

Malaria Bulletin: A Compendium of Current Literature

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ABSTRACTS

1: *Acta Trop.* 2008 Jun;106(3):207-212.

Changing patterns of forest malaria among the mobile adult male population in Chumkiri District, Cambodia.

Dyssoley L, Kaneko A, Eto H, Mita T, Socheat D, Børkman A, Kobayakawa T.

Department of International Affairs and Tropical Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan; The National Center for Parasitology, Entomology and Malaria Control, Ministry of Health, # 372, Boulevard Monivong, Corner Street 322, Phnom Penh, Cambodia.

Forest malaria remains a major problem in many parts of Southeast Asia and South America. In Cambodia, where a significant reduction of malaria morbidity and mortality has been observed in the last 20 years, the forest malaria situation was studied in Chumkiri District by analysing the available passive case detection data and conducting malariometric (n=1018) and questionnaire surveys (n=374) in four forest-fringe villages. There has been a decreasing trend of malaria incidence from 2001. Plasmodium falciparum was highly predominant and P. vivax was rare. The nearby-forest villages showed significantly higher parasite rates than the far-from-forest villages (9.0% vs. 1.2%, p<0.01). Malaria was highly restricted to the male adults but was nearly non-existent in other accompanying family members, including small children and females. Low income and working in forests were strongly associated with the malaria risk. Our results suggest that transmission has greatly reduced in forest-fringe villages, but remains active in forests, which is primarily maintained between the forest vector Anopheles dirus and ethnic minority inhabitants. Specific interventions directed to these previously neglected in-forest inhabitants to protect themselves and male adult villagers during their forest activities are necessary to achieve an ultimate goal of malaria elimination from Cambodia.

2: *Ann Trop Med Parasitol.* 2008 Jun;102(4):297-308.

Predictors of serum ferritin and haemoglobin during pregnancy, in a malaria-endemic area of western Kenya.

Alusala DN, Estambale BB, Magnussen P, Friis H, Luoba AI, Mwaniki D.

Division of Malaria Control, Ministry of Health, P.O. Box 20750 - 00202, Nairobi, Kenya.

Between 2000 and 2004, a cross-sectional survey was conducted, as part of a prospective cohort study, among the women attending antenatal-care clinics in Bondo district, a malaria-endemic area of western Kenya. The aim was to assess the prevalence of iron deficiency and determine the predictors of haemoglobin and serum ferritin concentrations in the women who had a gestational age between 14 and 24 weeks. A standardized questionnaire was used to collect and store the relevant bio-data for the study. Haemoglobin and ferritin concentrations were evaluated, sickle-cell status was determined, and malarial parasitaemias were detected and evaluated, using blood samples collected at enrolment. Multiple regression analysis was then used to test for significant predictors of the haemoglobin and serum ferritin concentrations. Although 842 women were enrolled in the prospective cohort study, haemoglobin concentrations were evaluated for only 828 of them, serum ferritin levels for 621, and levels of parasitaemia for 812. The mean haemoglobin concentration recorded was 10.9 g/dl. Although 37.9% of the subjects had mild-moderate anaemia (7.0-10.5 g haemoglobin/dl), only 0.5% were severely anaemic (<7.0 g haemoglobin/dl). The geometric mean serum ferritin concentration recorded was 18.9 mug/litre, and 32.3% of the subjects evaluated had low serum concentrations of ferritin (<12 mug/litre). Among the parasitaemic primigravidae (but not the parasitaemic multigravidae), those found positive for sickle-cell trait had significantly lower haemoglobin concentrations than those found negative in a sickling test (P=0.01). Among the pregnant women of Bondo district, gravidity, malarial infection and sickle cell appear to be key predictors of haemoglobin concentration.

3: *Ann Trop Paediatr.* 2008 Jun;28(2):103-9.

Taste-masked quinine sulphate pellets: bio-availability in adults and steady-state plasma concentrations in children with uncomplicated Plasmodium falciparum malaria.

Kayumba PC, Twagirumukiza M, Huyghebaert N, Ntawukuliryayo JD, van Bortel L,

Vervaet C, Remon JP.

Department of Pharmacy, National University of Rwanda, Butare, Rwanda; Laboratory of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium.

BACKGROUND: Quinine sulphate (QS), like most other antimalarials, is in tablet form designed for adults. In children, treatment is based on breaking the tablets to adapt the dose to the child's bodyweight. However, poor breakability owing to the tablet design or the absence of a score line can lead to inaccurate dosage. Furthermore, QS is very bitter which reduces its acceptability to children. QS taste-masked pellets have been developed which offer more flexibility in adapting dosage to a child's weight. **AIMS:** To evaluate the oral bio-availability of QS taste-masked pellets in healthy adult volunteers and to determine steady-state plasma concentrations in children aged <5 years with uncomplicated Plasmodium falciparum malaria. **METHODS:** Healthy adult volunteers at Kigali University Hospital received a single dose of 600 mg QS as tastemasked pellets or as commercially available tablets. A total of 56 children <5 years with uncomplicated P. falciparum malaria were recruited among patients attending Butare University Hospital and nearby health centres and treated with QS taste-masked pellets, 10-12.5 mg/kg every 8 h for 7 days. Quinine steady-state plasma concentrations were assessed on the 4th day of treatment. **RESULTS:** Following administration of taste-masked pellets to healthy adult volunteers, peak plasma concentration (C(max)) and area-under-the-curve (AUC) (C(max) 4.7 mug.ml(-1), AUC(0-24) 63.5 mug.h.ml(-1)) were significantly higher (p<0.05) than for tablets (C(max) 3.7 mug.ml(-1), AUC(0-24) 52.4 mug.h.ml(-1)), but still within the limits reported for quinine. The steady-state concentrations in children were in the therapeutic range for quinine. All the children recovered and completed the 14-day follow-up. **CONCLUSION:** QS taste-masked pellets offered the possibility to easily adjust the dose to the bodyweight of the child and can be used as an alternative to dividing tablets.

4: *Antimicrob Agents Chemother.* 2008 Jun 2.

The cell penetrating peptide TP10 shows broad spectrum activity against both Plasmodium falciparum and Trypanosoma brucei brucei.

Arrighi RB, Ebikeme C, Jiang Y, Ranford-Cartwright L, Barrett MP, Langel U, Faye I.

Department of Genetics, Microbiology, and Toxicology, Department of Neurochemistry, Stockholm University, 106 91 Stockholm, Sweden; Institute of Biomedical and Life Sciences, Division of Infection and Immunity, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow G12 8TA, UK.

Malaria and trypanosomiasis are diseases which afflict millions, for which novel therapies are urgently required. We have tested two well characterized cell penetrating peptides (CPPs) for anti-parasitic activity. One CPP designated TP10, has broad spectrum anti-parasitic activity against Plasmodium falciparum, both blood and mosquito stages, and blood stage Trypanosoma brucei brucei.

5: *Antimicrob Agents Chemother.* 2008 Jun;52(6):2212-22.

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

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

Center for Global Health and Diseases, Case Western Reserve University, School of Medicine, Wolstein Research Building, room no. 4204, 2103 Cornell Road,

Cleveland, OH 44106-7286, USA. rkm@case.edu

Mutations in the chloroquine resistance (CQR) transporter gene of *Plasmodium falciparum* (PfCRT; chromosome 7) play a key role in CQR, while mutations in the multidrug resistance gene (PfMDR1; chromosome 5) play a significant role in the parasite's resistance to a variety of antimalarials and also modulate CQR. To compare patterns of genetic variation at PfCRT and PfMDR1 loci, we investigated 460 blood samples from *P. falciparum*-infected patients from four Asian, three African, and three South American countries, analyzing microsatellite (MS) loci flanking PfCRT (five loci [approximately 40 kb]) and PfMDR1 (either two loci [approximately 5 kb] or four loci [approximately 10 kb]). CQR PfMDR1 allele-associated MS haplotypes showed considerably higher genetic diversity and higher levels of subdivision than CQR PfCRT allele-associated MS haplotypes in both Asian and African parasite populations. However, both PfCRT and PfMDR1 MS haplotypes showed similar levels of low diversity in South American parasite populations. Median-joining network analyses showed that the PfCRT MS haplotypes correlated well with geography and CQR PfCRT alleles, whereas there was no distinct PfMDR1 MS haplotype that correlated with geography and/or CQR PfMDR1 alleles. Furthermore, multiple independent origins of CQR PfMDR1 alleles in Asia and Africa were inferred. These results suggest that variation at PfCRT and PfMDR1 loci in both Asian and African parasite populations is generated and/or maintained via substantially different mechanisms. Since PfMDR1 mutations may be associated with resistance to artemisinin combination therapies that are replacing CQ, particularly in Africa, it is important to determine if, and how, the genetic characteristics of this locus change over time.

6: *Antimicrob Agents Chemother.* 2008 Jun;52(6):2130-7.

Antiparasitic activities and toxicities of individual enantiomers of the 8-aminoquinoline 8-[(4-amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy]quinoline succinate.

Nanayakkara NP, Ager AL Jr, Bartlett MS, Yardley V, Croft SL, Khan IA, McChesney JD, Walker LA.

National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. dhammika@olemiss.edu

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

7: *Apoptosis*. 2008 May 11.

Glycosylphosphatidylinositol-induced cardiac myocyte death might contribute to the fatal outcome of *Plasmodium falciparum* malaria.

Wennicke K, Debierre-Grockiego F, Wichmann D, Brattig NW, Pankuweit S, Maisch B, Schwarz RT, Ruppert V.

Department of Internal Medicine - Cardiology, Philipps-University of Marburg, University Hospital, Baldingerstrasse 1, 35043, Marburg/Lahn, Germany.

BACKGROUND: Glycosylphosphatidylinositol (GPI) purified from *Plasmodium falciparum* has been shown to play an important role as a toxin in the pathology of malaria. Previous studies demonstrated cardiac involvement in patients suffering from severe malaria due to *P. falciparum*. Therefore, we tested the hypothesis that GPI induces apoptosis in cardiomyocytes. **METHODS AND RESULTS:** By using TUNEL and caspase activity assays, we provided evidence for apoptosis induction in cardiomyocytes by *P. falciparum* GPI after 48 h of incubation. A similar result was obtained in heart cells of mice 48 h after *in vivo* injection of GPI. Gene expression analyses in GPI-treated cardiomyocytes showed an up-regulation of apoptotic genes (*apaf-1*, *bax*) and of a myocardial damage marker *bnp* (brain natriuretic peptide), while a down-regulation was observed for the anti-apoptotic gene *bcl-2* and for the heat shock protein *hsp70*. In spite of inflammatory cytokine gene up-regulation by GPI, co-culture with peripheral mononuclear cells (PMNCs) did not change the results obtained with cardiomyocytes alone, indicating a direct effect of GPI on cardiac myocytes. Co-culture with non-myocytic cardiac cells (NMCCs) resulted in up-regulation of *Hsp70* and *Bcl-2* genes in GPI-treated cardiomyocytes but without repercussion on the apoptosis level. A malaria-infected patient, presenting fulminant heart failure showed typical signs of cardiac myocyte apoptosis demonstrating the clinical relevance of toxin induced heart damage for the lethality of malaria. Our studies performed *in vitro* and in mice suggest that the GPI could be responsible for cardiomyocyte apoptosis that occurred in this patient. **CONCLUSION:** *Plasmodium falciparum* GPI-induced apoptosis might participate in the lethality of malaria.

8: *BMC Genomics*. 2008 May 23;9(1):244.

Cloning, characterization, and expression of microRNAs from the Asian malaria mosquito, *Anopheles stephensi*.

Mead EA, Tu Z.

ABSTRACT: **BACKGROUND:** microRNAs (miRNAs) are non-coding RNAs that are now recognized as a major class of gene-regulating molecules widely distributed in metazoans and plants. miRNAs have been found to play important roles in apoptosis, cancer, development, differentiation, inflammation, longevity, and viral infection. There are a few reports describing miRNAs in the African malaria mosquito, *Anopheles gambiae*, on the basis of similarity to known miRNAs from other species. *An. stephensi* is the most important malaria vector in Asia and it is becoming a model Anopheline species for physiological and genetics studies. **RESULTS:** We report the cloning and characterization of 27 distinct miRNAs from 17-day old *An. stephensi* female mosquitoes. Seventeen of the 27 miRNAs matched previously predicted *An. gambiae* miRNAs, offering the first experimental verification of miRNAs from mosquito species. Ten of the 27 are miRNAs previously unknown to mosquitoes, four of which did not match any known miRNAs in any organism. Twenty-five of the 27 *Anopheles* miRNAs had conserved sequences in the genome of a divergent relative, the yellow fever mosquito *Aedes aegypti*. Two clusters of miRNAs were found within introns of orthologous genes in *An. gambiae*, *Ae. aegypti*, and *Drosophila melanogaster*. Mature miRNAs were detected in *An. stephensi* for all of the nine selected miRNAs, including the four novel miRNAs (miR-x1- miR-x4), either by northern blot or by Ribonuclease Protection Assay.

Expression profile analysis of eight of these miRNAs revealed distinct expression patterns from early embryo to adult stages in *An. stephensi*. In both *An. stephensi* and *Ae. aegypti*, the expression of miR-x2 was restricted to adult females and predominantly in the ovaries. A significant reduction of miR-x2 level was observed 72 hrs after a blood meal. Thus miR-x2 is likely involved in female reproduction and its function may be conserved among divergent mosquitoes. A mosquito homolog of miR-14, a regulator of longevity and apoptosis in *D. melanogaster*, represented 25% of all sequenced miRNA clones from 17-day old *An. stephensi* female mosquitoes. *An. stephensi* miR-14 displayed a relatively strong signal from late embryonic to adult stages. miR-14 expression is consistent during the adult lifespan regardless of age, sex, and blood feeding status. Thus miR-14 is likely important across all mosquito life stages. CONCLUSIONS: This study provides experimental evidence for 23 conserved and four new microRNAs in *An. stephensi* mosquitoes. Comparisons between miRNA gene clusters in *Anopheles* and *Aedes* mosquitoes, and in *D. melanogaster* suggest the loss or significant change of two miRNA genes in *Ae. aegypti*. Expression profile analysis of eight miRNAs, including the four new miRNAs, revealed distinct patterns from early embryo to adult stages in *An. stephensi*. Further analysis showed that miR-x2 is likely involved in female reproduction and its function may be conserved among divergent mosquitoes. Consistent expression of miR-14 suggests that it is likely important across all mosquito life stages from embryos to aged adults. Understanding the functions of mosquito miRNAs will undoubtedly contribute to a better understanding of mosquito biology including longevity, reproduction, and mosquito-pathogen interactions, which are important to disease transmission.

9: *BMC Genomics*. 2008 May 19;9:227.

SNP discovery and molecular evolution in *Anopheles gambiae*, with special emphasis on innate immune system.

Cohuet A, Krishnakumar S, Simard F, Morlais I, Koutsos A, Fontenille D, Mindrinos M, Kafatos FC.

Institut de Recherche pour le Développement, UR 016, BP 64501, 911 Avenue Agropolis, 34394 Montpellier Cedex 5, France. cohuet@mpl.ird.fr

BACKGROUND: *Anopheles* innate immunity affects Plasmodium development and is a potential target of innovative malaria control strategies. The extent and distribution of nucleotide diversity in immunity genes might provide insights into the evolutionary forces that condition pathogen-vector interactions. The discovery of polymorphisms is an essential step towards association studies of susceptibility to infection. RESULTS: We sequenced coding fragments of 72 immune related genes in natural populations of *Anopheles gambiae* and of 37 randomly chosen genes to provide a background measure of genetic diversity across the genome. Mean nucleotide diversity (π) was 0.0092 in the *A. gambiae* S form, 0.0076 in the M form and 0.0064 in *A. arabiensis*. Within each species, no statistically significant differences in mean nucleotide diversity were detected between immune related and non immune related genes. Strong purifying selection was detected in genes of both categories, presumably reflecting strong functional constraints. CONCLUSION: Our results suggest similar patterns and rates of molecular evolution in immune and non-immune genes in *A. gambiae*. The 3,214 Single Nucleotide Polymorphisms (SNPs) that we identified are the first large set of *Anopheles* SNPs from fresh, field-collected material and are relevant markers for future phenotype-association studies.

10: *BMC Health Serv Res.* 2008 Jun 1;8(1):119.

A qualitative study of the feasibility and community perception on the effectiveness of artemether-lumefantrine use in the context of home management of malaria in south-west Nigeria.

Ajayi IO, Falade CO, Olley BO, Yusuf B, Gbotosho S, Iyiola O, Olaniyan OJ, Happi C, Munguti K, Pagnoni F.

ABSTRACT: **BACKGROUND:** In Nigeria ACT use at the community level has not been evaluated and the use of antimalarial drugs (commonly chloroquine (CQ) at home has been shown to be largely incorrect. The treatment regimen of ACT is however more complicated than that of CQ. There is thus a need to determine the feasibility of using ACT at the home level and determine community perception on its use. **METHODS:** A before and after qualitative study using key informant interviews (KII) and focus group discussions (FGDs) was conducted in selected villages in Ona-Ara local government area. At baseline, 14 FGDs and 14 KIIs were conducted. Thereafter, community medicine distributors (CMDs) were trained in each village to dispense artemether-lumefantrine (AL) to febrile children aged 6-59 months presumed to have uncomplicated malaria. After one year of drug distribution, nine KIIs and 10 FGDs were conducted. Participants and key informants were mothers and fathers with children under five years, traditional heads of communities, opinion leaders and health workers. **RESULTS:** None of the participants have heard of AL prior to study. Participants were favourably disposed to introduction of AL into the community. Mothers/caregivers were said to have used AL in place of the orthodox drugs and herbs reported commonly used prior to study after commencement of AL distribution. The use of CMDs for drug distribution was acceptable to the participants and they were judged to be efficient as they were readily available, distributed correct dose of AL and mobilised the community effectively. AL was perceived to be very effective and no significant adverse event was reported. Major concerns to the sustainability of the program were the negative attitudes of health workers towards discharge of their duties, support to the CMDs and the need to provide CMDs incentives. In addition regular supply of drugs and adequate supervision of CMDs were advised. **CONCLUSION:** Our findings showed that the use of AL at home and community level is feasible with adequate training of community medicine distributors and caregivers. Community members perceived AL to be effective thus fostering acceptability. The negative attitudes of the health workers and issue of incentives to CMDs need to be addressed for successful scaling-up of ACT use at community level.

11: *BMC Mol Biol.* 2008 May 14;9:47.

Distinct and stage specific nuclear factors regulate the expression of falcipains, Plasmodium falciparum cysteine proteases.

Sunil S, Chauhan VS, Malhotra P.

Malaria Group, International Centre for Genetic Engineering and Biotechnology, PO Box 10504, Aruna Asaf Ali Marg, New Delhi 110067, India. sujatha13@yahoo.com

BACKGROUND: Plasmodium falciparum cysteine proteases (falcipains) play indispensable roles in parasite infection and development, especially in the process of host erythrocyte rupture/invasion and hemoglobin degradation. No detailed molecular analysis of transcriptional regulation of parasite proteases especially cysteine proteases has yet been reported. In this study, using a combination of transient transfection assays and electrophoretic mobility shift assays (EMSA), we demonstrate the presence of stage specific nuclear factors that bind to unique sequence elements in the 5'upstream regions of the falcipains and probably modulate the expression of cysteine proteases. **RESULTS:** Falcipains differ in their timing of expression and exhibit ability to compensate each

other's functions at asexual blood stages of the parasite. Present study was undertaken to study the transcriptional regulation of falcipains. Transient transfection assay employing firefly luciferase as a reporter revealed that a ~1 kb sequence upstream of translational start site is sufficient for the functional transcriptional activity of falcipain-1 gene, while falcipain-2, -2' and -3 genes that exist within 12 kb stretch on chromosome 11 require ~2 kb upstream sequences for the expression of reporter luciferase activity. EMSA analysis elucidated binding of distinct nuclear factors to specific sequences within the 5'upstream regions of falcipain genes. Analysis of falcipains' 5'upstream regulatory regions did not reveal the presence of sequences known to bind general eukaryotic factors. However, we did find parasite specific sequence elements such as poly(dA) poly(dT) tracts, CCAAT boxes and a single 7 bp-G rich sequence, (A/G)NGGGG(C/A) in the 5' upstream regulatory regions of these genes, thereby suggesting the role(s) of Plasmodium specific transcriptional factors in the regulation of falcipain genes. CONCLUSION: Taken together, these results suggest that expression of Plasmodium cysteine proteases is regulated at the transcriptional level and parasite specific factors regulate the expression of falcipain genes. These findings open new venues for further studies in identification of parasite specific transcription factors.

12: *BMC Public Health*. 2008 May 9;8:157.

Malaria treatment in the retail sector: knowledge and practices of drug sellers in rural Tanzania.

Hetzel MW, Dillip A, Lengeler C, Obrist B, Msechu JJ, Makemba AM, Mshana C, Schulze A, Mshinda H.

Dept. of Public Health and Epidemiology, Swiss Tropical Institute, PO Box, CH-4002 Basel, Switzerland. manuel.hetzel@unibas.ch

BACKGROUND: Throughout Africa, the private retail sector has been recognised as an important source of antimalarial treatment, complementing formal health services. However, the quality of advice and treatment at private outlets is a widespread concern, especially with the introduction of artemisinin-based combination therapies (ACTs). As a result, ACTs are often deployed exclusively through public health facilities, potentially leading to poorer access among parts of the population. This research aimed at assessing the performance of the retail sector in rural Tanzania. Such information is urgently required to improve and broaden delivery channels for life-saving drugs. **METHODS:** During a comprehensive shop census in the districts of Kilombero and Ulanga, Tanzania, we interviewed 489 shopkeepers about their knowledge of malaria and malaria treatment. A complementary mystery shoppers study was conducted in 118 retail outlets in order to assess the vendors' drug selling practices. Both studies included drug stores as well as general shops. **RESULTS:** Shopkeepers in drug stores were able to name more malaria symptoms and were more knowledgeable about malaria treatment than their peers in general shops. In drug stores, 52% mentioned the correct child-dosage of sulphadoxine-pyrimethamine (SP) compared to only 3% in general shops. In drug stores, mystery shoppers were more likely to receive an appropriate treatment (OR = 9.6), but at an approximately seven times higher price. Overall, adults were more often sold an antimalarial than children (OR = 11.3). On the other hand, general shopkeepers were often ready to refer especially children to a higher level if they felt unable to manage the case. **CONCLUSION:** The quality of malaria case-management in the retail sector is not satisfactory. Drug stores should be supported and empowered to provide correct malaria-treatment with drugs they are allowed to dispense. At the same time, the role of general shops as first contact points for malaria patients needs to be re-considered. Interventions to improve availability of ACTs in the retail sector are urgently required within the given legal framework.

13: *Chemosphere*. 2008 May 19.

DDT and its metabolites in breast milk from the Madeira River basin in the Amazon, Brazil.

Azeredo A, Torres JP, de Freitas Fonseca M, Britto JL, Bastos WR, Azevedo E Silva CE, Cavalcanti G, Meire RO, Sarcinelli PN, Claudio L, Markowitz S, Malm O.

Colegiado de Ciências Farmacêuticas, Departamento de Saúde, UEFS, Brazil; Laboratório de Radioisótopos Eduardo Penna Franca, Instituto de Biofísica Carlos Chagas Filho, UFRJ, Brazil.

Until the 1990s the 1,1,1-trichloro-bis-2,2'-(4chlorophenyl) ethane (DDT) was sprayed in the walls of the house along the Madeira River basin, Brazilian Amazon, a region well known for its large number of malaria cases. In 1910, Oswaldo Cruz described the presence of malaria in 100% of the population living in some localities from the Madeira River basin. Data available in the literature point to the DDT contamination in fishes captured in Madeira River region. Fish is the major source of dietary protein to these people. DDT tends to accumulate in lipid rich tissues and is being eliminated by different events, including lactation. Considering the importance of feeding breast milk to the children, the associated risks of DDT exposure via breast milk intake to children must be assessed. This is the main objective of this work: to analyse the presence of the p,p'-DDT and its metabolites p,p'-DDE and p,p'-DDD in 69 human milk samples and to estimate the intake of DDT and its metabolite in terms of total DDT (total DDT=p,p'-DDE+p,p'-DDD+p,p'-DDT). All the samples showed contamination with DDT and its metabolites ranging from 25.4 to 9361.9ng of total DDT/g of lipid (median=369.6ng of total DDT/g of lipid) and 8.7% of the estimated daily intake (EDI), in terms of total DDT, which was higher than the acceptable daily intake proposed by the WHO.

14: *Diagn Microbiol Infect Dis*. 2008 May 21.

An 8-year survey on the occurrence of imported malaria in a nonendemic area by microscopy and molecular assays.

Calderaro A, Gorrini C, Peruzzi S, Piccolo G, Dettori G, Chezzi C.

Department of Pathology and Laboratory Medicine, Section of Microbiology, University of Parma, 43100 Parma, Italy.

Our study aimed to describe the occurrence of imported malaria in a nonendemic area (Parma, Italy) during the period 2000 to 2007, comparing the data obtained by microscopy and molecular assays targeting plasmodial 18S subunit rRNA gene. The prevalence of imported malaria in Parma was 21.8% by microscopy and 22.7% by polymerase chain reaction (PCR). *Plasmodium falciparum* accounted for 81.1% of the cases, followed by *Plasmodium ovale* (8.8%), *Plasmodium vivax* (3.8%), and *Plasmodium malariae* (1.9%). Mixed infections accounted for 4.4% of the cases. In this study, PCRs proved to be more sensitive and specific than microscopy and changed the picture of malaria epidemiology in Parma, detecting additional cases of malaria undiagnosed by microscopy and allowing speciation of plasmodia in cases misidentified by microscopy. Generally, imported malaria cases reflect the number of immigrants who visit their native countries, in particular, West Africa, explaining the increased prevalence of *P. ovale* cases among non-*P. falciparum* infections in Parma.

15: *Eukaryot Cell*. 2008 May 16.

The Histone Acetyltransferase Inhibitor Anacardic Acid Leads to Changes in Global Gene Expression During *in vitro* Plasmodium falciparum Development.

Cui L, Miao J, Furuya T, Fan Q, Li X, Rathod PK, Su XZ, Cui L.

Department of Entomology, The Pennsylvania State University, 501 ASI Building, University Park, Pennsylvania 16802; Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892; and Bagley Hall 192H, Department of Chemistry, University of Washington, Seattle, Washington 98195.

To better understand the role of histone lysine acetylation in transcription in *Plasmodium falciparum*, we sought to attenuate the histone acetyltransferase (HAT) activity using anacardic acid (AA). We showed that AA reversibly and noncompetitively inhibited the HAT activity of recombinant PfGCN5. To a lesser extent, AA inhibited the PfGCN5 activity in parasite nuclear extracts, but did not affect the histone deacetylase activity. AA blocked the growth of both chloroquine-sensitive and -resistant strains with a 50% inhibitory concentration of approximately 30 μM . Treatment of the parasites with 20 μM of AA for 12 h had no obvious effect on parasite growth or gross morphology, but induced hypoacetylation of histone H3 at K9 and K14, but not H4 at K5, K8, K12, and K16, suggesting inhibition of the PfGCN5 HAT. Microarray analysis showed that this AA treatment resulted in ≥ 2 -fold change in the expression of 271 (approximately 5%) parasite genes in late trophozoites, among which 207 genes were down-regulated. Cluster analysis of gene expression indicated that AA mostly down-regulated active genes, and this gene pool significantly overlapped with that enriched for H3K9 acetylation. We further demonstrated by chromatin immunoprecipitation and real-time polymerase chain reaction that AA treatment reduced acetylation near the putative promoters of a set of down-regulated genes. This study suggests that the parasitocidal effect of AA is at least partially associated with its inhibition of PfGCN5 HAT, resulting in the disturbance of the transcription program in the parasites.

16: *Exp Parasitol*. 2008 Jun;119(2):304-7.

Plasmodium chabaudi: Efficacy of artemisinin+curcumin combination treatment on a clone selected for artemisinin resistance in mice.

Martinelli A, Rodrigues LA, Cravo P.

Centro de Malária e Outras Doenças Tropicais/IHMT/UNL/Unidade de Biologia Molecular, Rua da Junqueira 96, 1349-008 Lisbon, Portugal.

Recent studies have proposed curcumin as a potential partner for artemisinin in artemisinin combination therapies to treat malaria infections. The efficacy of curcumin alone and in combination with artemisinin was evaluated on a clone of *Plasmodium chabaudi* selected for artemisinin resistance *in vivo*. The addition of piperine as an enhancer of curcumin activity was also tested. Results indicated that curcumin, both alone and in combination with piperine had only a modest antimalarial effect and was not able to reverse the artemisinin-resistant phenotype or significantly affect growth of the tested clone when used in combination with artemisinin. This is in contrast with previous *in vivo* work and calls for further experimental evaluation of the antimalarial potential of curcumin.

17: *Exp Parasitol.* 2008 Jun;119(2):272-8.

Counter-regulatory anti-parasite cytokine responses during concurrent *Plasmodium yoelii* and intestinal helminth infections in mice.

Noland GS, Urban JF Jr, Fried B, Kumar N.

Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe Street, Room E5144, Baltimore, MD 21205, USA.

Malaria and helminth infections are two of the most prevalent parasitic diseases globally. While concomitant infection is common, mechanisms contributing to altered disease outcomes during co-infection remain poorly defined. We have previously reported exacerbation of normally non-lethal *Plasmodium yoelii* malaria in BALB/c mice chronically infected with the intestinal trematode *Echinostoma caproni*. The goal of the present studies was to determine the effect of helminth infection on IFN-gamma and other key cytokines during malaria co-infection in the *P. yoelii*-*E. caproni* and *P. yoelii*-*Heligmosomoides polygyrus* model systems. Polyclonally stimulated spleen cells from both *E. caproni*- and *H. polygyrus*-infected mice produced significantly lower amounts of IFN-gamma during *P. yoelii* co-infection than malaria-only infected mice. Furthermore, the magnitude of IFN-gamma suppression was correlated with the relative amounts of IL-4 induced by these helminths (*E. caproni*=low; *H. polygyrus*=high), but not IL-10. Concurrent malaria infection also suppressed helminth-associated IL-4 responses, indicating that immunologic counter-regulation occurs during co-infection with malaria and intestinal helminths.

18: *Infect Immun.* 2008 Jun;76(6):2706-14.

Differences in human antibody reactivity to *Plasmodium falciparum* variant surface antigens are dependent on age and malaria transmission intensity in northeastern Tanzania.

Vestergaard LS, Lusingu JP, Nielsen MA, Mmbando BP, Dodoo D, Akanmori BD, Alifrangis M, Bygbjerg IC, Lemnge MM, Staalsoe T, Hviid L, Theander TG.

Centre for Medical Parasitology at Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), and Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark.
l.vestergaard@cmp.dk

Plasmodium falciparum variant surface antigens (VSA) are involved in the pathogenesis of malaria. Immunoglobulin G (IgG) with specificity for VSA (anti-VSA IgG) is therefore considered important for acquired immunity. To better understand the nature and dynamics of variant-specific IgG responses at population level, we conducted an immunoepidemiological study in nearby communities in northeastern Tanzania, situated at different altitudes and therefore exposed to different levels of *P. falciparum* transmission intensity. Samples of plasma and infected red blood cells (IRBC) were collected from 759 individuals aged 0 to 19 years. Plasma levels of IgG with specificity for VSA expressed by a panel of different parasite isolates were measured by flow cytometry, while the ability of plasma to inhibit IRBC adhesion to CD36 was examined in cellular assays. The level and repertoire of the heterologous anti-VSA IgG response developed dramatically in individuals at 1 to 2 years of age in the high-transmission area, reaching a maximum level at around 10 years of age; only a modest further increase was observed among older children and adults. In contrast, at lower levels of malaria transmission, anti-VSA IgG levels were lower and the repertoire was more narrow, and similar age- and transmission-dependent differences were observed with regard to the ability of the plasma samples to inhibit adhesion of IRBC to CD36. These differences

indicate a strong and dynamic relationship between malaria exposure and functional characteristics of the variant-specific antibody response, which is likely to be important for protection against malaria.

19: *Int J Parasitol.* 2008 Jun;38(7):743-7.

In vitro evaluations of antimalarial drugs and their relevance to clinical outcomes.

Ekland EH, Fidock DA.

Department of Microbiology, College of Physicians and Surgeons at Columbia University, 701 W 168th Street, New York, NY 10032, USA.

Plasmodium falciparum resistance to the former first-line antimalarials chloroquine and sulfadoxine/pyrimethamine has reached critically high levels in many malaria-endemic regions. This has spurred the introduction of several new artemisinin-based combination therapies (ACTs) that display excellent potency in treating drug-resistant malaria. Monitoring for the emergence of drug resistant *P. falciparum* is important for maximising the clinically effective lifespan of ACTs. Here, we provide a commentary on the article by Kaddouri et al., published in this issue of the International Journal of Parasitology, which documents the levels of susceptibility to ACT drugs and chloroquine in *P. falciparum* isolates from Mali. These authors report that some isolates approached a proposed in vitro threshold of resistance to monodesethyl-amodiaquine (the principal effective metabolite of amodiaquine, an important ACT partner drug), and establish baseline levels of susceptibility to the ACT drugs dihydroartemisinin and lumefantrine. The majority of clinical isolates manifested in vitro resistance to chloroquine. The authors also show good concordance between field-based assays employing a non-radioactive lactate dehydrogenase-based method of determining in vitro drug IC(50) values and the well-established [(3)H]hypoxanthine-based radioactive method. This work illustrates a good example of drug resistance surveillance, whose global coordination is being championed by the World Antimalarial Resistance Network. Our current opinion also more generally discusses the complexities inherent to conducting in vitro investigations with *P. falciparum* patient isolates and correlating these findings with treatment outcome data.

20: *Int J Parasitol.* 2008 Jun;38(7):819-27.

Vaccination with a *Plasmodium chabaudi adami* multivalent DNA vaccine cross-protects A/J mice against challenge with *P. c. adami* DK and virulent *Plasmodium chabaudi chabaudi* AS parasites.

Scorza T, Grubb K, Cambos M, Santamaria C, Tshikudi Malu D, Spithill TW.

Department of Biological Sciences, Université du Québec à Montréal, Case postale 8888, Succursale Centre-Ville, Montréal, Quebec, Canada.

A current goal of malaria vaccine research is the development of vaccines that will cross-protect against multiple strains of malaria. In the present study, the breadth of cross-reactivity induced by a 30K multivalent DNA vaccine has been evaluated in susceptible A/J mice (H-2a) against infection with the *Plasmodium chabaudi adami* DK strain and a virulent parasite subspecies, *Plasmodium chabaudi chabaudi* AS. Immunized A/J mice were significantly protected against infection with both *P. c. adami* DK (31-40% reduction in cumulative parasitemia) and *P. c. chabaudi* AS parasites, where a 30-39% reduction in cumulative parasitemia as well as enhanced survival was observed. The 30K vaccine-induced specific IFN-gamma production by splenocytes in response to native antigens from both *P. c. chabaudi* AS and *P. c. adami* DK. Specific antibodies reacting with surface antigens expressed on *P. c. adami* DS and *P. c. chabaudi* AS infected red blood cells, and with opsonizing properties, were detected. These results suggest that multivalent

vaccines encoding conserved antigens can feasibly induce immune cross-reactivity that span Plasmodium strains and subspecies and can protect hosts of distinct major histocompatibility complex haplotypes.

21: *J Ethnopharmacol.* 2008 May 22;117(3):507-511.

Anti-protozoan activities of Harungana madagascariensis stem bark extract on trichomonads and malaria.

Iwalewa EO, Omisore NO, Adewunmi CO, Gbolade AA, Ademowo OG, Nneji C, Agboola OI, Daniyan OM.

Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria; Department of Paraclinical Sciences (Phytomedicine Programme), Faculty of Veterinary Sciences, University of Pretoria, PB 0X4, Onderstepoort 0110, Pretoria, South Africa.

AIM OF THE STUDY: The ethanolic stem bark extract of *Harungana madagascariensis* (Hypericaceae), (Choisy) Poir were evaluated for their activities on *Trichomonas gallinae* (Rivolta) Stabler isolated from the pigeon (*Columba livia*). It was also tested for their anti-malarial activity on N67 *Plasmodium yoelii nigeriensis* (in vivo) in mice and on *Plasmodium falciparum* isolates in vitro. **MATERIALS AND METHODS:** The anti-trichomonal screening was performed in vitro using *Trichomonas gallinae* culture. The minimum lethal concentration (MLC) is the lowest concentration of the test extract in which no motile organisms were observed. The anti-malarial effects were determined in-vivo for suppressive, curative and prophylactic activities in mice receiving a standard inoculum size of 1×10^7 (0.2ml) infected erythrocytes of *Plasmodium yoelii nigeriensis* intraperitoneally, and the in vitro was performed against 3 isolates of *Plasmodium falciparum* in a candle jar procedures. **RESULTS:** The IC(50) of the extract and metronidazole (MDZ) (Flagyl) on *Trichomonas gallinae* at 48h are 187 and 1.56µg/ml. The IC(50) of the extract, chloroquine (CQ) and artemether (ART) on *Plasmodium falciparum* are between 0.052 and 0.517µg/ml for the extract and 0.021 and 0.0412µg/ml for ART and CQ, respectively. The actions of the extract in in vivo study on *Plasmodium yoelii nigeriensis* showed that in both suppressive and prophylactic tests the percentages chemo-suppressive were between 28.6-44.8% and 30.2-78.2% respectively, while only 80mg/kg of the extract reduced the parasitaemia level when compared to the control and the standard drugs in curative test. **CONCLUSIONS:** *Harungana madagascariensis* stem bark extract therefore exhibited significant anti-protozoan effects against *Trichomonas* and *Plasmodium* both in vivo and in vitro.

22: *J Exp Med.* 2008 May 12;205(5):1133-43.

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

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

Tropical Disease Unit, Department of Medicine, McLaughlin-Rotman Centre for Global Health, Toronto General Hospital, Toronto, Ontario M5G 2C4, Canada.

Experimental infection of mice with *Plasmodium berghei* ANKA (PbA) provides a powerful model to define genetic determinants that regulate the development of cerebral malaria (CM). Based on the hypothesis that excessive activation of the complement system may confer susceptibility to CM, we investigated the role of C5/C5a in the development of CM. We show a spectrum of susceptibility to PbA in a panel of inbred mice; all CM-susceptible mice examined were found to be C5 sufficient, whereas all C5-deficient strains were resistant to CM. Transfer of the C5-defective allele from an A/J (CM resistant) onto a C57BL/6 (CM-susceptible) genetic background in a congenic strain conferred increased

resistance to CM; conversely, transfer of the C5-sufficient allele from the C57BL/6 onto the A/J background recapitulated the CM-susceptible phenotype. The role of C5 was further explored in B10.D2 mice, which are identical for all loci other than C5. C5-deficient B10.D2 mice were protected from CM, whereas C5-sufficient B10.D2 mice were susceptible. Antibody blockade of C5a or C5a receptor (C5aR) rescued susceptible mice from CM. In vitro studies showed that C5a-potentiated cytokine secretion induced by the malaria product *P. falciparum* glycosylphosphatidylinositol and C5aR blockade abrogated these amplified responses. These data provide evidence implicating C5/C5a in the pathogenesis of CM.

23: *J Immunol.* 2008 May 15;180(10):6877-84.

Coinfection with nonlethal murine malaria parasites suppresses pathogenesis caused by *Plasmodium berghei* NK65.

Niikura M, Kamiya S, Kita K, Kobayashi F.

Institute of Laboratory Animals, Graduate School of Medicine, Faculty of Medicine, Kyorin University, Tokyo, Japan. mniikura@ks.kyorin-u.ac.jp

Mixed infection with different *Plasmodium* species is often observed in endemic areas, and the infection with benign malaria parasites such as *Plasmodium vivax* or *P. malariae* has been considered to reduce the risk of developing severe pathogenesis caused by *P. falciparum*. However, it is still unknown how disease severity is reduced in hosts during coinfection. In the present study, we investigated the influence of coinfection with nonlethal parasites, *P. berghei* XAT (Pb XAT) or *P. yoelii* 17X (Py 17X), on the outcome of *P. berghei* NK65 (Pb NK65) lethal infection, which caused high levels of parasitemia and severe pathogenesis in mice. We found that the simultaneous infection with nonlethal Pb XAT or Py 17X suppressed high levels of parasitemia, liver injury, and body weight loss caused by Pb NK65 infection, induced high levels of reticulocytopenia, and subsequently prolonged survival of mice. In coinfecting mice, the immune response, including the expansion of B220(int)CD11c(+) cells and CD4(+) T cells and expression of IL-10 mRNA, was comparable to that in nonlethal infection. Moreover, the suppression of liver injury and body weight loss by coinfection was reduced in IL-10(-/-) mice, suggesting that IL-10 plays a role for a reduction of severity by coinfection with nonlethal malaria parasites.

24: *J Infect Dis.* 2008 Jun 3.

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

Clark TD, Greenhouse B, Njama-Meya D, Nzarubara B, Maiteki-Sebuguzi C, Staedke SG, Seto E, Kanya MR, Rosenthal PJ, Dorsey G.

1Department of Medicine, University of California, San Francisco, and 2School of Public Health, University of California, Berkeley; 3Makerere University Medical School, Kampala, Uganda; 4London School of Hygiene and Tropical Medicine, London, United Kingdom.

Background. Malaria risk may be heterogeneous in urban areas of Africa. Identifying those at highest risk for malaria may lead to more targeted approaches to malaria control. Methods. A representative sample of 558 children aged 1-10 years were recruited from a census population in a single parish of Kampala and followed up for 2 years. Malaria was diagnosed when a child presented with a new episode of fever and a thick blood smear positive for parasites. Multivariate analysis was used to identify independent predictors of malaria incidence. Results. A total of 695 episodes of uncomplicated malaria were diagnosed after 901 person years of follow-up. Sick cell trait

(relative risk [RR], 0.68 [95% confidence interval {CI}, 0.52-0.90]), glucose-6-phosphate dehydrogenase deficiency in female children (RR, 0.48 [95% CI, 0.31-0.75]), and use of an insecticide-treated bed net (RR, 0.52 [95% CI, 0.32-0.83]) were associated with a lower risk of malaria. The distance of the subject's residence from a swamp bordering the parish showed a strong "dose-response" relationship; living in the swamp was the strongest predictor of malaria risk (RR, 3.94 [95% CI, 2.61-5.97]). Conclusion. Malaria incidence was highly heterogeneous in this urban cohort of children. Malaria control interventions in urban areas should target populations living in pockets of high malaria risk.

25: *J Infect Dis.* 2008 Jun 1;197(11):1605-1613.

Increased Gametocytemia after Treatment: An Early Parasitological Indicator of Emerging Sulfadoxine-Pyrimethamine Resistance in Falciparum Malaria.

Barnes KI, Little F, Mabuza A, Mngomezulu N, Govere J, Durrheim D, Roper C, Watkins B, White NJ.

1Division of Clinical Pharmacology, Department of Medicine, and 2Department of Statistical Sciences, University of Cape Town, Cape Town, and 3Malaria Control Programme/Communicable Disease Control, Mpumalanga Provincial Department of Health, Nelspruit, South Africa; 4World Health Organization Intercountry Programme for Southern Africa, Harare, Zimbabwe; 5London School of Hygiene and Tropical Medicine, London, and 6Centre for Vaccinology and Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom; and 7Wellcome Trust-Mahidol University Oxford Tropical Medicine Research Programme, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Background. Although malaria treatment aims primarily to eliminate the asexual blood stages that cause illness, reducing the carriage of gametocytes is critical for limiting malaria transmission and the spread of resistance. Methods. Clinical and parasitological responses to the fixed-dose combination of sulfadoxine and pyrimethamine in patients with uncomplicated falciparum malaria were assessed biannually since implementation of this treatment policy in 1998 in Mpumalanga Province, South Africa. Results. Despite sustained cure rates of >90% ([Formula: see text]), the duration of gametocyte carriage increased from 3 to 22 weeks (per 1000 person-weeks) between 1998 and 2002 ([Formula: see text]). The dhfr and dhps mutations associated with sulfadoxine-pyrimethamine resistance were the most important drivers of the increased gametocytemia, although these mutations were not associated with increased pretreatment asexual parasite density or slower asexual parasite clearance times. The geometric mean gametocyte duration and area under the gametocyte density time curve (per 1000 person-weeks) were 7.0 weeks and 60.8 gametocytes/ μ L per week, respectively, among patients with wild-type parasites, compared with 45.4 weeks ([Formula: see text]) and 1212 gametocytes/ μ L per week ([Formula: see text]), respectively, among those with parasites containing 1-5 dhfr/dhps mutations. Conclusions. An increased duration and density of gametocyte carriage after sulfadoxine-pyrimethamine treatment was an early indicator of drug resistance. This increased gametocytemia among patients who have primary infections with drug-resistant Plasmodium falciparum fuels the spread of resistance even before treatment failure rates increase significantly.

26: *J Infect Dis.* 2008 Jun 1;197(11):1598-1604.

Emergence of a dhfr Mutation Conferring High-Level Drug Resistance in Plasmodium falciparum Populations from Southwest Uganda.

Lynch C, Pearce R, Pota H, Cox J, Abeku TA, Rwakimari J, Naidoo I, Tibenderana J, Roper C.

1Disease Control and Vector Biology Unit and 2Pathogen Molecular Biology Unit, Department of Infectious Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom; 3National Malaria Control Programme, Ministry of Health of Uganda, Kampala, Uganda; and 4Malaria Lead Programme, Medical Research Council, Durban, South Africa.

The S108N, C59R, and N51I mutations in the Plasmodium falciparum gene that encodes dihydrofolate reductase, dhfr, confer resistance to pyrimethamine and are common in Africa. However, the I164L mutation, which confers high-level resistance, is rarely seen. We found a 14% prevalence of the I164L mutation among a sample of 51 patients with malaria in Kabale District in southwest Uganda in 2005 and a 4% prevalence among 72 patients with malaria in the neighboring district of Rukungiri during the same year. Surveillance at 6 sites across Uganda during 2002-2004 reported a single case of infection involving an I164L mutant, also in the southwest, suggesting that this is a regional hot spot. The spatial clustering and increasing prevalence of the I164L mutation is indicative of local transmission of the mutant. Targeted surveillance is needed to confirm the extent of the spread of the I164L mutation and to monitor the impact of I164L on the efficacy of antifolates for intermittent preventive treatment of pregnant women and/or infants with falciparum malaria.

27: *J Infect Dis.* 2008 May 23.

Decreased Susceptibility to Plasmodium falciparum Infection in Pregnant Women with Iron Deficiency.

Kabyemela ER, Fried M, Kurtis JD, Mutabingwa TK, Duffy PE.

1Mother Offspring Malaria Studies Project, Seattle Biomedical Research Institute, and 2University of Washington, Seattle, Washington; 3Center for International Health Research, Rhode Island Hospital, Brown University, Providence, Rhode Island; and 4Muheza Designated District Hospital, Muheza, 5Tumaini University, Moshi, and 6National Institute for Medical Research, Dar es Salaam, Tanzania.

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

Antibodies to Plasmodium falciparum and Plasmodium vivax Merozoite Surface Protein 5 in Indonesia: Species-Specific and Cross-Reactive Responses.

Woodberry T, Minigo G, Piera KA, Hanley JC, Silva HD, Salwati E, Kenangalem E, Tjitra E, Coppel RL, Price RN, Anstey NM, Plebanski M.

1International Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, and Departments of 2Immunology and 3Microbiology, Monash University, Victoria, Australia; 4National Institute of Health Research and Development (NIHRD), Ministry of Health, Jakarta, and 5NIHRD-Menzies Research Program and District Health Authority, Timika, Papua, Indonesia; 6Centre for Vaccinology and Tropical Medicine, Nuffield Department of Clinical Medicine, Churchill Hospital, Oxford, United Kingdom.

Background. Merozoite surface protein (MSP) 5 is a candidate antigen for a malaria vaccine. In cross-sectional and longitudinal studies, we measured MSP5 antibody responses in Papuans with acute *Plasmodium falciparum* malaria, *Plasmodium vivax* malaria, and mixed *P. falciparum* and *P. vivax* malaria and in those with past exposure. **Methods.** Enzyme-linked immunosorbent assay (ELISA) was used to quantitate antibody responses to *P. falciparum* MSP5 (PfMSP5) and *P. vivax* MSP5 (PvMSP5) in 82 subjects with *P. falciparum* infection, 86 subjects with *P. vivax* infection, 85 subjects with mixed infection, and 87 asymptomatic individuals. Longitudinal responses through day 28 were tested in 20 persons. Cross-reactivity was tested by competition ELISA. **Results.** PfMSP5 or PvMSP5 immunoglobulin (Ig) G was detected in 39%-52% of subjects, and IgM was detected in 44%-72%. IgG responses were distributed equally between IgG(3) and IgG(1) for PfMSP5 but were predominantly IgG(3) for PvMSP5. Although IgG responses were generally specific for PfMSP5 or PvMSP5, cross-species reactivity was found in 7 of 107 dual-positive responders. No significant difference was seen in the magnitude, frequency, or subclass of PfMSP5 or PvMSP5 IgG antibodies between groups. There was no significant association between antibody responses and therapeutic response. **Conclusion.** PfMSP5 and PvMSP5 were frequently recognized by short-lived, species-specific antibodies. Although infrequent, the cross-reactive MSP5 antibodies indicate that an appropriately formulated vaccine may elicit and/or enhance cross-species recognition, which may be very useful in areas where both parasites are endemic.

29: *J Trop Pediatr.* 2008 Jun;54(3):184-91.

In-hospital risk estimation in children with malaria--early predictors of morbidity and mortality.

Winkler AS, Salmhofer G, Helbok R, Kalokola F, Matuja W, Schmutzhard E.

Department of Neurology, University of Ulm, Ulm, Germany. drawinkler@yahoo.com.au

BACKGROUND: Rapid diagnosis and adequate therapy are crucial to prevent development of severe disease and death in children suffering from malaria. A reliable but easy system for disease severity assessment would help to fast track seriously ill children and provide suitable therapies for different patient groups. **OBJECTIVES:** To examine risk factors and appropriate scoring systems in children suffering from malaria for outcome in terms of morbidity and mortality. **Methods:** A prospective, consecutive study in children admitted to the Muhimbili Medical Centre in Dar es Salaam was conducted to evaluate risk factors and test appropriate scoring systems. The simplified Multi-Organ Dysfunction Score (sMODS), a severity of disease classification consisting mainly of clinical data, was applied. Chosen outcome parameters were morbidity and mortality. Results were compared to those obtained from the World Health Organisation (WHO) classification of severe malaria, the Blantyre Coma Scale (BCS) and selected

single clinical parameters. RESULTS: Seventy-five children were recruited into the study. Mean age was 28 months ranging from 6 months to 8 years. 'Severe Malaria', according to WHO criteria was evident in 57 patients (76%). Mean sMODS on admission was 15.6 +/- 2. Seven patients (9%) died. Among single symptoms, impaired consciousness and respiratory distress predicted both, fatal outcome and morbidity. In terms of scoring systems, the sMODS correlated with both outcome parameters. In comparison, the WHO criteria did not correlate with any of the two parameters, the BCS correlated with mortality only. CONCLUSION: In our study, sMODS has been shown to represent a useful quantitative approach towards disease severity classification in resource poor settings and can be used for risk estimation in children suffering from malaria in terms of both morbidity and mortality.

30: *Lancet Infect Dis.* 2008 Jun;8(6):369-378.

Measuring malaria endemicity from intense to interrupted transmission.

Hay SI, Smith DL, Snow RW.

Malaria Public Health and Epidemiology Group, Centre for Geographic Medicine, Kenya Medical Research Institute/University of Oxford/Wellcome Trust Collaborative Programme, Nairobi, Kenya; Spatial Ecology and Epidemiology Group, Tinbergen Building, Department of Zoology, University of Oxford, Oxford, UK.

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

31: *Lancet Infect Dis.* 2008 Jun;8(6):387-9.

Lessons from the past: managing insecticide resistance in malaria control and eradication programmes.

Kelly-Hope L, Ranson H, Hemingway J.

Vector Group, Liverpool School of Tropical Medicine, Liverpool, UK.

The distribution of insecticide-treated bednets to help combat the burden of malaria in sub-Saharan Africa has accelerated in the past 5 years. Additionally, many countries are also considering, or have already begun, indoor residual spraying campaigns. These are positive developments, since vector control has repeatedly proven to be an effective means of reducing malaria transmission. However, the sustainability of these insecticide-based interventions relies on the continuing susceptibility of the anopheles vectors to the limited number of available insecticides. Continual monitoring for early signs of insecticide resistance and the adoption of carefully considered resistance management strategies are therefore required. Regrettably, this essential monitoring component is frequently given a low priority in the push to meet ambitious coverage targets. We outline the key requirements for establishing an insecticide resistance surveillance system and urge all those involved in malaria vector control, either directly or as facilitators, to ensure that these measures are incorporated into control programmes. Failure to act now will inevitably lead to a future breakdown in disease control and jeopardise hopes of eradicating this major public-health problem.

32: *Malar J.* 2008 Jun 2;7(1):100.

Malaria transmission pattern resilience to climatic variability is mediated by insecticide-treated nets.

Chaves LF, Kaneko A, Taleo G, Pascual M, Wilson ML.

ABSTRACT: **BACKGROUND:** Malaria is an important public-health problem in the archipelago of Vanuatu and climate has been hypothesized as important influence on transmission risk. Beginning in 1988, a major intervention using insecticide-treated bed nets (ITNs) was implemented in the country in an attempt to reduce Plasmodium transmission. To date, no study has addressed the impact of ITN intervention in Vanuatu, how it may have modified the burden of disease, and whether there were any changes in malaria incidence that might be related to climatic drivers. **Methods and findings** Monthly time series (January 1983 through December 1999) of confirmed Plasmodium falciparum and Plasmodium vivax infections in the archipelago were analysed. During this 17 year period, malaria dynamics underwent a major regime shift around May 1991, following the introduction of bed nets as a control strategy in the country. By February of 1994 disease incidence from both parasites was reduced by at least 50%, when at most 20% of the population at risk was covered by ITNs. Seasonal cycles, as expected, were strongly correlated with temperature patterns, while inter-annual cycles were associated with changes in precipitation. Following the bed net intervention, the influence of environmental drivers of malaria dynamics was reduced by 30-80% for climatic forces, and 33-54 % for other factors. A time lag of about five months was observed for the qualitative change ("regime shift") between the two parasites, the change occurring first for P. falciparum. The latter might be explained by interspecific interactions between the two parasites within the human hosts and their distinct biology, since P. vivax can relapse after a primary infection. **CONCLUSION:** The Vanuatu ITN programme represents an excellent example of implementing an infectious disease control programme. The distribution was undertaken to cover a large, local proportion (~80%) of people in villages where malaria was present. The successful coverage was possible because of the strategy for distribution of ITNs by prioritizing the free distribution to groups with restricted means for their acquisition, making the access to this resource equitable across the population. These results emphasize the need to implement infectious disease control programmes focusing on the most vulnerable populations.

33: *Malar J.* 2008 Jun 2;7(1):99.

Longitudinal study of Plasmodium falciparum and Plasmodium vivax in a Karen population in Thailand.

Phimpraphi W, Paul RE, Yimsamran S, Puangsa-Art S, Thanyavanich N, Maneeboonyang W, Prommongkol S, Sornklom S, Chaimungkun W, Chavez IF, Blanc H, Looareesuwan S, Sakuntabhai A, Singhasivanon P.

ABSTRACT: **BACKGROUND:** Clinical case treatment of malaria infections where Plasmodium falciparum and Plasmodium vivax are sympatric has achieved effective reductions in P. falciparum prevalence and incidence rates, but has been less successful for P. vivax. The high transmissibility of P. vivax and its capacity to relapse have been suggested to make it a harder parasite species to control. **METHODS:** A clinical malaria case treatment programme was carried out over a decade in a Karen community composed of seven hamlets on the Thai-Myanmar border. **RESULTS:** From 1994 to 2004, prevalence rates of both P. falciparum and P. vivax decreased by 70-90% in six of the seven study hamlets, but were unchanged in one hamlet. Overall, incidence rates decreased by 72% and 76% for P. falciparum and P. vivax respectively over the period 1999-2004. The age-incidence and prevalence curves suggested that P. vivax was more transmissible than P. falciparum despite

a greater overall burden of infection with *P. falciparum*. Male gender was associated with increased risk of clinical presentation with either parasite species. Children (<15 years old) had an increased risk of presenting with *P. vivax* but not *P. falciparum*. CONCLUSIONS: There was a considerable reduction in incidence rates of both *P. vivax* and *P. falciparum* over a decade following implementation of a case treatment programme. The concern that intervention methods would inadvertently favour one species over another, or even lead to an increase in one parasite species, does not appear to be fulfilled in this case.

34: *Malar J.* 2008 May 30;7(1):97.

Acute pancreatitis and subdural haematoma in a patient with severe falciparum malaria. Case report and review of literature.

Seshadri P, Dev AV, Viggewarpu S, Sathyendra S, Peter JV.

ABSTRACT: Plasmodium falciparum infection is known to be associated with a spectrum of systemic complications ranging from mild and self-limiting to life-threatening. This case report illustrates a patient who had a protracted course in hospital due to several rare complications of falciparum malaria. A 21-year old man presented with a five-day history of high-grade fever, jaundice and abdominal pain and a two-day history of altered conscious state. A diagnosis of severe falciparum malaria was made based on the clinical presentation and a positive blood smear with parasitaemia of 45%. Despite adequate anti-malarial therapy with artesunate, the patient had persistent and worsening abdominal pain. Investigations suggested a diagnosis of acute pancreatitis, a rare association with falciparum malaria. However, in spite of supportive therapy for acute pancreatitis and a 10-day course of intravenous artesunate and oral doxycycline at recommended doses, he continued to be febrile with peripheral blood smear showing persistence of ring forms. Antimalarial therapy was, therefore, changed to quinine on the suspicion of possible artesunate resistance. On the 17th day of stay in hospital, the patient developed generalized tonic-clonic seizures. Computerized tomography of the brain showed bilateral fronto-parietal subdural haematomas that were surgically drained. His fever persisted beyond 30-days despite broad-spectrum antibiotics, quinine therapy and negative malarial smears. A possibility of drug fever was considered and all drugs were ceased. He subsequently became afebrile and was discharged on the 38th hospital admission day. Recognition of complications and appropriate management at each stage facilitated successful outcome. This report has been presented to highlight the occurrence of several rare complications of falciparum malaria in the same patient.

35: *Malar J.* 2008 May 29;7(1):96.

Access to artemisinin combination therapy for malaria in remote areas of Cambodia.

Yeung S, Van Damme W, Socheat D, White NJ, Mills A.

ABSTRACT: BACKGROUND: Malaria-endemic countries are switching antimalarial drug policy to artemisinin combination therapies (ACTs) and the global community are considering the setting up of a global subsidy mechanism in order to make them accessible and affordable. However, specific interventions may be needed to reach remote at-risk communities and to ensure that they are used appropriately. This analysis documents the coverage with ACTs versus artemisinin monotherapies, the effectiveness of malaria outreach teams (MOTs) and Village Malaria Workers (VMWs) in increasing access to appropriate diagnosis and treatment with ACTs in Cambodia, the first country to switch national antimalarial drug policy to an ACT of artesunate and mefloquine (A+M) in 2000. METHODS: A cross-sectional survey was carried out in three different types of intervention area: with VMWs, MOTs and no specific interventions. Individuals with a history of fever in the last three

weeks were included in the study and completed a questionnaire on their treatment seeking and drug usage behaviour. Blood was taken for a rapid diagnostic test (RDT) and data on the households socio-economic status were also obtained. RESULTS: In areas without specific interventions, only 17% (42/251) of respondents received a biological diagnosis, 8% (17/206) of respondents who received modern drug did so from a public health facility, and only 8% of them (17/210) received A+M. Worryingly, 78% (102/131) of all artemisinin use in these areas was as a monotherapy. However, both the VMW scheme and MOT scheme significantly increased the likelihood of being seen by a trained provider (Adjusted Odds Ratios (AOR) of 148 and 4 respectively) and of receiving A+M (AORs of 2.7 and 7.7 respectively). CONCLUSION: The coverage rates of appropriate diagnosis and treatment of malaria were disappointingly low and the use of artemisinin monotherapy alarmingly high. This reflects the fragmented nature of Cambodia's health system in remote areas and the reliance placed by these communities on informal vendors from whom artemisinin monotherapies are widely available. However VMWs in particular are an effective means of improving access to malaria diagnosis and treatment. The VMW scheme and the social marketing of RDTs and blister-packaged artesunate and mefloquine in the private sector have both been scaled up nationally. Case management in the public sector has also reportedly improved. Given recent concerns regarding the development of artemisinin drug resistance on the Thai-Cambodia border, the effectiveness of these measures in reducing the use of artemisinin monotherapy needs to be urgently re-evaluated.

36: *Malar J.* 2008 May 27;7(1):94.

Plasmodium vivax trophozoites insensitive to chloroquine.

Sharrock WW, Suwanarusk R, Lek-Uthai U, Edstein MD, Kosiavasee V, Travers T, Jaidee A, Sriprawat K, Price RN, Nosten F, Russell B.

ABSTRACT: BACKGROUND: *Plasmodium vivax* is a major cause of malaria and is still primarily treated with chloroquine. Chloroquine inhibits the polymerization of haem to inert haemozoin. Free haem monomers are thought to catalyze oxidative damage to the *Plasmodium* spp. trophozoite, the stage when haemoglobin catabolism is maximal. However preliminary in vitro observations on *P. vivax* clinical isolates suggest that only ring stages (early trophozoites) are sensitive to chloroquine. In this study, the stage specific action of chloroquine was investigated in synchronous cryopreserved isolates of *P. vivax*. METHODS: The in vitro chloroquine sensitivity of paired ring and trophozoite stages from 11 cryopreserved *P. vivax* clinical isolates from Thailand and two *Plasmodium falciparum* clones (chloroquine resistant K1 and chloroquine sensitive FC27) was measured using a modified WHO microtest method and fluorometric SYBR Green I Assay. The time each stage was exposed to chloroquine treatment was controlled by washing the chloroquine off at 20 hours after the beginning of treatment. RESULTS: *Plasmodium vivax* isolates added to the assay at ring stage had significantly lower median IC50s to chloroquine than the same isolates added at trophozoite stage (median IC50 12 nM vs 415nM p<0.01). Although only 36% (4/11) of the SYBR Green I assays for *P. vivax* were successful, both microscopy and SYBR Green I assays indicated that only *P. vivax* trophozoites were able to develop to schizonts at chloroquine concentrations above 100nM. CONCLUSIONS: Data from this study confirms the diminished sensitivity of *P. vivax* trophozoites to chloroquine, the stage thought to be the target of this drug. These results raise important questions about the pharmacodynamic action of chloroquine, and highlight a fundamental difference in the activity of chloroquine between *P. vivax* and *P. falciparum*.

37: *Malar J.* 2008 May 26;7(1):92.

Reduced susceptibility to pyrethroid insecticide-treated nets by the malaria vector *Anopheles gambiae* s.l. in western Uganda.

John R, Ephraim T, Andrew A.

ABSTRACT: **BACKGROUND:** Pyrethroid insecticide-treated mosquito nets are massively being scaled-up for malaria prevention particularly in children under five years of age and pregnant mothers in sub-Saharan Africa. However, there is serious concern of the likely evolution of widespread pyrethroid resistance in the malaria vector *Anopheles gambiae* s.l. due to the extensive use of pyrethroid insecticide-treated mosquito nets. The purpose of this study was to ascertain the status of pyrethroid resistance in *An. gambiae* s.l. in western Uganda. **METHODS:** Wild mosquitoes (1-2 days old) were exposed in 10 replicates to new nets impregnated with K-othrine (Deltamethrin 25mg/m²), Solfac EW50 (Cyfluthrin 50mg/m²) and Fendona 6SC (Cypermethrin 50mg/m²) and observed under normal room temperature and humidity (Temperature 24.8oC -27.4 oC, Humidity 65.9-45.7). A similar set of mosquitoes collected from the control area 80 km away were exposed to a deltamethrin 25mg/m² impregnated net at the same time and under the same conditions. The 10-year mean KDT50 and mortality rates for each of the three pyrethroid insecticides were compared using the Student t-test. **RESULTS:** A significant increase in the mean knockdown time (KDT50) and mean mortality rate were observed in almost all cases an indication of reduced susceptibility. The overall results showed a four-fold increase in the mean knockdown time (KDT50) and 1.5-fold decrease in mortality rate across the three pyrethroid insecticides. There was a significant difference in the 10-year mean KDT50 between deltamethrin and cyfluthrin; deltamethrin and cypermethrin, but no significant difference between cyfluthrin and cypermethrin. The 10-year mean KDT50 for mosquitoes exposed to deltamethrin from the control site was significantly different from that of mosquitoes from the intervention site (p<0.05, t=3.979, 9df). The 10-year mean difference in mortality rate between deltamethrin (84.64%) ; cyfluthrin (74.18%) ;cypermethrin (72.19%) and the control (90.45%) showed a significant decline in mortality across all the three insecticides. **CONCLUSION:** Generally the results showed a trend of increase in mosquito resistance status with cross-resistance against all the three pyrethroid insecticides. This study reveals for the first time the development of pyrethroid resistance in *An. gambiae* s.l. in Western Uganda. It is therefore strongly recommended that the impact of this development on malaria control efforts be closely monitored and alternative fabric treatments be considered before this problem curtails community wide implementation of this malaria control strategy in Uganda.

38: *Malar J.* 2008 May 26;7:91.

Antipyretic effect of ibuprofen in Gabonese children with uncomplicated falciparum malaria: a randomized, double-blind, placebo-controlled trial.

Matsiégui PB, Missinou MA, Necek M, Mavoungou E, Issifou S, Lell B, Kremsner PG.

Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon.
matsiegui@yahoo.fr

BACKGROUND: Antipyretic drugs are widely used in children with fever, though there is a controversy about the benefit of reducing fever in children with malaria. In order to assess the effect of ibuprofen on fever compared to placebo in children with uncomplicated *Plasmodium falciparum* malaria in Gabon, a randomized double blind placebo controlled trial, was designed. **METHODS:** Fifty children between two and seven years of age with uncomplicated malaria were included in the study. For the treatment of fever, all patients "received" mechanical treatment when the temperature rose above 37.5 degrees C. In addition to the mechanical treatment, continuous fanning and cooling blanket, patients

were assigned randomly to receive ibuprofen (7 mg/kg body weight, every eight hours) or placebo. RESULTS: The fever clearance time using a fever threshold of 37.5 degrees C was similar in children receiving ibuprofen compared to those receiving placebo. The difference was also not statistically significant using a fever threshold of 37.8 degrees C or 38.0 degrees C. However, the fever time and the area under the fever curve were significantly smaller in the ibuprofen group compared to the placebo group. CONCLUSION: Ibuprofen is effective in reducing the time with fever. The effect on fever clearance is less obvious and depends on definition of the fever threshold. TRIAL REGISTRATION: The trial registration number is: NCT00167713.

39: *Malar J.* 2008 May 23;7(1):90.

A structural annotation resource for the selection of putative target proteins in the malaria parasite.

Joubert Y, Joubert F.

ABSTRACT: BACKGROUND: Protein structure plays a pivotal role in elucidating mechanisms of parasite functioning and drug resistance. Moreover, protein structure aids the determination of protein function, which can together with the structure be used to identify novel drug targets in the parasite. However, various structural features in Plasmodium falciparum proteins complicate the experimental determination of protein structures. Limited similarity to proteins in the Protein Data Bank and the shortage of solved protein structures in the malaria parasite necessitate genome-scale structural annotation of P. falciparum proteins. Additionally, the annotation of a range of structural features facilitates the identification of suitable targets for experimental and computational studies. METHODS: An integrated structural annotation system was developed and applied to P. falciparum, Plasmodium vivax and Plasmodium yoelii. The annotation included searches for sequence similarity, patterns and domains in addition to the following predictions: secondary structure, transmembrane helices, protein disorder, low complexity, coiled-coils and small molecule interactions. Subsequently, candidate proteins for further structural studies were identified based on the annotated structural features. RESULTS: The annotation results are accessible through a web interface, enabling users to select groups of proteins which fulfil multiple criteria pertaining to structural and functional features. Analysis of features in the P. falciparum proteome showed that protein-interacting proteins contained a higher percentage of predicted disordered residues than non-interacting proteins. Proteins interacting with 10 or more proteins have a disordered content concentrated in the range of 60-100%, while the disorder distribution for proteins having only one interacting partner, was more evenly spread. CONCLUSIONS: A series of P. falciparum protein targets for experimental structure determination, comparative modelling and in silico docking studies were putatively identified. The system is available for public use, where researchers may identify proteins by querying with multiple physico-chemical, sequence similarity and interaction features.

40: *Malar J.* 2008 May 22;7(1):89.

Performance and usefulness of the Hexagon rapid diagnostic test in children with asymptomatic malaria living in the Mount Cameroon region.

Wanji S, Kimbi HK, Eyong JE, Tendongfor N, Ndamukong JL.

ABSTRACT: BACKGROUND: Rapid and correct diagnosis of malaria is considered an important strategy in the control of the disease. However, it remains to be determined how well these tests can perform in those who harbour the parasite, but are asymptomatic, so that rapid diagnostic tests (RDTs) could be used in rapid mass surveillance in malaria control programmes. METHODS: Microscopic and immunochromatographic diagnosis of malaria were performed on blood samples from

the hyperendemic Mount Cameroon region. Thin and thick blood films were stained with Giemsa and examined under light microscopy for malaria parasites. The RDT was performed on the blood samples for the detection of Plasmodium species. In addition, the performance characteristics of the test were determined using microscopy as gold standard. RESULTS: Results revealed 40.32% to be positive for microscopy and 34.41% to be positive for the RDT. Parasites were detected in a greater proportion of samples as the parasite density increase. Plasmodium falciparum was the predominant Plasmodium species detected in the study population either by microscopy or by the RDT. Overall, the test recorded a sensitivity and specificity of 85.33% and 95.05% respectively, and an accuracy of 91.40%. The sensitivity and specificity of the RDT increased as parasite densities increased. CONCLUSION: The Hexagon Malaria Combi™ test showed a high sensitivity and specificity in diagnosing malaria in asymptomatic subjects and so could be suitable for use in mass surveillance programmes for the management and control of malaria.

41: *Malar J.* 2008 May 21;7(1):88.

Interactions between dendritic cells and CD4+ T cells during Plasmodium infection.

Ocana-Morgner C, Wong KA, Rodriguez A.

ABSTRACT: BACKGROUND: During infection, dendritic cells (DCs) encounter pathogenic microorganisms that can modulate their function and shape the T cell responses generated. During the process of T cell activation, DCs normally establish strong, long-lasting interactions with naive T cells. METHODS: Using a mouse malaria model, the interactions of DCs and naive CD4+ T cells have been analysed. RESULTS: DCs, either incubated in vitro with infected erythrocytes or isolated from infected mice, are able to present exogenous antigens by MHC-II, but are not able to establish prolonged effective interactions with naive CD4+ T cells and do not induce T cell activation. It was also found that effective T cell activation of naive CD4+ T cells is impaired during late Plasmodium yoelii infection. CONCLUSION: These data may provide a mechanism for the lack of effective adaptive immune responses induced by the Plasmodium parasite.

42: *Malar J.* 2008 May 21;7:87.

Escalating Plasmodium falciparum antifolate drug resistance mutations in Macha, rural Zambia.

Mkulama MA, Chishimba S, Sikalima J, Rouse P, Thuma PE, Mharakurwa S.

The Malaria Institute at Macha, Namwala Road, Choma, Zambia.

mtawa.mkulama@macha.org.zm

BACKGROUND: In Zambia the first-line treatment for uncomplicated malaria is artemisinin combination therapy (ACT), with artemether-lumefantrine currently being used. However, the antifolate regimen, sulphadoxine-pyrimethamine (SP), remains the treatment of choice in children weighing less than 5 kg and also in expectant mothers. SP is also the choice drug for intermittent preventive therapy in pregnancy and serves as stand-by treatment during ACT stock outs. The current study assessed the status of Plasmodium falciparum point mutations associated with antifolate drug resistance in the area around Macha. METHODS: A representative sample of 2,780 residents from the vicinity of Macha was screened for malaria by microscopy. At the same time, blood was collected onto filter paper and dried for subsequent P. falciparum DNA analysis. From 188 (6.8%) individuals that were thick film-positive, a simple random sub-set of 95 P. falciparum infections were genotyped for DHFR and DHPS antifolate resistance mutations, using nested PCR and allele-specific restriction enzyme digestion. RESULTS: Plasmodium falciparum field samples exhibited a high prevalence of

antifolate resistance mutations, including the DHFR triple (Asn-108 + Arg-59 + Ile-51) mutant (41.3%) and DHPS double (Gly-437 + Glu-540) mutant (16%). The quintuple (DHFR triple + DHPS double) mutant was found in 4 (6.5%) of the samples. Levels of mutated parasites showed a dramatic escalation, relative to previous surveys since 1988. However, neither of the Val-16 and Thr-108 mutations, which jointly confer resistance to cycloguanil, was detectable among the human infections. The Leu-164 mutation, associated with high grade resistance to both pyrimethamine and cycloguanil, as a multiple mutant with Asn-108, Arg-59 and (or) Ile-51, was also absent. CONCLUSION: This study points to escalating levels of *P. falciparum* antifolate resistance in the vicinity of Macha. Continued monitoring is recommended to ensure timely policy revisions before widespread resistance exacts a serious public health toll.

43: *Malar J.* 2008 May 20;7:86.

A regulatable transgene expression system for cultured *Plasmodium falciparum* parasites.

Epp C, Raskolnikov D, Deitsch KW.

Department of Microbiology and Immunology, Weill Medical College of Cornell University, 1300 York Avenue, W-704, Box 62, New York, NY 10021, USA.
christian.epp@med.uni-heidelberg.de

BACKGROUND: The ability to transfect and create transgenic cultured malaria parasites has transformed the study of *Plasmodium falciparum* over the last decade. With the completion of the annotated genome sequence, the process of gene discovery now routinely includes gene knockouts, over-expression and complementation analysis. However, while this technology has proven extremely valuable, significant limitations exist. In particular, *P. falciparum* DNA is often unstable and difficult to clone because of its AT-rich, repetitive nature. As a result, transgene expression constructs can be difficult to assemble due to the need to include two expression cassettes on a single plasmid, one to drive expression of the transgene of interest and a second for expression of the selectable marker. In addition, transgene expression levels are usually not regulatable, making it difficult to assess phenotypes that are sensitive to the amount of protein expressed. RESULTS: A plasmid based system for transgene expression is described that uses a single, bidirectional promoter to drive expression of both the transgene and the selectable marker, thus greatly reducing the size of the construct and enhancing stability. Further, by altering the concentration of drug used for selection, it is possible to modulate the copy number of the concatameric episomes and thereby regulate the expression level of the transgene through a range greater than 10 fold. CONCLUSION: The transgene expression system described here should prove useful for both routine protein over-expression and complementation experiments as well as for experiments in which precisely manipulating the expression level of candidate proteins is desirable. This should provide an additional level of precision to the tools used to study the molecular biology of malaria parasites.

44: *Malar J.* 2008 May 20;7:85.

Retention and efficacy of long-lasting insecticide-treated nets distributed in eastern Sudan: a two-step community-based study.

Hassan Sel-D, Malik EM, Okoued SI, Eltayeb EM.

Emergency and Humanitarian Action Directorate, Federal Ministry of Health, Khartoum, Sudan. saadhussein@gmail.com

BACKGROUND: In order to assess the effectiveness of long-lasting insecticide-treated nets (LLINs) as a method for malaria control, there is a need

to determine how high is the retention of bed nets, how they are utilized, and how efficacious they are against the mosquitoes that transmit the disease. This is especially important in case of Sudan after emergence of resistance to pyrethroids in use. METHODS: This two-step study aimed to assess the retention and efficacy of LLINs (Olysettrade mark) distributed in the year 2006 in Kassala district in eastern Sudan. In the first step, using a cluster sample technique, heads of 210 households (30 by 7) were interviewed, and six LLINs were collected and later tested for efficacy. In the second step, eight focus group discussion sessions were conducted to complement the results from the first step. RESULTS: Results showed that the retention of LLINs was 92.9% one-and-half years after distribution. Some bed nets were distributed against a price. Utilization of bed nets by children under five years of age and by pregnant women was found to be 55% and 42.1% respectively. For the bioassay efficacy tests, mean knock down after 60 minutes was 91.1%, while mortality after 24 hours was 99.4%. CONCLUSION: LLINs (Olysettrade mark) were efficacious at the time of the study. People appreciated the usefulness but were not fully aware of their importance and were not motivated enough to use them. The retention of the bed nets was quite high but the utilization of the nets needs more focus from the National Malaria Control Programme. Bed net distribution activities should be accompanied by wide health education campaigns and followed up with tracking surveys to evaluate their effectiveness.

45: *Malar J.* 2008 May 20;7(1):84.

Cost of increasing access to artemisinin combination therapy: the Cambodian experience.

Yeung S, Van Damme W, Socheat D, White NJ, Mills A.

ABSTRACT: BACKGROUND: Malaria-endemic countries are switching antimalarial drug policy from cheap ineffective monotherapies to artemisinin combination therapies (ACTs) for the treatment of Plasmodium falciparum malaria and the global community are considering setting up a global subsidy to fund their purchase. However, in order to ensure that ACTs are correctly used and are accessible to the poor and remote communities who need them, specific interventions will be necessary and the additional costs need to be considered. METHODS: This paper presents an incremental cost analysis of some of these interventions in Cambodia, the first country to change national antimalarial drug policy to an ACT of artesunate and mefloquine. These costs include the cost of rapid diagnostic tests (RDTs), the cost of blister-packaging the drugs locally and the costs of increasing access to diagnosis and treatment to remote communities through malaria outreach teams (MOTs) and Village Malaria Workers (VMW). RESULTS: At optimum productive capacity, the cost of blister-packaging cost under \$0.20 per package but in reality was significantly more than this because of the low rate of production. The annual fixed cost (exclusive of RDTs and drugs) per capita of the MOT and VMW schemes was \$0.44 and \$0.69 respectively. However because the VMW scheme achieved a higher rate of coverage than the MOT scheme, the cost per patient treated was substantially lower at \$5.14 compared to \$12.74 per falciparum malaria patient treated. The annual cost per capita inclusive of the RDTs and drugs was \$19.31 for the MOT scheme and \$11.28 for the VMW scheme given similar RDT positivity rates of around 22% and good provider compliance to test results. CONCLUSION: In addition to the cost of ACTs themselves, substantial additional investments are required in order to ensure that they reach the targeted population via appropriate delivery systems and to ensure that they are used appropriately. In addition, differences in local conditions, in particular the prevalence of malaria and the pre-existing infrastructure, need to be considered in choosing appropriate diagnostic and delivery strategies.

46: *Malar J.* 2008 May 19;7:83.

Plasma IP-10, apoptotic and angiogenic factors associated with fatal cerebral malaria in India.

Jain V, Armah HB, Tongren JE, Ned RM, Wilson NO, Crawford S, Joel PK, Singh MP, Nagpal AC, Dash AP, Udhayakumar V, Singh N, Stiles JK.

National Institute of Malaria Research (ICMR), Jabalpur, India.

vidhanjain78@yahoo.com

BACKGROUND: Plasmodium falciparum in a subset of patients can lead to cerebral malaria (CM), a major contributor to malaria-associated mortality. Despite treatment, CM mortality can be as high as 30%, while 10% of survivors of the disease may experience short- and long-term neurological complications. The pathogenesis of CM is mediated by alterations in cytokine and chemokine homeostasis, inflammation as well as vascular injury and repair processes although their roles are not fully understood. The hypothesis for this study is that CM-induced changes in inflammatory, apoptotic and angiogenic factors mediate severity of CM and that their identification will enable development of new prognostic markers and adjunctive therapies for preventing CM mortalities. **METHODS:** Plasma samples (133) were obtained from healthy controls (HC, 25), mild malaria (MM, 48), cerebral malaria survivors (CMS, 48), and cerebral malaria non-survivors (CMNS, 12) at admission to the hospital in Jabalpur, India. Plasma levels of 30 biomarkers ((IL-1beta, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, Eotaxin, FGF basic protein, G-CSF, GM-CSF, IFN-gamma, IP-10, MCP-1 (MCAF), MIP-1alpha, MIP-1beta, RANTES, TNF-alpha, Fas-ligand (Fas-L), soluble Fas (sFas), soluble TNF receptor 1 (sTNF-R1) and soluble TNF receptor 2 (sTNFR-2), PDGF bb and VEGF)) were simultaneously measured in an initial subset of ten samples from each group. Only those biomarkers which showed significant differences in the pilot analysis were chosen for testing on all remaining samples. The results were then compared between the four groups to determine their role in CM severity. **RESULTS:** IP-10, sTNF-R2 and sFas were independently associated with increased risk of CM associated mortality. CMNS patients had a significantly lower level of the neuroprotective factor VEGF when compared to other groups ($P < 0.0045$). The ratios of VEGF to IP-10, sTNF-R2, and sFas distinguished CM survivors from non survivors ($P < 0.0001$). **CONCLUSION:** The results suggest that plasma levels of IP-10, sTNF-R2 and sFas may be potential biomarkers of CM severity and mortality. VEGF was found to be protective against CM associated mortality and may be considered for adjunctive therapy to improve the treatment outcome in CM patients.

47: *Malar J.* 2008 May 19;7:81.

Malaria and obesity: obese mice are resistant to cerebral malaria.

Robert V, Bourgoignie C, Depoix D, Thouvenot C, Lombard MN, Grellier P.

Institut de Recherche pour Développement, Unité de Recherche 77 and Unité de Recherche 16, 213 rue La Fayette, 75480 Paris cedex 10, France. v.robert@mnhn.fr

BACKGROUND: The relationship between malaria and obesity are largely unknown. This is partly due to the fact that malaria occurs mainly in tropical areas where, until recently, obesity was not prevalent. It now appears, however, that obesity is emerging as a problem in developing countries. To investigate the possible role of obesity on the host-parasite response to malarial infection, this study applied a murine model, which uses the existence of genetically well characterized obese mice. **METHODS:** The receptivity of obese homozygous ob/ob mice was compared to the receptivity of control heterozygous ob/+ lean mice after a single injection of Plasmodium berghei ANKA sporozoites. Both parasitaemia and mortality in response to infection were recorded. **RESULTS:** The control mice

developed the expected rapid neurological syndromes associated with the ANKA strain, leading to death after six days, in absence of high parasitaemia. The obese mice, on the other hand, did not develop cerebral malaria and responded with increasing parasitaemia, which produced severe anemia leading to death 18-25 days after injection. CONCLUSION: The observed major differences in outward symptoms for malarial infection in obese versus control mice indicate a link between obesity and resistance to the infection which could be addressed by malariologists studying human malaria.

48: *Malar J.* 2008 May 9;7:79.

Timing of intermittent preventive treatment for malaria during pregnancy and the implications of current policy on early uptake in north-east Tanzania.

Anders K, Marchant T, Chambo P, Mapunda P, Reyburn H.

London School of Hygiene and Tropical Medicine, London, WC1E 7HT, UK.

katie.anders@hotmail.co.uk

BACKGROUND: Intermittent preventive treatment (IPTp) is efficacious in reducing the adverse outcomes associated with pregnancy-associated malaria, however uptake of the recommended two doses is low in Tanzania, and little is known of the timepoint during pregnancy at which it is delivered. This study investigated the timing of delivery of IPTp to pregnant women attending antenatal clinics (ANC), and the potential determinants of timely uptake. METHODS: Structured interviews were conducted with staff and pregnant women at antenatal clinics in northeast Tanzania, and antenatal consultations were observed. Facility-based and individual factors were analysed for any correlation with timing of IPTp uptake. RESULTS: Almost half the women interviewed first attended ANC during or before the fourth month of gestation, however 86% of these early attendees did not receive IPTp on their first visit. The timing of IPTp delivery complied closely with the national guidelines which stipulate giving the first dose at 20-24 weeks gestation. Uptake of at least one dose of IPTp among women who had reached this gestation age was 67%, although this varied considerably between clinics. At one facility, IPTp was not delivered because SP was out of stock. CONCLUSION: Early uptake of IPTp was found to be hampered by factors external to health worker performance or women's individual preferences. These include insufficient drug stocks and an apparent lack of information to health workers on the reasoning for continued use of SP for IPTp when it has been replaced as a first-line treatment. In addition, an unexpectedly high proportion of women attend antenatal clinics before 20 weeks of pregnancy. While current policy denies the use of IPTp at this time, there is emerging, but incomplete, evidence that malaria in early pregnancy may contribute considerably to the burden of pregnancy-related malaria. Current policy may thus result in a missed opportunity for maximising the benefit of this intervention, and efforts to encourage earlier attendance at ANC alone are unlikely to improve uptake of IPTp. More evidence is needed to weigh the benefits of early IPTp use against theoretical risks of antifolate drugs in early pregnancy.

49: *Mol Biol Evol.* 2008 Jun;25(6):1245-52.

Local adaptation and vector-mediated population structure in *Plasmodium vivax* malaria.

Joy DA, Gonzalez-Ceron L, Carlton JM, Gueye A, Fay M, McCutchan TF, Su XZ.

Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

djoy@mail.nih.gov

Plasmodium vivax in southern Mexico exhibits different infectivities to 2 local

mosquito vectors, *Anopheles pseudopunctipennis* and *Anopheles albimanus*. Previous work has tied these differences in mosquito infectivity to variation in the central repeat motif of the malaria parasite's circumsporozoite (csp) gene, but subsequent studies have questioned this view. Here we present evidence that *P. vivax* in southern Mexico comprised 3 genetic populations whose distributions largely mirror those of the 2 mosquito vectors. Additionally, laboratory colony feeding experiments indicate that parasite populations are most compatible with sympatric mosquito species. Our results suggest that reciprocal selection between malaria parasites and mosquito vectors has led to local adaptation of the parasite. Adaptation to local vectors may play an important role in generating population structure in *Plasmodium*. A better understanding of coevolutionary dynamics between sympatric mosquitoes and parasites will facilitate the identification of molecular mechanisms relevant to disease transmission in nature and provide crucial information for malaria control.

50: *Mol Biol Evol.* 2008 Jun;25(6):1192-8.

Origins of human malaria: rare genomic changes and full mitochondrial genomes confirm the relationship of *Plasmodium falciparum* to other mammalian parasites but complicate the origins of *Plasmodium vivax*.

Roy SW, Irimia M.

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA. scottwroy@gmail.com

Despite substantial work, the phylogeny of malaria parasites remains debated. The matter is complicated by concerns about patterns of evolution in potentially strongly selected genes as well as the extreme AT bias of some *Plasmodium* genomes. Particularly contentious has been the position of the most virulent human parasite *Plasmodium falciparum*, whether grouped with avian parasites or within a larger clade of mammalian parasites. Here, we study 3 classes of rare genomic changes, as well as the sequences of mitochondrial ribosomal RNA (rRNA) genes. We report 3 lines of support for a clade of mammalian parasites: 1) we find no instances of spliceosomal intron loss in a hypothetical ancestor of *P. falciparum* and the avian parasite *Plasmodium gallinaceum*, suggesting against a close relationship between those species; 2) we find 4 genomic mitochondrial indels supporting a mammalian clade, but none grouping *P. falciparum* with avian parasites; and 3) slowly evolving mitochondrial rRNA sequences support a mammalian parasite clade with 100% posterior probability. We further report a large deletion in the mitochondrial large subunit rRNA gene, which suggests a subclade including both African and Asian parasites within the clade of closely related primate malarias. This contrasts with previous studies that provided strong support for separate Asian and African clades, and reduces certainty about the historical and geographic origins of *Plasmodium vivax*. Finally, we find a lack of synapomorphic gene losses, suggesting a low rate of ancestral gene loss in *Plasmodium*.

51: *Nature.* 2008 May 29;453(7195):609-14.

Sex ratio adjustment and kin discrimination in malaria parasites.

Reece SE, Drew DR, Gardner A.

Institute of Evolutionary Biology, Ashworth Laboratories, School of Biological Science, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK. sarah.reece@ed.ac.uk

Malaria parasites and related Apicomplexans are the causative agents of some of the most serious infectious diseases of humans, companion animals, livestock and wildlife. These parasites must undergo sexual reproduction to transmit from

vertebrate hosts to vectors, and their sex ratios are consistently female-biased. Sex allocation theory, a cornerstone of evolutionary biology, is remarkably successful at explaining female-biased sex ratios in multicellular taxa, but has proved controversial when applied to malaria parasites. Here we show that, as predicted by theory, sex ratio is an important fitness-determining trait and *Plasmodium chabaudi* parasites adjust their sex allocation in response to the presence of unrelated conspecifics. This suggests that *P. chabaudi* parasites use kin discrimination to evaluate the genetic diversity of their infections, and they adjust their behaviour in response to environmental cues. Malaria parasites provide a novel way to test evolutionary theory, and support the generality and power of a darwinian approach.

52: *Parasitol Int.* 2008 Jun;57(2):150-7.

High mobility group box (HMGB) proteins of *Plasmodium falciparum*: DNA binding proteins with pro-inflammatory activity.

Kumar K, Singal A, Rizvi MM, Chauhan VS.

International Centre of Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi-110067, India.

High mobility group box chromosomal protein 1 (HMGB1), known as an abundant, non-histone architectural chromosomal protein, is highly conserved across different species. Homologues of HMGB1 were identified and cloned from malaria parasite, *Plasmodium falciparum*. Sequence analyses showed that the *P. falciparum* HMGB1 (PfHMGB1) exhibits 45, 23 and 18%, while PfHMGB2 shares 42, 21 and 17% homology with *Saccharomyces cerevisiae*, human and mouse HMG box proteins respectively. Parasite PfHMGB1 and PfHMGB2 proteins contain one HMG Box domain similar to B-Box of mammalian HMGB1. Electrophoretic Mobility Shift Assay (EMSA) showed that recombinant PfHMGB1 and PfHMGB2 bind to DNA. Immunofluorescence Assay using specific antibodies revealed that these proteins are expressed abundantly in the ring stage nuclei. Significant levels of PfHMGB1 and PfHMGB2 were also present in the parasite cytosol at trophozoite and schizont stages. Both, PfHMGB1 and PfHMGB2 were found to be potent inducers of pro-inflammatory cytokines such as TNF α from mouse peritoneal macrophages as analyzed by both reverse transcription PCR and by ELISA. These results suggest that secreted PfHMGB1 and PfHMGB2 may be responsible for eliciting/ triggering host inflammatory immune responses associated with malaria infection.

53: *Parasitol Int.* 2008 Jun;57(2):143-9.

A survey of malarial infection in endemic areas of Savannakhet province, Lao PDR and comparative diagnostic efficiencies of Giemsa staining, acridine orange staining, and semi-nested multiplex PCR.

Khaminsou N, Kritpetcharat O, Daduang J, Kritpetcharat P.

College of Health Technology, Vientiane, Lao Democratic People's Republic.

Malaria remains one of the most important parasitic diseases in Lao PDR, especially in forested rural areas. Knowing the rate of infection using highly sensitive and specific methods, and the factors related to malarial infection, may be helpful in reducing the infection and mortality rates. We aimed to study the malarial infection rate by comparing three detection methods, i.e., Giemsa staining, acridine orange (AO) staining and semi-nested multiplex PCR. The study also included some factors related to malarial infection in the endemic areas of Savannakhet province, Lao PDR. The respective malarial infection rates by Giemsa staining, AO staining and semi-nested multiplex PCR in Houy Jang vs. Keng Thong villages were 13.1 vs. 20.8, 16.2 vs. 25.4 and 20.8 vs. 30.8%. The infection rate among children not over 10 years of age was higher than infection rate among the

older ages ($p=0.002$, Z-test for two proportions). The higher infection rates by semi-nested multiplex PCR over Giemsa and AO staining suggest the existence of many subclinical cases with low level parasitemia, undetected by microscopic techniques. We found no mixed infections using Giemsa or AO staining, but using semi-nested multiplex PCR we found 1.2% (3/260) mixed *P. falciparum* and *P. vivax* infections, suggesting that semi-nested multiplex PCR is suitable for detecting malarial infection from endemic areas whose cases may have low parasitemia and/or mixed infection. The factors significantly related to malarial infection from 260 questionnaires were: (1) children and young adults, (2) not having lived in the area more than 5 years, and (3) not using a mosquito net over the bed, indicating an increased risk of new residents of contracting malaria and a need to promote bed nets.

54: *Parasitol Int.* 2008 Jun;57(2):223-8.

Cloning and characterization of Plasmodium vivax serine hydroxymethyltransferase.

Leartsakulpanich U, Kongkasuriyachai D, Imwong M, Chotivanich K, Yuthavong Y.

National Center for Genetic Engineering and Biotechnology, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand. ubolsree@biotec.or.th

Serine hydroxymethyltransferase (SHMT), which catalyzes the reversible reaction of serine and tetrahydrofolate to glycine and methylenetetrahydrofolate, is one of the three enzymes in dTMP synthesis pathway that is highly active during cell division and has been proposed as a potential chemotherapeutic target in infectious diseases and cancer. This is the first study to describe nucleotide and amino acid sequences of SHMT from the malaria parasite *Plasmodium vivax*. Sequencing of 12 *P. vivax* isolates revealed limited polymorphisms in 3 noncoding regions. Its biological function is also reported.

55: *Parasitol Res.* 2008 Jun 5.

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

Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K.

Division of Vector Biology, Department of Zoology, Annamalai University, Annamalai Nagar, 608 002, Tamil Nadu, India, drgovind1979@rediffmail.com.

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

56: *Parasitol Res.* 2008 Jun 5.

Ribozyme cleavage of Plasmodium falciparum gyrase A gene transcript affects the parasite growth.

Ahmed A, Sharma YD.

Department of Biotechnology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, 110029, India.

Deoxyribonucleic acid (DNA) gyrase is an important enzyme that facilitates the movement of replication and transcription complexes through DNA by creating negative supercoils ahead of the complex. Its presence in *Plasmodium falciparum* is now established and considered a good drug target since it is absent in the human host. The sequence of *P. falciparum* gyrase A subunit was analyzed for its messenger ribonucleic acid (mRNA) folding as well as target accessibility for ribozymes. The four GUC triplet sites identified at 334, 491, 1907, and 2642 nucleotide positions of the Gyrase A mRNA were also accessible to oligos by RNase H assay. Site GUC(491) was optimally accessible followed by GUC(1907), GUC(334), and GUC(2642) sites. Ribozymes were produced against all these sites and tested for their in vitro transcript cleavage potentials where RZ(491) showed the maximum cleavage rate. Therefore, this ribozyme (RZ(491)) was chemically synthesized albeit with modifications so as to make it resistant against ribonuclease attack. The modified ribozyme retained its cleavage potential and was able to inhibit the *P. falciparum* parasite growth up to 49.54% and 74.77% at 20 and 30 μ M ribozyme concentrations, respectively, as compared to the untreated culture. However, up to 20% and 24.32% parasite growth inhibition was observed at the same ribozyme concentrations of 20 and 30 μ M when compared with control ribozyme-treated cultures. This ribozyme as well as other targets identified here can be investigated further to develop the effective chemotherapeutic agents against malaria.

57: *Parasitol Res.* 2008 Jun 5.

Mutations in PFCRT K76T do not correlate with sulfadoxine-pyrimethamine-amodiaquine failure in Pikine, Senegal.

Sarr O, Ahouidi AD, Ly O, Daily JP, Ndiaye D, Ndir O, Mboup S, Wirth DF.

Laboratory of Bacteriology and Virology, Hospital Aristide Le Dantec, BP 7325, Dakar, Senegal, osarr@hsph.harvard.edu

In 2003, the high level of chloroquine (CQ) treatment failure for uncomplicated *Plasmodium falciparum* malaria cases has led Senegal to adopt a new combination therapy with sulfadoxine-pyrimethamine and amodiaquine (SP-AQ). From September through November 2004, we used the 14-day World Health Organization follow-up protocol to assess the therapeutic response in patients with uncomplicated *P. falciparum* malaria in an area of high prevalence of *pfprt* T76 mutant allele and SP resistance mutations. Of the 82 patients who were recruited, 68 (82.9%) completed follow-up. The response of the patients to treatment was adequate clinical response for 63 out of 68 patients (92.6%), while five (7.4%) clinical failures were recorded, four early treatment failures, and one late treatment failure. The prevalence of the *pfprt* T76 allele at day 0 was 59.5%. The two-sided Fisher's exact test did not show an association between *pfprt* T76 allele and treatment failure ($p = 0.167$). The transitory treatment is effective and safe. However, the presence of high levels of mutant alleles points out the need to closely monitor the new therapeutic regimen.

58: *Parasitol Res.* 2008 May 21.

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

Karunamoorthi K, Mulelam A, Wassie F.

Unit of Vector Biology & Control, School of Environmental Health Science, Public Health Faculty, Jimma University, Jimma, Ethiopia, k_karunamoorthi@yahoo.com

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

59: *Parasitology.* 2008 May 12:1-6.

Immune responses during helminth-malaria co-infection: a pilot study in Ghanaian school children.

Hartgers FC, Obeng BB, Boakye D, Yazdanbakhsh M.

Department of Parasitology, Leiden University Medical Centre, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.

SUMMARY Malaria and helminth infections have a shared geographical distribution and therefore co-infections are frequent in tropical areas of the world. Human populations of helminth and malaria co-infection have shown contradictory results for the course of malarial infection and disease, possibly depending on the type of helminth studied, the intensity of helminth infection and the age of the study population. Although immunological studies might clarify the underlying mechanisms of protection or increased susceptibility, there are very few studies that have looked at immunological parameters in helminth and malaria co-infection. After discussing the available immunological data on co-infection, we describe a pilot study performed in Ghanaian school children where we compare anti-malarial responses in children living in an urban area, where the prevalence of helminth and *Plasmodium falciparum* infections was low, with that of children living in a rural area with high prevalence of helminth and *Plasmodium falciparum* infections.

60: *PLoS ONE*. 2008 Jun 4;3(6):e2335.

Marine actinomycetes: a new source of compounds against the human malaria parasite.

Prudhomme J, McDaniel E, Ponts N, Bertani S, Fenical W, Jensen P, Le Roch K.

Department of Cell Biology and Neuroscience, University of California Riverside, Riverside, California, United States of America.

BACKGROUND: Malaria continues to be a devastating parasitic disease that causes the death of 2 million individuals annually. The increase in multi-drug resistance together with the absence of an efficient vaccine hastens the need for speedy and comprehensive antimalarial drug discovery and development. Throughout history, traditional herbal remedies or natural products have been a reliable source of antimalarial agents, e.g. quinine and artemisinin. Today, one emerging source of small molecule drug leads is the world's oceans. Included among the source of marine natural products are marine microorganisms such as the recently described actinomycete. Members of the genus *Salinispora* have yielded a wealth of new secondary metabolites including salinosporamide A, a molecule currently advancing through clinical trials as an anticancer agent. Because of the biological activity of metabolites being isolated from marine microorganisms, our group became interested in exploring the potential efficacy of these compounds against the malaria parasite. **METHODS:** We screened 80 bacterial crude extracts for their activity against malaria growth. We established that the pure compound, salinosporamide A, produced by the marine actinomycete, *Salinispora tropica*, shows strong inhibitory activity against the erythrocytic stages of the parasite cycle. Biochemical experiments support the likely inhibition of the parasite 20S proteasome. Crystal structure modeling of salinosporamide A and the parasite catalytic 20S subunit further confirm this hypothesis. Ultimately we showed that salinosporamide A protected mice against deadly malaria infection when administered at an extremely low dosage. **CONCLUSION:** These findings underline the potential of secondary metabolites, derived from marine microorganisms, to inhibit *Plasmodium* growth. More specifically, we highlight the effect of proteasome inhibitors such as salinosporamide A on in vitro and in vivo parasite development. Salinosporamide A (NPI-0052) now being advanced to phase I trials for the treatment of refractory multiple myeloma will need to be further explored to evaluate the safety profile for its use against malaria.

61: *PLoS ONE*. 2008 May 28;3(5):e2280.

***Plasmodium falciparum* antigens on the surface of the gametocyte-infected erythrocyte.**

Saeed M, Roeffen W, Alexander N, Drakeley CJ, Targett GA, Sutherland CJ.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom.

BACKGROUND: The asexual blood stages of the human malaria parasite *Plasmodium falciparum* produce highly immunogenic polymorphic antigens that are expressed on the surface of the host cell. In contrast, few studies have examined the surface of the gametocyte-infected erythrocyte. **METHODOLOGY/PRINCIPAL FINDINGS:** We used flow cytometry to detect antibodies recognising the surface of live cultured erythrocytes infected with gametocytes of *P. falciparum* strain 3D7 in the plasma of 200 Gambian children. The majority of children had been identified as carrying gametocytes after treatment for malaria, and each donated blood for mosquito-feeding experiments. None of the plasma recognised the surface of erythrocytes infected with developmental stages of gametocytes (I-IV), but 66 of 194 (34.0%) plasma contained IgG that recognised the surface of erythrocytes infected with mature (stage V) gametocytes. Thirty-four (17.0%) of 200 plasma

tested recognised erythrocytes infected with trophozoites and schizonts, but there was no association with recognition of the surface of gametocyte-infected erythrocytes (odds ratio 1.08, 95% C.I. 0.434-2.57; P = 0.851). Plasma antibodies with the ability to recognise gametocyte surface antigens (GSA) were associated with the presence of antibodies that recognise the gamete antigen Pfs 230, but not Pfs48/45. Antibodies recognising GSA were associated with donors having lower gametocyte densities 4 weeks after antimalarial treatment.

CONCLUSIONS/SIGNIFICANCE: We provide evidence that GSA are distinct from antigens detected on the surface of asexual 3D7 parasites. Our findings suggest a novel strategy for the development of transmission-blocking vaccines.

62: *PLoS ONE*. 2008 May 28;3(5):e2287.

The transmembrane isoform of Plasmodium falciparum MAEBL is essential for the invasion of Anopheles salivary glands.

Saenz FE, Balu B, Smith J, Mendonca SR, Adams JH.

Global Health and Infectious Diseases Research, Department of Global Health, College of Public Health, University of South Florida, Tampa, Florida, United States of America.

Malaria transmission depends on infective stages in the mosquito salivary glands. Plasmodium sporozoites that mature in midgut oocysts must traverse the hemocoel and invade the mosquito salivary glands in a process thought to be mediated by parasite ligands. MAEBL, a homologue of the transmembrane EBP ligands essential in merozoite invasion, is expressed abundantly in midgut sporozoites. Alternative splicing generates different MAEBL isoforms and so it is unclear what form is functionally essential. To identify the MAEBL isoform required for *P. falciparum* (NF54) sporozoite invasion of salivary glands, we created knockout and allelic replacements each carrying CDS of a single MAEBL isoform. Only the transmembrane form of MAEBL is essential and is the first *P. falciparum* ligand validated as essential for invasion of *Anopheles* salivary glands. MAEBL is the first *P. falciparum* ligand experimentally determined to be essential for this important step in the life cycle where the vector becomes infectious for transmitting sporozoites to people. With an increasing emphasis on advancing vector-based transgenic methods for suppression of malaria, it is important that this type of study, using modern molecular genetic tools, is done with the agent of the human disease. Understanding what *P. falciparum* sporozoite ligands are critical for mosquito transmission will help validate targets for vector-based transmission-blocking strategies.

63: *PLoS ONE*. 2008 May 21;3(5):e2252.

A murine model of falciparum-malaria by in vivo selection of competent strains in non-myelodepleted mice engrafted with human erythrocytes.

Angulo-Barturen I, Jiménez-Díaz MB, Mulet T, Rullas J, Herreros E, Ferrer S, Jiménez E, Mendoza A, Regadera J, Rosenthal PJ, Bathurst I, Pompliano DL, Gómez de las Heras F, Gargallo-Viola D.

Diseases of the Developing World, Infectious Diseases-Centre for Excellence in Drug Discovery, GlaxoSmithKline, Tres Cantos, Madrid, Spain.
inigo.x.angulo@gsk.com

To counter the global threat caused by *Plasmodium falciparum* malaria, new drugs and vaccines are urgently needed. However, there are no practical animal models because *P. falciparum* infects human erythrocytes almost exclusively. Here we describe a reliable *falciparum* murine model of malaria by generating strains of *P. falciparum* in vivo that can infect immunodeficient mice engrafted with human erythrocytes. We infected NOD(scid/beta2m-/-) mice engrafted with human

erythrocytes with *P. falciparum* obtained from in vitro cultures. After apparent clearance, we obtained isolates of *P. falciparum* able to grow in peripheral blood of engrafted NOD(scid/beta2m-/-) mice. Of the isolates obtained, we expanded in vivo and established the isolate Pf3D7(0087/N9) as a reference strain for model development. Pf3D7(0087/N9) caused productive persistent infections in 100% of engrafted mice infected intravenously. The infection caused a relative anemia due to selective elimination of human erythrocytes by a mechanism dependent on parasite density in peripheral blood. Using this model, we implemented and validated a reproducible assay of antimalarial activity useful for drug discovery. Thus, our results demonstrate that *P. falciparum* contains clones able to grow reproducibly in mice engrafted with human erythrocytes without the use of myeloablative methods.

64: *PLoS ONE*. 2008 May 21;3(5):e2227.

Duration of protection against malaria and anaemia provided by intermittent preventive treatment in infants in Navrongo, Ghana.

Cairns M, Carneiro I, Milligan P, Owusu-Agyei S, Awine T, Gosling R, Greenwood B, Chandramohan D.

Department of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, United Kingdom. matthew.cairns@lshtm.ac.uk

BACKGROUND: Intermittent preventive treatment for malaria in Infants (IPTi) has been shown to give effective and safe protection against malaria. It has been suggested that IPTi might have long-lasting beneficial effects but, in most settings, the protection provided by IPTi appears to be short-lived. Knowledge of the duration of protection given by IPTi would help interpret the results of existing trials and suggest optimal delivery schedules for IPTi. This study investigated how the protective efficacy of IPTi against malaria and anaemia changes over time. **METHODS AND FINDINGS:** A secondary analysis of data from a cluster-randomised, placebo-controlled trial of IPTi using sulfadoxine-pyrimethamine (SP) in Ghana was conducted. In this trial IPTi was given to 2485 infants at 3, 4, 9 and 12 months of age; children remained in follow-up until two years of age. Poisson regression with a random effect to adjust for the cluster-randomised design was used to determine protective efficacy of IPTi against clinical malaria and anaemia in defined time strata following administration of IPTi. Analysis of first-or-only clinical malaria episode following the individual IPTi doses showed that some protection against malaria lasted between 4 to 6 weeks. A similar pattern was seen when the incidence of all malaria episodes up to 2 years of age was analysed in relation to the most recent IPT, by pooling the incidence of malaria after the individual IPTi doses. Protective efficacy within four weeks of IPTi was 75.2% (95% CI: 66-82) against malaria, 78.9% (95% CI: 69-86) against high parasite density malaria, and 93.8% (95% CI: 73-99) against anaemia. Protection against these outcomes was short-lived, with evidence of any effect lasting for only 6, 6 and 4 weeks respectively. Protection in children who were parasitaemic when receiving IPTi appeared to be of shorter duration than in uninfected children. There was no evidence of any benefit of IPTi after the immediate period following the IPTi doses. **CONCLUSIONS:** Intermittent preventive treatment provides considerable protection against malaria and anaemia for short periods, even in an area of intense seasonal transmission. Due to the relatively short duration of protection provided by each dose of IPTi, this treatment will be of most benefit when delivered at the time of peak malaria incidence.

65: *PLoS ONE*. 2008 May 14;3(5):e2172.

Evidence of introgression of the ace-1(R) mutation and of the ace-1 duplication in West African Anopheles gambiae s. s.

Djogbénou L, Chandre F, Berthomieu A, Dabiré R, Koffi A, Alout H, Weill M.

Institut de Recherche pour le Développement, Cotonou, Benin.

BACKGROUND: The role of inter-specific hybridisation is of particular importance in mosquito disease vectors for predicting the evolution of insecticide resistance. Two molecular forms of *Anopheles gambiae* s.s., currently recognized as S and M taxa, are considered to be incipient sibling species. Hybrid scarcity in the field was suggested that differentiation of M and S taxa is maintained by limited or absent gene flow. However, recent studies have revealed shared polymorphisms within the M and S forms, and a better understanding of the occurrence of gene flow is needed. One such shared polymorphism is the G119S mutation in the ace-1 gene (which is responsible for insecticide resistance); this mutation has been described in both the M and S forms of *A. gambiae* s.s. **METHODS AND RESULTS:** To establish whether the G119S mutation has arisen independently in each form or by genetic introgression, we analysed coding and non-coding sequences of ace-1 alleles in M and S mosquitoes from representative field populations. Our data revealed many polymorphic sites shared by S and M forms, but no diversity was associated with the G119S mutation. These results indicate that the G119S mutation was a unique event and that genetic introgression explains the observed distribution of the G119S mutation within the two forms. However, it was impossible to determine from our data whether the mutation occurred first in the S form or in the M form. Unexpectedly, sequence analysis of some resistant individuals revealed a duplication of the ace-1 gene that was observed in both *A. gambiae* s.s. M and S forms. Again, the distribution of this duplication in the two forms most likely occurred through introgression. **CONCLUSIONS:** These results highlight the need for more research to understand the forces driving the evolution of insecticide resistance in malaria vectors and to regularly monitor resistance in mosquito populations of Africa.

66: *PLoS Pathog*. 2008 May 23;4(5):e1000070.

Transmission blocking immunity in the malaria non-vector mosquito Anopheles quadriannulatus species A.

Habtewold T, Povelones M, Blagborough AM, Christophides GK.

Immunology and Infection, Division of Cell and Molecular Biology, Faculty of Natural Sciences, Imperial College London, London, United Kingdom.

Despite being phylogenetically very close to *Anopheles gambiae*, the major mosquito vector of human malaria in Africa, *Anopheles quadriannulatus* is thought to be a non-vector. Understanding the difference between vector and non-vector mosquitoes can facilitate development of novel malaria control strategies. We demonstrate that *An. quadriannulatus* is largely resistant to infections by the human parasite *Plasmodium falciparum*, as well as by the rodent parasite *Plasmodium berghei*. By using genetics and reverse genetics, we show that resistance is controlled by quantitative heritable traits and manifested by lysis or melanization of ookinetes in the mosquito midgut, as well as by killing of parasites at subsequent stages of their development in the mosquito. Genes encoding two leucine-rich repeat proteins, LRIM1 and LRIM2, and the thioester-containing protein, TEPl, are identified as essential in these immune reactions. Their silencing completely abolishes *P. berghei* melanization and dramatically increases the number of oocysts, thus transforming *An. quadriannulatus* into a highly permissive parasite host. We hypothesize that the mosquito immune system is an important cause of natural refractoriness to malaria

and that utilization of this innate capacity of mosquitoes could lead to new methods to control transmission of the disease.

67: *PLoS Pathog.* 2008 May 16;4(5):e1000069.

Conserved mosquito/parasite interactions affect development of *Plasmodium falciparum* in Africa.

Mendes AM, Schlegelmilch T, Cohuet A, Awono-Ambene P, De Iorio M, Fontenille D, Morlais I, Christophides GK, Kafatos FC, Vlachou D.

Imperial College London, Division of Cell and Molecular Biology, Faculty of Natural Sciences, South Kensington Campus, London, United Kingdom.

In much of sub-Saharan Africa, the mosquito *Anopheles gambiae* is the main vector of the major human malaria parasite, *Plasmodium falciparum*. Convenient laboratory studies have identified mosquito genes that affect positively or negatively the developmental cycle of the model rodent parasite, *P. berghei*. Here, we use transcription profiling and reverse genetics to explore whether five disparate mosquito gene regulators of *P. berghei* development are also pertinent to *A. gambiae*/*P. falciparum* interactions in semi-natural conditions, using field isolates of this parasite and geographically related mosquitoes. We detected broadly similar albeit not identical transcriptional responses of these genes to the two parasite species. Gene silencing established that two genes affect similarly both parasites: infections are hindered by the intracellular local activator of actin cytoskeleton dynamics, WASP, but promoted by the hemolymph lipid transporter, ApoII/I. Since *P. berghei* is not a natural parasite of *A. gambiae*, these data suggest that the effects of these genes have not been drastically altered by constant interaction and co-evolution of *A. gambiae* and *P. falciparum*; this conclusion allowed us to investigate further the mode of action of these two genes in the laboratory model system using a suite of genetic tools and infection assays. We showed that both genes act at the level of midgut invasion during the parasite's developmental transition from ookinete to oocyst. ApoII/I also affects the early stages of oocyst development. These are the first mosquito genes whose significant effects on *P. falciparum* field isolates have been established by direct experimentation. Importantly, they validate for semi-field human malaria transmission the concept of parasite antagonists and agonists.

68: *Proc Biol Sci.* 2008 May 22;275(1639):1171-9.

CD4+T cells do not mediate within-host competition between genetically diverse malaria parasites.

Barclay VC, Råberg L, Chan BH, Brown S, Gray D, Read AF.

School of Biological Sciences, University of Edinburgh, Edinburgh, UK.
victoria.barclay@ed.ac.uk

Ecological interactions between microparasite populations in the same host are an important source of selection on pathogen traits such as virulence and drug resistance. In the rodent malaria model *Plasmodium chabaudi* in laboratory mice, parasites that are more virulent can competitively suppress less virulent parasites in mixed infections. There is evidence that some of this suppression is due to immune-mediated apparent competition, where an immune response elicited by one parasite population suppress the population density of another. This raises the question whether enhanced immunity following vaccination would intensify competitive interactions, thus strengthening selection for virulence in *Plasmodium* populations. Using the *P. chabaudi* model, we studied mixed infections of virulent and avirulent genotypes in CD4+T cell-depleted mice. Enhanced efficacy of CD4+T cell-dependent responses is the aim of several candidate

malaria vaccines. We hypothesized that if immune-mediated interactions were involved in competition, removal of the CD4+T cells would alleviate competitive suppression of the avirulent parasite. Instead, we found no alleviation of competition in the acute phase, and significant enhancement of competitive suppression after parasite densities had peaked. Thus, the host immune response may actually be alleviating other forms of competition, such as that over red blood cells. Our results suggest that the CD4+-dependent immune response, and mechanisms that act to enhance it such as vaccination, may not have the undesirable affect of exacerbating within-host competition and hence the strength of this source of selection for virulence.

69: *Proc Natl Acad Sci U S A.* 2008 Jun 3.

Naturally acquired Duffy-binding protein-specific binding inhibitory antibodies confer protection from blood-stage Plasmodium vivax infection.

King CL, Michon P, Shakri AR, Marcotty A, Stanistic D, Zimmerman PA, Cole-Tobian JL, Mueller I, Chitnis CE.

Case Western Reserve University Center for Global Health and Diseases, Cleveland, OH 44106;

Individuals residing in malaria-endemic regions acquire protective immunity after repeated infection with malaria parasites; however, mechanisms of protective immunity and their immune correlates are poorly understood. Blood-stage infection with Plasmodium vivax depends completely on interaction of P. vivax Duffy-binding protein (PvDBP) with the Duffy antigen on host erythrocytes. Here, we performed a prospective cohort treatment/reinfection study of children (5-14 years) residing in a P. vivax-endemic region of Papua New Guinea (PNG) in which children were cleared of blood-stage infection and then examined biweekly for reinfection for 25 weeks. To test the hypothesis that naturally acquired binding inhibitory antibodies (BIAbs) targeting PvDBP region II (PvDBPII) provide protection against P. vivax infection, we used a quantitative receptor-binding assay to distinguish between antibodies that merely recognize PvDBP and those that inhibit binding to Duffy. The presence of high-level BIAbs (>90% inhibition of PvDBPII-Duffy binding, n = 18) before treatment was associated with delayed time to P. vivax reinfection diagnosed by light microscopy (P = 0.02), 55% reduced risk of P. vivax reinfection (Hazard's ratio = 0.45, P = 0.04), and 48% reduction in geometric mean P. vivax parasitemia (P < 0.001) when compared with children with low-level BIAbs (n = 148). Further, we found that stable, high-level BIAbs displayed strain-transcending inhibition by reducing reinfection with similar efficiency of PNG P. vivax strains characterized by six diverse PvDBPII haplotypes. These observations demonstrate a functional correlate of protective immunity in vivo and provide support for developing a vaccine against P. vivax malaria based on PvDBPII.

70: *Proc Natl Acad Sci U S A.* 2008 Jun 3;105(22):7857-62.

Population structure of the genes encoding the polymorphic Plasmodium falciparum apical membrane antigen 1: Implications for vaccine design.

Duan J, Mu J, Thera MA, Joy D, Kosakovsky Pond SL, Diemert D, Long C, Zhou H, Miura K, Ouattara A, Dolo A, Doumbo O, Su XZ, Miller L.

Malaria Vaccine Development Branch and Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-0425.

Immunization with the highly polymorphic Plasmodium falciparum apical membrane antigen 1 (PfAMA1) induces protection in animals but primarily against parasites that express the same or similar alleles. One strategy to overcome the obstacle

of polymorphism is to combine PfAMA1 proteins representing major haplotypes into one vaccine. To determine the minimum number of haplotypes that would confer broad protection, we sequenced the coding region of PfAMA1 from 97 clones from around the world and 61 isolates from Mali, identifying 150 haplotypes for domains 1 to 3 that included previous sequences. A clustering algorithm grouped the 150 haplotypes into six populations that were independent of geographic location. Each of the six populations contained haplotypes predominantly of that population (predominant haplotypes) and haplotypes that were a mixture of haplotypes represented in other populations (admixed haplotypes). To determine the biological relevance of the populations identified through the clustering algorithm, antibodies induced against one predominant haplotype of population 1 (3D7) and one admixed haplotype of population 5 (FVO) were tested for their ability to block parasite invasion of erythrocytes. Parasites expressing PfAMAs belonging to population 1 were efficiently inhibited by 3D7-specific antibodies, whereas parasites expressing PfAMAs belonging to other populations were not. For FVO-specific antibodies, we observed growth inhibition against itself as well as isolates belonging to populations 3 and 6. Our data suggests that the inclusion of PfAMA1 sequences from each of the six populations may result in a vaccine that induces protective immunity against a broad range of malaria parasites.

71: *Soc Sci Med.* 2008 Jun;66(12):2486-94.

When to seek health care: A duration analysis for malaria patients in Nepal.

Sharma VR.

Department of Economics, University of Colorado Denver, 1380 Lawrence Street, Suite 460, Campus Box 181, Post Box 173364, Denver, CO 80217-3364, United States.

We find that the log-normal distribution of care-seeking time - the number of days from the onset of symptoms of malaria to when a patient seeks treatment from a provider - best described the treatment-seeking behavior of malaria patients in rural areas of two districts of Nepal. The care-seeking rate, or the probability of seeking care, was low on the first day of the symptoms; it increased sharply over the first five days and then gradually declined. Since at the time of the research there was a system of malaria workers taking monthly surveillance rounds of each house to detect and treat malaria cases, patients, instead of traveling to a provider for care, generally waited for malaria workers to arrive at home when the wait for malaria workers was short. But, the probability of seeking care on any day rose if the wait was longer. Women generally tended to wait longer for the malaria workers in order to receive treatment at home. Patient's age, household size, education, and the type of malaria species infecting the patient had no significant effect on care-seeking rate. Given an assumption that a wait of 100 days for a malaria worker would effectively represent total absence of surveillance program, the estimated model predicted higher care-seeking rates under no surveillance program than under the monthly surveillance program.

72: *Trans R Soc Trop Med Hyg.* 2008 Jun;102(6):591-598.

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

Okoye PN, Brooke BD, Koekemoer LL, Hunt RH, Coetzee M.

Vector Control Reference Unit, National Institute for Communicable Diseases, National Health Laboratory Service (NHLS), Private Bag X4, Sandringham 2131, South Africa; School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Indoor-resting anopheline mosquitoes were collected from Obuasi, Ghana, and were identified morphologically and by PCR as *Anopheles funestus* Giles. Wild-caught

females were induced to lay eggs. Samples of F1 progeny from each family were divided into cohorts and were either exposed to DDT and permethrin or were stored for biochemical analysis. Bioassay data by family show evidence of DDT and pyrethroid resistance in the parent *A. funestus* population. The sodium channel gene of DDT survivors and DDT-susceptible individuals was PCR amplified and sequenced to determine whether any *kdr*-type mutations were present. Molecular analysis of the IIS5-IIS6 segment of the sodium channel gene gave no indication of any *kdr*-type mutations associated with resistance phenotypes. Biochemical analysis suggests that DDT and pyrethroid resistance may be metabolically mediated, although there were no clear correlations between enzyme levels/activities and insecticide resistance across families. Furthermore, an altered acetylcholinesterase conferring carbamate resistance was evident. These results can be used to plan an effective malaria control strategy in the Obuasi region.

73: *Trans R Soc Trop Med Hyg.* 2008 May 29.

Improving quantitation of malaria parasite burden with digital image analysis.

Frean J. Parasitology Reference Unit, National Institute for Communicable Diseases and University of the Witwatersrand, Johannesburg, South Africa.

Quantitation of malaria parasite burden has prognostic value as well as providing objective evidence of response to treatment or, potentially, to vaccination against malaria. Estimation of parasite load by microscopy is prone to inaccuracy and inconsistency. Digital image analysis is well suited to this application rather than to the more difficult task of malaria diagnosis and species identification. Preliminary work has shown the feasibility of using off-the-shelf hardware and software. Standardised banks of slides for comparing human and machine counts, cheaper imaging methods for laboratories with limited resources, and customisation of readily available image analysis software are proposed as priority needs.

74: *Trans R Soc Trop Med Hyg.* 2008 May 28.

Point-of-care testing for malaria outbreak management.

Ratnawati , Hatta M, Smits HL.

Department of Parasitology, Hasanuddin University, Makassar, Indonesia.

A rapid antigen assay for malaria was performed on blood samples collected during a simultaneous outbreak of falciparum malaria and vivax malaria on a remote island in the Indonesian archipelago. During the outbreak, a total of 89 patients (4.3% of the population) were diagnosed with malaria within a week. Microscopic examination revealed 78 malaria slide-positive cases, of whom 49 (62.8%) were identified as *P. falciparum*, 7 (9.0%) as *P. vivax* and 22 (28.2%) as mixed *P. falciparum* and *P. vivax* infections. The rapid malaria assay showed excellent correlation with expert-confirmed routine microscopy for *P. falciparum* and *P. vivax* monoinfections and mixed infections with a parasite density >50parasites/mul. Several slide-negative blood samples collected from febrile patients with clinical malaria tested positive in the rapid test. The estimated sensitivity calculated for the rapid test (91.0%) was slightly higher than that of microscopy (87.6%). The result indicates that rapid antigen detection for malaria could be a useful alternative to microscopy to reduce the workload during emergency outbreak situations.

75: *Trans R Soc Trop Med Hyg.* 2008 May 28.

Intermittent preventive treatment of malaria in pregnancy: the incremental cost-effectiveness of a new delivery system in Uganda.

Mbonye AK, Hansen KS, Bygbjerg IC, Magnussen P.

Department of Community Health, Ministry of Health, Box 7272, Kampala, Uganda.

The main objective of this study was to assess whether traditional birth attendants, drug-shop vendors, community reproductive health workers and adolescent peer mobilisers could administer intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) to pregnant women. The study was implemented in 21 community clusters (intervention) and four clusters where health centres provided routine IPTp (control). The primary outcome measures were the proportion of women who completed two doses of SP; the effect on anaemia, parasitaemia and low birth weight; and the incremental cost-effectiveness of the intervention. The study enrolled 2785 pregnant women. The majority, 1404/2081 (67.5%) receiving community-based care, received SP early and adhered to the two recommended doses compared with 281/704 (39.9%) at health centres ($P < 0.001$). In addition, women receiving community-based care had fewer episodes of anaemia or severe anaemia and fewer low birth weight babies. The cost per woman receiving the full course of IPTp was, however, higher when delivered via community care at US\$2.60 compared with US\$2.30 at health centres, due to the additional training costs. The incremental cost-effectiveness ratio of the community delivery system was Uganda shillings 1869 (US\$1.10) per lost disability-adjusted life-year (DALY) averted. In conclusion, community-based delivery increased access and adherence to IPTp and was cost-effective.

76: *Trans R Soc Trop Med Hyg.* 2008 May 20.

Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000.

Ogbonna A, Uneke CJ.

Department of Pharmaceutical Services, Federal Medical Centre, P.O. Box 472, Abakaliki, Nigeria.

Following increased resistance of malaria parasites to conventional drugs in the malarial regions of the world, the WHO is promoting artemisinin-based combination therapy (ACT) for treating uncomplicated malaria. The objective of this report is to review the available scientific information on the efficacy, safety, resistance and policy implementation of ACT as it relates to sub-Saharan Africa since the Abuja 2000 Roll Back Malaria initiative. To achieve this, a Medline search was performed to identify scientific publications relevant to the review. The data reviewed indicated that ACT proved very effective in the treatment of uncomplicated *Plasmodium falciparum* malaria in the region. ACT was shown to be effective, safe and tolerable and no resistance has been detected so far. However, the major challenges to its widespread use in the region include its high cost, low drug quality and poor healthcare delivery systems, among others. It is absolutely imperative for sub-Saharan African countries to establish an effective national antimalarial drug policy which will provide safe, effective, high-quality, accessible and affordable antimalarial drugs such as ACT to the populations at risk of malaria but, at the same time, promote rational drug use in order to delay or prevent the development of antimalarial drug resistance.

77: *Trans R Soc Trop Med Hyg.* 2008 May 8.

Traditional birth home attendance and its implications for malaria control during pregnancy in Nigeria.

Idowu OA, Mafiana CF, Sotiloye D.

Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria.

A longitudinal survey was carried out to assess prevalence of malaria infection among attendees of a traditional birth home (TBH) in the metropolis of Abeokuta, Nigeria. Malaria prevalence was 62.4%, and various degrees of anaemia were recorded in 80.3% of pregnant women. Patronage by pregnant teenagers was 10.8%, with all of them anaemic and 73.9% infected with malarial parasites. Knowledge of malaria transmission and prevention were generally poor, with the emphasis placed on exposure to direct rays and heat from the sun. Avoidance of the sun's heat was therefore considered to be an effective preventive measure; another was the consumption of specially prepared and packaged herbal tea, which the pregnant women were expected to drink daily. Only 36.3% of the women associated malaria infection with mosquito bites. The use of insecticide-treated nets (ITNs) was not recorded among the women, although a large proportion (91.3%) showed a willingness to buy ITNs. The cost of receiving antenatal care at the TBH was higher than that in public hospitals. Patronage of TBHs was observed to be linked more with cultural beliefs than poverty. This study suggests that there is a need to extend malaria control interventions to women attending TBHs.

78: *Trends Parasitol.* 2008 Jun;24(6):264-71.

HIV and malaria co-infection: interactions and consequences of chemotherapy.

Skinner-Adams TS, McCarthy JS, Gardiner DL, Andrews KT.

University of Queensland, Brisbane, 4072, Australia; Queensland Institute of Medical Research and Australian Centre for International and Tropical Health, Herston, Queensland, 4006, Australia.

The global epidemiology of HIV/AIDS and malaria overlap because a significant number of HIV-infected individuals live in regions with different levels of malaria transmission. Although the consequences of co-infection with HIV and malaria parasites are not fully understood, available evidence suggests that the infections act synergistically and together result in worse outcomes. The importance of understanding chemotherapeutic interactions during malaria and HIV co-infection is now being recognized. We know that some antimalarial drugs have weak antiretroviral effects; however, recent studies have also demonstrated that certain antiretroviral agents can inhibit malaria-parasite growth. Here, we discuss recent findings on the impact of HIV/AIDS and malaria co-infection and the possible roles of chemotherapy in improving the treatment of these diseases.

79: *Trop Med Int Health.* 2008 Jun;13(6):784-7.

Effects of revised diagnostic recommendations on malaria treatment practices across age groups in Kenya.

Zurovac D, Njogu J, Akhwale W, Hamer DH, Larson BA, Snow RW.

Malaria Public Health and Epidemiology Group, KEMRI/Wellcome Trust Research Programme, Nairobi, Kenya. dzurovac@nairobi.kemri-wellcome.org

OBJECTIVE: The recent change of treatment policy for uncomplicated malaria from sulfadoxine-pyrimethamine to artemether-lumefantrine (AL) in Kenya was accompanied by revised malaria diagnosis recommendations promoting presumptive

antimalarial treatment in young children and parasitological diagnosis in patients 5 years and older. We evaluated the impact of these age-specific recommendations on routine malaria treatment practices 4-6 months after AL treatment was implemented. METHODS: Cross-sectional, cluster sample survey using quality-of-care assessment methods in all government facilities in four Kenyan districts. Analysis was restricted to the 64 facilities with malaria diagnostics and AL available on the survey day. Main outcome measures were antimalarial treatment practices for febrile patients stratified by age, use of malaria diagnostic tests, and test result. RESULTS: Treatment practices for 706 febrile patients (401 young children and 305 patients > or =5 years) were evaluated. 43.0% of patients > or =5 years and 25.9% of children underwent parasitological malaria testing (87% by microscopy). AL was prescribed for 79.7% of patients > or =5 years with positive test results, for 9.7% with negative results and for 10.9% without a test. 84.6% of children with positive tests, 19.2% with negative tests, and 21.6% without tests were treated with AL. At least one antimalarial drug was prescribed for 75.0% of children and for 61.3% of patients > or =5 years with a negative test result. CONCLUSIONS: Despite different recommendations for patients below and above 5 years of age, malaria diagnosis and treatment practices were similar in the two age groups. Parasitological diagnosis was under-used in older children and adults, and young children were still tested. Use of AL was low overall and alternative antimalarials were commonly prescribed; but AL prescribing largely followed the results of malaria tests. Malaria diagnosis recommendations differing between age groups appear complex to implement; further strengthening of diagnosis and treatment practices under AL policy is required.

80: *Trop Med Int Health*. 2008 Jun;13(6):762-70.

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

Al-Taiar A, Jaffar S, Assabri A, Al-Habori M, Azazy A, Al-Gabri A, Al-Ganadi M, Attal B, Whitty CJ.

Faculty of Medicine and Health Sciences, Sana'a University, Sana'a, Yemen.
[a m altaiar@yahoo.com](mailto:a_m_altaiar@yahoo.com)

OBJECTIVE: To investigate the impact of socio-economic and environmental factors on developing severe malaria in comparison with mild malaria in Yemen. METHOD: Case-control study comparing 343 children aged 6 months to 10 years diagnosed with WHO-defined severe malaria (cases) at the main children's hospital in Taiz and 445 children with mild malaria (controls) diagnosed in the health centres, which serve the areas where the cases came from. RESULTS: In univariate analysis, age <1 year, distance from health centre, delay to treatment and driving time to health centre were associated with progression from mild to severe malaria. In multivariate analysis, distance to nearest health centre >2 km was significantly associated with progression to severe disease. Environmental and vector control factors associated with protection from acquiring malaria (such as sleeping under bednets) were not associated with protection from moving from mild to severe disease. CONCLUSIONS: Innovative ways to improve access to antimalarial treatment for those living more than 2 km away from health centres such as home management of malaria, especially for infants and young children, should be explored in malaria-endemic areas of Yemen.

81: *Trop Med Int Health*. 2008 Jun;13(6):788-94.

Anaemia in a rural Ugandan HIV cohort: prevalence at enrolment, incidence, diagnosis and associated factors.

Mugisha JO, Shafer LA, Van der Paal L, Mayanja BN, Eotu H, Hughes P, Whitworth JA, Grosskurth H.

MRC/UVRI Uganda Research Unit on AIDS, Entebbe, Uganda.

joseph.mugisha@mrcuganda.org

OBJECTIVES: To determine the prevalence and incidence of anaemia in HIV-positive and negative individuals; to identify risk factors for anaemia, prior to the introduction of HAART; and to determine the validity of the clinical diagnosis of anaemia. **METHODS:** Between 1990 and 2003, we followed a rural population based cohort of HIV-infected and uninfected participants. Prevalence and incidence of anaemia were determined clinically and by laboratory measurements. The sensitivity, specificity and predictive values of clinical diagnosis were calculated. **RESULTS:** The prevalence of anaemia at enrolment was 18.9% among HIV-positive and 12.9% among HIV-negative participants ($P = 0.065$). Incidence of anaemia increased with HIV disease progression, from 103 per 1000 person-years of observation among those with CD4 counts >500 to 289 per 1000 person-years of observation among those with CD4 counts <200 . Compared to laboratory diagnosis, the clinical diagnosis of anaemia had a sensitivity of 17.8%, specificity of 96.8%, a positive predictive value of 50.6% and a negative predictive value of 86.4%. Being female, low CD4 cell counts, HIV-positive, wasting syndrome, WHO stage 3 or 4, malaria, fever, pneumonia and oral candidiasis were associated with prevalent anaemia. **CONCLUSIONS:** Anaemia prevalence and incidence were higher among HIV-positive than negative participants. Compared to laboratory diagnosis, clinical detection of anaemia had a low sensitivity. Clinicians working in settings with limited laboratory support must be conscious of the risk of anaemia when managing HIV/AIDS patients, particularly when using antiretroviral drugs which by themselves may cause anaemia as a side effect. We recommend that haemoglobin should be measured before starting ART and monthly for the first three months.

82: *Trop Med Int Health*. 2008 Jun;13(6):827-34.

Insecticide-treated net ownership and usage in Niger after a nationwide integrated campaign.

Thwing J, Hochberg N, Eng JV, Issifi S, Eliades MJ, Minkoulou E, Wolkon A, Gado H, Ibrahim O, Newman RD, Lama M.

Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30341, USA. fez3@cdc.gov

OBJECTIVES: In December 2005 and March 2006, Niger conducted nationwide integrated campaigns to distribute polio vaccine and long lasting insecticide-treated nets (LLINs) to children <5 years of age. We evaluated the campaign effectiveness, net retention, insecticide-treated net (ITN) ownership, and usage. **METHODS:** Two nationwide cross-sectional surveys in January 2006 (dry season) and September 2006 (rainy season), using a stratified two-stage cluster sampling design. We mapped selected communities, selected households by simple random sampling, and administered questionnaires by interviewers using personal digital assistants. **RESULTS:** The first survey showed that ITN ownership in all households was 6.3% prior to the campaign, increasing to 65.1% after the campaign in the second survey. The second survey also showed that 73.4% of households with children <5 received an LLIN and that 97.7% of households that received $>$ or $=$ one LLIN retained it. The wealth equity ratio for ITN ownership in households with children <5 increased from 0.17 prior to the campaign to 0.79 afterward.

During the dry season, 15.4% of all children <5 and 11.3% of pregnant women slept under an ITN, while during rainy season, 55.5% of children <5 and 48.2% of pregnant women slept under an ITN. CONCLUSIONS: Free distribution during the integrated campaign rapidly increased ITN ownership and decreased inequities between those in the highest and lowest wealth quintiles. Retention of ITNs was very high, and usage was high during malaria transmission season. However, ITN ownership and usage by vulnerable groups continues to fall short of RBM targets, and additional strategies are needed to increase ownership and usage.

83: *Trop Med Int Health*. 2008 Jun;13(6):771-83.

Estimates of the burden of malaria morbidity in Africa in children under the age of 5 years.

Roca-Feltrer A, Carneiro I, Armstrong Schellenberg JR.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK. arantxa.roca-feltrer@lshtm.ac.uk

OBJECTIVE: To estimate the direct burden of malaria among children younger than 5 years in sub-Saharan Africa (SSA) for the year 2000, as part of a wider initiative on burden estimates. METHODS: A systematic literature review was undertaken in June 2003. Severe malaria outcomes (cerebral malaria, severe malarial anaemia and respiratory distress) and non-severe malaria data were abstracted separately, together with information on the characteristics of each study and its population. Population characteristics were also collated at a national level. A meta-regression model was used to predict the incidence of malaria fevers at a national level. For severe outcomes, results were presented as median rates as data were too sparse for modelling. RESULTS: For the year 2000, an estimated 545,000 (uncertainty interval: 105,000-1,750,000) children under the age of 5 in SSA experienced an episode of severe malaria for which they were admitted to hospital. A total of 24,000 (interquartile range: 12,000-37,000) suffered from persistent neurological deficits as a result of cerebral malaria. The number of malaria fevers associated with high parasite density in under-5s in SSA in 2000 was estimated as 115,750,000 (uncertainty interval: 91,243,000-257,957,000). CONCLUSION: Our study predicts a lower burden than previous estimates of under-5 malaria morbidity in SSA. As there is a lack of suitable data to enable comprehensive estimates of annual malaria incidence, we describe the information needed to improve the validity of future estimates.

84: *Vaccine*. 2008 Jun 2;26(23):2818-23.

Adenovirus 5 and 35 vectors expressing Plasmodium falciparum circumsporozoite surface protein elicit potent antigen-specific cellular IFN-gamma and antibody responses in mice.

Shott JP, McGrath SM, Pau MG, Custers JH, Ophorst O, Demoitié MA, Dubois MC, Komisar J, Cobb M, Kester KE, Dubois P, Cohen J, Goudsmit J, Heppner DG, Stewart VA.

Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, 20910, USA.

Falciparum malaria vaccine candidates have been developed using recombinant, replication-deficient serotype 5 and 35 adenoviruses (Ad5, Ad35) encoding the Plasmodium falciparum circumsporozoite surface protein (CSP) (Ad5.CS, Ad35.CS) (Crucell Holland BV, Leiden, The Netherlands). To evaluate the immunogenicity of these constructs, BALB/cJ mice were immunized twice with either Ad5.CS, Ad35.CS, empty Ad5-vector (eAd5), empty Ad35 vector (eAd35), or saline. Another group received the CSP-based RTS,S malaria vaccine formulated in the proprietary Adjuvant System AS01B (GlaxoSmithKline Biologicals, Rixensart, Belgium). Here we

report that Ad5.CS, Ad35.CS, and RTS,S/AS01B, elicited both cellular and serologic CSP antigen-specific responses in mice. These adenoviral vectors induce strong malaria-specific immunity and warrant further evaluation.

85: Vaccine. 2008 May 12;26(20):2521-7.

Addition of CpG ODN to recombinant *Pseudomonas aeruginosa* ExoProtein A conjugates of AMA1 and Pfs25 greatly increases the number of responders.

Qian F, Rausch KM, Muratova O, Zhou H, Song G, Diouf A, Lambert L, Narum DL, Wu Y, Saul A, Miller LH, Long CA, Mullen GE.

Malaria Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852, USA.

Both the blood-stage protein apical membrane antigen 1 (AMA1) and the 25-kDa sexual-stage protein (Pfs25) of *Plasmodium falciparum* are two leading candidates in malarial vaccine development. We have previously demonstrated that conjugation of these malarial antigens to recombinant *Pseudomonas aeruginosa* ExoProtein A (rEPA) significantly increased the mean-specific functional antibody responses in mice; however, some mice responded poorly and were unable to demonstrate a functional response. We hypothesized that the immunogenicities of these two malarial antigens could be further enhanced by the inclusion of a CpG oligodeoxynucleotide in the formulation. Mice were immunized with either rEPA-conjugated or unconjugated AMA1 and Pfs25 formulated on Alhydrogel with or without the addition of CPG 7909. Mice received the formulations on days 0 and 28, and mouse sera were collected on day 42. ELISA analyses on these sera showed that the addition of CPG 7909 to AMA1-rEPA and Pfs25-rEPA formulated on Alhydrogel induced significantly higher mean antibody titers than the formulations without CPG 7909, and led to a mixed Th1/Th2 response as demonstrated by the production of mouse IgG1 and IgG2a subclasses. The presence of CPG 7909 in the formulations of both conjugated antigens greatly increased the proportion of responders with antibody titers sufficient to inhibit blood-stage parasite growth in vitro or block transmission of sexual-stage parasites to mosquitoes. The results obtained in this study indicate the potential use of a combination strategy to increase the number of responders to malarial antigens in humans.