in which the changes in interventions operate. This paper reflects on how people in an area of seasonal malaria perceive the causes and transmission of the disease, and what prevention and treatment measures they practise to cope with the disease. It also highlights some of the challenges of malaria treatment for health care providers. The study was undertaken in 2003 in Adami Tulu District in south-central Ethiopia, where malaria is a major health problem. Pre-tested structured questionnaires and focus group discussions were conducted among men and women. Malaria, locally known as busa, was perceived as the most important cause of ill health in the area. Respondent's perception and knowledge about the cause and transmission of the disease were relatively high. The newly introduced insecticide-treated nets were not popular in the area, and only 6.4% of households possessed at least one. The results showed that patients use multiple sources of health care for malaria treatment. Public health facilities, private clinics and community health workers were the main providers of malaria treatment. Despite higher treatment costs, people preferred to use private health care providers for malaria treatment due to the higher perceived quality of care they offer. In conclusion, effort in the prevention and control of malaria should be intensified through addressing not only public facilities, but also the private sector and community-based control interventions. Appropriate and relevant information on malaria should be disseminated to the local community. The authors propose the provision of effective antimalarial drugs and malaria prevention tools such as subsidized or free insecticide-treated

nets.

19: *J Cell Mol Med.* 2007 Dec 5

Strategies for developing multi-epitope, subunit-based, chemically-synthesized antimalarial vaccines.

Patarroyo ME, Cifuentes G, Bermúdez A, Patarroyo MA.

Fundación Instituto de Immunología de Colombia (FIDIC), Bogotá, Colombia.

An anti-malarial vaccine against the extremely lethal *P. falciparum* is desperately needed. Peptides from this parasite's proteins involved in invasion and having high red blood cell binding ability were identified; these conserved peptides were not immunogenic or protection-inducing when used for immunizing Aotus monkeys. Modifying some critical binding residues in these high activity binding peptides' (HABPs) attachment to red blood cells (RBC) allowed them to induce immunogenicity and protection against experimental challenge and acquire the ability to bind to specific HLA-DRbeta1* alleles. These modified HABPs adopted certain characteristic structural configurations as determined by circular dichroism (CD) and (1)H-nuclear magnetic resonance (NMR) associated with certain HLA-DR haplotype binding activities and characteristics, such as a 2A distance difference between amino acids fitting into HLA-DRbeta1* Pockets 1 to 9, residues participating in binding to HLA-DR pockets and residues making contact with the TCR, suggesting haplotype- and allele-conscious TCR. This data has been demonstrated in HLA-DR-like genotyped monkeys and provided the basis for designing highly-effective, subunit-based, multi-antigen, multistage, synthetic vaccines, for immediate human use, malaria being one of them.

20: *J Chromatogr B Analyt Technol Biomed Life Sci.* 2007 Dec 15;860(2):160-5.

High-performance liquid chromatographic assay for the determination of sulfadoxine and N-acetyl sulfadoxine in plasma from patients infected with sensitive and resistant Plasmodium falciparum malaria.

Dua VK, Gupta NC, Sethi P, Edwards G, Dash AP. National Institute of Malaria Research, Field Unit, Sector-III, BHEL, Hardwar 249403, India.

A reversed-phase high-performance liquid chromatographic method using a mobile phase of acetonitrile-methanol-trifluoroacetic acid-water (16.1:7.2:0.1:76.6, v/v/v/v) at a flow rate of 1.0mlmin⁻¹ on a LiChrosphertrade mark RP-18 column with UV (254nm) detection has been developed for the separation of sulfadoxine and its metabolite N-acetyl sulfadoxine in plasma. No interferences due to endogenous compounds or common antimalarial drugs were noticed. The limit of detection for sulfadoxine and N-acetyl sulfadoxine was 0.01µgml⁻¹ with a signal-to-noise ratio of 5:1 while the limit of quantification was 2.5µgml⁻¹. Intra-day mean relative standard deviations (RSD's) for sulfadoxine and N-acetyl sulfadoxine were 2.6 and 2.8%, respectively, while mean inter-day RSD's for sulfadoxine and N-acetyl sulfadoxine were 2.4 and 2.8%, respectively. Extraction recoveries averaged 90.6% for sulfadoxine and 86.9% for N-acetyl sulfadoxine. The method was applied for the assay of sulfadoxine and its metabolite N-acetyl sulfadoxine in plasma from Plasmodium falciparum malaria patients. Mean plasma sulfadoxine concentrations on day 2 (51h) from samples collected from sensitive and resistant *P. falciparum* patients treated with three tablets of Fansidartrade mark were 62.8 and 60.5µgml⁻¹, respectively. Mean ratio of N-acetyl sulfadoxine to sulfadoxine was 9.1% for responders and 13.9% for non-responders which revealed that higher amounts of the metabolite N-acetyl sulfadoxine were present in non-responders. The method described should find an application in the therapeutic monitoring of malaria patients.

Variation in use of erythrocyte invasion pathways by *Plasmodium falciparum* mediates evasion of human inhibitory antibodies.

Persson KE, McCallum FJ, Reiling L, Lister NA, Stubbs J, Cowman AF, Marsh K, Beeson JG.

The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia. Department of Medical Biology, University of Melbourne, Parkville, Victoria, Australia. Centre for Geographic Medicine Research – Coast, Kenya Medical Research Institute, Kilifi, Kenya.

Antibodies that inhibit *Plasmodium falciparum* invasion of erythrocytes are believed to be an important component of immunity against malaria. During blood-stage infection, *P. falciparum* can use different pathways for erythrocyte invasion by varying the expression and/or utilization of members of 2 invasion ligand families: the erythrocyte-binding antigens (EBAs) and reticulocyte-binding homologs (PfRhs). Invasion pathways can be broadly classified into 2 groups based on the use of sialic acid (SA) on the erythrocyte surface by parasite ligands. We found that inhibitory antibodies are acquired by malaria-exposed Kenyan children and adults against ligands of SA-dependent and SA-independent invasion pathways, and the ability of antibodies to inhibit erythrocyte invasion depended on the pathway used by *P. falciparum* isolates. Differential inhibition of *P. falciparum* lines that varied in their use of specific EBA and PfRh proteins pointed to these ligand families as major targets of inhibitory antibodies. Antibodies against recombinant EBA and PfRh proteins were acquired in an age-associated manner, and inhibitory antibodies against EBA175 appeared prominent among some individuals. These findings suggest that variation in invasion phenotype might have evolved as a mechanism that facilitates immune evasion by *P. falciparum* and that a broad inhibitory response against multiple ligands may be required for effective immunity.

Rapid Diagnosis of Vivax Malaria by SD BIOLINE Malaria Antigen test and Thrombocytopenia.

Lee SW, Jeon K, Jeon BR, Park I.

Department of Internal Medicine, Armed Forces Yangju Hospital; Department of Laboratory Medicine, Armed Forces Yangju Hospital; Office of Preventive Medicine, Armed Forces Medical Command.

An easy and reliable diagnostic method for malaria is highly desirable. We examined the recently introduced SD BIOLINE Malaria Antigen test, which detects *Plasmodium lactate dehydrogenase*, with the additional aid of the presence/absence of thrombocytopenia to diagnose vivax malaria. We enrolled 732 clinically suspected malaria patients in an area endemic for vivax malaria. We performed microscopic examination of thin film, applied the SD BIOLINE Malaria Antigen test, and checked their platelet count. One hundred and ninety-five patients were smear-positive for vivax malaria. The sensitivity of the SD BIOLINE Malaria Antigen test was 96.4% and its specificity was 98.9%. We found that 95.4% of malaria patients had thrombocytopenia and the proportion with malaria increased as platelet counts decreased. A positive SD BIOLINE Malaria Antigen test with thrombocytopenia showed a 100% positive predictive value for vivax malaria. In conclusion, the SD BIOLINE Malaria Antigen test is a rapid and accurate diagnostic method for vivax malaria, and a platelet count can facilitate a rapid diagnosis of malaria.

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

Benoit-Vical F, Soh PN, Saléry M, Harguem L, Poupat C, Nongonierma R.

Laboratoire de Chimie de Coordination du CNRS, UPR8241, 31077 Toulouse 4, France; Service de Parasitologie-Mycologie, Centre Hospitalier Universitaire de Rangueil, 31059 Toulouse 9, France.

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

24: *Malar J.* 2007 Dec 21;6(1):170

Adding artesunate to sulphadoxine-pyrimethamine greatly improves the treatment efficacy in children with uncomplicated falciparum malaria on the coast of Benin, West Africa.

Nahum A, Erhart A, Gazard D, Agbowai C, Van Overmeir C, Van Loen H, Menten J, Akogbeto M, Coosemans M, Massougbdji A, D'Alessandro U.

ABSTRACT: BACKGROUND: Benin has recently shifted its national antimalarial drug policy from monotherapies to combinations containing artemisinin derivatives. When this decision was taken, the available information on alternatives to chloroquine and sulphadoxine-pyrimethamine, the first- and second-line treatment, was sparse. METHODS: In 2003 - 2005, before the drug policy change, a randomized, open-label, clinical trial was carried out on the efficacy of chloroquine, and sulphadoxine-pyrimethamine alone or combined with artesunate, with the aim of providing policy makers with the information needed to formulate a new antimalarial drug policy. Children between six and 59 months of age, with uncomplicated malaria and living in the lagoon coastal area in southern Benin, were randomly allocated to one of the three study arms and followed up for 28 days. RESULTS: Treatment failure (PCR corrected) was significantly lower in the artesunate + sulphadoxine-pyrimethamine group (4/77, 5.3%) than in chloroquine group (51/71, 71.8%) or the sulphadoxine-pyrimethamine alone group (30/70, 44.1%) ($p < 0.001$). Despite high sulphadoxine-pyrimethamine failure, its combination with artesunate greatly improved treatment efficacy. CONCLUSIONS: In Benin, artesunate + sulphadoxine-pyrimethamine is efficacious and could be used when the recommended artemisinin-based combinations (artemether-lumefantrine and amodiaquine-artesunate) are not available. However, because sulphadoxine-pyrimethamine is also used in pregnant women as intermittent preventive treatment, its combination with artesunate should not be widely employed in malaria patients as this may compromise the efficacy of intermittent preventive treatment.

25: Malar J. 2007 Dec 19;6(1):168

Estimated financial and human resources requirements for the treatment of malaria in Malawi.

Muula AS, Rudatsikira E, Siziya S, Mataya RH.

ABSTRACT: **BACKGROUND:** Malaria fever is a common medical presentation and diagnosis in Malawi. The national malaria policy supports self-diagnosis and self-medication for uncomplicated malaria with first line anti-malaria drugs. While a qualitative appreciation of the burden of malaria on the health system is recognized, there is limited quantitative estimation of the burden malaria exacts on the health system, especially with regard to human resources and financial burden on Malawi. **METHODS:** The burden of malaria was assessed based on estimated incidence rates for a high endemic country of which Malawi is one. Data on the available human resources and financial resources committed towards malaria from official Malawi government documents and programme reports were obtained. The amount of human and financial resources that would be required to treat 65% or 85% of symptomatic malaria cases as per the Roll Back Malaria partnership and the US President's Malaria Initiative targets were estimated. **RESULTS:** Based on a malaria incidence rate of 1.4 episodes per year per person it was estimated that there would be 3.71 million symptomatic episodes of malaria among children <5 years of age based on mid-2007 census projections. At 0.59 episodes each year per person there would be 2.13 million episodes in the 5 to 14 year age group and 1.02 million episodes in. There would be 761,848 malaria cases when HIV is not factored in among those 15 years of age or above; this figure rose to 2.2 million when the impact of HIV in increasing malaria incidence was considered. The prevalence of HIV has resulted in 42.3% increase in symptomatic malaria cases. Treating 65% to 85% of cases would result in using 8.9% to 12.2% of the national health budget or 22.2% to 33.2% of the national drug budget. Furthermore, having 65% to 85% of cases treated at a health facility would consume 55.5% to 61.1% of full-time equivalents of all the clinicians registered in the country. While this study's estimated time of 5 and 10 minutes per consultation may differ in actual practice, due to time constraints patients may not be seen for longer consultation in resources limited settings. **CONCLUSION:** Malaria exacts a heavy toll on the health system in Malawi. The national recommendation of self-medication with first-line drug for uncomplicated malaria is justified as there are not enough clinicians to provide clinical care for all cases. The Malawi Ministry of Health's promotion of malaria drug prescription including other lower cadre health workers may be justified.

26: Malar J. 2007 Dec 19;6(1):167

Duffy blood group gene polymorphisms among malaria vivax patients in four areas of the Brazilian Amazon region.

Cavasini CE, Mattos LC, D'Almeida Couto AA, D'Almeida Couto VS, Gollino Y, Moretti LJ, Bonini-Domingos CR, Rossit AR, Castilho LM, Machado RL.

ABSTRACT: **BACKGROUND:** Duffy blood group polymorphisms are important in areas where Plasmodium vivax predominates, because this molecule acts as a receptor for this protozoan. In the present study, Duffy blood group genotyping in P. vivax malaria patients from four different Brazilian endemic areas is reported, exploring significant associations between blood group variants and susceptibility or resistance to malaria. **METHODS:** The P. vivax identification was determined by non-genotypic and genotypic screening tests. The Duffy blood group was genotyped by PCR/RFLP in 330 blood donors and 312 malaria patients from four Brazilian Amazon areas. In order to assess the variables significance and to obtain independence among the proportions, the Fisher's exact test was used. **RESULTS:** The data show a high frequency of the FYA/FYB genotype, followed by

FYB/FYB, FYA/FYA, FYA/FYB-33 and FYB/FYB-33. Low frequencies were detected for the FYA/FYX, FYB/FYX, FYX/FYX and FYB-33/FYB-33 genotypes. Negative Duffy genotype (FYB-33/FYB-33) was found in both groups: individuals infected and non-infected (blood donors). No individual carried the FYX/FYB-33 genotype. Some of the Duffy genotypes frequencies showed significant differences between donors and malaria patients. CONCLUSIONS: The obtained data suggest that individuals with the FYA/FYB genotype have higher susceptibility to malaria. The presence of the FYB-33 allele may be a selective advantage in the population, reducing the rate of infection by *P. vivax* in this region. Additional efforts may contribute to better elucidate the physiopathologic differences in this parasite/host relationship in regions endemic for *P. vivax* malaria, in particular the Brazilian Amazon region.

27: *Malar J.* 2007 Dec 17;6(1):166

Haemoglobin and haematocrit: the threefold conversion is also non valid for assessing anaemia in Plasmodium vivax malaria-endemic settings.

Rodriguez-Morales AJ, Sanchez E, Arria M, Vargas M, Piccolo C, Colina R, Franco-Paredes C.

ABSTRACT: It has been recently reported that the standard threefold conversion from haematocrit to haemoglobin underestimates the prevalence of anaemia and low levels of haemoglobin in children living in areas endemic for *Plasmodium falciparum* malaria. The data presented herein describes the experience in a malaria-endemic zone in northeastern Venezuela (state of Sucre), where a similar bias between haematocrit and haemoglobin in patients with *Plasmodium vivax* infection was found. In summary, the relationship between haematocrit and haemoglobin needs to be specifically evaluated according to each particular region or epidemiological setting.

28: *Malar J.* 2007 Dec 17;6(1):165

Complement activation in Ghanaian children with severe Plasmodium falciparum malaria.

Helegbe GK, Goka BQ, Kurtzhals JA, Addae MM, Ollaga E, Tetteh JK, Dodoo D, Ofori MF, Obeng-Adjei G, Hirayama K, Awandare GA, Akanmori BD.

ABSTRACT: BACKGROUND: Severe anaemia (SA), intravascular haemolysis (IVH) and respiratory distress (RD) are severe forms of *Plasmodium falciparum* malaria, with RD reported to be of prognostic importance in African children with malarial anaemia. Complement factors have been implicated in the mechanism leading to excess anaemia in acute *P. falciparum* infection. METHODS: The direct Coombs test (DCT) and flow cytometry were used to investigate the mean levels of RBC-bound complement fragments (C3d and C3b alphabeta) and the regulatory proteins [complement receptor-1 (CD35) and decay accelerating factor (CD55)] in children with discrete clinical forms of *P. falciparum* malaria. The relationship between the findings and clinical parameters including coma, haemoglobin (Hb) levels and RD were investigated. RESULTS: Of the 484 samples tested, 131(27%) were positive in DCT, out of which 115/131 (87.8%) were positive for C3d alone while 16/131 (12.2%) were positive for either IgG alone or both. 67.4% of the study population were below five years of age and DCT positivity was more common in this age group relative to children who were five years or older (Odds ratio, OR=3.8; 95%CI, 2.2-6.7, $p<0.001$). DCT correlated significantly with RD (beta=-304, $p=0.006$), but multiple regression analysis revealed that, Hb (beta=-0.341, $p=0.012$) and coma (beta=-0.256, $p=0.034$) were stronger predictors of RD than DCT (beta=0.228, $p=0.061$). DCT was also not associated with IVH, $p=0.19$, while spleen size was inversely correlated with Hb ($r=-402$, $p=0.001$). Flow cytometry showed similar mean fluorescent intensity (MFI) values of CD35, CD55 and C3balphabeta levels on the surfaces of RBC in patients and asymptomatic controls (AC). However, binding

of C3b alphabeta correlated significantly with CD35 or CD55 ($p < 0.001$).
CONCLUSIONS: These results suggest that complement activation contributed to anaemia in acute childhood *P. falciparum* malaria, possibly through induction of erythrophagocytosis and haemolysis. In contrast to other studies, this study did not find association between levels of the complement regulatory proteins, CD35 and CD55 and malarial anaemia. These findings suggest that complement activation could also be involved in the pathogenesis of RD but larger studies are needed to confirm this finding.

29: *Malar J.* 2007 Dec 17;6(1):164

Sequence analysis of Plasmodium falciparum cytochrome b in multiple geographic sites.

Ekala MT, Khim N, Legrand E, Randrianarivelojosia M, Jambou R, Fandeur T, Menard D, Assi SB, Henry MC, Rogier C, Bouchier C, Mercereau-Puijalon O.

ABSTRACT: BACKGROUND: The antimalarial drug atovaquone specifically targets *Plasmodium falciparum* cytochrome b (Pfcytb), a mitochondrial gene with uniparental inheritance. Cases of resistance to atovaquone associated with mutant Pfcytb have been reported, justifying efforts to better document the natural polymorphism of this gene. To this end, a large molecular survey was conducted in several malaria endemic areas where atovaquone was not yet in regular use. METHODS: The polymorphism of the Pfcytb was analysed by direct sequencing of PCR products corresponding to the full length coding region. Sequence was generated for 671 isolates originating from three continents: Africa (Senegal, Ivory Coast, Central African Republic, and Madagascar), Asia (Cambodia) and South America (French Guiana). RESULTS: Overall, 11 polymorphic sites were observed, of which eight were novel mutations. There was a large disparity in the geographic distribution of the mutants. All isolates from Senegal, Central African Republic and Madagascar displayed a Camp/3D7 wild type Pfcytb sequence, as did most samples originating from Cambodia and Ivory Coast. One synonymous (t759a at codonV253V) and two non-synonymous (t553g and a581g at codons F185V and H194R, respectively) singletons were detected in Ivory Coast. Likewise, two synonymous (a126t and c793t at codons -T42T and L265L, respectively) singletons were observed in Cambodia. In contrast, seven mutated sites, affecting seven codons and defining four mutant haplotypes were observed in French Guiana. The wild type allele was observed in only 14% of the French Guiana isolates. The synonymous c688t mutation at position L230L was highly prevalent; the most frequent allele was the c688t single mutant observed in 84% of the isolates. The other alleles were singletons (a126t/a165c, a4g/a20t/a1024c and a20t/t341c/c688t corresponding to T42T/S55S, N2D/N71I/I342L, N71I/L114S/L230L, respectively). The codon 268 polymorphisms associated with atovaquone resistance were not observed in the panel the isolates studied. Overall, the wild type PfcYTb protein isoform was highly predominant in all study areas, including French Guiana, suggesting stringent functional constraints. CONCLUSIONS: These data along with previously identified Pfcytb field polymorphisms indicate a clustering of molecular signatures, suggesting different ancestral types in South America and other continents. The absence of mutations associated with most atovaquone-proguanil clinical failures indicates that the atovaquone-proguanil association is an interesting treatment option in the study areas.

30: *Malar J.* 2007 Dec 9;6(1):163

Malaria incidence and efficacy of intermittent preventive treatment in infants (IPTi).

Kobbe R, Adjei S, Kreuzberg C, Kreuels B, Thompson B, Thompson PA, Marks F, Busch W, Tosun M, Schreiber N, Opoku E, Adjei O, Meyer CG, May J.

ABSTRACT: BACKGROUND: Intermittent preventive antimalarial treatment in infants

(IPTi) is currently evaluated as a malaria control strategy. Among the factors influencing the extent of protection that is provided by IPTi are the transmission intensity, seasonality, drug resistance patterns, and the schedule of IPTi administrations. The aim of this study was to determine how far the protective efficacy of IPTi depends on spatio-temporal variations of the prevailing incidence of malaria. METHODS: One thousand seventy infants were enrolled in a registered controlled trial on the efficacy of IPTi with sulphadoxine-pyrimethamine (SP) in the Ashanti Region, Ghana, West Africa (ClinicalTrials.gov: NCT00206739). Stratification for the village of residence and the month of birth of study participants demonstrated that the malaria incidence was dependent on spatial (range of incidence rates in different villages 0.6-2.0 episodes/year) and temporal (range of incidence rates in children of different birth months 0.8-1.2 episodes/year) factors. The range of spatio-temporal variation allowed ecological analyses of the correlation between malaria incidence rates, anti-Plasmodium falciparum lysate IgG antibody levels and protective efficacies provided by IPTi. RESULTS: Protective efficacy of the first SP administration was positively correlated with malaria incidences in children living in a distinct village or born in a distinct month (R^2 0.48, $p < 0.04$ and R^2 0.63, $p < 0.003$, respectively). Corresponding trends were seen after the second and third study drug administration. Accordingly, IgG levels against parasite lysate increased with malaria incidence. This correlation was stronger in children who received IPTi, indicating an effect modification of the intervention. CONCLUSIONS: The spatial and temporal variations of malaria incidences in a geographically and meteorologically homogeneous study area exemplify the need for close monitoring of local incidence rates in all types of intervention studies. The increase of the protective efficacy of IPTi with malaria incidences may be relevant for IPTi implementation strategies and, possibly, for other malaria control measures.

31: Malar J. 2007 Dec 6;6(1):162

Climate prediction of El Nino malaria epidemics in north-west Tanzania.

Jones AE, Uddenfeldt Wort U, Morse AP, Hastings IM, Gagnon AS.

ABSTRACT: BACKGROUND: Malaria is a significant public health problem in Tanzania. Approximately 16 million malaria cases are reported every year and 100,000 to 125,000 deaths occur. Although most of Tanzania is endemic to malaria, epidemics occur in the highlands, notably in Kagera, a region that was subject to widespread malaria epidemics in 1997 and 1998. This study examined the relationship between climate and malaria incidence in Kagera with the aim of determining whether seasonal forecasts may assist in predicting malaria epidemics. METHODS: A regression analysis was performed on retrospective malaria and climatic data during each of the two annual malaria seasons to determine the climatic factors influencing malaria incidence. The ability of the DEMETER seasonal forecasting system in predicting the climatic anomalies associated with malaria epidemics was then assessed for each malaria season. RESULTS: It was found that malaria incidence is positively correlated with rainfall during the first season (Oct-Mar) (R -squared=0.73, $p < 0.01$). For the second season (Apr-Sep), high malaria incidence was associated with increased rainfall, but also with high maximum temperature during the first rainy season (multiple R -squared=0.79, $p < 0.01$). The robustness of these statistical models was tested by excluding the two epidemic years from the regression analysis. DEMETER would have been unable to predict the heavy El Nino rains associated with the 1998 epidemic. Nevertheless, this epidemic could still have been predicted using the temperature forecasts alone. The 1997 epidemic could have been predicted from observed temperatures in the preceding season, but the consideration of the rainfall forecasts would have improved the temperature-only forecasts over the remaining years. CONCLUSIONS: These results demonstrate the potential of a seasonal forecasting system in the development of a malaria early warning system in Kagera region.

32: *Malar J.* 2007 Dec 4;6:161.

How is childhood development of immunity to *Plasmodium falciparum* enhanced by certain antimalarial interventions?

Sutherland CJ, Drakeley CJ, Schellenberg D. Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, UK. colin.sutherland@lshtm.ac.uk

ABSTRACT: The development of acquired protective immunity to *Plasmodium falciparum* infection in young African children is considered in the context of three current strategies for malaria prevention: insecticide-impregnated bed nets or curtains, anti-sporozoite vaccines and intermittent preventive therapy. Evidence is presented that each of these measures may permit attenuated *P. falciparum* blood-stage infections, which do not cause clinical malaria but can act as an effective blood-stage "vaccine". It is proposed that the extended serum half-life, and rarely considered liver-stage prophylaxis provided by the anti-folate combination sulphadoxine-pyrimethamine frequently lead to such attenuated infections in high transmission areas, and thus contribute to the sustained protection from malaria observed among children receiving the combination as intermittent preventative therapy or for parasite clearance in vaccine trials.

33: *Malar J.* 2007 Dec 4;6:160.

Intermittent preventive treatment for the prevention of malaria during pregnancy in high transmission areas.

Briand V, Cottrell G, Massougbodji A, Cot M. Mother and Child Health in the Tropics (UR010), Development Research Institute (IRD), Paris, France. valerie.briand@gmail.com

ABSTRACT: Malaria in pregnancy is one of the major causes of maternal morbidity and adverse birth outcomes. In high transmission areas, its prevention has recently changed, moving from a weekly or bimonthly chemoprophylaxis to intermittent preventive treatment (IPTp). IPTp consists in the administration of a single curative dose of an efficacious anti-malarial drug at least twice during pregnancy - regardless of whether the woman is infected or not. The drug is administered under supervision during antenatal care visits. Sulphadoxine-pyrimethamine (SP) is the drug currently recommended by the WHO. While SP-IPTp seems an adequate strategy, there are many issues still to be explored to optimize it. This paper reviewed data on IPTp efficacy and discussed how to improve it. In particular, the determination of both the optimal number of doses and time of administration of the drug is essential, and this has not yet been done. As both foetal growth and deleterious effects of malaria are maximum in late pregnancy women should particularly be protected during this period. Monitoring of IPTp efficacy should be applied to all women, and not only to primi- and secondigravidae, as it has not been definitively established that multigravidae are not at risk for malaria morbidity and mortality. In HIV-positive women, there is an urgent need for specific information on drug administration patterns (need for higher doses, possible interference with sulpha-based prophylaxis of opportunistic infections). Because of the growing level of resistance of parasites to SP, alternative drugs for IPTp are urgently needed. Mefloquine is presently one of the most attractive options because of its long half life, high efficacy in sub-Saharan Africa and safety during pregnancy. Also, efforts should be made to increase IPTp coverage by improving the practices of health care workers, the motivation of women and their perception of malaria complications in pregnancy. Because IPTp is not applicable in early pregnancy, which is a period when malaria may also be deleterious for women and their offspring, there is a necessity to integrate this strategy with other preventive

measures which can be applied earlier in pregnancy such as insecticide-treated nets.

34: *Med Mal Infect.* 2007 Dec 5

Assessing the application of Rwanda's national protocol for uncomplicated malaria treatment in healthcare institutions in Kigali city, Rwanda. [Article in French]

Nzayirambaho M, Freund RJ, Millet P, Lombraill P, Malvy D, Potel G. École de santé publique, université nationale du Rwanda, B.P. 5229, Kigali, Rwanda.

In November 2001, the National Health Ministry of Rwanda advocated a new therapeutic protocol replacing chloroquine by an amodiaquine+sulfadoxine-pyrimethamine combination for the treatment of uncomplicated malaria. OBJECTIVES: This study had for aim to assess the application of this new protocol in Kigali healthcare institutions. POPULATION AND METHODS: A knowledge, attitudes and practices study (KAP) was carried out between June and August 2003. A questionnaire was answered by 120 care providers working in 15 healthcare institutions selected randomly in health facilities treating uncomplicated malaria. Antimalarial treatments prescribed to 150 patients were also reviewed from consultation files and analyzed. RESULTS: After analysis, 63.3% prescriptions were in line with the national protocol. Factors associated to the nonobservance of the national protocol were: the carer's ignorance of any recommended treatment, his doubt of efficacy of recommended drugs, and his fear of adverse effects. CONCLUSIONS: The authors recommend informing the care providers about the national protocol. Findings also demonstrate the need to include care providers in any modifications of the national policy in terms of drug efficacy and potential adverse effects of the new strategy.

35: *Mol Med.* 2007 Dec 8

Reduced immune complex binding capacity and increased complement susceptibility of red cells from children with severe malaria-associated anemia.

Owuor BO, Odhiambo CO, Otieno WO, Adhiambo C, Makawiti DW, Stoute JA. The US Army Medical Research Unit, Kenya, and the Kenya Medical Research Institute, Nairobi, Kenya.

Plasmodium falciparum malaria causes 1-2 million deaths per year. Most deaths occur as a result of complications such as severe anemia and cerebral malaria (CM) (coma). Red cells of children with severe malaria-associated anemia (SMA) have acquired deficiencies in the complement regulatory proteins complement receptor 1 (CR1, CD35) and decay accelerating factor (DAF, CD55). We investigated whether these deficiencies affect the ability of erythrocytes to bind immune complexes (ICs) and regulate complement activation. We recruited 75 children with SMA (Hb \leq 6 g/dL) from the holoendemic malaria region of the Lake Victoria basin, western Kenya, and 74 age and gender-matched uncomplicated malaria controls. In addition, we recruited 32 children with CM and 52 age and gender-matched controls. Deficiencies in red cell CR1 and CD55 in children with SMA were accompanied by a marked decline in IC binding capacity and increased C3b deposition in vivo and ex vivo. Importantly, these changes were specific since they were not seen in red cells of children with CM or their controls. These data suggest that the declines in red cell CR1 and CD55 seen in children with SMA are of physiologic significance and may predispose erythrocytes to complement-mediated damage and phagocytosis in vivo.

36: *Mol Microbiol.* 2008 Jan;67(1):78-87.

Mapping a common interaction site used by *Plasmodium falciparum* Duffy binding-like domains to bind diverse host receptors.

Howell DP, Levin EA, Springer AL, Kraemer SM, Phippard DJ, Schief WR, Smith JD.

Seattle Biomedical Research Institute, 307 Westlake Ave N, Ste 500, Seattle, WA 98109-5219, USA, and Department of Pathobiology, University of Washington, Box 357238, Seattle, WA 98195, USA.

The Duffy binding-like (DBL) domain is a key adhesive module in *Plasmodium falciparum*, present in both erythrocyte invasion ligands (EBLs) and the large and diverse *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family of cytoadherence receptors. DBL domains bind a variety of different host receptors, including intercellular adhesion molecule 1 (ICAM-1), a receptor interaction that may have a role in infected erythrocyte binding to cerebral blood vessels and cerebral malaria. In this study, we expressed the nearly full complement of DBLbeta-C2 domains from the IT4/25/5 (IT4) parasite isolate and showed that ICAM-1-binding domains (DBLbeta-C2(ICAM-1)) were confined to group B and group C PfEMP1 proteins and were not present in group A, suggesting that ICAM-1 selection pressure differs between PfEMP1 groups. To further dissect the molecular determinants of binding, we modelled a DBLbeta-C2(ICAM-1) domain on a solved DBL structure and created alanine substitution mutants in two DBLbeta-C2(ICAM-1) domains. This analysis indicates that the DBLbeta-C2::ICAM-1 interaction maps to the equivalent glycan binding region of EBLs, and suggests a general model for how DBL domains evolve under dual selection for host receptor binding and immune evasion.

37: *Mol Microbiol.* 2007 Dec 18

An atypical orthologue of 6-pyruvoyltetrahydropterin synthase can provide the missing link in the folate biosynthesis pathway of malaria parasites.

Dittrich S, Mitchell SL, Blagborough AM, Wang Q, Wang P, Sims PF, Hyde JE.

Manchester Interdisciplinary Biocentre, Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK.

Folate metabolism in malaria parasites is a long-standing, clinical target for chemotherapy and prophylaxis. However, despite determination of the complete genome sequence of the lethal species *Plasmodium falciparum*, the pathway of de novo folate biosynthesis remains incomplete, as no candidate gene for dihydroneopterin aldolase (DHNA) could be identified. This enzyme catalyses the third step in the well-characterized pathway of plants, bacteria, and those eukaryotic microorganisms capable of synthesizing their own folate. Utilizing bioinformatics searches based on both primary and higher protein structures, together with biochemical assays, we demonstrate that *P. falciparum* cell extracts lack detectable DHNA activity, but that the parasite possesses an unusual orthologue of 6-pyruvoyltetrahydropterin synthase (PTPS), which simultaneously gives rise to two products in comparable amounts, the predominant of which is 6-hydroxymethyl-7,8-dihydropterin, the substrate for the fourth step in folate biosynthesis (catalysed by 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase; PPPK). This can provide a bypass for the missing DHNA activity and thus a means of completing the biosynthetic pathway from GTP to dihydrofolate. Supported by site-directed mutagenesis experiments, we ascribe the novel catalytic activity of the malarial PTPS to a Cys to Glu change at its active site relative to all previously characterized PTPS molecules, including that of the human host.

The role of osmiophilic bodies and Pfg377 expression in female gametocyte emergence and mosquito infectivity in the human malaria parasite *Plasmodium falciparum*.

de Koning-Ward TF, Olivieri A, Bertuccini L, Hood A, Silvestrini F, Charvalias K, Berzosa Díaz P, Camarda G, McElwain TF, Papenfuss T, Healer J, Baldassarri L, Crabb BS, Alano P, Ranford-Cartwright LC.

The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, Vic. 3050, Australia.

Osmiophilic bodies are membrane-bound vesicles, found predominantly in *Plasmodium* female gametocytes, that become progressively more abundant as the gametocyte reaches full maturity. These vesicles lie beneath the subpellicular membrane of the gametocyte, and the release of their contents into the parasitophorous vacuole has been postulated to aid in the escape of gametocytes from the erythrocyte after ingestion by the mosquito. Currently, the only protein known to be associated with osmiophilic bodies in *Plasmodium falciparum* is Pfg377, a gametocyte-specific protein expressed at the onset of osmiophilic body development. Here we show by targeted gene disruption that Pfg377 plays a fundamental role in the formation of these organelles, and that female gametocytes lacking the full complement of osmiophilic bodies are significantly less efficient both *in vitro* and *in vivo* in their emergence from the erythrocytes upon induction of gametogenesis, a process whose timing is critical for fertilization with the short-lived male gamete. This reduced efficiency of emergence explains the significant defect in oocyst formation in mosquitoes fed blood meals containing Pfg377-negative gametocytes, resulting in an almost complete blockade of infection.

39: *Nature.* 2007 Dec 13;450(7172):1091-5.

Distinct physiological states of *Plasmodium falciparum* in malaria-infected patients.

Daily JP, Scanfeld D, Pochet N, Le Roch K, Plouffe D, Kamal M, Sarr O, Mboup S, Ndir O, Wypij D, Levasseur K, Thomas E, Tamayo P, Dong C, Zhou Y, Lander ES, Ndiaye D, Wirth D, Winzeler EA, Mesirov JP, Regev A.

Department of Immunology and Infectious Disease, [Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115, USA.

Infection with the malaria parasite *Plasmodium falciparum* leads to widely different clinical conditions in children, ranging from mild flu-like symptoms to coma and death. Despite the immense medical implications, the genetic and molecular basis of this diversity remains largely unknown. Studies of *in vitro* gene expression have found few transcriptional differences between different parasite strains. Here we present a large study of *in vivo* expression profiles of parasites derived directly from blood samples from infected patients. The *in vivo* expression profiles define three distinct transcriptional states. The biological basis of these states can be interpreted by comparison with an extensive compendium of expression data in the yeast *Saccharomyces cerevisiae*. The three states *in vivo* closely resemble, first, active growth based on glycolytic metabolism, second, a starvation response accompanied by metabolism of alternative carbon sources, and third, an environmental stress response. The glycolytic state is highly similar to the known profile of the ring stage *in vitro*, but the other states have not been observed *in vitro*. The results reveal a previously unknown physiological diversity in the *in vivo* biology of the malaria parasite, in particular evidence for a functional mitochondrion in the asexual-stage parasite, and indicate *in vivo* and *in vitro* studies to determine

how this variation may affect disease manifestations and treatment.

40: *Parasitol Res.* 2008 Jan;102(2):175-81.

Are coinfections of malaria and filariasis of any epidemiological significance?

Muturi EJ, Jacob BG, Kim CH, Mbogo CM, Novak RJ. Department of Medicine, William C. Gorgas Center for Geographic Medicine, University of Alabama at Birmingham, 206C Bevill Biomedical Research Building, 845 19th Street South, Birmingham, AL, 35294, USA, emuturi@uab.edu

Africa accounts for about 33 and 90% of the world's burden of lymphatic filariasis (LF) and malaria, respectively. Despite tremendous progress in the approach to their diagnosis, epidemiology, and treatment, and global campaigns for their control and/or elimination, their global burden and economic costs have continued to rise. In most rural areas of the tropics, both diseases co-occur in the same human population and share common mosquito vectors. It is therefore conceived that control of the two diseases can be integrated using tools that have been proven effective recently or in the past. Before implementation of control programs in areas co-endemic for both diseases, it is deemed necessary to understand how the two diseases interact in the vector and human hosts. Here, we summarize available knowledge on coinfections of malaria and LF and provide an insight on how they can be managed.

41: *Parasitol Res.* 2007 Dec 8

Blood coagulation in falciparum malaria-a review.

Ghosh K, Shetty S. Institute of Immunohaematology (ICMR), KEM Hospital, Parel, Mumbai, 400 012, India, kanjakshaghosh@hotmail.com

Falciparum malaria infection influences blood coagulation by various interacting pathobiological mechanisms, the most important being the overwhelming response of the host to sepsis resulting in a cytokine storm. In addition, the parasite infects the red cells leading to changes in the red cell phospholipid composition which supports blood coagulation. Red cells infected with Plasmodium falciparum also adhere to deeper tissue capillary endothelium leading to profound damage to endothelial cells leading to further activation. This results in widespread consumption of platelets and activation of blood coagulation which at times culminates in a clinically and pathologically detectable disseminated intravascular coagulation (DIC). Monocyte-macrophage system also gets activated in this infection compounding the hypercoagulable state. Heavy parasitaemia leading to occlusion of hepatic microcirculation leads to abnormalities in synthesis and secretion of coagulation factors and their inhibitors. Drugs used in the treatment for falciparum malaria can cause thrombocytopaenia, bone marrow suppression and haemolytic anaemia, all of which can interfere indirectly with blood coagulation. Microparticle formation from platelets, red cells and macrophages also causes widespread activation of blood coagulation, and this recently observed mechanism is the focus of intense research in many other inflammatory and neoplastic conditions where there is activation of blood coagulation system. Thus, in severe falciparum malaria, there is activation of blood coagulation system along with thrombocytopaenia, even before widespread DIC and coagulation failure occur.

42: *PLoS Comput Biol.* 2007 Dec 28;3(12):e255.

Determination of the Processes Driving the Acquisition of Immunity to Malaria Using a Mathematical Transmission Model.

Filipe JA, Riley EM, Drakeley CJ, Sutherland CJ, Ghani AC.

Acquisition of partially protective immunity is a dominant feature of the epidemiology of malaria among exposed individuals. The processes that determine the acquisition of immunity to clinical disease and to asymptomatic carriage of malaria parasites are poorly understood, in part because of a lack of validated immunological markers of protection. Using mathematical models, we seek to better understand the processes that determine observed epidemiological patterns. We have developed an age-structured mathematical model of malaria transmission in which acquired immunity can act in three ways ("immunity functions"): reducing the probability of clinical disease, speeding the clearance of parasites, and increasing tolerance to subpatent infections. Each immunity function was allowed to vary in efficacy depending on both age and malaria transmission intensity. The results were compared to age patterns of parasite prevalence and clinical disease in endemic settings in northeastern Tanzania and The Gambia. Two types of immune function were required to reproduce the epidemiological age-prevalence curves seen in the empirical data; a form of clinical immunity that reduces susceptibility to clinical disease and develops with age and exposure (with half-life of the order of five years or more) and a form of anti-parasite immunity which results in more rapid clearance of parasitaemia, is acquired later in life and is longer lasting (half-life of >20 y). The development of anti-parasite immunity better reproduced observed epidemiological patterns if it was dominated by age-dependent physiological processes rather than by the magnitude of exposure (provided some exposure occurs). Tolerance to subpatent infections was not required to explain the empirical data. The model comprising immunity to clinical disease which develops early in life and is exposure-dependent, and anti-parasite immunity which develops later in life and is not dependent on the magnitude of exposure, appears to best reproduce the pattern of parasite prevalence and clinical disease by age in different malaria transmission settings. Understanding the effector mechanisms underlying these two immune functions will assist in the design of transmission-reducing interventions against malaria.

43: *PLoS Genet.* 2007 Dec 7;3(12):e217

Localization of Candidate Regions Maintaining a Common Polymorphic Inversion (2La) in *Anopheles gambiae*.

White BJ, Hahn MW, Pombi M, Cassone BJ, Lobo NF, Simard F, Besansky NJ.

Chromosomal inversion polymorphisms are thought to play a role in adaptive divergence, but the genes conferring adaptive benefits remain elusive. Here we study 2La, a common polymorphic inversion in the African malaria vector *Anopheles gambiae*. The frequency of 2La varies clinally and seasonally in a pattern suggesting response to selection for aridity tolerance. By hybridizing genomic DNA from individual mosquitoes to oligonucleotide microarrays, we obtained a complete map of differentiation across the *A. gambiae* genome. Comparing mosquitoes homozygous for the 2La gene arrangement or its alternative (2L+(a)), divergence was highest at loci within the rearranged region. In the 22 Mb included within alternative arrangements, two approximately 1.5 Mb regions near but not adjacent to the breakpoints were identified as being significantly diverged, a conclusion validated by targeted sequencing. The persistent association of both regions with the 2La arrangement is highly unlikely given known recombination rates across the inversion in 2La heterozygotes, thus implicating selection on genes underlying these regions as factors responsible for the maintenance of 2La. Polymorphism and divergence data are consistent with a model in which the inversion is maintained by migration-selection balance between multiple alleles inside these regions, but further experiments will be needed to fully distinguish between the epistasis (coadaptation) and local adaptation models for the maintenance of 2La.

44: *PLoS ONE*. 2007 Dec 26;2(12):e1371.

Sterile Protection against Malaria Is Independent of Immune Responses to the Circumsporozoite Protein.

Grüner AC, Mauduit M, Tewari R, Romero JF, Depinay N, Kayibanda M, Lallemand E, Chavatte JM, Crisanti A, Sinnis P, Mazier D, Corradin G, Snounou G, Rénia L.

Institut Cochin, Department of Immunology, Université Paris Descartes, Centre National de la Recherche Scientifique (CNRS) UMR 8104, Paris, France.

BACKGROUND: Research aimed at developing vaccines against infectious diseases generally seeks to induce robust immune responses to immunodominant antigens. This approach has led to a number of efficient bacterial and viral vaccines, but it has yet to do so for parasitic pathogens. For malaria, a disease of global importance due to infection by *Plasmodium* protozoa, immunization with radiation-attenuated sporozoites uniquely leads to long lasting sterile immunity against infection. The circumsporozoite protein (CSP), an important component of the sporozoite's surface, remains the leading candidate antigen for vaccines targeting the parasite's pre-erythrocytic stages. Difficulties in developing CSP-based vaccines that reproduce the levels of protection afforded by radiation-attenuated sporozoites have led us to question the role of CSP in the acquisition of sterile immunity. We have used a parasite transgenic for the CSP because it allowed us to test whether a major immunodominant *Plasmodium* antigen is indeed needed for the induction of sterile protective immunity against infection. **METHODOLOGY/MAIN FINDINGS:** We employed a *P. berghei* parasite line that expresses a heterologous CSP from *P. falciparum* in order to assess the role of the CSP in the protection conferred by vaccination with radiation-attenuated *P. berghei* parasites. Our data demonstrated that sterile immunity could be obtained despite the absence of immune responses specific to the CSP expressed by the parasite used for challenge. **CONCLUSIONS:** We conclude that other pre-erythrocytic parasite antigens, possibly hitherto uncharacterised, can be targeted to induce sterile immunity against malaria. From a broader perspective, our results raise the question as to whether immunodominant parasite antigens should be the favoured targets for vaccine development.

45: *PLoS ONE*. 2007 Dec 12;2(12):e1311.

A Randomized Open-Label Trial of Artesunate- Sulfadoxine-Pyrimethamine with or without Primaquine for Elimination of Sub-Microscopic *P. falciparum* Parasitaemia and Gametocyte Carriage in Eastern Sudan.

El-Sayed B, El-Zaki SE, Babiker H, Gadalla N, Ageep T, Mansour F, Baraka O, Milligan P, Babiker A.

Department of Epidemiology, Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan.

BACKGROUND: In areas of seasonal malaria transmission, treatment of asymptomatic carriers of malaria parasites, whose parasitaemia persists at low densities throughout the dry season, could be a useful strategy for malaria control. We carried out a randomized trial to compare two drug regimens for clearance of parasitaemia in order to identify the optimum regimen for use in mass drug administration in the dry season. **METHODOLOGY AND PRINCIPAL FINDINGS:** A two-arm open-label randomized controlled trial was conducted during the dry season in an area of distinct seasonal malaria in two villages in Gedarif State in eastern Sudan. Participants were asymptomatic adults and children aged over 6 months, with low-density *P. falciparum* infection detected by PCR. Participants were randomized to receive artesunate/sulfadoxine-pyrimethamine (AS+SP) combination for three days with or without a dose of primaquine (PQ) on the fourth day. Parasitaemia detected by PCR on days 3, 7 and 14 after the start of treatment and

gametocytes detected by RT-PCR on days 7 and 14 were then recorded. 104 individuals who had low density parasitaemia at screening were randomized and treated during the dry season. On day 7, 8.3% were positive by PCR in the AS+SP+PQ group and 6.5% in the AS+SP group (risk difference 1.8%, 95%CI -10.3% to +13.8%). At enrolment, 12% (12/100) were carrying gametocytes. This was reduced to 6.4% and 4.4% by day 14 (Risk difference 1.9% (95%CI -9.3% to +13.2%) in AS+SP+PQ and AS+SP groups, respectively. CONCLUSION: Addition of primaquine to artemisinin combination treatment did not improve elimination of parasitaemia and prevention of gametocyte carriage in carriers with low-density parasitaemia in the dry season. TRIAL REGISTRATION: ClinicalTrials.gov NCT00330902.

46: *PLoS ONE*. 2007 Dec 5;2(12):e1278.

A virosomal malaria Peptide vaccine elicits a long-lasting sporozoite-inhibitory antibody response in a phase 1a clinical trial.

Okitsu SL, Silvie O, Westerfeld N, Curcic M, Kammer AR, Mueller MS, Sauerwein RW, Robinson JA, Genton B, Mazier D, Zurbriggen R, Pluschke G.

Molecular Immunology, Swiss Tropical Institute, Basel, Switzerland.

OBJECTIVES: Peptides delivered on the surface of influenza virosomes have been shown to induce solid humoral immune responses in experimental animals. High titers of peptide-specific antibodies were also induced in a phase 1a clinical trial in volunteers immunized with virosomal formulations of two peptides derived from the circumsporozoite protein (CSP) and the apical membrane antigen 1 (AMA-1) of *Plasmodium falciparum*. The main objective of this study was to perform a detailed immunological and functional analysis of the CSP-specific antibodies elicited in this phase 1a trial. METHODOLOGY/PRINCIPAL FINDINGS: 46 healthy malaria-naïve adults were immunized with virosomal formulations of two peptide-phosphatidylethanolamine conjugates, one derived from the NANP repeat region of *P. falciparum* CSP (designated UK-39) the other from *P. falciparum* AMA-1 (designated AMA49-C1). The two antigens were delivered in two different concentrations, alone and in combination. One group was immunized with empty virosomes as control. In this report we show a detailed analysis of the antibody response against UK-39. Three vaccinations with a 10 microg dose of UK-39 induced high titers of sporozoite-binding antibodies in all volunteers. This IgG response was affinity matured and long-lived. Co-administration of UK-39 and AMA49-C1 loaded virosomes did not interfere with the immunogenicity of UK-39. Purified total IgG from UK-39 immunized volunteers inhibited sporozoite migration and invasion of hepatocytes in vitro. Sporozoite inhibition closely correlated with titers measured in immunogenicity assays. CONCLUSIONS: Virosomal delivery of a short, conformationally constrained peptide derived from *P. falciparum* CSP induced a long-lived parasite-inhibitory antibody response in humans. Combination with a second virosomally-formulated peptide derived from *P. falciparum* AMA-1 did not interfere with the immunogenicity of either peptide, demonstrating the potential of influenza virosomes as a versatile, human-compatible antigen delivery platform for the development of multivalent subunit vaccines. TRIAL REGISTRATION: ClinicalTrials.gov NCT00400101.

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

Progression of *Plasmodium berghei* through *Anopheles stephensi* Is Density-Dependent.

Sinden RE, Dawes EJ, Alavi Y, Waldock J, Finney O, Mendoza J, Butcher GA, Andrews L, Hill AV, Gilbert SC, Basáñez MG.

It is well documented that the density of *Plasmodium* in its vertebrate host modulates the physiological response induced; this in turn regulates parasite survival and transmission. It is less clear that parasite density in the mosquito

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

48: *PLoS Pathog.* 2007 Dec 21;3(12):e192

Hemolytic C-Type Lectin CEL-III from Sea Cucumber Expressed in Transgenic Mosquitoes Impairs Malaria Parasite Development.

Yoshida S, Shimada Y, Kondoh D, Kouzuma Y, Ghosh AK, Jacobs-Lorena M, Sinden RE.

The midgut environment of anopheline mosquitoes plays an important role in the development of the malaria parasite. Using genetic manipulation of anopheline mosquitoes to change the environment in the mosquito midgut may inhibit development of the malaria parasite, thus blocking malaria transmission. Here we generate transgenic *Anopheles stephensi* mosquitoes that express the C-type lectin CEL-III from the sea cucumber, *Cucumaria echinata*, in a midgut-specific manner. CEL-III has strong and rapid hemolytic activity toward human and rat erythrocytes in the presence of serum. Importantly, CEL-III binds to ookinetes, leading to strong inhibition of ookinete formation in vitro with an IC50 of 15 nM. Thus, CEL-III exhibits not only hemolytic activity but also cytotoxicity toward ookinetes. In these transgenic mosquitoes, sporogonic development of *Plasmodium berghei* is severely impaired. Moderate, but significant inhibition was found against *Plasmodium falciparum*. To our knowledge, this is the first demonstration of stably engineered anophelines that affect the *Plasmodium* transmission dynamics of human malaria. Although our laboratory-based research does not have immediate applications to block natural malaria transmission, these findings have significant implications for the generation of refractory mosquitoes to all species of human *Plasmodium* and elucidation of mosquito-parasite interactions.

49: *Prog Drug Res.* 2008;65:45, 47-118.

Drug discovery and development with plant-derived compounds.

Potterat O, Hamburger M. University of Basel, Institute of Pharmaceutical Biology, Klingelbergstrasse 50, 4056 Basel, Switzerland.

An overview is given on current efforts in drug development based on plant-derived natural products. Emphasis is on projects which have advanced to clinical development. Therapeutic areas covered include cancer, viral infections including HIV, malaria, inflammatory diseases, nociception and vaccine adjuvants, metabolic disorders, and neurodegenerative diseases. Aspects which are specific to plant-based drug discovery and development are also addressed, such as supply issues in the commercial development, and the Convention on Biological Diversity.

50: *Prog Lipid Res.* 2008 Jan;47(1):50-61.

New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents.

Carballeira NM.

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, San Juan 00931-3346, Puerto Rico.

This review deals with the most recent findings on the antimalarial, antimycobacterial, and antifungal properties of fatty acids, with particular emphasis on novel marine fatty acids. The first section deals with the most recent and some background literature on what has been the latest developments with respect to fatty acids as antimalarial agents and the importance of enzyme inhibition, in particular the inhibition of the enoyl-ACP reductase (FabI) of *Plasmodium falciparum*, the principal agent responsible for malaria. This section of the review also emphasizes the latest antimalarial research with the very long-chain Delta5,9 fatty acids from sponges. The second section of the review deals with the recent literature on the antimycobacterial activity of fatty acids and the importance of enzyme inhibition, in particular the inhibition of the enoyl-ACP reductase (InhA) of *Mycobacterium tuberculosis* for antimycobacterial activity. The inhibitory activities of the Delta5,9 fatty acids against InhA as well as that of the alpha-methoxylated fatty acids are also discussed. The importance of Delta5,9 fatty acids as topoisomerase I inhibitors and its connection to cancer is also reviewed. The last part of the review, the antifungal section, also emphasizes the most recent research with antifungal fatty acids and the importance of enzyme inhibition, in particular N-myristoyltransferase (NMT) inhibition, for antifungal activity. This last section of the review emphasizes the latest research with the alpha-methoxylated fatty acids but the importance of acetylenic fatty acids is also considered.

51: *Scand J Immunol.* 2007 Dec 12

Naturally Acquired Immunity and Reduced Susceptibility to falciparum Malaria in Two Subpopulations of Endemic Eastern India.

Biswas S, Seth RK, Tyagi PK, Sharma SK, Dash AP.

National Institute of Malaria Research (Indian Council of Medical Research), 22 Sham Nath Marg, Delhi, India.

This study was aimed to assess the prevalence of naturally acquired humoral immune responses and their association with reduced susceptibility to malaria in children and adults with differential clinical conditions from an Indian zone where malaria is endemic. The study was undertaken in an eastern province of India (Keonjhar, Orissa) in a group of 341 children (both younger and older) and 98 adults living in two different areas, Town area and Forest area. They were studied for their parasitological and immunological profiles. Sera from different age-matched groups were screened by ELISA to measure IgG reactivities for characterizing humoral immune responses to the B-cell epitopes of *Plasmodium falciparum* MSP1, AMA1, RAP1 and EBA175 peptides and *P. falciparum*-infected erythrocyte lysate. In Town area, overall *P. falciparum* cases were 5.5%, whereas those in Forest area were 26.7%. We observed an age-wise increasing trend of immunity in these two populations. It was also noticed that the frequency of responders to stage-specific antigens was higher in individuals from the Town area where the frequency of malaria was lower. The naturally acquired humoral immune responses to different stage-specific antigens of *P. falciparum* reflect the reduced risk of malaria in the study groups. The higher frequency of seroresponders showed correlation with lower risk of developing malaria.

52: *Trans R Soc Trop Med Hyg.* 2008 Jan;102(1):25-31.

Assessment of three new parasite lactate dehydrogenase (pan-pLDH) tests for diagnosis of uncomplicated malaria.

Fogg C, Twesigye R, Batwala V, Piola P, Nabasumba C, Kiguli J, Mutebi F, Hook C, Guillerm M, Moody A, Guthmann JP.

Epicentre, 8 rue Saint Sabin, 75011, Paris, France; Médecins sans Frontières, 8 rue Saint Sabin, 75011, Paris, France.

A study to assess the diagnostic capabilities of three parasite lactate dehydrogenase (pan-pLDH) tests, Vistapan((R)), Carestarttrade mark and Parabank((R)), was conducted in Uganda. An HRP2 test, Paracheck-Pf((R)), and a Giemsa-stained blood film were performed with the pLDH tests for outpatients with suspected malaria. In total, 460 subjects were recruited: 248 with positive blood films and 212 with negative blood films. Plasmodium falciparum was present in 95% of infections. Sensitivity above 90% was shown by two pLDH tests, Carestart (95.6%) and Vistapan (91.9%), and specificity above 90% by Parabank (94.3%) and Carestart (91.5%). Sensitivity decreased with low parasitaemia (chi(2) trend, P<0.001); however, all tests achieved sensitivity >90% with parasitaemia >=100/mul. All tests had good inter-reader reliability (kappa>0.95). Two weeks after diagnosis, 4-10% of pLDH tests were still positive compared with 69.7% of the HRP2 tests. All tests had similar ease of use. In conclusion, two pLDH tests performed well in diagnosing P. falciparum malaria, and all pLDH tests became negative after treatment more quickly than the HRP2. Therefore the rapid test of choice for use with artemisinin-combination therapies in this area would be one of these new pLDH tests.

53: *Trans R Soc Trop Med Hyg.* 2008 Jan;102(1):20-24.

Malaria diagnosis under field conditions in the Venezuelan Amazon.

Metzger WG, Vivas-Martínez S, Rodriguez I, Gonçalves J, Bongard E, Fanello CI, Vivas L, Magris M.

Centro Amazónico de Investigación y Control de Enfermedades Tropicales 'Simon Bolívar' (CAICET), Puerto Ayacucho, Venezuela; Eberhard Karls Universität Tübingen, Institut für Tropenmedizin, Humanparasitologie, Wilhelmstrasse 27, 72074 Tübingen, Germany.

To improve practical, accurate diagnosis of malaria in the Amazon rainforest of Venezuela, two rapid diagnostic tests (RDT) (OptiMAL-IT((R)) and FalciVax((R))) and a laboratory light microscope, used in the field with a battery-operated head lamp as an external light source, were evaluated against the standard laboratory microscope procedure for malaria detection. One hundred and thirty-six Yanomami patients were studied for the presence of malaria parasites. Thirty-three patients (24%) were positive for malaria (Plasmodium falciparum, P. vivax, P. malariae). Twenty-one (64%) of the positive patients had <100 parasites/mul. Both RDTs showed poor sensitivity (24.2% for OptiMAL-IT((R)) and 36.4% for FalciVax((R))) but good specificity (99% both for OptiMAL-IT((R)) and FalciVax((R))). Field and laboratory microscopy showed sensitivities of 94% and 91%, respectively. The kappa coefficient was 0.90, indicating a high agreement between field and laboratory microscopy. We conclude that (i) adequate slide reading cannot be substituted by either of the two RDTs in the Venezuelan Amazon and (ii) the use of a light source such as that described above makes slide reading more feasible than hitherto in remote areas without electricity.

54: *Trans R Soc Trop Med Hyg.* 2008 Jan;102(1):7-10.

Malaria drug and vaccine trials in Africa: obstacles and opportunities.

Lang TA, Kokwaro GO.

KEMRI/Wellcome Trust Programme, P.O. Box 230, Kilifi, Kenya.

There are several new treatments and vaccine technologies in clinical development for childhood malaria that have arrived in the clinical phase of evaluation during the past 5-10 years. This is a long-awaited change as until this time there had been little in the pipeline. As these products progress, evaluating them in the populations for whom they are being developed is becoming increasingly challenging. Many more capable trial sites are required and thousands of children and their parents need to be willing to take part in all the clinical trials that will be necessary if even a handful of these products make it through to obtaining a marketing approval license. Then, beyond licensure, these products will need to be assessed in more 'real-life' phase IV trials to establish whether they can truly impact the high level of mortality that malaria brings to the under-five population in Africa. Here we explore the issues that face both the trial sites and the product developers and present how this opportunity should be utilised to develop experienced African clinical researchers and facilities alongside getting these products through into public health use.

55: *Trans R Soc Trop Med Hyg.* 2007 Dec 13

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

N'guessan R, Knols BG, Pannetier C, Rowland M.

London School of Hygiene and Tropical Medicine, London, UK; Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin.

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

56: *Trans R Soc Trop Med Hyg.* 2007 Dec 12

Antimalarial resistance and DHFR/DHPS genotypes of Plasmodium falciparum three years after introduction of sulfadoxine-pyrimethamine and amodiaquine in rural Tanzania.

Eriksen J, Mwankusye S, Mduma S, Veiga MI, Kitua A, Tomson G, Petzold MG, Swedberg G, Gustafsson LL, Warsame M.

Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden; Division of International Health (IHCAR), Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden.

We assessed the efficacy of sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ)

and DHFR/DHPS genotypes of *Plasmodium falciparum* in rural Tanzania, 3 years after their introduction as first- and second-line treatments for uncomplicated malaria, respectively. Underfive children with uncomplicated malaria were given standard treatments of either SP (n=66) or AQ (n=30) and treatment outcomes after 14 and 28 days were determined. Total treatment failure of 18 and 42.5% was observed for SP on days 14 and 28, respectively. For AQ, total treatment failure of 27 and 53% was found on day 14 and 28, respectively. On day 14, significantly lower SP total treatment failures were observed in 2004 compared with results from a study conducted in 1999 in the same location. No relationship was detected between clinical outcome and DHFR/DHPS genotypes, but the point mutation prevalence in parasites was higher than in 1999. Pre-treatment blood levels of SP were detected in a quarter of the study children: less than expected. We report unacceptably high levels of total treatment failures, both for first- and second-line treatments for uncomplicated malaria in Tanzania 3 years after their introduction, supporting the decision to replace them with artemisinin-based combination therapy.

57: *Vaccine*. 2007 Dec 21;26(1):96-107.

Expression and purification of a *Plasmodium vivax* antigen - PvTARAg55 tryptophan- and alanine-rich antigen and its immunological responses in human subjects.

Siddiqui AA, Singh N, Sharma YD. Department of Biotechnology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India.

Despite the immense global efforts, the malaria vaccine is not yet available and requires the identification of newer target molecules. Since tryptophan-rich proteins of *P. yoelii* have been proposed as vaccine candidates, we describe here the expression, purification and immunological characterization of a 55kDa *Plasmodium vivax* tryptophan- and alanine-rich antigen (PvTARAg55). This protein consists of 480 aa residues with a calculated molecular mass of 55.0kDa. It shows 42% aa sequence identity (64% homology) with PyPAg1 of *P. yoelii* and shares positional conservation of tryptophan residues. Sequence analysis of PvTARAg55 from different *P. vivax* isolates revealed that tryptophan-rich domain which contains most of the B-cell epitopes was highly conserved in the parasite population while the alanine-rich domain showed polymorphism. Exon-2 covering major part (420 aa) of the protein including both the domains was PCR amplified, cloned, expressed in *Escherichia coli*, and the recombinant protein purified to its homogeneity. Majority of *P. vivax*-infected individuals (82.5%, n=40) produced antibodies against this antigen. Proliferative responses to the recombinant PvTARAg55 were observed in 60% (n=20) of individuals who had recently been exposed to the *P. vivax* infection. Measurement of Th1- (IFN-gamma, TNF-alpha, and IL-12) and Th2-type (IL-4 and IL-10) cytokine production in response to this recombinant antigen revealed a mixed type T-cell response with a Th2 response being more pronounced. These results demonstrate that PvTARAg55 elicits high humoral and cellular immune responses thus establishes its immunogenicity in humans.

58: *Vaccine*. 2007 Dec 12;25(51):8549-56.

The requirement of CD80, CD86, and ICAM-1 on the ability of adjuvant formulations to potentiate antibody responses to a *Plasmodium falciparum* blood-stage vaccine.

Hui G, Hashimoto C.

Department of Tropical Medicine and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, United States.

Many adjuvants are known to enhance expression of co-stimulatory and adhesion molecules secondarily to the activation of immune cells. Whether interactions via these molecules are obligatory in adjuvants' ability to potentiate vaccine

immunogenicity is less clear. We investigated the ability of eight adjuvant formulations to potentiate the immunogenicity of a malaria vaccine in mice deficient in the prominent co-stimulatory molecules, CD80 and CD86; and the adhesion ligand, ICAM-1. While no adjuvants could bypass co-stimulatory requirements, more formulations exhibited dependency for CD86 than for CD80. In CD80 or CD86 KO mice, formulations with the saponin derivative, QS21 could efficiently default to the other B7 molecule. This effect was dominant over other adjuvant constituents. The requirement for ICAM-1 could be readily bypassed using adjuvant formulations containing immunomodulators; whereas this was not the case with emulsion-type adjuvants in which reduction in adjuvanticity was associated with decreases in antigen-specific IFN-gamma responses. These studies may help to guide the formulation of vaccine adjuvants to maintain effectiveness in hosts with altered immunological environment that often result from infections.