

## Malaria Bulletin: A Compendium of Current Literature

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The November 2008 issue contains citations, abstracts and links to the full-text of 38 studies and citations and abstracts to an additional 64 recently published malaria studies. The entries are alphabetical by journal title.

[Part A – Articles with links to the full-text](#)  
(38 studies)

[Part B – Citations and Abstracts](#) – (64 studies)

### Part A – Articles with links to full-text (Alphabetical by journal title)

1: *BMC Public Health*. 2008 Oct 14;8(1):356.

**Spatial effects of mosquito bednets on child mortality.**

<http://www.biomedcentral.com/1471-2458/8/356>

Gosoni L, Vounatsou P, Tami A, Nathan R, Grundmann H, Lengeler C.

**ABSTRACT:** **BACKGROUND:** Insecticide treated nets (ITN) have been proven to be an effective tool in reducing the burden of malaria. Few randomized clinical trials examined the spatial effect of ITNs on child mortality at a high coverage level, hence it is essential to better understand these effects in real-life situation with varying levels of coverage. We analyzed for the first time data from a large follow-up study in an area of high perennial malaria transmission in southern Tanzania to describe the spatial effects of bednets on all-cause child mortality. **METHODS:** The study was carried out between October 2001 and September 2003 in 25 villages in Kilombero Valley, southern Tanzania. Bayesian geostatistical models were fitted to assess the effect of different bednet density measures on child mortality adjusting for possible confounders. **RESULTS:** In the multivariate model addressing potential confounding, the only measure significantly associated with child mortality was the bed net density at household level; we failed to observe additional community effect benefit from bed net coverage in the community. **CONCLUSIONS:** In this multiyear, 25 village assessment, despite substantial known inadequate insecticide-treatment for bed nets, the density of household bed net ownership was significantly associated with all cause child mortality reduction. The absence of community effect of bednets in our study area might be explained by (1) the small proportion of nets which are treated with insecticide, and (2) the relative homogeneity of coverage with nets in the area. To reduce malaria transmission for both users and non-users it is important to increase the ITNs and long-lasting nets coverage to at least the present untreated nets coverage.

2: *Emerg Infect Dis*. 2008 Nov;14(11):1750-2.

**Use of malaria rapid diagnostic test to identify Plasmodium knowlesi infection.**

<http://www.cdc.gov/eid/content/14/11/1750.htm>

McCutchan TF, Piper RC, Makler MT.

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Reports of human infection with *Plasmodium knowlesi*, a monkey malaria, suggest that it and other nonhuman malaria species may be an emerging health problem. We report the use of a rapid test to supplement microscopic analysis in

distinguishing the 5 malaria species that infect humans.

3: *Emerg Infect Dis.* 2008 Nov;14(11):1707-14.

**Mixture for controlling insecticide-resistant malaria vectors.**

<http://www.cdc.gov/eid/content/14/11/1707.htm>

Pennetier C, Costantini C, Corbel V, Licciardi S, Dabiré RK, Lapied B, Chandre F, Hougard JM.

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The spread of resistance to pyrethroids in the major Afrotropical malaria vectors *Anopheles gambiae* s.s. necessitates the development of new strategies to control resistant mosquito populations. To test the efficacy of nets treated with repellent and insecticide against susceptible and insecticide-resistant *An. gambiae* mosquito populations, we impregnated mosquito bed nets with an insect repellent mixed with a low dose of organophosphorous insecticide and tested them in a rice-growing area near Bobo-Dioulasso, Burkina Faso. During the first 2 weeks posttreatment, the mixture was as effective as deltamethrin alone and was more effective at killing *An. gambiae* that carried knockdown resistance (kdr) or insensitive acetylcholinesterase resistance (AcelR) genes. The mixture seemed to not kill more susceptible genotypes for the kdr or AcelR alleles. Mixing repellents and organophosphates on bed nets could be used to control insecticide-resistant malaria vectors if residual activity of the mixture is extended and safety is verified.

4: *J Biol Chem.* 2008 Oct 28.

**Plasmodium falciparum purine nucleoside phosphorylase is critical for viability of malaria parasites.**

<http://www.jbc.org/cgi/reprint/M807218200v1>

Madrid DC, Ting LM, Waller KL, Schramm VL, Kim K.

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Human malaria infections resulting from *Plasmodium falciparum* have become increasingly difficult to treat due to the emergence of drug resistant parasites. The *Plasmodium falciparum* purine salvage enzyme purine nucleoside phosphorylase (PfPNP) is a potential drug target. Previous studies, in which PfPNP was targeted by transition state analogue inhibitors, found that those inhibiting human PNP and PfPNPs killed *P. falciparum* in vitro. However, many drugs have off-target interactions, and genetic evidence is required to demonstrate single target action for this class of potential drugs. We used targeted gene disruption in *P. falciparum* strain 3D7 to ablate PNP expression, yielding transgenic 3D7 parasites (pfpnp). Lysates of the pfpnp parasites showed no PNP activity, but activity of another purine salvage enzyme, adenosine deaminase (PfADA), was normal. When compared with wild type 3D7, the pfpnp parasites showed a greater requirement for exogenous purines and a severe growth defect at physiological concentrations of hypoxanthine. Drug assays using immucillins, specific transition state inhibitors of PNP, were performed on wild type and pfpnp parasites. The pfpnp parasites were more sensitive to PNP inhibitors that bound hPNP tighter and less sensitive to MT-ImmH, an inhibitor with 100-fold preference for PfPNP over hPNP. The results demonstrate the importance of purine salvage in *P. falciparum* and validate PfPNP as the target of immucillins.

5: *J Biol Chem.* 2008 Oct 28.

**ATP/ADP-binding to a novel nucleotide binding domain of the reticulocyte binding protein Py235 of *Plasmodium yoelii*.**

<http://www.jbc.org/cgi/reprint/M803102200v1>

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The mechanism by which a malaria merozoite recognizes a suitable host cell is mediated by a cascade of receptor ligand interactions. In addition to the availability of the appropriate receptors, intracellular ATP plays an important role in determining if erythrocytes are suitable for merozoite invasion. Recent work has shown that ATP secreted from erythrocytes signals a number of cellular processes. To determine whether ATP-signaling might be involved in merozoite invasion, we investigated whether known plasmodium invasion proteins contain nucleotide binding motifs. Domain mapping identified a putative nucleotide binding region within all members of the reticulocyte binding protein homologue (RBL) family analyzed. A representative domain termed here Nucleotide Binding Domain 94 (NBD94), has been expressed and demonstrated to specifically bind to ATP. Nucleotide affinities of NBD94 were determined by fluorescence correlation spectroscopy (FCS), showing increased binding of ATP- compared to ADP-analogues. ATP-binding was reduced by the known F1FO ATP synthase inhibitor 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. Fluorescence quenching and circular dichroism spectroscopy (CD) of NBD94 after binding of different nucleotides provide evidence for structural changes in this protein. Our data suggests that different structural changes induced by ATP/ADP binding to RBL could play an important role during the invasion process.

6: *J Biol Chem.* 2008 Oct 10;283(41):27604-11.

**IMC1b is a putative membrane skeleton protein involved in cell shape, mechanical strength, motility, and infectivity of malaria ookinetes.**

<http://www.jbc.org/cgi/content/full/283/41/27604>

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Membrane skeletons are cytoskeletal elements that have important roles in cell development, shape, and structural integrity. Malaria parasites encode a conserved family of putative membrane skeleton proteins related to articulins. One member, IMC1a, is expressed in sporozoites and localizes to the pellicle, a unique membrane complex believed to form a scaffold onto which the ligands and glideosome are arranged to mediate parasite motility and invasion. IMC1b is a closely related structural paralogue of IMC1a, fostering speculation that it could be functionally homologous but in a different invasive life stage. Here we have generated genetically modified parasites that express IMC1b tagged with green fluorescent protein, and we show that it is targeted exclusively to the pellicle of ookinetes. We also show that IMC1b-deficient ookinetes display abnormal cell shape, reduced gliding motility, decreased mechanical strength, and reduced infectivity. These findings are consistent with a membrane skeletal role of IMC1b and provide strong experimental support for the view that membrane skeletons form an integral part of the pellicle of apicomplexan zoites and function to provide rigidity to the pellicular membrane complex. The similarities observed between the loss-of-function phenotypes of IMC1a and IMC1b show that

membrane skeletons of ookinetes and sporozoites function in an overall similar way. However, the fact that ookinetes and sporozoites do not use the same IMC1 protein implies that different mechanical properties are required of their respective membrane skeletons, likely reflecting the distinct environments in which these life stages must operate.

7: *J Biol Chem.* 2008 Oct 8.

**Identification and characterization of small molecule inhibitors of plasmodium falciparum dihydroorotate dehydrogenase.**

<http://www.jbc.org/cgi/reprint/M804990200v1>

Patel V, Booker M, Kramer M, Ross L, Kennedy LM, Dvorin JD, Duraisingh MT, Sliz P, Wirth DF, Clardy J.

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*Plasmodium falciparum* causes the most deadly form of malaria and accounts for over one million deaths annually. The malaria parasite is unable to salvage pyrimidines and relies on de novo biosynthesis for survival. Dihydroorotate dehydrogenase (DHOD), a mitochondrially localized flavoenzyme, catalyzes the rate-limiting step of this pathway and is therefore an attractive antimalarial chemotherapeutic target. Using a target-based high-throughput screen, we have identified a series of potent, species-specific inhibitors of *P. falciparum* DHOD (pfdHOD) that are also efficacious against three cultured strains (3D7, HB3, and Dd2) of *P. falciparum*. The primary antimalarial mechanism of action of these compounds was confirmed to be inhibition of pfdHOD through a secondary assay with transgenic malaria parasites and, the structural basis for enzyme inhibition was explored through in silico structure-based docking and site-directed mutagenesis. Compound-mediated cytotoxicity was not observed with human dermal fibroblasts or renal epithelial cells. These data validate pfdHOD as an antimalarial drug target and provide chemical scaffolds with which to begin medicinal chemistry efforts.

8: *J Infect Dis.* 2008 Nov 1;198(9):1265-1275.

**Sickle Cell Trait Is Associated with a Delayed Onset of Malaria: Implications for Time-to-Event Analysis in Clinical Studies of Malaria.**

<http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=18752444>

Crompton PD, Traore B, Kayentao K, Doumbo S, Ongoiba A, Diakite SA, Krause MA, Doumtable D, Kone Y, Weiss G, Huang CY, Doumbia S, Guindo A, Fairhurst RM, Miller LH, Pierce SK, Doumbo OK.

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**Background.** The World Health Organization (WHO) recently recommended that the time to first malaria episode serve as the primary end point in phase III malaria vaccine trials—the first of which will be held in Africa. Although common red blood cell (RBC) polymorphisms such as sickle hemoglobin (HbS) are known to protect against malaria in Africa, their impact on this end point has not been investigated. **Methods.** A longitudinal study of 225 individuals aged 2–25 years was conducted in Mali. The association between common RBC polymorphisms and the time to first malaria episode was evaluated. **Results.** Among children

aged 2-10 years, sickle cell trait (HbAS) was associated with a 34-day delay in the median time to first malaria episode ([Formula: see text]). Cox regression analysis showed that greater age (hazard ratio [HR], 0.87 [95% CI, 0.80-0.94]; [Formula: see text]), HbAS (HR, 0.48 [95% CI, 0.26-0.91]; [Formula: see text]), and asymptomatic parasitemia at enrollment (HR, 0.35 [95% CI, 0.14-0.85]; [Formula: see text]) were associated with decreased malaria risk. Conclusion. @nbsp; Given the delay in the time to first malaria episode associated with HbAS, it would be advisable for clinical trials and observational studies that use this end point to include Hb typing in the design of studies conducted in areas where HbAS is prevalent.

9: *Malar J.* 2008 Nov 3;7(1):230.

**Evaluation of two counterflow traps for testing behaviour-mediating compounds for the malaria vector *Anopheles gambiae* s.s. under semi-field conditions in Tanzania.**

<http://www.malariajournal.com/content/7/1/230>

Schmied WH, Takken W, Killeen GF, Knols BG, Smallegange RC.

ABSTRACT: BACKGROUND: Evaluation of mosquito responses towards different trap-bait combinations in field trials is a time-consuming process that can be shortened by experiments in contained semi-field systems. Possible use of the BG Sentinel (BGS) trap to sample *Anopheles gambiae* s.s. was evaluated. The efficiency of this trap was compared with that of the Mosquito Magnet-X (MM-X) trap when baited with foot odour alone or combinations of foot odour with carbon dioxide (CO<sub>2</sub>) or lemongrass as behaviour-modifying cues. METHODS: Female *An. gambiae* s.s. were released in an experimental flight arena that was placed in a semi-field system and left overnight. Catch rates for the MM-X and BGS traps were recorded. Data were analysed by fitting a generalized linear model to the (n+1) transformed catches. RESULTS: Both types of traps successfully captured mosquitoes with all odour cues used. When the BGS trap was tested against the MM-X trap in a choice assay with foot odour as bait, the BGS trap caught about three times as many mosquitoes as the MM-X trap (P=0.002). Adding CO<sub>2</sub> (500ml/min) to foot odour increased the number of mosquitoes caught by 268% for the MM-X (P<0.001) and 34% (P=0.051) for the BGS trap, compared to foot odour alone. When lemongrass leaves were added to foot odour, mosquito catches were reduced by 39% (BGS, P<0.001) and 38% (MM-X, P=0.353), respectively. CONCLUSIONS: The BGS trap shows high potential for field trials due to its simple construction and high catch rate when baited with human foot odour only. However, for rapid screening of different baits in a contained semi-field system the superior discriminatory power of the MM-X trap is advantageous.

10: *Malar J.* 2008 Nov 2;7(1):229.

**Spread of anti-malarial drug resistance: Mathematical model with implications for ACT drug policies.**

<http://www.malariajournal.com/content/7/1/229>

Pongtavornpinyo W, Yeung S, Hastings IM, Dondorp AM, Day NP, White NJ.

ABSTRACT: BACKGROUND: Most malaria-endemic countries are implementing a change in anti-malarial drug policy to artemisinin-based combination therapy (ACT). The impact of different drug choices and implementation strategies is uncertain. Data from many epidemiological studies in different levels of malaria endemicity and in areas with the highest prevalence of drug resistance like borders of Thailand are certainly valuable. Formulating an appropriate dynamic data-driven model is a powerful predictive tool for exploring the impact of these strategies quantitatively. METHODS: A comprehensive model was constructed incorporating

important epidemiological and biological factors of human, mosquito, parasite and treatment. The iterative process of developing the model, identifying data needed, and parameterization has been taken to strongly link the model to the empirical evidence. The model provides quantitative measures of outcomes, such as malaria prevalence/incidence and treatment failure, and illustrates the spread of resistance in low and high transmission settings. The model was used to evaluate different anti-malarial policy options focusing on ACT deployment. RESULTS: The model predicts robustly that in low transmission settings drug resistance spreads faster than in high transmission settings, and treatment failure is the main force driving the spread of drug resistance. In low transmission settings, ACT slows the spread of drug resistance to a partner drug, especially at high coverage rates. This effect decreases exponentially with increasing delay in deploying the ACT and decreasing rates of coverage. In the high transmission settings, however, drug resistance is driven by the proportion of the human population with a residual drug level, which gives resistant parasites some survival advantage. The spread of drug resistance could be slowed down by controlling presumptive drug use and avoiding the use of combination therapies containing drugs with mismatched half-lives, together with reducing malaria transmission through vector control measures. CONCLUSIONS: This paper has demonstrated the use of a comprehensive mathematical model to describe malaria transmission and the spread of drug resistance. The model is strongly linked to the empirical evidence obtained from extensive data available from various sources. This model can be a useful tool to inform the design of treatment policies, particularly at a time when ACT has been endorsed by WHO as first-line treatment for falciparum malaria worldwide.

11: *Malar J.* 2008 Oct 31;7(1):228.

**Spatio-seasonal modeling of the incidence rate of malaria in Mozambique.**

<http://www.malariajournal.com/content/7/1/228>

Abellana R, Ascaso C, Aponte J, Saute F, Nhalungo D, Delino A, Alonso P.

ABSTRACT: BACKGROUND: The objective was to study the seasonal effect on the spatial distribution of the incidence of malaria in children under 10 years old living in the Manhica district, Mozambique. METHODS: The data of the clinical malaria incidence were obtained from a study of two cohorts of children followed from December 1996 to July 1999. The cases were obtained by the active detection method. Hierarchical Bayesian models were used to model the incidence of malaria, including spatial correlation nested to climatic season. The models were compared with the deviance information criterion. The age and gender of the children were also taken into account. RESULTS: The incidence of malaria is associated with age, period and climate season. The incidence presents a clear spatial pattern, with a higher incidence in the neighbourhoods situated in the north and northeast of the Manhica area. The transmission of malaria is highest during the wet season but the spatial pattern of malaria does not differ from that during the dry season. CONCLUSIONS: The incidence of malaria in Manhica presents a spatial pattern which is independent of the seasonal climatic conditions. The climate modifies the incidence of malaria in the entire region but does not change the spatial pattern of the incidence of this disease. These findings may be useful for the planning of malaria control activities. These activities can be performed taking account that the neighbourhoods with more incidence of malaria do not change over the annual climate seasons.

12: *Malar J.* 2008 Oct 31;7(1):227.

**Willingness and ability to pay for artemisinin-based combination therapy in rural Tanzania.**

<http://www.malariajournal.com/content/7/1/227>

Saulo EC, Forsberg BC, Premji Z, Montgomery SM, Bjorkman A.

**ABSTRACT: BACKGROUND:** The aim of this study was to analyse willingness to pay (WTP) and ability to pay (ATP) for ACT for children below five years of age in a rural setting in Tanzania before the introduction of artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated malaria. Socio-economic factors associated with WTP and expectations on anti-malaria drugs, including ACT, were also explored. **METHODS:** Structured interviews and focus group discussions were held with mothers, household heads, health-care workers and village leaders in Ishozi, Gera and Ishunju wards in north-west Tanzania in 2004. Contingent valuation method (CVM) was used with "take-it-or-leave-it" as the eliciting method, expressed as WTP for a full course of ACT for a child and households' opportunity cost of ACT was used to assess ATP. The study included descriptive analyses with multivariate adjustment for potential confounding factors. **RESULTS:** Among 265 mothers and household heads, 244 (92%, CI=88%-95%) were willing to pay Tanzanian Shillings (TSh) 500 (US\$ 0.46) for a child's dose of ACT, but only 55% (49%-61%) were willing to pay more than TSh 500. Mothers were more often willing to pay than male household heads (adjusted odds ratio=2.1, CI=1.2-3.6). Socio-economic status had no significant effect on WTP. The median annual non-subsidized ACT cost for clinical malaria episodes in an average household was calculated as US\$ 6.0, which would represent 0.9% of the average total consumption expenditures as estimated from official data in 2001. The cost of non-subsidized ACT represented 7.0% of reported total annual expenditure on food and 33.0% of total annual expenditure on health care. "Rapid effect," "no adverse effect" and "inexpensive" were the most desired features of an anti-malarial drug. **CONCLUSIONS:** WTP for ACT in this study was less than its real cost and a subsidy is, therefore, needed to enable its equitable affordability. The decision taken in Tanzania to subsidize Coartema fully at governmental health care facilities and at a consumer price of TSh 300-500 (US\$ 0.28-0.46) at special designated shops through the programme of Accredited Drug Dispensing Outlets (ADDOS) appears to be well founded.

13: *Malar J.* 2008 Oct 31;7(1):226.

**The effect of a single blood meal on the phenotypic expression of insecticide resistance in the major malaria vector *Anopheles funestus*.**

<http://www.malariajournal.com/content/7/1/226>

Spillings BL, Coetzee M, Koekemoer LL, Brooke BD.

**ABSTRACT: BACKGROUND:** *Anopheles funestus* is a major malaria vector in southern Africa. Vector control efforts rely on the use of insecticide chemicals to significantly reduce the number of malaria vectors by targeting that portion of the female population that takes blood meals and subsequently rests indoors. It has been suggested that the intake of a blood meal may assist female mosquitoes to tolerate higher doses of insecticide through vigour tolerance. It is hypothesized that during the process of blood digestion, detoxification mechanisms required for the neutralizing of harmful components in the blood meal may also confer an increased ability to tolerate insecticide intoxication through increased enzyme regulation. **METHODS:** Bottle bioassays using a range of concentrations of permethrin were performed on pyrethroid susceptible and

resistant laboratory strains of *An. funestus* in order to detect differences in insecticide susceptibility following a single blood meal. Based on these results, a discriminating dosage was identified (double the lowest dosage that resulted in 100% mortality of the susceptible strain). Blood-fed and unfed females drawn from the resistant strain of *An. funestus* were then assayed against this discriminating dose, and the percentage mortality for each sample was scored and compared. RESULTS: In the insecticide dose response assays, neither the fully susceptible nor the resistant strain of *An. funestus* showed any significant difference in insecticide susceptibility following a blood meal, regardless of the stage of blood meal digestion. A significant increase in the level of resistance was however detected in the resistant *An. funestus* strain following a single blood meal, based on exposure to a discriminating dose of permethrin. CONCLUSIONS: The fully susceptible *An. funestus* strain did not show any significant alteration in susceptibility to insecticide following a blood meal, suggesting that vigour tolerance through increased body mass (and increased dilution of internalized insecticide) does not play a significant role in tolerance to insecticide intoxication. The increase in insecticide tolerance in the pyrethroid-resistant strain of *An. funestus* following a blood meal suggests that insecticide detoxification mechanisms involved in insecticide resistance are stimulated by the presence of a blood meal prior to insecticide exposure, leading to enhanced expression of the resistance phenotype. This finding may be significant in terms of the methods used to control indoor resting populations of *An. funestus*, if the mass killing effect of insecticide application proves increasingly inadequate against blood-feeding females already carrying the insecticide resistance phenotype.

14: *Malar J.* 2008 Oct 31;7(1):225.

**Assessment of the pharmacokinetics and dynamics of two combination regimens of fosmidomycin-clindamycin in patients with acute uncomplicated falciparum malaria.**

<http://www.malariajournal.com/content/7/1/225>

Ruengweerayut R, Looareesuwan S, Hutchinson D, Chauemung A, Banmairuroi V, Na-Bangchang K.

ABSTRACT: BACKGROUND: This study investigated the pharmacokinetics of fosmidomycin when given in combination with clindamycin at two dosage regimens in patients with acute uncomplicated falciparum malaria. METHODS: A total of 70 patients with acute uncomplicated Plasmodium falciparum malaria who fulfilled the enrolment criteria were recruited in the pharmacokinetic study. Patients were treated with two different dosage regimens of fosmidomycin in combination with clindamycin as follows: Group I: fosmidomycin (900 mg) and clindamycin (300 mg) every 6 hours for 3 days (n= 25); and Group II: fosmidomycin (1,800 mg) and clindamycin (600 mg) every 12 hours for 3 days (n=54). RESULTS: Both regimens were well tolerated with no serious adverse events. The 28-day cure rates for Group I and Group II were 91.3 and 89.7%, respectively. Steady-state plasma concentrations of fosmidomycin and clindamycin were attained at about 24 hr after the first dose. The pharmacokinetics of both fosmidomycin and clindamycin analysed by model-independent and model-dependent approaches were generally in broad agreement. There were marked differences in the pharmacokinetic profiles of fosmidomycin and clindamycin when given as two different combination regimens. In general, most of the dose-dependent pharmacokinetic parameters (model-independent C<sub>max</sub>: 3.74 vs 2.41 ug/ml; C<sub>max-ss</sub>: 2.80 vs 2.08 ug/ml; C<sub>max-min-ss</sub>: 2.03 vs 0.71 ug/ml; AUC: 23.31 vs 10.63 ug.hr/ml (median values) were significantly higher in patients who received the high dose regimen (Group II). However, C<sub>min-ss</sub> was lower in this group (0.80 vs 1.37 ug/ml), resulting in significantly higher fluctuations in the plasma concentrations of both fosmidomycin and clindamycin following multiple dosing (110.0 vs 41.9%). Other pharmacokinetic parameters, notably total clearance (CL/F), apparent volume of distribution (V/F, V<sub>z</sub>/F) and elimination half-life (t<sub>1/2z</sub>, t<sub>1/2e</sub>) were also significantly different between

the two dosage regimens. In addition, the dose-dependent pharmacokinetics of both fosmidomycin and clindamycin tended to be lower in patients with recrudescence responses in both groups. CONCLUSIONS: The findings may suggest that dosing frequency and duration have a significant impact on outcome. The combination of fosmidomycin (900 mg) and clindamycin (300-600 mg) administered every six hours for a minimum of five days would constitute the lowest dose regimen with the shortest duration of treatment and which could result in a cure rate greater than 95%.

15: *Malar J.* 2008 Oct 30;7(1):224.

**Social and cultural aspects of 'malaria' and its control in central Cote d'Ivoire.**

<http://www.malariajournal.com/content/7/1/224>

Esse-Diby C, Utzinger J, Tschannen AB, Raso G, Pfeiffer C, Granado S, Koudou BG, N'goran EK, Cisse G, Girardin O, Tanner M, Obrist B.

ABSTRACT: BACKGROUND: A sound local understanding of preventive measures and health-seeking behaviour is important for the effective control of malaria. The purpose of this study was to assess the knowledge, attitudes, practices and beliefs of 'malaria' and its control in two rural communities of central Cote d'Ivoire, and to examine associations between 'malaria' and the households' socioeconomic status. METHODS: A cross-sectional household survey was carried out, using a combination of qualitative and quantitative methods. People's socioeconomic status was estimated, employing a household asset-based approach. RESULTS: Malaria was identified as djekouadjo, the local folk name of the disease. Although people were aware of malaria-related symptoms and their association with mosquitoes, folk perceptions were common. In terms of treatment, a wide array of modern and traditional remedies was employed, often in combination. Individuals with a sound knowledge of the causes and symptoms of malaria continued to use traditional treatments and only a few people sleep under bed nets, whereas folk beliefs did not necessarily translate into refusal of modern treatments. Perceived causes of malaria were linked to the household's socioeconomic status with wealthier individuals reporting mosquitoes more frequently than poorer households. Bed nets were more frequently used in wealthier social strata, whereas other protective measures - perceived to be cheaper - were more prominent among the poorest. CONCLUSION: Equitable access to resources at household, community and health system levels are essential in order to enable community members to prevent and treat malaria. There is a need for community-based approaches that match health care services with poor people's needs and resources.

16: *Malar J.* 2008 Oct 29;7(1):222.

**A highly sensitive, PCR-based method for the detection of Plasmodium falciparum clones in microtiter plates.**

<http://www.malariajournal.com/content/7/1/222>

Maher SP, Balu B, Shoue DA, Weissenbach ME, Adams JH.

ABSTRACT: BACKGROUND: Cloning of parasites by limiting dilution is an essential and rate-limiting step in many aspects of malaria research including genomic and genetic manipulation studies. The standard Giemsa-stained blood smears to detect parasites is time-consuming, whereas the more sensitive parasite lactate dehydrogenase assay involves multiple steps and requires fresh reagents. A simple PCR-based method was therefore tested for parasite detection that can be adapted to high throughput studies. METHODS: Approximately 1µL of packed erythrocytes

from each well of a microtiter cloning plate was directly used as template DNA for a PCR reaction with primers for the parasite 18s rRNA gene. Positive wells containing parasites were identified after rapid separation of PCR products by gel electrophoresis. RESULTS: The PCR-based method can consistently detect a parasitaemia as low as 0.0005%, which is equivalent to 30 parasite genomes in a single well of a 96-well plate. Parasite clones were easily detected from cloning plates using this method and a comparison of PCR results with Giemsa-stained blood smears showed that PCR not only detected all the positive wells identified in smears, but also detected wells not identified otherwise, thereby confirming its sensitivity. CONCLUSIONS: The PCR-based method reported here is a simple, sensitive and efficient method for detecting parasite clones in culture. This method requires very little manual labor and can be completely automated for high throughput studies. The method is sensitive enough to detect parasites a week before they can be seen in Giemsa smears and is highly effective in identifying slow growing parasite clones.

17: *Malar J.* 2008 Oct 29;7(1):221.

**Operational accuracy and comparative persistent antigenicity of HRP2 rapid diagnostic tests for *Plasmodium falciparum* malaria in a hyperendemic region of Uganda.**

<http://www.malariajournal.com/content/7/1/221>

Kyabayinze DJ, Tibenderana JK, Odong GW, Rwakimari JB, Counihan H.

ABSTRACT: BACKGROUND: Parasite-based diagnosis of malaria by microscopy requires laboratory skills that are generally unavailable at peripheral health facilities. Rapid diagnostic tests (RDTs) require less expertise, but accuracy under operational conditions has not been fully evaluated in Uganda. There are also concerns about RDTs that use the antigen histidine-rich protein 2 (HRP2) to detect *Plasmodium falciparum*, because this antigen can persist after effective treatment, giving false positive test results in the absence of infection. An assessment of the accuracy of Malaria Pf immuno-chromatographic test (ICT) and description of persistent antigenicity of HRP2 RDTs was undertaken in a hyperendemic area of Uganda. METHODS: Using a cross-sectional design, a total of 357 febrile patients of all ages were tested using ICT, and compared to microscopy as the gold standard reference. Two independent RDT readings were used to assess accuracy and inter-observer reliability. With a longitudinal design to describe persistent antigenicity of ICT and Paracheck, 224 children aged 6-59 months were followed up at 7-day intervals until the HRP2 antigens were undetectable by the RDTs. RESULTS: Of the 357 patients tested during the cross-sectional component, 40% (139) had positive blood smears for asexual forms of *P. falciparum*. ICT had an overall sensitivity of 98%, a specificity of 72%, a negative predictive value (NPV) of 98% and a positive predictive value (PPV) of 69%. ICT showed a high inter-observer reliability under operational conditions, with 95% of readings having assigned the same results (kappa statistics 0.921,  $p < 0.001$ ). In children followed up after successful antimalaria treatment, the mean duration of persistent antigenicity was 32 days, and this duration varied significantly depending on pre-treatment parasitaemia. In patients with parasite density  $> 50,000/\mu\text{l}$ , the mean duration of persistent antigenicity was 37 days compared to 26 days for parasitaemia less than  $1,000/\mu\text{l}$  (log rank 21.9,  $p < 0.001$ ). CONCLUSIONS: ICT is an accurate and appropriate test for operational use as a diagnostic tool where microscopy is unavailable. However, persistent antigenicity reduces the accuracy of this and other HRP2-based RDTs. The low specificity continues to be of concern, especially in children below five years of age. These pose limitations that need consideration, such as their use for diagnosis of patients returning with symptoms within two to four weeks of treatment. Good clinical skills are essential to interpret test results.

18: *Malar J.* 2008 Oct 29;7(1):220.

**Chloroquine-resistant *Plasmodium vivax* malaria in Debre Zeit, Ethiopia.**

<http://www.malariajournal.com/content/7/1/220>

Teka H, Petros B, Yamuah L, Tesfaye G, Elhassan I, Muchohi S, Kokwaro G, Aseffa A, Engers H.

**ABSTRACT: BACKGROUND:** *Plasmodium vivax* accounts for about 40% of all malaria infection in Ethiopia. Chloroquine (CQ) is the first line treatment for confirmed *P. vivax* malaria in the country. The first report of CQ treatment failure in *P. vivax* was from Debre Zeit, which suggested the presence of chloroquine resistance. **METHODS:** An in vivo drug efficacy study was conducted in Debre Zeit from June to August 2006. Eighty-seven patients with microscopically confirmed *P. vivax* malaria, aged between 8 months and 52 years, were recruited and treated under supervision with CQ (25 mg/kg over three days). Clinical and parasitological parameters were assessed during the 28 day follow-up period. CQ and desethylchloroquine (DCQ) blood and serum concentrations were determined with high performance liquid chromatography (HPLC) in patients who showed recurrent parasitaemia. **RESULTS:** Of the 87 patients recruited in the study, one was lost to follow-up and three were excluded due to *P. falciparum* infection during follow-up. A total of 83 (95%) of the study participants completed the follow-up. On enrolment, 39.8% had documented fever and 60.2% had a history of fever. The geometric mean parasite density of the patients was 6,614.6 parasites/microlitre. Among these, four patients had recurrent parasitaemia on Day 28. The blood CQ plus DCQ concentrations of these four patients were all above the minimal effective concentration (>100 ng/ml). **CONCLUSIONS:** Chloroquine-resistant *P. vivax* parasites are emerging in Debre Zeit, Ethiopia. A multi-centre national survey is needed to better understand the extent of *P. vivax* resistance to CQ in Ethiopia.

19: *Malar J.* 2008 Oct 28;7(1):219.

**Country-wide assessment of the genetic polymorphism in *Plasmodium falciparum* and *Plasmodium vivax* antigens detected with rapid diagnostic tests for malaria.**

<http://www.malariajournal.com/content/7/1/219>

Mariette N, Barnadas C, Bouchier C, Tichit M, Menard D.

**ABSTRACT: BACKGROUND:** Rapid diagnostic tests (RDTs) are becoming increasingly indispensable in malaria management, as a means of increasing the accuracy of diagnosis. The WHO has issued recommendations, but the selection of the most suitable RDT remains difficult for users in endemic countries. The genetic variability of the antigens detected with RDTs has been little studied, but may affect the sensitivity of RDTs. This factor has been studied by comparisons between countries at continental level, but little information is available concerning antigen variability within a given country. **METHODS:** A country-wide assessment of polymorphism of the PfHRP2, PfHRP3, pLDH and aldolase antigens was carried out in 260 *Plasmodium falciparum* and 127 *Plasmodium vivax* isolates, by sequencing the genes encoding these antigens in parasites originating from the various epidemiological strata for malaria in Madagascar. **RESULTS:** Higher levels of polymorphism were observed for the pfhrp2 and pfhrp3 genes than for the *P. falciparum* and *P. vivax* aldolase and pldh genes. Pfhrp2 sequence analysis predicted that 9% of Malagasy isolates would not be detected at parasite densities [less than or equal to] 250 parasites/ul (ranging from 6% in the north to 14% in the south), although RDTs based on PfHRP2 detection are now recommended in Madagascar. **CONCLUSIONS:** These findings highlight the importance of training of health workers and the end users of RDTs in the provision of information about

the possibility of false-negative results for patients with clinical symptoms of malaria, particularly in the south of Madagascar.

20: *Malar J.* 2008 Oct 27;7(1):218.

**Human population, urban settlement patterns and their impact on Plasmodium falciparum malaria endemicity.**

<http://www.malariajournal.com/content/7/1/218>

Tatem AJ, Guerra CA, Kabaria CW, Noor AM, Hay SI.

**ABSTRACT: BACKGROUND:** The efficient allocation of financial resources for malaria control and the optimal distribution of appropriate interventions require accurate information on the geographic distribution of malaria risk and of the human populations it affects. Low population densities in rural areas and high population densities in urban areas can influence malaria transmission substantially. Here, the Malaria Atlas Project (MAP) global database of Plasmodium falciparum parasite rate (PfPR) surveys, medical intelligence and contemporary population surfaces are utilized to explore these relationships and other issues involved in combining malaria risk maps with those of human population distribution in order to define populations at risk more accurately. **METHODS:** First, an existing population surface was examined to determine if it was sufficiently detailed to be used reliably as a mask to identify areas of very low and very high population density as malaria free regions. Second, the potential of international travel and health guidelines (ITHGs) for identifying malaria free cities was examined. Third, the differences in PfPR values between surveys conducted in author-defined rural and urban areas were examined. Fourth, the ability of various global urban extent maps to reliably discriminate these author-based classifications of urban and rural in the PfPR database was investigated. Finally, the urban map that most accurately replicated the author-based classifications was analysed to examine the effects of urban classifications on PfPR values across the entire MAP database. **RESULTS:** Masks of zero population density excluded many non-zero PfPR surveys, indicating that the population surface was not detailed enough to define areas of zero transmission resulting from low population densities. In contrast, the ITHGs enabled the identification and mapping of 53 malaria free urban areas within endemic countries. Comparison of PfPR survey results showed significant differences between author-defined 'urban' and 'rural' designations in Africa, but not for the remainder of the malaria endemic world. The Global Rural Urban Mapping Project (GRUMP) urban extent mask proved most accurate for mapping these author-defined rural and urban locations, and further sub-divisions of urban extents into urban and peri-urban classes enabled the effects of high population densities on malaria transmission to be mapped and quantified. **CONCLUSION:** The availability of detailed, contemporary census and urban extent data for the construction of coherent and accurate global spatial population databases is often poor. These known sources of uncertainty in population surfaces and urban maps have the potential to be incorporated into future malaria burden estimates. Currently, insufficient spatial information exists globally to identify areas accurately where population density is low enough to impact upon transmission. Medical intelligence does however exist to reliably identify malaria free cities. Moreover, in Africa, urban areas that have a significant effect on malaria transmission can be mapped.

21: *Malar J.* 2008 Oct 26;7(1):217.

**History of malaria control in Tajikistan and rapid malaria appraisal in an agro-ecological setting.**

<http://www.malariajournal.com/content/7/1/217>

Matthys B, Sherkanov T, Karimov SS, Khabirov Z, Mostowlansky T, Utzinger J, Wyss K.

**ABSTRACT: BACKGROUND:** Reported malaria cases in rice growing areas in western Tajikistan were at the root of a rapid appraisal of the local malaria situation in a selected agro-ecological setting where only scarce information was available. The rapid appraisal was complemented by a review of the epidemiology and control of malaria in Tajikistan and Central Asia from 1920 until today. Following a resurgence in the 1990s, malaria transmission has been reduced considerably in Tajikistan as a result of concerted efforts by the government and international agencies. The goal for 2015 is transmission interruption, with control interventions and surveillance currently concentrated in the South, where foci of *Plasmodium vivax* and *Plasmodium falciparum* persist. **METHODS:** The rapid malaria appraisal was carried out in six communities of irrigated rice cultivation during the peak of malaria transmission (August/September 2007) in western Tajikistan. In a cross-sectional survey, blood samples were taken from 363 schoolchildren and examined for *Plasmodium* under a light microscope. A total of 56 farmers were interviewed about agricultural activities and malaria. Potential *Anopheles* breeding sites were characterized using standardized procedures. A literature review on the epidemiology and control of malaria in Tajikistan was conducted. **RESULTS:** One case of *P. vivax* was detected among the 363 schoolchildren examined (0.28%). The interviewees reported to protect themselves against mosquito bites and used their own concepts on fever conditions, which do not distinguish between malaria and other diseases. Three potential malaria vectors were identified, i.e. *Anopheles superpictus*, *Anopheles pulcherrimus* and *Anopheles hyrcanus* in 58 of the 73 breeding sites examined (79.5%). Rice paddies, natural creeks and man-made ponds were the most important *Anopheles* habitats. **CONCLUSION:** The presence of malaria vectors and parasite reservoirs, low awareness of, and protection against malaria in the face of population movements and inadequate surveillance may render local communities vulnerable to potential epidemics. To attain malaria transmission interruption in Tajikistan by 2015, there is a need for rigorous surveillance along with strengthening of primary health care facilities for effective case management, and possibly a more differentiated vector control strategy based on additional local evidence.

22: *Malar J.* 2008 Oct 24;7(1):216.

**A country-wide malaria survey in Mozambique. I. *Plasmodium falciparum* infection in children in different epidemiological settings.**

<http://www.malariajournal.com/content/7/1/216>

Mabunda S, Casimiro S, Quinto L, Alonso P.

**ABSTRACT: BACKGROUND:** Across tropical Africa the bulk of malaria-related morbidity and mortality is particularly high during childhood. Classical malariometric surveys have relied on assessing malaria infection prevalence. The last comprehensive evaluation of the malaria situation in Mozambique was carried out during the 1950s. This aims to characterize the malaria transmission intensities and to estimate the disease burden that may help guide control programme. **METHODS:** Between February 2002 and April 2003, a house-to-house survey, was carried out in 24 districts randomly selected. A total of 8,816 children aged below 10 years old were enrolled. Finger prick and blood collection were performed to prepare thick and thin films for malaria parasite species identification, density and haemoglobin concentration. Axillary temperature was also measured. Prevalence of infection, parasite density and anaemia were estimated for age groups category in each region/stratum. Comparisons between proportions were made using Chi-square test or Fisher exact. Relationship between age groups, region/stratum and parasite prevalence, density was determined using

linear regression. All survey mean estimations were adjusted for sampling weights, clustering and stratification. RESULTS: Malaria parasite prevalence was 58.9% (5.190/8.816), the majority of blood smears 52.4% (4,616/8,816) were due to Plasmodium falciparum and geometric mean parasite density was 1,211 parasites/ $\mu$ l (95% CI, 1,141 - 1,286). Gametocytes prevalence, only for P. falciparum was 5.6% (518/8,816). The burden was highest in the northern regions and in the coastal stratum. Parasite infection and geometric mean parasite density peaked during the second year of life and thereafter decreased with increasing age. Mean haemoglobin concentrations was 9.9 g/dl (95% CI 9.5 - 10.2). Anaemia prevalence was 69.8% (6.257/8.816) and among anaemic children 11.5% (743/6.257) were severely anaemic. Anaemia rose dramatically during the first year of life to peak among children in the 12 - 23 months age group. Highest levels of anaemia were recorded in both northern and central-northern regions 77.9% and 79.4% respectively. CONCLUSIONS: This survey confirms that malaria especially that caused by P. falciparum, remains endemic throughout the country. The burden of malaria disease and anaemia-related malaria during childhood constitute a major public health problem and warrant integrated and collaborative interventions towards its control.

23: Malar J. 2008 Oct 24;7(1):215.

**Trends in malaria morbidity following the introduction of artesunate plus amodiaquine combination in M'lomp village dispensary, south-western Senegal.**

<http://www.malariajournal.com/content/7/1/215>

Sarrassat S, Senghor P, Le Hesran JY.

ABSTRACT: BACKGROUND: In Thailand, South Africa and Zanzibar, a decrease in malaria morbidity was observed following the introduction of artemisinin-based combination therapy (ACT). In Senegal, therapeutic trials supervised the in vivo efficacy of artesunate plus amodiaquine from 1999 to 2005 at the M'lomp village dispensary. The trends in malaria morbidity in this village were evaluated from 2000 to 2002. METHODS: Each year, between July and December inclusive, fevers treated with antimalarials and slide-proven, uncomplicated malaria cases were collected from dispensary health records. Data were also collected in 1998, just prior to ACT introduction. Pearson's chi square tests and Student tests were used to compare two percentages or two means respectively ( $\alpha=0.05$ ). RESULTS: Between 1998 and 2002, the total number of fevers treated with antimalarials and their repetitiveness progressively decreased: From 2824 to 945 fevers and from 17.6% to 9.7% (RR1998-2002=0.55; [0.44-0.69];  $p<0.0001$ ) respectively. Considering uncomplicated malaria cases only, a decrease was observed in their total number between 2001 and 2002, from 953 to 570 cases. The incidence rate and repetitiveness also decreased. The incidence rate fell from 46.1% in 2001 to 37.5% in 2002 ( $p<0.0001$ ) and the repetitiveness decreased from 13.0% in 2000 to 6.6% in 2002 (RR2000-2002=0.51; [0.35-0.72];  $p=0.0001$ ). CONCLUSION: The percentage of uncomplicated malaria cases treated with ACT increased, from 18.9% in 2000 to 64.0% in 2002, making it tempting to conclude an impact on malaria morbidity. Nonetheless, the decline in incidence rate of uncomplicated malaria was slight and a lower recorded rainfall was reported in 2002 which could also explain this decline. The context in which ACT is introduced affects the impact on malaria morbidity. In M'lomp, in contrast to studies in Thailand, South Africa and Zanzibar, ACT coverage of malaria cases was low and no vector control measure was deployed. Moreover, the malaria transmission level is higher. In sub-Saharan countries, in order to optimize the impact on malaria morbidity, ACT deployment must be supported, on the one hand, by a strengthening of public health system to ensure a high ACT coverage and, on the other hand, by others measures, such vector control measures.

24: *Malar J.* 2008 Oct 21;7(1):214.

**Intra-specific variation of sperm length in the malaria vector *Anopheles gambiae*: males with shorter sperm have higher reproductive success.**

<http://www.malariajournal.com/content/7/1/214>

Voordouw MJ, Koella JC, Hurd H.

**ABSTRACT:** **BACKGROUND:** Intra-specific variation in sperm length influences male reproductive success in several species of insects. In males of the malaria vector *Anopheles gambiae*, sperm length is highly variable but the significance of this variation is unknown. Understanding what determines the reproductive success of male mosquitoes is critical for controlling malaria, and in particular for replacing natural populations with transgenic, malaria-resistant mosquitoes. **METHODS:** A laboratory population of *A. gambiae* males was tested for intra-specific variation in sperm length. A full-sib quantitative genetic design was used to test for a genetic component of sperm length in *A. gambiae* males and estimate its heritability. This study also tested for a relationship between sperm length and male reproductive success in *A. gambiae*. Male reproductive success was measured as the proportions of inseminated and ovipositing females. **RESULTS:** There was intra-specific variation of sperm length in *A. gambiae*. There was no significant genetic variation in sperm length and its heritability was low ( $h^2 = 0.18$ ) compared to other insects. Sperm length was correlated with male body size (measured as wing length). Males with short sperm had significantly higher reproductive success than males with long sperm and this was independent of body size. **CONCLUSIONS:** This is the first study to demonstrate intra-specific variation in sperm length in *A. gambiae* and that males with short sperm have higher reproductive success. That sperm length influences female oviposition is important for any strategy considering the release of transgenic males.

25: *Malar J.* 2008 Oct 21;7:213.

**The acceptability of intermittent preventive treatment of malaria in infants (IPTi) delivered through the expanded programme of immunization in southern Tanzania.**

<http://www.malariajournal.com/content/7/1/213>

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**BACKGROUND:** Intermittent preventive treatment of malaria in infants (IPTi) reduces the incidence of clinical malaria. However, before making decisions about implementation, it is essential to ensure that IPTi is acceptable, that it does not adversely affect attitudes to immunization or existing health seeking behaviour. This paper reports on the reception of IPTi during the first implementation study of IPTi in southern Tanzania. **METHODS:** Data were collected through in-depth interviews, focus group discussions and participant observation carried out by a central team of social scientists and a network of key informants/interviewers who resided permanently in the study sites. **RESULTS:** IPTi was generally acceptable. This was related to routinization of immunization and resonance with traditional practices. Promoting "health" was considered more important than preventing specific diseases. Many women thought that immunization was obligatory and that health staff might be unwilling to assist in the future if they were non-adherent. Weighing and socialising were important reasons for clinic attendance. Non-adherence was due largely to practical, social and structural factors, many of which could be overcome. Reasons for non-adherence

were sometimes interlinked. Health staff and "road to child health" cards were the main source of information on the intervention, rather than the specially designed posters. Women did not generally discuss child health matters outside the clinic, and information about the intervention percolated slowly through the community. Although there were some rumours about sulphadoxine pyrimethamine (SP), it was generally acceptable as a drug for IPTi, although mothers did not like the way tablets were administered. There is no evidence that IPTi had a negative effect on attitudes or adherence to the expanded programme on immunisation (EPI) or treatment seeking or existing malaria prevention. CONCLUSION: In order to improve adherence to both EPI and IPTi local priorities should be taken into account. For example, local women are often more interested in weighing than in immunization, and they view vaccination and IPTi as vaguely "healthy" rather preventing specific diseases. There should be more emphasis on these factors and more critical consideration by policy makers of how much local knowledge and understanding is minimally necessary in order to make interventions successful.

26: *Malar J.* 2008 Oct 21;7:212.

**Differences in genetic population structures of *Plasmodium falciparum* isolates from patients along Thai-Myanmar border with severe or uncomplicated malaria.**

<http://www.malariajournal.com/content/7/1/212>

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BACKGROUND: There have been many reports on the population genetic structures of *Plasmodium falciparum* from different endemic regions, but few studies have examined the characteristics of isolates from patients with different clinical outcomes. The population genetic structures of *P. falciparum* isolates from patients with either severe or uncomplicated malaria were examined. METHODS: Twelve microsatellite DNA loci from *P. falciparum* were used to assess the population genetic structures of 50 isolates (i.e., 25 isolates from patients with severe malaria and 25 from patients with uncomplicated malaria) collected in the Thai-Myanmar border area between 2002 and 2005. RESULTS: Genetic diversity and effective population sizes were greater in the uncomplicated malaria group than in the severe malaria group. Evidence of genetic bottlenecks was not observed in either group. Strong linkage disequilibrium was observed in the uncomplicated malaria group. The groups demonstrated significant genetic differentiation ( $P < 0.05$ ), and allele frequencies for 3 of the 12 microsatellite loci differed significantly between the two groups. CONCLUSION: These findings suggest that the genetic structure of *P. falciparum* populations in patients with severe malaria differs from that in patients with uncomplicated malaria. The microsatellite loci used in this study were presumably unrelated to antigenic features of the parasites, but, these findings suggest that some loci may influence the clinical outcome of malaria.

27: *Malar J.* 2008 Oct 20;7:211.

**Interaction of an atypical *Plasmodium falciparum* ETRAMP with human apolipoproteins.**

<http://www.malariajournal.com/content/7/1/211>

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**BACKGROUND:** In order to establish a successful infection in the human host, the malaria parasite *Plasmodium falciparum* must establish interactions with a variety of human proteins on the surface of different cell types, as well as with proteins inside the host cells. To better understand this aspect of malaria pathogenesis, a study was conducted with the goal of identifying interactions between proteins of the parasite and those of its human host. **METHODS:** A modified yeast two-hybrid methodology that preferentially selects protein fragments that can be expressed in yeast was used to conduct high-throughput screens with *P. falciparum* protein fragments against human liver and cerebellum libraries. The resulting dataset was analyzed to exclude interactions that are not likely to occur in the human host during infection. **RESULTS:** An initial set of 2,200 interactions was curated to remove proteins that are unlikely to play a role in pathogenesis based on their annotation or localization, and proteins that behave promiscuously in the two-hybrid assay, resulting in a final dataset of 456 interactions. A cluster that implicates binding between *P. falciparum* PFE1590w/ETRAMP5, a putative parasitophorous vacuole membrane protein, and human apolipoproteins ApoA, ApoB and ApoE was selected for further analysis. Different isoforms of ApoE, which are associated with different outcomes of malaria infection, were shown to display differential interactions with PFE1590w. **CONCLUSION:** A dataset of interactions between proteins of *P. falciparum* and those of its human host was generated. The preferential interaction of the *P. falciparum* PFE1590w protein with the human ApoE epsilon3 and ApoE epsilon4 isoforms, but not the ApoE epsilon2 isoform, supports the hypothesis that ApoE genotype affects risk of malaria infection. The dataset contains other interactions of potential relevance to disease that may identify possible vaccine candidates and drug targets.

28: *Malar J.* 2008 Oct 17;7:210.

**Artemisinin derivatives versus quinine in treating severe malaria in children: a systematic review.**

<http://www.malariajournal.com/content/7/1/210>

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**BACKGROUND:** The efficacy of intravenous quinine, which is the mainstay for treating severe malaria in children, is decreasing in South East Asia and Africa. Artemisinin derivatives are a potential alternative to quinine. However, their efficacy compared to quinine in treating severe malaria in children is not clearly understood. The objective of this review was to assess the efficacy of parenteral artemisinin derivatives versus parenteral quinine in treating severe malaria in children. **METHODS:** All randomized controlled studies comparing parenteral artemisinin derivatives with parenteral quinine in treating severe malaria in children were included in the review. Data bases searched were: The Cochrane Central Register of Controlled Trials (The Cochrane Library Issue 4, 2007), MEDLINE (1966 to February 2008), EMBASE (1980 to February 2008), and LILACS (1982 to February 2008). Dichotomous variables were compared using risk ratios (RR) and the continuous data using weighted mean difference (WMD). **RESULTS:** Twelve trials were included (1,524 subjects). There was no difference in mortality between artemisinin derivatives and quinine (RR = 0.90, 95% CI 0.73 to 1.12). The artemisinin derivatives resolved coma faster than quinine (WMD = -4.61, 95% CI: -7.21 to -2.00, fixed effect model), but when trials with adequate concealment only were considered this differences disappeared. There was no statistically significant difference between the two groups in parasite clearance time, fever clearance time, incidence of neurological sequelae and 28th day cure rate. One trial reported significantly more local reactions at the injection site

with intramuscular quinine compared to artemether. None of the trials was adequately powered to demonstrate equivalence. CONCLUSION: There was no evidence that treatment of children with severe malaria with parenteral artemisinin derivatives was associated with lower mortality or long-term morbidity compared to parenteral quinine. Future studies require adequately powered equivalence trial design to decide whether both drugs are equally effective.

29: *Malar J.* 2008 Oct 10;7:206.

**Spatial analysis of malaria in Anhui province, China.**

<http://www.malariajournal.com/content/7/1/206>

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BACKGROUND: Malaria has re-emerged in Anhui Province, China, and this province was the most seriously affected by malaria during 2005-2006. It is necessary to understand the spatial distribution of malaria cases and to identify highly endemic areas for future public health planning and resource allocation in Anhui Province. METHODS: The annual average incidence at the county level was calculated using malaria cases reported between 2000 and 2006 in Anhui Province. GIS-based spatial analyses were conducted to detect spatial distribution and clustering of malaria incidence at the county level. RESULTS: The spatial distribution of malaria cases in Anhui Province from 2000 to 2006 was mapped at the county level to show crude incidence, excess hazard and spatial smoothed incidence. Spatial cluster analysis suggested 10 and 24 counties were at increased risk for malaria ( $P < 0.001$ ) with the maximum spatial cluster sizes at  $< 50\%$  and  $< 25\%$  of the total population, respectively. CONCLUSION: The application of GIS, together with spatial statistical techniques, provide a means to quantify explicit malaria risks and to further identify environmental factors responsible for the re-emerged malaria risks. Future public health planning and resource allocation in Anhui Province should be focused on the maximum spatial cluster region.

30: *Malar J.* 2008 Oct 10;7(1):205.

**Spatial distribution of the chromosomal forms of anopheles gambiae in Mali.**

<http://www.malariajournal.com/content/7/1/205>

Sogoba N, Vounatsou P, Bagayoko MM, Doumbia S, Dolo G, Gosoniu L, Traore SF, Smith TA, Toure YT.

ABSTRACT: BACKGROUND: Maps of the distribution of malaria vectors are useful tools for stratification of malaria risk and for selective vector control strategies. Although the distribution of members of the *Anopheles gambiae* complex is well documented in Africa, a continuous map of the spatial distribution of the chromosomal forms of *An. gambiae* s.s. is not yet available at country level to support control efforts. METHODS: Bayesian geostatistical methods were used to produce continuous maps of the spatial distribution of the chromosomal forms of *An. gambiae* s.s. (Mopti, Bamako, Savanna and their hybrids/recombinants) based on their relative frequencies in relation to climatic and environmental factors in Mali. RESULTS: The maps clearly show that each chromosomal form favours a particular defined eco-climatic zone. The Mopti form prefers the dryer northern Savanna and Sahel and the flooded/irrigated areas of the inner delta of the Niger River. The Savanna form favours the Sudan savanna areas, particularly the South and South-Eastern parts of the country (Kayes and Sikasso regions). The Bamako

form has a strong preference for specific environmental conditions and it is confined to the Sudan savanna areas around urban Bamako and the Western part of Sikasso region. The hybrids/recombinants favour the Western part of the country (Kayes region) bordering the Republic of Guinea Conakry. CONCLUSIONS: The maps provide valuable information for selective vector control in Mali (insecticide resistance management) and may serve as a decision support tool for the basis for future malaria control strategies including genetically manipulated mosquitoes.

31: *Malar J.* 2008 Oct 9;7:204.

**CD36 selection of 3D7 Plasmodium falciparum associated with severe childhood malaria results in reduced VAR4 expression.**

<http://www.malariajournal.com/content/7/1/204>

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BACKGROUND: A subset of the Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1(SM)) is involved in the cytoadherence of P. falciparum-infected red blood cells (iRBC) contributing to the pathogenesis of severe disease among young children in malaria endemic areas. The PfEMP1(SM) are encoded by group A var genes that are composed of a more constrained range of amino acid sequences than groups B and C var genes encoding PfEMP1(UM) associated with uncomplicated malaria. Also, unlike var genes from groups B and C, those from group A do not have sequences consistent with CD36 binding--a major cytoadhesion phenotype of P. falciparum isolates. METHODS: A 3D7 PfEMP1(SM) sub-line (3D7(SM)) expressing VAR4 (PFD1235w/MAL8P1.207) was selected for binding to CD36. The protein expression of this parasite line was monitored by surface staining of iRBC using VAR4-specific antibodies. The serological phenotype of the 3D7(SM) parasites was determined by flow cytometry using malaria semi-immune and immune plasma and transcription of the 59 var genes in 3D7 were analysed by real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using var-specific primers. RESULTS: A selection-induced increased adhesion of 3D7(SM) iRBC to CD36 resulted in a reduced var4 transcription and VAR4 surface expression. CONCLUSION: VAR4 is not involved in CD36 adhesion. The current findings are consistent with the notion that CD36 adhesion is not associated with particular virulent parasite phenotypes, such as those believed to be exhibited by VAR4 expressing parasites.

32: *Parasit Vectors.* 2008 Oct 22;1(1):42.

**Longitudinal evaluation of Ocimum and other plants effects on the feeding behavioral response of mosquitoes (Diptera: Culicidae) in the field in Tanzania.**

<http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=pubmed&pubmedid=18945343>

Kweka EJ, Mosha FW, Lowassa A, Mahande AM, Mahande MJ, Massenga CP, Tenu F, Lyatuu EE, Mboya MA, Temu EA.

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ABSTRACT: BACKGROUND: The use of repellent materials from plants against nuisance insects is common with great potential to compliment existing malaria control programmes and this requires evaluation in the field. Ocimum plant species, Ocimum suave (Willd) and O. kilimandscharicum (Guerke) materials and their essential oils extracted by steam distillation were evaluated in the field and experimental huts for repellence, exophily and feeding inhibition effects against

three mosquito species, *Anopheles arabiensis* (Patton), *An. gambiae* ss (Giles) and *Culex quinquefasciatus* (Say). The protective effect of essential oils from *Ocimum* plants were compared with N, N-diethyl-3-methylbenzamide (DEET), a standard synthetic repellent. Also, the protective effect of fumigation by burning of repellent plants; *Ocimum suave*, *Ocimum kilimandscharicum*, *Azadirachta indica*, *Eucalyptus globules* and *Lantana camara* were tested in experimental huts and selected local houses. RESULTS: In the field, protection by *Ocimum* plants from mosquito bites was high and there was small variation among different mosquito species. Protection efficiency was 93.4%, 91.98% and 89.75% for *An. arabiensis* while for *Cx. quinquefasciatus* it was 91.30%, 88.65% and 90.50% for DEET, *Ocimum suave* and *O. kilimandscharicum* respectively. In the experimental hut, deterrence induced by burning of *Ocimum* and other plants ranged from 73.1.0% to 81.9% for *An. arabiensis* and 56.5% to 67.8% for *Cx. quinquefasciatus*, while feeding inhibition was 61.1% to 100% for *An. arabiensis* and 50% to 100% for *Cx. quinquefasciatus*. Evaluations under field conditions confirmed high protective efficacy, enhanced feeding inhibition and house entry inhibition (Deterrence). CONCLUSION: This study shows the potential of *Ocimum suave* and *Ocimum kilimandscharicum* crude extracts and whole plants of *Ocimum suave*, *Ocimum kilimandscharicum*, *Azadirachta indica*, *Eucalyptus globules* and *Lantana camara* for use in protecting against human biting while the burning of plants reduces significantly the indoor resting mosquitoes.

33: *PLoS ONE*. 2008;3(10):e3557.

**Antibody-mediated growth inhibition of *Plasmodium falciparum*: relationship to age and protection from parasitemia in Kenyan children and adults.**

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003557>

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BACKGROUND: Antibodies that impair *Plasmodium falciparum* merozoite invasion and intraerythrocytic development are one of several mechanisms that mediate naturally acquired immunity to malaria. Attempts to correlate anti-malaria antibodies with risk of infection and morbidity have yielded inconsistent results. Growth inhibition assays (GIA) offer a convenient method to quantify functional antibody activity against blood stage malaria. METHODS: A treatment-time-to-infection study was conducted over 12-weeks in a malaria holoendemic area of Kenya. Plasma collected from healthy individuals (98 children and 99 adults) before artemether-lumefantrine treatment was tested by GIA in three separate laboratories. RESULTS: Median GIA levels varied with *P. falciparum* line (D10, 8.8%; 3D7, 34.9%; FVO, 51.4% inhibition). The magnitude of growth inhibition decreased with age in all *P. falciparum* lines tested with the highest median levels among children <4 years compared to adults (e.g. 3D7, 45.4% vs. 30.0% respectively,  $p = 0.0003$ ). Time-to-infection measured by weekly blood smears was significantly associated with level of GIA controlling for age. Upper quartile inhibition activity was associated with less risk of infection compared to individuals with lower levels (e.g. 3D7, hazard ratio = 1.535, 95% CI = 1.012-2.329;  $p = 0.0438$ ). Various GIA methodologies had little effect on measured parasite growth inhibition. CONCLUSION: Plasma antibody-mediated growth inhibition of blood stage *P. falciparum* decreases with age in residents of a malaria holoendemic area. Growth inhibition assay may be a useful surrogate of protection against infection when outcome is controlled for age.

34: *PLoS ONE*. 2008;3(10):e3571.

**Acquisition of growth-inhibitory antibodies against blood-stage *Plasmodium falciparum*.**

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003571>

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**BACKGROUND:** Antibodies that inhibit the growth of blood-stage *Plasmodium falciparum* may play an important role in acquired and vaccine-induced immunity in humans. However, the acquisition and activity of these antibodies is not well understood. **METHODS:** We tested dialysed serum and purified immunoglobulins from Kenyan children and adults for inhibition of *P. falciparum* blood-stage growth in vitro using different parasite lines. Serum antibodies were measured by ELISA to blood-stage parasite antigens, extracted from *P. falciparum* schizonts, and to recombinant merozoite surface protein 1 (42 kDa C-terminal fragment, MSP1-42). **RESULTS:** Antibodies to blood-stage antigens present in schizont protein extract and to recombinant MSP1-42 significantly increased with age and were highly correlated. In contrast, growth-inhibitory activity was not strongly associated with age and tended to decline marginally with increasing age and exposure, with young children demonstrating the highest inhibitory activity. Comparison of growth-inhibitory activity among samples collected from the same population at different time points suggested that malaria transmission intensity influenced the level of growth-inhibitory antibodies. Antibodies to recombinant MSP1-42 were not associated with growth inhibition and high immunoglobulin G levels were poorly predictive of inhibitory activity. The level of inhibitory activity against different isolates varied. **CONCLUSIONS:** Children can acquire growth-inhibitory antibodies at a young age, but once they are acquired they do not appear to be boosted by on-going exposure. Inhibitory antibodies may play a role in protection from early childhood malaria.

35: *PLoS ONE*. 2008;3(10):e3549.

**Gene disruption of *Plasmodium falciparum* p52 results in attenuation of malaria liver stage development in cultured primary human hepatocytes.**

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003549>

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Difficulties with inducing sterile and long lasting protective immunity against malaria with subunit vaccines has renewed interest in vaccinations with attenuated *Plasmodium* parasites. Immunizations with sporozoites that are attenuated by radiation (RAS) can induce strong protective immunity both in humans and rodent models of malaria. Recently, in rodent parasites it has been shown that through the deletion of a single gene, sporozoites can also become attenuated in liver stage development and, importantly, immunization with these sporozoites results in immune responses identical to RAS. The promise of vaccination using these genetically attenuated sporozoites (GAS) depends on translating the results in rodent malaria models to human malaria. In this study,

we perform the first essential step in this transition by disrupting, p52, in *P. falciparum* an ortholog of the rodent parasite gene, p36p, which we had previously shown can confer long lasting protective immunity in mice. These *P. falciparum* P52 deficient sporozoites demonstrate gliding motility, cell traversal and an invasion rate into primary human hepatocytes in vitro that is comparable to wild type sporozoites. However, inside the host hepatocyte development is arrested very soon after invasion. This study reveals, for the first time, that disrupting the equivalent gene in both *P. falciparum* and rodent malaria *Plasmodium* species generates parasites that become similarly arrested during liver stage development and these results pave the way for further development of GAS for human use.

36: *PLoS ONE*. 2008;3(10):e3403.

**A nationwide survey of the quality of antimalarials in retail outlets in Tanzania.**

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003403>

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**INTRODUCTION:** Retail pharmaceutical products are commonly used to treat fever and malaria in sub-Saharan African countries. Small scale studies have suggested that poor quality antimalarials are widespread throughout the region, but nationwide data are not available that could lead to generalizable conclusions about the extent to which poor quality drugs are available in African communities. This study aimed to assess the quality of antimalarials available from retail outlets across mainland Tanzania. **METHODS AND FINDINGS:** We systematically purchased samples of oral antimalarial tablets from retail outlets across 21 districts in mainland Tanzania in 2005. A total of 1080 antimalarial formulations were collected including 679 antifol antimalarial samples (394 sulfadoxine/pyrimethamine and 285 sulfamethoxypyrazine/pyrimethamine), 260 amodiaquine samples, 63 quinine samples, and 51 artemisinin derivative samples. A systematic subsample of 304 products was assessed for quality by laboratory based analysis to determine the amount of the active ingredient and dissolution profile by following the published United States Pharmacopoeia (USP) monogram for the particular tablet being tested. Products for which a published analytical monogram did not exist were assessed on amount of active ingredient alone. Overall 38 or 12.2% of the samples were found to be of poor quality. Of the antifolate antimalarial drugs tested 13.4% were found to be of poor quality by dissolution and content analysis using high-performance liquid chromatography (HPLC). Nearly one quarter (23.8%) of quinine tablets did not comply within the tolerance limits of the dissolution and quantification analysis. Quality of amodiaquine drugs was relatively better but still unacceptable as 7.5% did not comply within the tolerance limits of the dissolution analysis. Formulations of the artemisinin derivatives all contained the stated amount of active ingredient when analysed using HPLC alone. **CONCLUSIONS:** Substandard antimalarial formulations were widely available in Tanzania at the time of this study. No products were detected that did not contain any amount of the stated active ingredient. Quinine and sulfadoxine/pyrimethamine products were the most widely available and also the most likely to be of poor quality. Substandard products were identified in all parts of the country and were labeled as made by both domestic and international manufacturers. With the expansion of the retail pharmaceutical sector as a delivery channel for antimalarial formulations the need for regular nationwide monitoring of their quality will become increasingly important.

37: *PLoS ONE*. 2008;3(10):e3366.

**amal genes of sympatric *Plasmodium vivax* and *P. falciparum* from Venezuela differ significantly in genetic diversity and recombination frequency.**

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003366>

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**BACKGROUND:** We present the first population genetic analysis of homologous loci from two sympatric human malaria parasite populations sharing the same human hosts, using full-length sequences of amal genes from *Plasmodium vivax* and *P. falciparum* collected in the Venezuelan Amazon. **METHODOLOGY/PRINCIPAL FINDINGS:** Significant differences between the two species were found in genetic diversity at the amal locus, with 18 distinct haplotypes identified among the 73 Pvamal sequences obtained, compared to 6 unique haplotypes from 30 Pfamal sequences, giving overall diversity estimates of  $h = 0.9091$ , and  $h = 0.538$  respectively. Levels of recombination were also found to differ between the species, with *P. falciparum* exhibiting very little recombination across the 1.77 kb sequence. In contrast, analysis of patterns of nucleotide substitutions provided evidence that polymorphisms in the amal gene of both species are maintained by balancing selection, particularly in domain I. The two distinct population structures observed are unlikely to result from different selective forces acting upon the two species, which share both human and mosquito hosts in this setting. Rather, the highly structured *P. falciparum* population appears to be the result of a population bottleneck, while the much less structured *P. vivax* population is likely to be derived from an ancient pool of diversity, as reflected in a larger estimate of effective population size for this species. Greatly reduced mosquito transmission in 1997, due to low rainfall prior to the second survey, was associated with far fewer *P. falciparum* infections, but an increase in *P. vivax* infections, probably due to hypnozoite activation. **CONCLUSIONS/SIGNIFICANCE:** The relevance of these findings to putative competitive interactions between these two important human pathogen species is discussed. These results highlight the need for future control interventions to employ strategies targeting each of the parasite species present in endemic areas.

38: *Proc Natl Acad Sci U S A*. 2008 Nov 4;105(44):17097-102.

**Angiopoietin-2 is associated with decreased endothelial nitric oxide and poor clinical outcome in severe falciparum malaria.**

<http://hwmaint.pnas.org/cgi/content/full/105/44/17097>

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Adherence of parasitized erythrocytes to activated endothelium causes microvascular obstruction, tissue ischemia, and clinical complications in severe malaria (SM); however, the mechanisms leading to endothelial activation remain unclear. The angiogenic factors, angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) are modulators of endothelial activation, with Ang-2 release from Weibel-Palade bodies (WPBs) being regulated by endothelial nitric oxide (NO). We explored the relationships between endothelial NO bioavailability, Ang-2, VEGF, tissue perfusion, and clinical outcomes in SM. We measured plasma Ang-2 and VEGF, together with biomarkers of severity from 146 adults with and

without SM, in parallel with longitudinal measures of endothelial function by using reactive hyperemia peripheral arterial tonometry (a measure of endothelial NO bioavailability). Regression was used to relate concentrations of Ang-2/VEGF with malaria disease severity, biomarkers of perfusion, endothelial activation, and parasite biomass. The longitudinal relationship between Ang-2 and endothelial function was assessed by using a mixed-effects model. Ang-2 concentrations were elevated in SM and associated with increased venous lactate, plasma intercellular cell adhesion molecule-1 concentrations, parasite biomass, and mortality. In contrast, VEGF concentrations were inversely associated with these biomarkers. Ang-2 concentrations were significantly better predictors of death than venous lactate ( $P = 0.03$ ). Recovery of endothelial function was associated with falling concentrations of Ang-2. Ang-2 release from endothelial cells with reduced NO bioavailability is likely to contribute to endothelial activation, sequestered parasite biomass, impaired perfusion, and poor outcome in severe falciparum malaria. Agents that improve endothelial NO, reduce WPB exocytosis, and/or antagonize Ang-2 may have therapeutic roles in SM.

## **Part B – Citations and abstracts (Alphabetical by journal title)**

1: *Am J Trop Med Hyg.* 2008 Nov;79(5):670-2.

### **Evaluation of two new immunochromatographic assays for diagnosis of malaria.**

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We assessed the performance of two new commercially available rapid diagnostic tests (RDTs) for malaria (SD Bioline Malaria Ag Pf test and Ag Pf/Pan test) in 200 patients with uncomplicated malaria between August and October 2007 in Madagascar. Results of the two RDTs were compared with those obtained by microscopy and real-time polymerase chain reaction. The sensitivity and specificity for detection of *Plasmodium falciparum* were 93% and 98.9%, respectively, for the SD Bioline Malaria Ag Pf test and 92.9% and 98.9% for the SD Bioline Malaria Ag Pf/Pan test. The sensitivity of the SD Bioline Malaria Ag Pf/Pan test was much lower for detection of other species (63.6%). The sensitivity of the two new assays decreased to 77.3% at parasitemia levels < 100 parasites/microL for detection of *P. falciparum*.

2: *Am J Trop Med Hyg.* 2008 Nov;79(5):662-9.

### **Characterization of "Yaa Chud" Medicine on the Thailand-Myanmar border: selecting for drug-resistant malaria and threatening public health.**

Newton PN, Hampton CY, Alter-Hall K, Teerwarakulpana T, Prakongpan S, Ruangveerayuth R, White NJ, Day NP, Tudino MB, Mancuso N, Fernández FM.

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Multidrug-resistant *Plasmodium falciparum* malaria is a severe public health problem on the Thailand-Myanmar border. Many villagers buy packets of 4-5 mixed medicines ("yaa chud") from shops without medical assessment as their first-line malaria treatment. In 2000-2001 a local researcher purchased 50 yaa chud from 44 shops around Mae Sot, Thailand and Myawaddy, Myanmar (Burma), for his wife who was said to be pregnant with fever and drowsiness. The tablets/capsules were provisionally identified by appearance and active ingredients determined in a subset by using mass and atomic spectrometry. The most frequently detected active ingredients were acetaminophen (22%), chlorpheniramine (13.4%), chloroquine

(12.6%), tetracycline/doxycycline (11.4%), and quinine (5.1%). Only seven bags contained potentially curative medicine for malaria. A total of 82% of the bags contained medicines contraindicated in pregnancy. Inappropriate, ineffective antimalarial drugs on the Thailand-Myanmar border are likely to increase malaria morbidity, mortality and health costs and engender the emergence and spread of antimalarial drug resistance.

3: *Am J Trop Med Hyg.* 2008 Nov;79(5):655-61.

**A randomized trial of artesunate-mefloquine versus artemether-lumefantrine for treatment of uncomplicated Plasmodium falciparum malaria in Mali.**

Sagara I, Diallo A, Kone M, Coulibaly M, Diawara SI, Guindo O, Maiga H, Niamebele MB, Sissoko M, Dicko A, Djimde A, Doumbo OK.

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The choice of appropriate artemisinin-based combination therapy depends on several factors (cost, efficacy, safety, reinfection rate, and simplicity of administration). In this study, we tested the hypothesis that artesunate-mefloquine (Artequin) is as efficacious as artemether-lumefantrine (Coartem) in treatment of uncomplicated Plasmodium falciparum malaria. The study was carried out from August 2004 through February 2005 in Kambila, Mali. Subjects with weights  $\geq 10$  kg and uncomplicated malaria were enrolled.

Artesunate-mefloquine was given once a day for three days and artemether/lumefantrine twice a day for three days. A total of 470 (235 in each arm) patients were enrolled. The unadjusted 28-day cure rate was higher in artesunate-mefloquine arm than in the artemether-lumefantrine arm (79.7% versus 67.8%;  $P < 0.004$ ). After correction for reinfection, the 28-day cure rates were similar in the two groups (96.04% versus 96.93%). Artesunate-mefloquine is well-tolerated and is as effective as artemether-lumefantrine for the treatment of P. falciparum malaria. Artesunate-mefloquine also prevented more new infections.

4: *Am J Trop Med Hyg.* 2008 Nov;79(5):652-4.

**Forest malaria in central Vietnam.**

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Studies were conducted in a village in central Vietnam to explain the existence of a forest malaria cycle of transmission external to the village. The findings suggested no malaria transmission in the village because of the absence of a suitable vector, but suggested evidence for transmission in villagers when attending garden plots in the forested hills surrounding the village. A sizeable population residing near these garden plots, the presence of Anopheles dirus (a highly efficient vector), and a degree of malaria immunity within the inhabitants created suitable conditions to sustain malaria transmission outside the village.

5: *Antimicrob Agents Chemother.* 2008 Nov;52(11):3868-74.

**Synthesis, antimalarial activity, and intracellular targets of MEFAS, a new hybrid compound derived from mefloquine and artesunate.**

de Pilla Varotti F, Botelho AC, Andrade AA, de Paula RC, Fagundes EM, Valverde A, Mayer LM, Mendonça JS, de Souza MV, Boechat N, Krettli AU.

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A new synthetic antimalarial drug, a salt derived from two antimalarial molecules, mefloquine (MQ) and artesunate (AS), here named MEFAS, has been tested for its pharmacological activity. Combinations of AS plus MQ hydrochloride are currently being used in areas with drug-resistant *Plasmodium falciparum* parasites; although AS clears parasitemia in shorter time periods than any other antimalarial drug, it does not cure infected patients; in addition, MQ causes side effects and is rather expensive, important problems considering that malaria affects mostly populations in poor countries. Here, we show that MEFAS is more effective than the combination of AS and MQ, tested in parallel at different mass proportions, against *P. falciparum* (chloroquine-resistant clone W2 and chloroquine-sensitive clone 3D7) in vitro and in mice infected with *Plasmodium berghei*, promoting cure of this infection. MEFAS tested against HepG2 hepatoma cells exhibited lower toxicity than the antimalarials AS and MQ alone or combined. Possible targets of MEFAS have been studied by confocal microscopy using fluorescent probes (Fluo-4 AM and BCECF-AM) in *P. falciparum* synchronous culture of W2-infected red blood cells. Dynamic images show that MEFAS exhibited intracellular action increasing cytoplasmic Ca(2+) at 1.0 ng/ml. This effect was also observed in the presence of tapsigargin, an inhibitor of SERCA, suggesting an intracellular target distinct from the endoplasmic reticulum. Trophozoites loaded with BCECF-AM, when treated with MEFAS, were still able to mobilize protons from the digestive vacuole (DV), altering the pH gradient. However, in the presence of bafilomycin A1, an inhibitor of the H(+) pump from acidic compartments of eukaryotic cells, MEFAS had no action on the DV. In conclusion, the endoplasmic reticulum and DV are intracellular targets for MEFAS in *Plasmodium* sp., suggesting two modes of action of this new salt. Our data support MEFAS as a candidate for treating human malaria.

6: *Cochrane Database Syst Rev.* 2008 Oct 8;(4):CD004912.

#### **Drugs for treating uncomplicated malaria in pregnant women.**

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**BACKGROUND:** Women are more vulnerable to malaria during pregnancy, and malaria infection may have adverse consequences for the fetus. Identifying safe and effective treatments is important. **OBJECTIVES:** To compare the effects of drug regimens for treating uncomplicated *falciparum* malaria in pregnant women. **SEARCH STRATEGY:** We searched the Cochrane Infectious Diseases Group Specialized Register (February 2008), CENTRAL (The Cochrane Library 2008, Issue 1), MEDLINE (1966 to February 2008), EMBASE (1974 to February 2008), LILACS (February 2008), mRCT (February 2008), reference lists, and conference abstracts. We also contacted researchers in the field, organizations, and pharmaceutical companies. **SELECTION CRITERIA:** Randomized and quasi-randomized controlled trials of antimalarial drugs for treating uncomplicated malaria in pregnant women. **DATA COLLECTION AND ANALYSIS:** Two authors assessed trial eligibility and risk of bias, and extracted data. We performed a quantitative analysis only where we could combine the data. We combined dichotomous data using the risk ratio (RR) and presented each result with a 95% confidence interval (CI). **MAIN RESULTS:** Ten trials (1805 participants) met the inclusion criteria. Two were quasi-randomized, seven did not describe allocation concealment, and all adjusted treatment failure to exclude new infections. One trial reported fewer treatment failures at day 63 with artesunate plus mefloquine compared with quinine (RR 0.09, 95% CI 0.02 to 0.38; 106 participants). One trial reported fewer treatment failures at day 63 with artesunate plus atovaquone-proguanil compared with quinine (RR 0.14, 95% CI 0.03 to 0.57; 80 participants). One trial reported fewer treatment failures at day 28

when amodiaquine was compared with chloroquine (RR 0.20, 95% CI 0.08 to 0.46; 420 participants) and when amodiaquine plus sulfadoxine-pyrimethamine was compared with chloroquine (RR 0.02, 95% CI 0.00 to 0.26; 418 participants). Compared with sulfadoxine-pyrimethamine given alone, one trial reported fewer treatment failures at delivery (or day 40) with artesunate plus sulfadoxine-pyrimethamine (RR 0.15, 95% CI 0.04 to 0.59; 79 participants) and azithromycin plus sulfadoxine-pyrimethamine (RR 0.27, 95% CI 0.10 to 0.76; 82 participants).  
AUTHORS' CONCLUSIONS: Data are scant. Some combination treatments appear to be effective at treating malaria in pregnancy; however, safety data are limited.

7: *Gene*. 2008 Oct 15;423(1):63-71.

**Topi, an IS630/Tc1/mariner-type transposable element in the African malaria mosquito, *Anopheles gambiae*.**

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IS630/Tc1/mariner elements are diverse and widespread within insects. The African malaria mosquito, *Anopheles gambiae*, contains over 30 families of IS630/Tc1/mariner elements although few have been studied in any detail. To examine the history of Topi elements in *An. gambiae* populations, Topi elements (n=73) were sampled from five distinct populations of *An. gambiae* from eastern and western Africa and evaluated with respect to copy number, nucleotide diversity and insertion site-occupancy frequency. Topi 1 and 2 elements were abundant (10-34 per diploid genome) and highly diverse ( $\pi=0.051$ ). Elements from mosquitoes collected in Nigeria were Topi 2 elements and those from mosquitoes collected in Mozambique were Topi 1 elements. Of the 49 Topi transposase open reading frames sequenced none were found to be identical. Intact elements with complete transposase open reading frames were common, although based on insertion site-occupancy frequency data it appeared that genetic drift was the major force acting on these IS630/Tc1/mariner-type elements. Topi 3 elements were not recovered from any of the populations sampled in this study and appear to be rare elements in *An. gambiae*, possibly due to a recent introduction.

8: *Infect Immun*. 2008 Nov;76(11):5149-57.

**Enhanced Toll-like receptor responsiveness associated with mitogen-activated protein kinase activation in *Plasmodium falciparum*-infected children.**

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Acute *Plasmodium falciparum* infection is associated with strongly upregulated cytokine responses that are at least partly the result of activation of Toll-like receptors (TLRs). Whether and how TLR expression/responsiveness changes upon malarial infection is, however, currently not well understood. To assess this, we examined expression of TLRs and used the TLR ligand lipopolysaccharide (LPS) and Pam(3)Cys to stimulate peripheral blood mononuclear cells (PBMCs) from Ghanaian schoolchildren who live in a rural area where *P. falciparum* is endemic. Expression of TLR2 was higher, and responses to its ligand, Pam(3)Cys, were enhanced in *P. falciparum*-infected children compared to their uninfected counterparts. In cells from the same children, stimulation by Pam(3)Cys resulted in higher p38 mitogen-activated protein kinase activation and higher cytokine production. In vitro experiments confirmed that preincubation of PBMCs with *P. falciparum*-infected red blood cells enhanced responsiveness to TLR ligands. Taken

together, the data indicate that *P. falciparum*-infected children in areas where malaria is endemic have an altered innate immune system, which might be important for the balance between immunity and pathology when new infections are encountered or when novel vaccines are introduced.

9: *Infect Immun.* 2008 Nov;76(11):4876-82.

**Identification and characterization of the Plasmodium yoelii PyP140/RON4 protein, an orthologue of Toxoplasma gondii RON4, whose cysteine-rich domain does not protect against lethal parasite challenge infection.**

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Previously, we identified a *Plasmodium yoelii* YM 140-kDa merozoite protein, designated PyP140, which formed a complex with apical membrane antigen 1 (AMA1). Furthermore, we produced a nonprotective monoclonal antibody (MAB), 48F8, that immunoprecipitated metabolically labeled PyP140 and localized the protein to the merozoite's apical end and, less frequently, to the merozoite surface, as observed by immunofluorescence assay (IFA). Here, using MAB 48F8, we have identified the *pyp140* gene by screening a *P. yoelii* lambda-Zap cDNA expression library. The *pyp140* cDNA covers approximately 90% of the putative open reading frame (ORF) of PY02159 from the *P. yoelii* NL genome sequencing project. Analysis of the complete gene identified the presence of two introns. The ORF encodes a 102,407-Da protein with an amino-terminal signal sequence, a series of three unique types of repeats, and a cysteine-rich region. The binding site of MAB 48F8 was also identified. A BLAST search with the deduced amino acid sequence shows significant similarity with the *Toxoplasma gondii* RON4 protein and the *Plasmodium falciparum* RON4 protein, and the sequence is highly conserved in other *Plasmodium* species. We produced the cysteine-rich domain of PyP140/RON4 by using the *Pichia pastoris* expression system and characterized the recombinant protein biochemically and biophysically. BALB/c mice immunized with the protein formulated in oil-in-water adjuvants produced antibodies that recognize parasitized erythrocytes by IFA and native PyP140/RON4 by immunoblotting but failed to protect against a lethal *P. yoelii* YM infection. Our results show that PyP140/RON4 is located within the rhoptries or micronemes. It may associate in part with AMA1, but the conserved cysteine-rich domain does not appear to elicit inhibitory antibodies, a finding that is supported by the marked sequence conservation in this protein within *Plasmodium* spp., suggesting that it is not under immune pressure.

10: *Int J Biometeorol.* 2008 Nov;52(8):747-53.

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

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Water temperature is an important determinant in many aquatic biological processes, including the growth and development of malaria mosquito (*Anopheles arabiensis* and *A. gambiae*) immatures. Water turbidity affects water temperature, as suspended particles in a water column absorb and scatter sunlight and hence determine the extinction of solar radiation. To get a better understanding of the relationship between water turbidity and water temperature, a series of semi-natural larval habitats (diameter 0.32 m, water depth 0.16 m) with

increasing water turbidity was created. Here we show that at midday (1300 hours) the upper water layer (thickness of 10 mm) of the water pool with the highest turbidity was on average 2.8 degrees C warmer than the same layer of the clearest water pool. Suspended soil particles increase the water temperature and furthermore change the temperature dynamics of small water collections during daytime, exposing malaria mosquito larvae, which live in the top water layer, longer to higher temperatures.

11: *Int J Infect Dis.* 2008 Oct 14.

**Mutation in pfmdr1 gene in chloroquine-resistant Plasmodium falciparum isolates, Southeast Iran.**

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**OBJECTIVES:** The main objective of the present study was to detect point mutations at positions 86, 184, 1034, 1042, and 1246 of the Plasmodium falciparum multidrug resistance gene (pfmdr1) in blood samples collected from malaria patients in Chabahar, a harbor city located in Southeast Iran. **METHODS:** Twenty-six blood samples from patients infected with P. falciparum, who had a chloroquine (CQ) response failure, were collected pre-treatment. Following treatment with CQ, drug susceptibility was assessed using an in vivo test. Molecular detection of single nucleotide polymorphisms (SNPs) was carried out using the LightCycler hybridization probe assay. **RESULTS:** The pfmdr1 N86Y mutation was found in six isolates (23.1%). Mutations at the four other positions were not observed in any isolates. **CONCLUSION:** The present study showed no mutation at codon positions 184, 1034, 1042, and 1246 of pfmdr1 in any of the Iranian P. falciparum isolates; thus these alleles cannot serve as markers for CQ resistance in Iran.

12: *Int J Parasitol.* 2008 Oct 11.

**Morphology and kinetics of the three distinct phases of red blood cell invasion by Plasmodium falciparum merozoites.**

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The invasion of red blood cells (RBCs) is an essential event in the life cycle of all malaria-causing Plasmodium parasites; however, there are major gaps in our knowledge of this process. Here, we use video microscopy to address the kinetics of RBC invasion in the human malaria parasite Plasmodium falciparum. Under in vitro conditions merozoites generally recognise new target RBCs within 1min of their release from their host RBC. Parasite entry ensues and is complete on average 27.6s after primary contact. This period can be divided into two distinct phases. The first is an approximately 11s 'pre-invasion' phase that involves an often dramatic RBC deformation and recovery process. The second is the classical 'invasion' phase where the merozoite becomes internalised within the RBC in a approximately 17s period. After invasion, a third 'echinocytosis' phase commences when about 36s after every successful invasion a dramatic dehydration-type morphology was adopted by the infected RBC. During this phase, the echinocytotic effect reached a peak over the next 23.4s, after which the infected RBC recovered over a 5-11min period. By then the merozoite had assumed an amoeboid-like state and was apparently free in the cytoplasm. A comparison of our data with that of an earlier study of the distantly related primate parasite Plasmodium knowlesi indicated remarkable similarities, suggesting that the kinetics of invasion are conserved across the Plasmodium genus. This study provides a morphological and

kinetic framework onto which the invasion-associated physiological and molecular events can be overlaid.

13: *J Antimicrob Chemother.* 2008 Nov;62(5):872-8.

**Pharmacokinetics and pharmacodynamics of drug interactions involving rifampicin, rifabutin and antimalarial drugs.**

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Malaria and tuberculosis (TB) are two major global diseases mostly affecting the developing countries. Their treatment is often complex because of the drugs used, multidrug resistance, drug interactions and logistic problems such as drug availability and access. Patients are treated for TB for a minimum of 6 months and may concomitantly develop and be treated for malaria, especially during the rainy season. Rifampicin, a standard component of combination regimens for treating TB, is a potent inducer of hepatic cytochrome and other metabolic enzymes and is able to influence the pharmacokinetics of many drugs. Rifabutin, another rifamycin used less frequently than rifampicin, can also interact with drugs metabolized through the hepatic cytochromes. The mechanisms of any interaction of rifamycins with drugs used in malaria are not well defined. To complicate matters, acute malaria also plays a role in the pharmacokinetics and pharmacodynamics of drugs (i.e. quinine). The aim of this paper is to review known and potential drug-drug interactions between rifampicin, rifabutin and antimalarial drugs.

14: *J Antimicrob Chemother.* 2008 Nov;62(5):921-8.

**Accumulation of CVIET Pfcrt allele of Plasmodium falciparum in placenta of pregnant women living in an urban area of Dakar, Senegal.**

Niang M, Marrama L, Ekala MT, Alioune G, Tall A, Ndiaye JL, Sarr D, Dangou JM, Lehesran JY, Bouchier C, Mercereau-Puijalon O, Jambou R.

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**OBJECTIVES:** Chemoprophylaxis is recommended during pregnancy to reduce the risk of placental infection. However, in areas with increasing drug resistance, it can trigger selection of resistant parasites in the placenta and increase the frequency of placental malaria. The objective of this study was to analyse the selection of drug-resistant parasites in the placenta in an area where chloroquine was still recommended as prophylaxis. **PATIENTS AND METHODS:** We analysed the polymorphism of parasites from matched placental and venous blood samples at the time of delivery from women in Dakar. Polymorphism of the isolates was studied using nested PCR typing of MSA1 and MSA2 genes, and full sequence of PfcRT exon 2. **RESULTS:** Of 692 women recruited at delivery, 72 had placental malaria. Two Pfcrt exon 2 genotypes were found, and 86% of the placentas had monoallelic CVIET infection compared with 39% that had peripheral blood infection. Mixed parasite populations of CVIET/CVMNK occurred in 53% of the peripheral blood samples but only in 7% of the infected placentas. This selection of CVIET in placenta was not related to a decreased polymorphism of the parasites, as a large diversity of MSA1 and MSA2 was found in both placenta and venous blood. This diversity confirms that a multiplicity of circulation isolates can occur at low parasite transmission. msp1 and msp2 genotyping revealed mostly distinct populations of parasites in venous and placental blood. **CONCLUSIONS:** These data suggest that, even in low transmission areas, diverse parasite populations can accumulate in the placenta during pregnancy despite strong selection at the PfcRT locus due to chemoprophylaxis with chloroquine.

15: *J Chromatogr B Analyt Technol Biomed Life Sci.* 2008 Oct 18.

**Major pitfalls in the measurement of artemisinin derivatives in plasma in clinical studies.**

Lindegardh N, Hanpithakpong W, Kamanikom B, Singhasivanon P, Socheat D, Yi P, Dondorp AM, McGready R, Nosten F, White NJ, Day NP.

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A bioanalytical method for the analysis of artesunate (ARS) and its metabolite dihydroartemisinin (DHA) in human plasma using protein precipitation and liquid chromatography coupled to positive tandem mass spectroscopy was developed. The method was validated according to published US FDA-guidelines and showed excellent performance. However, when it was applied to clinical pharmacokinetic studies in malaria, variable degradation of the artemisinins introduced an unacceptable large source of error, rendering the assay useless. Haemolytic products related to sample collection and malaria infection degraded the compounds. Addition of organic solvents during sample processing and even low volume addition of the internal standard in an organic solvent caused degradation. A solid phase extraction method avoiding organic solvents eliminated problems arising from haemolysis induced degradation. Plasma esterases mediated only approximately 20% of ex vivo hydrolysis of ARS into DHA. There are multiple sources of major preventable error in measuring ARS and DHA in plasma samples from clinical trials. These various pitfalls have undoubtedly contributed to the large inter-subject variation in plasma concentration profiles and derived pharmacokinetic parameters for these important antimalarial drugs.

16: *J Clin Microbiol.* 2008 Nov;46(11):3759-65.

**Highly sensitive amperometric immunosensor for detection of Plasmodium falciparum histidine-rich protein 2 in serum of humans with malaria: comparison with a commercial kit.**

Sharma MK, Rao VK, Agarwal GS, Rai GP, Gopalan N, Prakash S, Sharma SK, Vijayaraghavan R.

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A disposable amperometric immunosensor was developed for the detection of Plasmodium falciparum histidine-rich protein 2 (PfHRP-2) in the sera of humans with P. falciparum malaria. For this purpose, disposable screen-printed electrodes (SPEs) were modified with multiwall carbon nanotubes (MWCNTs) and Au nanoparticles. The electrodes were characterized by cyclic voltammetry, scanning electron microscopy, and Raman spectroscopy. In order to study the immunosensing performances of modified electrodes, a rabbit anti-PfHRP-2 antibody (as the capturing antibody) was first immobilized on an electrode. Further, the electrode was exposed to a mouse anti-PfHRP-2 antibody from a serum sample (as the revealing antibody), followed by a rabbit anti-mouse immunoglobulin G-alkaline phosphatase conjugate. The immunosensing experiments were performed on bare SPEs, MWCNT-modified SPEs, and Au nanoparticle- and MWCNT-modified SPEs (Nano-Au/MWCNT/SPEs) for the amperometric detection of PfHRP-2 in a solution of 0.1 M diethanolamine buffer, pH 9.8, by applying a potential of 450 mV at the working electrode. Nano-Au/MWCNT/SPEs yielded the highest-level immunosensing performance among the electrodes, with a detection limit of 8 ng/ml. The analytical results of immunosensing experiments with human serum samples were compared with the results of a commercial Paracheck Pf test, as well as the results of microscopy. The specificities, sensitivities, and positive and

negative predictive values of the Paracheck Pf and amperometric immunosensors were calculated by taking the microscopy results as the "gold standard." The Paracheck Pf kit exhibited a sensitivity of 79% (detecting 34 of 43 positive samples; 95% confidence interval [CI], 75 to 86%) and a specificity of 81% (correctly identifying 57 of 70 negative samples; 95% CI, 76 to 92%), whereas the developed amperometric immunosensor showed a sensitivity of 96% (detecting 41 of 43 positive samples; 95% CI, 93 to 98%) and a specificity of 94% (correctly identifying 66 of 70 negative samples; 95% CI, 92 to 99%). The developed method is more sensitive and specific than the Paracheck Pf kit.

17: *J Community Health*. 2008 Oct 29.

**Does Insecticide Treated Mosquito Nets (ITNs) Prevent Clinical Malaria in Children Aged Between 6 and 59 Months Under Program Setting?**

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Inconsistent use of the mosquito nets and other social and technical factors were shown to influence efficacy of mosquito nets at field trials. But to date, experience with local factors influencing effectiveness of ITN programs remain very limited. The objective of this study was to assess the effectiveness of ITNs for preventing clinical malaria in under-five children of Omo Nada Woreda, Jimma Zone South West Ethiopia. Matched case-control study was conducted in the catchments population of Asendabo and Nada health centers, Omo Nada Woreda, South West Ethiopia on a sample of 273 under-five children. Each case of fever and parasitemia in a child was paired with two controls. Cases and controls were compared with regard to ITN ownership and other factors assessed by a pre-coded, pre-tested structured questionnaire. Data was analyzed using EPI-INFO version 3.3.2 software. To control the effect of confounding variables, conditional logistic regression model was used. Sleeping under the mosquito net the night (OR = 8.28 95% CI: 0.96, 71.1) and the week (OR = 2.41 95% CI: 0.41, 14.0) before the survey date were strongly, but not significantly associated with clinical malaria. Mosquito net possession and appropriate utilization of mosquito net were not associated with clinical malaria. In the comparison of cases with all the controls rolling out of mosquito net & corrugated iron roof were found to be independent predictors of clinical malaria. Knowledge about the sign and symptoms of malaria and its modes of transmission were also independent predictors of clinical malaria in comparison of cases with health center and community controls, respectively. With the presence of many programmatic deficiencies like poor ITN distribution and re-treatment services, ITNs were not significantly associated with clinical malaria in under-five children when used during low-transmission period. Further research using a large sample size is required. In line with ITN scale up, information Education Communication (IEC) about the preventive practices against malaria, causes of malaria, treatment and sign and symptoms of malaria should be given to the community.

18: *J Infect Dis*. 2008 Nov 4.

**Imbalanced Distribution of GM Immunoglobulin Allotypes According to the Clinical Presentation of Plasmodium falciparum Malaria in Beninese Children.**

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Selection pressure exerted by pathogens contributes to the persistence of polymorphisms in GM and KM allotypes, which are antigenic determinants of immunoglobulins. This study investigated the impact of GM and KM allotypes on the clinical response to Plasmodium falciparum infection among Beninese children, including 65 with severe malaria, 37 with uncomplicated malaria, and 53 with asymptomatic carriage. An inverse relationship was found between the GM 5,6,13,14; 1,17 phenotype and uncomplicated malaria. Genetic markers implicated in the composition and activity of immunoglobulins may be associated with the genetic control of both malaria infection and morbidity.

19: *J Infect Dis.* 2008 Oct 27.

**A Functional Single-Nucleotide Polymorphism in the CR1 Promoter Region Contributes to Protection against Cerebral Malaria.**

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**Background.** Although the level of erythrocyte complement receptor type 1 (E-CR1) expression in patients with malaria has been extensively studied, whether the level of expression of E-CR1 is associated with severe malaria remains controversial. The present study examined a possible association of polymorphisms in the CR1 gene with the severity of malaria, and it evaluated the influence of the associated polymorphism on expression of E-CR1. **Methods.** Seventeen single-nucleotide polymorphisms in CR1 were genotyped in 477 Thai patients who had Plasmodium falciparum malaria (203 had mild malaria, 165 had noncerebral severe malaria, and 109 had cerebral malaria). The E-CR1 expression level was measured by flow cytometry in 24 healthy Thai subjects. **Results.** The T allele of the reference single-nucleotide polymorphism rs9429942 in the CR1 promoter region was strongly associated with protection against cerebral malaria (2.2% of patients with mild malaria vs. 7.8% of patients with cerebral malaria; [Formula: see text]; Bonferroni-adjusted [Formula: see text]). The E-CR1 expression level was significantly higher in individuals with the TT genotype of rs9429942 than in individuals with the TC genotype of rs9429942 ([Formula: see text]). **Conclusions.** We identified a CR1 promoter allele, associated with higher E-CR1 expression, that conferred protection against cerebral malaria. Previous studies have shown that the rate of clearance of immune complexes (ICs) from the circulation is related to the E-CR1 level. These results lead to the hypothesis that the clearance of ICs regulated by E-CR1 therefore plays a crucial role in the pathogenesis of cerebral malaria.

20: *J Infect Dis.* 2008 Oct 17.

**Effect of Placental Malaria and HIV Infection on the Antibody Responses to Plasmodium falciparum in Infants.**

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1Malaria Branch, 2Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Chamblee, and 3Atlanta Research and Educational Foundation, Decatur, Georgia; 4Walter Reed Army Institute of Research, Silver Spring, Maryland; 5Kenya Medical Research Institute, and 6New

Nyanza Provincial Hospital, Kisumu, Kenya; 7Roll Back Malaria, World Health Organization, Geneva, Switzerland.

**Background.** Placental malaria (PM) and maternal infection with human immunodeficiency virus (HIV) type 1 have been shown to affect infant morbidity and immune responses to Plasmodium falciparum. We studied the effects of PM and HIV infection on the antimalarial antibody responses and morbidity outcomes of infants throughout the first year of life. **Methods.** A total of 411 Kenyan infants who were born to mothers who were singly or dually infected with PM and/or HIV had their levels of immunoglobulin G antibody to 6 P. falciparum antigens/epitopes (apical membrane antigen-1, erythrocyte-binding antigen-175; liver-stage antigen-1 [LSA-1], circumsporozoite protein [CSP], merozoite surface protein-2, and rhoptry-associated protein-1 [RAP-1]) and to tetanus toxoid (TT) tested using enzyme-linked immunosorbent assay. **Results.** PM had little effect on the antibody responses of infants, whereas maternal HIV infection resulted in decreased levels of antibody to LSA-1, CSP, and RAP-1 epitopes at birth, compared with the absence of PM and maternal HIV infection ([Formula: see text]). Levels of antibodies to TT were significantly reduced in infants born to mothers coinfecting with HIV and PM, compared with the levels noted in infants born to HIV-negative mothers ([Formula: see text]). In HIV-infected infants, levels of antibody to TT were reduced, but levels of antibody to malarial antigens were not. Antimalarial antibody levels were positively associated with malaria-related morbidity outcomes. **Conclusion.** Infant HIV infection and maternal coinfection with HIV and PM negatively influence antibody responses to TT, but not those to malarial antigens, in infants. Antimalarial antibodies rarely showed protective associations with morbidity in infants and were more often a marker for malaria exposure and risk of infection.

21: *J Infect Dis.* 2008 Oct 15;198(8):1219-26.

**Polymorphic variability in the interleukin (IL)-1beta promoter conditions susceptibility to severe malarial anemia and functional changes in IL-1beta production.**

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Interleukin (IL)-1beta is a cytokine released as part of the innate immune response to Plasmodium falciparum. Because the role played by IL-1beta polymorphic variability in conditioning the immunopathogenesis of severe malarial anemia (SMA) remains undefined, relationships between IL-1beta promoter variants (-31C/T and -511A/G), SMA (hemoglobin [Hb] level <6.0 g/dL), and circulating IL-1beta levels were investigated in children with parasitemia (n= 566) from western Kenya. The IL-1beta promoter haplotype -31C/-511A (CA) was associated with increased risk of SMA (Hb level <6.0 g/dL; odds ratio [OR], 1.98 [95% confidence interval {CI}, 1.55-2.27]; [Formula: see text]) and reduced circulating IL-1beta levels (p <.05). The TA (-31T/-511A) haplotype was nonsignificantly associated with protection against SMA (OR, 0.52 [95% CI, 0.18-1.16]; p =.11) and elevated IL-1beta production (p <.05). Compared with the non-SMA group, children with SMA had significantly lower IL-1beta levels and nonsignificant elevations in both IL-1 receptor antagonist (IL-1Ra) and the ratio of IL-1Ra to IL-1beta. The results presented demonstrate that variation in IL-1beta promoter conditions susceptibility to SMA and functional changes in circulating IL-1beta levels.

22: *J Infect Dis.* 2008 Oct 15;198(8):1202-11.

**A randomized, controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine, amodiaquine, or the combination in pregnant women in Ghana.**

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**BACKGROUND:** The use of sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp) is threatened by the spread of resistance to SP. Therefore, we studied the efficacy, safety, and tolerance of amodiaquine (AQ) or the combination of AQ and SP (SPAQ) as possible alternative treatments. **METHODS:** The study was performed in Ghana from June 2004 through February 2007. Women were individually randomized to receive IPTp with SP (n=1328), AQ (n= 986), or SPAQ (n=1328). Incidences of anemia, peripheral anemia, and placental parasitemia at delivery were assessed for paucigravidae, as were the birth weights of their infants. Delivery outcomes and the incidence of adverse events were investigated for all women. **RESULTS:** The prevalences of anemia (as defined by a hemoglobin concentration of <11.0 g/dL) at delivery were comparable between the SP and AQ groups and between the SP and SPAQ groups. Similarly, there was no significant difference between the SP and AQ groups or between the SP and SPAQ groups with regard to the incidences of low birth weight (LBW). Women who received AQ or SPAQ were more likely to report adverse events than were those who received SP. **CONCLUSION:** The effects of IPTp with AQ or SPAQ on maternal anemia and LBW were comparable to the effects of IPTp with SP; however, IPTp regimens that contain AQ are unlikely to be useful as an alternative to IPTp with SP in Ghana, because of a high frequency of associated adverse events. **TRIAL REGISTRATION:** Clinicaltrials.gov identifier: NCT00146783 .

23: *J Infect Dis.* 2008 Oct 15;198(8):1212-8.

**Acquisition of invasion-inhibitory antibodies specific for the 19-kDa fragment of merozoite surface protein 1 in a transmigrant population requires multiple infections.**

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Antibodies against the 19 kDa C-terminal fragment of merozoite surface protein 1 (MSP1(19)) are a major component of the invasion-inhibitory response in individuals immune to malaria. We report here the acquisition of MSP1(19)-specific invasion-inhibitory antibodies in a group of transmigrants who experienced their sequential malaria infections during settlement in an area of Indonesia where malaria is highly endemic. We used 2 transgenic *Plasmodium falciparum* parasite lines that expressed either endogenous MSP1(19) or the homologous region from *P. chabaudi* to measure the MSP1(19)-specific invasion-inhibitory antibodies. The results revealed that the acquisition of MSP1(19)-specific invasion-inhibitory antibodies required 2 or more *P. falciparum* infections. In contrast, enzyme-linked immunosorbent assays on the same serum samples showed that MSP1(19)-specific antibodies are present after the first malaria infection. This delay in the acquisition of functional antibodies by residents of areas where malaria is endemic is consistent with the observation that multiple malaria infections are required before clinical immunity is acquired.

24: *Lancet*. 2008 Nov 1;372(9649):1555-62.

**Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya.**

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International Center, National Institutes of Health, Bethesda, MD, USA.

**BACKGROUND:** As efforts to control malaria are expanded across the world, understanding the role of transmission intensity in determining the burden of clinical malaria is crucial to the prediction and measurement of the effectiveness of interventions to reduce transmission. Furthermore, studies comparing several endemic sites led to speculation that as transmission decreases morbidity and mortality caused by severe malaria might increase. We aimed to assess the epidemiological characteristics of malaria in Kilifi, Kenya, during a period of decreasing transmission intensity. **METHODS:** We analyse 18 years (1990-2007) of surveillance data from a paediatric ward in a malaria-endemic region of Kenya. The hospital has a catchment area of 250 000 people. Clinical data and blood-film results for more than 61 000 admissions are reported. **FINDINGS:** Hospital admissions for malaria decreased from 18.43 per 1000 children in 2003 to 3.42 in 2007. Over 18 years of surveillance, the incidence of cerebral malaria initially increased; however, malaria mortality decreased overall because of a decrease in incidence of severe malarial anaemia since 1997 (4.75 to 0.37 per 1000 children) and improved survival among children admitted with non-severe malaria. Parasite prevalence, the mean age of children admitted with malaria, and the proportion of children with cerebral malaria began to change 10 years before hospitalisation for malaria started to fall. **INTERPRETATION:** Sustained reduction in exposure to infection leads to changes in mean age and presentation of disease similar to those described in multisite studies. Changes in transmission might not lead to immediate reductions in incidence of clinical disease. However, longitudinal data do not indicate that reductions in transmission intensity lead to transient increases in morbidity and mortality.

25: *Lancet*. 2008 Nov 1;372(9649):1545-54.

**Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis.**

Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, Fulford AJ, Sesay SS, Abubakar I, Dunyo S, Sey O, Palmer A, Fofana M, Corrah T, Bojang KA, Whittle HC, Greenwood BM, Conway DJ.

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**BACKGROUND:** Malaria is a major cause of morbidity and mortality in Africa. International effort and funding for control has been stepped up, with substantial increases from 2003 in the delivery of malaria interventions to pregnant women and children younger than 5 years in The Gambia. We investigated the changes in malaria indices in this country, and the causes and public-health significance of these changes. **METHODS:** We undertook a retrospective analysis of original records to establish numbers and proportions of malaria inpatients, deaths, and blood-slide examinations at one hospital over 9 years (January, 1999-December, 2007), and at four health facilities in three different administrative regions over 7 years (January, 2001-December, 2007). We obtained additional data from single sites for haemoglobin concentrations in paediatric admissions and for age distribution of malaria admissions. **FINDINGS:** From 2003 to 2007, at four sites with complete slide examination records, the proportions of malaria-positive slides decreased by 82% (3397/10861 in 2003 to 337/6142 in

2007), 85% (137/1259 to 6/368), 73% (3664/16932 to 666/11333), and 50% (1206/3304 to 336/1853). At three sites with complete admission records, the proportions of malaria admissions fell by 74% (435/2530 to 69/1531), 69% (797/2824 to 89/1032), and 27% (2204/4056 to 496/1251). Proportions of deaths attributed to malaria in two hospitals decreased by 100% (seven of 115 in 2003 to none of 117 in 2007) and 90% (22/122 in 2003 to one of 58 in 2007). Since 2004, mean haemoglobin concentrations for all-cause admissions increased by 12 g/L (85 g/L in 2000-04 to 97 g/L in 2005-07), and mean age of paediatric malaria admissions increased from 3.9 years (95% CI 3.7-4.0) to 5.6 years (5.0-6.2). INTERPRETATION: A large proportion of the malaria burden has been alleviated in The Gambia. Our results encourage consideration of a policy to eliminate malaria as a public-health problem, while emphasising the importance of accurate and continuous surveillance.

26: *Lancet*. 2008 Oct 14.

**Efficacy and safety of artemether-lumefantrine dispersible tablets compared with crushed commercial tablets in African infants and children with uncomplicated malaria: a randomised, single-blind, multicentre trial.**

Abdulla S, Sagara I, Borrmann S, D'Alessandro U, González R, Hamel M, Ogutu B, Mårtensson A, Lyimo J, Maiga H, Sasi P, Nahum A, Bassat Q, Juma E, Otieno L, Björkman A, Beck HP, Andriano K, Cousin M, Lefèvre G, Ubben D, Premji Z.

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BACKGROUND: Combination treatments, preferably containing an artemisinin derivative, are recommended to improve efficacy and prevent *Plasmodium falciparum* drug resistance. Our aim was to show non-inferiority of a new dispersible formulation of artemether-lumefantrine to the conventional crushed tablet in the treatment of young children with uncomplicated malaria. METHODS: We did a randomised non-inferiority study on children weighing 5-35 kg with uncomplicated *P falciparum* malaria in Benin, Kenya, Mali, Mozambique, and Tanzania. The primary outcome measure was PCR-corrected 28-day parasitological cure rate. We aimed to show non-inferiority (with a margin of -5%) of dispersible versus crushed tablet. We constructed an asymptotic one-sided 97.5% CI on the difference in cure rates. A computer-generated randomisation list was kept centrally and investigators were unaware of the study medication administered. We used a modified intention-to-treat analysis. This trial is registered with ClinicalTrials.gov, number NCT00386763. FINDINGS: 899 children aged 12 years or younger were randomly assigned to either dispersible (n=447) or crushed tablets (n=452). More than 85% of patients in each treatment group completed the study. 812 children qualified for the modified intention-to-treat analysis (n=403 vs n=409). The PCR-corrected day-28 cure rate was 97.8% (95% CI 96.3-99.2) in the group on dispersible formulation and 98.5% (97.4-99.7) in the group on crushed formulation. The lower bound of the one-sided 97.5% CI was -2.7%. The most common drug-related adverse event was vomiting (n=33 [7%] and n=42 [9%], respectively). No signs of ototoxicity or relevant cardiotoxicity were seen. INTERPRETATION: A six-dose regimen of artemether-lumefantrine with the new dispersible formulation is as efficacious as the currently used crushed tablet in infants and children, and has a similar safety profile. FUNDING: Novartis Pharma, Basel, Switzerland, and Medicines for Malaria Venture (MMV), Geneva, Switzerland.

27: *Malar J*. 2008 Nov 4;7(1):231.

**Variations in entomological indices in relation to weather patterns and malaria incidence in East African highlands: implications for epidemic prevention and control.**

Kristan M, Abeku TA, Beard J, Okia M, Rapuoda B, Sang J, Cox J.

**ABSTRACT: BACKGROUND:** Malaria epidemics remain a significant public health issue in the East African highlands. The aim of this study was to monitor temporal variations in vector densities in relation to changes in meteorological factors and malaria incidence at four highland sites in Kenya and Uganda and to evaluate the implications of these relationships for epidemic prediction and control. **METHODS:** Mosquitoes were collected weekly over a period of 47 months while meteorological variables and morbidity data were monitored concurrently. Mixed-effects Poisson regression was used to study the temporal associations of meteorological variables to vector densities and of the latter to incidence rates of *Plasmodium falciparum*. **RESULTS:** *Anopheles gambiae* s.s. was the predominant vector followed by *Anopheles arabiensis*. *Anopheles funestus* was also found in low densities. Vector densities remained low even during periods of malaria outbreaks. Average temperature in previous month and rainfall in previous two months had a quadratic and linear relationship with *An. gambiae* s.s. density, respectively. A significant statistical interaction was also observed between average temperature and rainfall in the previous month. Increases in densities of this vector in previous two months showed a linear relationship with increased malaria incidence. **CONCLUSIONS:** Although epidemics in highlands often appear to follow abnormal weather patterns, interactions between meteorological, entomological and morbidity variables are complex and need to be modelled mathematically to better elucidate the system. This study showed that routine entomological surveillance is not feasible for epidemic monitoring or prediction in areas with low endemicity. However, information on unusual increases in temperature and rainfall should be used to initiate rapid vector surveys to assess transmission risk.

*28: Matern Child Health J. 2008 Nov;12(6):692-8.*

**Efficacy of Intermittent Preventive Treatment of Malaria with Sulphadoxine-pyrimethamine in Preventing Anaemia in Pregnancy among Nigerian Women.**

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**Objective** To evaluate the efficacy of intermittent preventive treatment of malaria using sulphadoxine-pyrimethamine (SP) in the prevention of anaemia in women of low parity in a low socio-economic, malaria endemic setting. **Method** The study design was an open randomized control trial comparing anaemia incidence among pregnant women on intermittent presumptive treatment of malaria with SP with those on chloroquine (CQ). A total of 352 primigravid and secondigravid women between 16 and 30 weeks gestation receiving antenatal care at the Primary Health Care Center, Enuwa in Ile-Ife, Osun State, Nigeria were serially recruited and randomly allocated into experimental and control groups of 176 each. The experimental group received SP (to a maximum of three doses depending on the gestational age at enrollment into the study) while the control group had treatment doses of CQ at recruitment and subsequently only if they had symptoms suggestive of malaria. The primary outcome measure was anaemia (haematocrit < 30) at 34 weeks of gestation. **Result** At recruitment and 34 weeks gestation, there was no statistically significant difference between the experimental and control group in terms of socio-demographic characteristics and past medical history. Thirty-three (22.6%) and 52 (37.1%) women in the study and control groups, respectively, had anaemia (protective efficacy 49.5%,  $p = 0.01$ ). With multivariate analysis, controlling for the possible confounding effects of education, parity, haemoglobin level at booking and malaria parasitaemia in peripheral blood, the difference in the incidence of anaemia in the two groups remained significant ( $p = 0.01$ ; odds ratio = 0.5; 95% confidence interval = 0.29-0.85). **Conclusion** The IPT regime with sulphadoxine-pyrimethamine is an effective, practicable strategy to decrease risk of anaemia in women of low

parity residing in areas endemic for malaria.

29: *Med Mal Infect.* 2008 Oct 23.

**[Can the thick drop/smear examination for malaria be replaced by a rapid diagnostic test in first intention? The Mayotte experience.]**

[Article in French]

de Carsalade GY, Lam Kam R, Lepere JF, de Brettes A, Peyramond D.

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SETTINGS: Malaria is a public health problem in the French island of Mayotte (160,000 inhabitants) in the Indian Ocean. In the late 1990, resistance to chloroquine greatly increased, and so did the number of malaria cases, so that a new health policy had to be adopted. Since 2001, the initial smear/thick drop examination, the results of which took too long to obtain, has systematically been replaced by a rapid diagnosis test (Optimal IT((R)) Diamed) in all hospitals and public health centers. METHOD: Epidemiological data of malaria on the island was collected and a prospective study was made from March 2005 to February 2006, on two sites (the emergency department of the main hospital and a rural health centre) on all patients presenting with malaria (104 and 139 cases respectively). RESULTS: The first Optimal IT((R)) test diagnosed the condition accurately in 88 and 96% of the cases, respectively. Every time symptoms would persist after negative test results and an Optimal IT((R)) test was repeated within three days, the parasitemia level was low (0.08 to 0.66%). Very low parasitemia level was very likely to account for a false negative (test result). CONCLUSIONS: These results concerning malaria (and its epidemiological data) in Mayotte show that the initial use of an Optimal IT((R)) test instead of the thin/thick blood smear results in a faster management of patients with malaria, although the Optimal IT((R)) test is slightly less sensitive and requires training/practice.

30: *Mol Biochem Parasitol.* 2008 Nov;162(1):40-51.

**5' sequence- and chromatin modification-dependent gene expression in Plasmodium falciparum erythrocytic stage.**

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*Plasmodium falciparum*, the human malaria parasite, is evolutionarily distant from other eukaryotes. Genome-wide analyses of transcription-associated proteins have revealed a relative paucity of putative regulatory transcription factors and an abundance of putative chromatin remodeling machinery, suggesting that this parasite has a transcription regulatory system that is distinct from those of other eukaryotes. Here, we have analyzed transcriptional regulation of the peroxiredoxin genes, *pfl-cys-prx* and *pftpx-1*, which show different expression patterns in *P. falciparum*. The reporter assays revealed the presence of putative enhancers in the 5' regions of these genes. Although *pfl-cys-prx* shows trophozoite/schizont stage-specific transcription, a putative cis-acting enhancer sequence in *pfl-cys-prx* was constitutively active when inserted into the 5' region of *pftpx-1*. Electrophoretic mobility shift and DNase I footprinting assays showed that this enhancer region is the target of trophozoite/schizont stage-specific DNA binding proteins. In addition, chromatin immunoprecipitation assays showed that the increased levels of histone acetylation in the 5' region

of pfl-cys-prx and pftpx-1 correlate with the transcriptional activity of these genes. Recruitment of PfGCN5 histone acetyltransferase to the pfl-cys-prx enhancer in trophozoite/schizont stage was observed. These results suggest that *P. falciparum* possesses a sophisticated system of transcriptional regulation during intraerythrocytic stages that is managed by coordinated interactions of unique cis-elements and trans-acting factors and chromatin modifications.

31: *Mol Biochem Parasitol.* 2008 Nov;162(1):96-9.

**Acid extrusion from the intraerythrocytic malaria parasite is not via a Na(+)/H(+) exchanger.**

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The intraerythrocytic malaria parasite, *Plasmodium falciparum* maintains an intracellular pH (pH(i)) of around 7.3. If subjected to an experimentally imposed acidification the parasite extrudes H(+), thereby undergoing a pH(i) recovery. In a recent study, Bennett et al. [Bennett TN, Patel J, Ferdig MT, Roepe PD. *P. falciparum* Na(+)/H(+) exchanger activity and quinine resistance. *Mol Biochem Parasitol* 2007;153:48-58] used the H(+) ionophore nigericin, in conjunction with an acidic medium, to acidify the parasite cytosol, and then used bovine serum albumin (BSA) to scavenge the nigericin from the parasite membrane. The ensuing Na(+)-dependent pH(i) recovery, seen following an increase in the extracellular pH, was attributed to a plasma membrane Na(+)/H(+) exchanger. This is at odds with previous reports that the primary H(+) extrusion mechanism in the parasite is a plasma membrane V-type H(+)-ATPase. Here we present evidence that the Na(+)-dependent efflux of H(+) from parasites acidified using nigericin/BSA is attributable to Na(+)/H(+) exchange via residual nigericin remaining in the parasite plasma membrane, rather than to endogenous transporter activity.

32: *Mol Biochem Parasitol.* 2008 Oct 8.

**Myristoylated adenylate kinase-2 of *Plasmodium falciparum* forms a heterodimer with myristoyltransferase.**

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Adenylate kinases (AK; ATP+AMP $\rightleftharpoons$ 2 ADP; E.C. 2.7.4.3.) are enzymes essentially involved in energy metabolism and macromolecular biosynthesis. As we reported previously, the malarial parasite *Plasmodium falciparum* possesses one genuine AK and one GTP-AMP phosphotransferase. Analysis of the *P. falciparum* genome suggested the presence of one additional adenylate kinase, which we designated AK2. Recombinantly produced AK2 was found to be a monomeric protein of 33kDa showing a specific activity of 10U/mg with ATP and AMP as a substrate pair and interacts with the AK-specific inhibitor P(1),P(5)-(diadenosine-5')-pentaphosphate (IC(50)=200nM). At its N-terminus AK2 carries a predicted myristoylation sequence. Interestingly this sequence is only present in AK2 of *P. falciparum* causing the severe tropical malaria and not in other malarial parasites. We heterologously coexpressed AK2 and *P. falciparum* N-myristoyltransferase (NMT) in the presence of myristate in *Escherichia coli*. As demonstrated by protein purification and mass spectrometry, AK2 is indeed myristoylated under catalysis of the parasites' transferase. The modification significantly enhances the stability of the kinase. Furthermore, AK2 and NMT were shown to interact strongly with each other forming a heterodimeric protein in vitro. To our knowledge this is the first direct evidence that *P. falciparum* NMT

myristoylates an intact malarial protein.

33: *Nat Rev Microbiol.* 2008 Nov;6(11):864-70.

### **What really happens to dendritic cells during malaria?**

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As dendritic cells (DCs) initiate all adaptive and some innate immune responses, it is not surprising that DC function during malaria is the subject of intensive investigations. However, the results of these investigations have so far been controversial. Here, we discuss various aspects of these studies, including the influence of the species and strain of *Plasmodium* on DC function, the effects of *Plasmodium* infection on the activation of CD8(+) T cells by DCs, the effects of haemozoin and the effects of *Plasmodium* infections on DC Toll-like-receptor signalling.

34: *Nature.* 2008 Oct 9;455(7214):799-803.

### **The genome of the simian and human malaria parasite *Plasmodium knowlesi*.**

Pain A, Böhme U, Berry AE, Mungall K, Finn RD, Jackson AP, Mourier T, Mistry J, Pasini EM, Aslett MA, Balasubrammaniam S, Borgwardt K, Brooks K, Carret C, Carver TJ, Cherevach I, Chillingworth T, Clark TG, Galinski MR, Hall N, Harper D, Harris D, Hauser H, Ivens A, Janssen CS, Keane T, Larke N, Lapp S, Marti M, Moule S, Meyer IM, Ormond D, Peters N, Sanders M, Sanders S, Sargeant TJ, Simmonds M, Smith F, Squares R, Thurston S, Tivey AR, Walker D, White B, Zuiderwijk E, Churcher C, Quail MA, Cowman AF, Turner CM, Rajandream MA, Kocken CH, Thomas AW, Newbold CI, Barrell BG, Berriman M.

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*Plasmodium knowlesi* is an intracellular malaria parasite whose natural vertebrate host is *Macaca fascicularis* (the 'kra' monkey); however, it is now increasingly recognized as a significant cause of human malaria, particularly in southeast Asia. *Plasmodium knowlesi* was the first malaria parasite species in which antigenic variation was demonstrated, and it has a close phylogenetic relationship to *Plasmodium vivax*, the second most important species of human malaria parasite (reviewed in ref. 4). Despite their relatedness, there are important phenotypic differences between them, such as host blood cell preference, absence of a dormant liver stage or 'hypnozoite' in *P. knowlesi*, and length of the asexual cycle (reviewed in ref. 4). Here we present an analysis of the *P. knowlesi* (H strain, Pk1(A+) clone) nuclear genome sequence. This is the first monkey malaria parasite genome to be described, and it provides an opportunity for comparison with the recently completed *P. vivax* genome and other sequenced *Plasmodium* genomes. In contrast to other *Plasmodium* genomes, putative variant antigen families are dispersed throughout the genome and are associated with intrachromosomal telomere repeats. One of these families, the KIRs, contains sequences that collectively match over one-half of the host CD99 extracellular domain, which may represent an unusual form of molecular mimicry.

35: *Nature*. 2008 Oct 9;455(7214):757-63.

**Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*.**

Carlton JM, Adams JH, Silva JC, Bidwell SL, Lorenzi H, Caler E, Crabtree J, Angiuoli SV, Merino EF, Amedeo P, Cheng Q, Coulson RM, Crabb BS, Del Portillo HA, Essien K, Feldblyum TV, Fernandez-Becerra C, Gilson PR, Gueye AH, Guo X, Kang'a S, Kooij TW, Korsinczky M, Meyer EV, Nene V, Paulsen I, White O, Ralph SA, Ren Q, Sargeant TJ, Salzberg SL, Stoeckert CJ, Sullivan SA, Yamamoto MM, Hoffman SL, Wortman JR, Gardner MJ, Galinski MR, Barnwell JW, Fraser-Liggett CM.

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The human malaria parasite *Plasmodium vivax* is responsible for 25-40% of the approximately 515 million annual cases of malaria worldwide. Although seldom fatal, the parasite elicits severe and incapacitating clinical symptoms and often causes relapses months after a primary infection has cleared. Despite its importance as a major human pathogen, *P. vivax* is little studied because it cannot be propagated continuously in the laboratory except in non-human primates. We sequenced the genome of *P. vivax* to shed light on its distinctive biological features, and as a means to drive development of new drugs and vaccines. Here we describe the synteny and isochore structure of *P. vivax* chromosomes, and show that the parasite resembles other malaria parasites in gene content and metabolic potential, but possesses novel gene families and potential alternative invasion pathways not recognized previously. Completion of the *P. vivax* genome provides the scientific community with a valuable resource that can be used to advance investigation into this neglected species.

36: *Nature*. 2008 Oct 9;455(7214):751-6.

**Malaria research in the post-genomic era.**

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For many pathogens the availability of genome sequence, permitting genome-dependent methods of research, can partially substitute for powerful forward genetic methods (genome-independent) that have advanced model organism research for decades. In 2002 the genome sequence of *Plasmodium falciparum*, the parasite causing the most severe type of human malaria, was completed, eliminating many of the barriers to performing state-of-the-art molecular biological research on malaria parasites. Although new, licensed therapies may not yet have resulted from genome-dependent experiments, they have produced a wealth of new observations about the basic biology of malaria parasites, and it is likely that these will eventually lead to new therapeutic approaches. This review will focus on the basic research discoveries that have depended, in part, on the availability of the *Plasmodium* genome sequences.

37: *Parasitol Res*. 2008 Nov;103(6):1435-43.

**Genetic characterization, distribution and prevalence of avian pox and avian malaria in the Berthelot's pipit (*Anthus berthelotii*) in Macaronesia.**

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Exotic pathogens have been implicated in the decline and extinction of various native-island-bird species. Despite the fact that there is increasing concern about the introduction of diseases in island ecosystems, little is known about parasites in the islands of Macaronesia. We focus on Berthelot's pipit (*Anthus berthelotii*), an endemic and widespread Macaronesian bird species, using a combination of field studies and molecular techniques to determine: (1) the range and prevalence of avian pox and malaria in Berthelot's pipits throughout the species' distribution, (2) the genetic characterization of both parasites in order to ascertain the level of host specificity. We sampled 447 pipits across the 12 islands inhabited by this species. Overall, 8% of all individuals showed evidence of pox lesions and 16% were infected with avian malaria, respectively. We observed marked differences in the prevalence of parasites among islands both within and between archipelagos. Avian pox prevalence varied between 0-54% within and between archipelagos and avian malaria prevalence varied between 0-64% within and between archipelagos. The diversity of pathogens detected was low: only two genetic lineages of avian malaria and one lineage of avian pox were found to infect the pipit throughout its range. Interestingly, both avian malaria parasites found were *Plasmodium* spp. that had not been previously reported in the Macaronesian avifauna (but that had been observed in the lesser kestrel *Falco naumannii*), while the avian pox was a host specific lineage that had previously been reported on two of the Canary Islands.

38: *Parasitol Res.* 2008 Nov;103(6):1361-8.

**Screening for antifeedant and larvicidal activity of plant extracts against *Helicoverpa armigera* (Hübner), *Sylepta derogata* (F.) and *Anopheles stephensi* (Liston).**

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Plant extracts, especially botanical insecticides, are currently studied more and more because of the possibility of their use in plant protection. Biological activity of five solvent plant extracts were studied using fourth instar larvae of gram pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae), cotton leaf roller *Sylepta derogata* (Lepidoptera: Pyralidae) and malaria vector *Anopheles stephensi* (Diptera: Culicidae). Antifeedant and larvicidal activity of acetone, chloroform, ethyl acetate, hexane and methanol peel, leaf and flower extracts of *Citrus sinensis*, *Ocimum canum*, *Ocimum sanctum* and *Rhinacanthus nasutus* were used in this study. During preliminary screening, the extracts were tested at 1,000 ppm concentration. The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in peel chloroform extract of *C. sinensis*, flower methanol extract of *O. canum* against the larvae of *H. armigera* (LC50 = 65.10, 51.78, LC90 = 277.39 and 218.18 ppm), peel methanol extract of *C. sinensis*, flower ethyl acetate extract of *O. canum* and leaf acetone extract of *O. sanctum* against the larvae of *S. derogata* (LC50 = 20.27, 58.21, 36.66, LC90 = 113.15, 285.70 and 668.02 ppm), peel methanol extract of *C. sinensis*, leaf and flower ethyl acetate extracts of *O. canum* against the larvae of *A. stephensi* (LC50 = 95.74, 101.53, 28.96, LC90 = 303.20, 492.43 and 168.05 ppm), respectively. These results suggest that the chloroform and methanol extract of *C. sinensis*, ethyl acetate flower extracts of *O. canum* and acetone extract of *O. sanctum* have the potential to be used as an ideal eco-friendly approach for the control of the agricultural pests *H. armigera*, *S. derogata* and medically important vector *A. stephensi*.

39: *Parasitol Res.* 2008 Oct 31.

**Reduced CD3/TCR complex expression leads to immunosuppression during Plasmodium falciparum malaria.**

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Inhibition of T cell function is an important pathological feature in malaria. We investigated which T cell population is reduced contributing to immunosuppression. We examined protein and RNA level of various cell receptors, specific for T cells in children having Plasmodium falciparum infection and compared those to healthy children of the same age. We observe reduced levels of cluster of differentiation (CD)3 and T cell receptor (TCR)alpha-beta in both RNA and protein expression level. This reduced expression was associated with a collapsed membrane asymmetry as determined by enhanced annexinV binding. Also human leukocyte antigen (HLA)-A,B,C- and HLA-DR-positive cells increasingly bound annexinV. The enhanced binding of annexinV was paralleled by a reduced expression of transcription factors such as T cell transcription factor 7 and GATA binding protein 3, which are involved in the expression of T cell specific genes. Also the expression of the transcription factors major histocompatibility complex class II transactivator and regulatory factor X 5, which are part of the HLA transcription machinery, is reduced during infection. We show that two mechanisms may lead to a suppression of the immune system during malaria: cell damage and reduction of gene expression of the CD3/TCR complex.

40: *Parasitology.* 2008 Nov 4:1-7.

**Differential inhibition of high and low Mr thioredoxin reductases of parasites by organotelluriums supports the concept that low Mr thioredoxin reductases are good drug targets.**

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**SUMMARY**Thioredoxin reductase (TrxR), a NADPH-dependent disulfide oxidoreductase, is vital in numerous cellular processes including defence against reactive oxygen species, cell proliferation and signal transduction. TrxRs occur in 2 forms, a high Mr enzyme characterized by those of mammals, the malaria parasite Plasmodium falciparum and some worms, and a low Mr form is present in bacteria, fungi, plants and some protozoan parasites. Our hypothesis is that the differences between the forms can be exploited in the development of selective inhibitors. In this study, cyclodextrin- and sulfonic acid-derived organotelluriums known to inhibit mammalian TrxR were investigated for their relative efficacy against P. falciparum TrxR (PfTrxR), a high Mr enzyme, and Trichomonas vaginalis TrxR (TvTrxR), a low Mr form of TrxR. The results suggest that selective inhibition of low Mr TrxRs is a feasible goal.

41: *Pharmacoepidemiol Drug Saf.* 2008 Oct 15.

**Drug utilisation study of antimalarials for the treatment of hospitalised children under five in south-eastern Nigeria.**

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Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu, Nigeria.

**PURPOSE:** This study aimed at describing the trend in the use of antimalarials for the treatment of malaria in children under 5 years from year 2000 to 2006 in south-eastern Nigeria. Adherence to the 2005 National Antimalarial Treatment Policy was assessed. Quality of drug use was also evaluated. Quality indices studied were the use of international non-proprietary name (INN) in prescription, number antimalarials per episode and use of drugs from essential drug list.

**METHODS:** The study was retrospective and longitudinal, using data obtained from in-patients folders of children under 5 years, hospitalised for malaria infection in 11 secondary health care centres in south-eastern Nigeria. **RESULTS:** The result of the study showed that chloroquine was mostly used for treating severe malaria in children less than 5 years despite the indication of a switch to quinine and parenteral artemisinins by the National Treatment Policy. Prescriptions of drugs were also not by INN names. However, many prescribers do not practice polypharmacy and most of the drugs used in secondary health care centres for treatment of severe malaria were in the essential drug list. **CONCLUSION:** There is a need for further studies to establish factors that affect the dissemination and use of treatment guidelines in Nigeria. Copyright (c) 2008 John Wiley & Sons, Ltd.

42: *Proc Natl Acad Sci U S A.* 2008 Oct 21;105(42):16290-5.

**The transcriptome of Plasmodium vivax reveals divergence and diversity of transcriptional regulation in malaria parasites.**

Bozdech Z, Mok S, Hu G, Imwong M, Jaidee A, Russell B, Ginsburg H, Nosten F, Day NP, White NJ, Carlton JM, Preiser PR.

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*Plasmodium vivax* causes over 100 million clinical infections each year. Primarily because of the lack of a suitable culture system, our understanding of the biology of this parasite lags significantly behind that of the more deadly species *P. falciparum*. Here, we present the complete transcriptional profile throughout the 48-h intraerythrocytic cycle of three distinct *P. vivax* isolates. This approach identifies strain specific patterns of expression for subsets of genes predicted to encode proteins associated with virulence and host pathogen interactions. Comparison to *P. falciparum* revealed significant differences in the expression of genes involved in crucial cellular functions that underpin the biological differences between the two parasite species. These data provide insights into the biology of *P. vivax* and constitute an important resource for the development of therapeutic approaches.

43: *Proteins.* 2008 Nov 1;73(2):440-57.

**Predicting functional residues in Plasmodium falciparum plasmepsins by combining sequence and structural analysis with molecular dynamics simulations.**

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Plasmepsins are aspartic proteases involved in the initial steps of the hemoglobin degradation pathway, a critical stage in the *Plasmodium falciparum* life cycle during human infection. Thus, they are attractive targets for novel therapeutic compounds to treat malaria, which remains one of the world's biggest health problems. The three-dimensional structures available for *P. falciparum* plasmepsins II and IV make structure-based drug design of antimalarial compounds

that focus on inhibiting plasmepsins possible. However, the structural flexibility of the plasmepsin active site cavity combined with insufficient knowledge of the functional residues and of those determining the specificity of parasitic enzymes is a drawback when designing specific inhibitors. In this study, we have combined a sequence and structural analysis with molecular dynamics simulations to predict the functional residues in *P. falciparum* plasmepsins. The careful analysis of X-ray structures and 3D models carried out here suggests that residues Y17, V105, T108, L191, L242, Q275, and T298 are important for plasmepsin function. These seven amino acids are conserved across the malarial strains but not in human aspartic proteases. Residues V105 and T108 are localized in a flap of an interior pocket and they only establish contacts with a specific non-peptide achiral inhibitor. We also observed a rapid conformational change in the L3 region of plasmepsins that closes the active site of the enzyme, which explains earlier experimental findings. These results shed light on the role of V105 and T108 residues in plasmepsin specificities, and they should be useful in structure-based design of novel, selective inhibitors that may serve as antimalarial drugs. (c) 2008 Wiley-Liss, Inc.

44: *Proteomics*. 2008 Oct 20.

**Profiling humoral immune responses to *P. falciparum* infection with protein microarrays.**

Doolan DL, Mu Y, Unal B, Sundaresh S, Hirst S, Valdez C, Randall A, Molina D, Liang X, Freilich DA, Oloo JA, Blair PL, Aguiar JC, Baldi P, Davies DH, Felgner PL.

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A complete description of the serological response following exposure of humans to complex pathogens is lacking and approaches suitable for accomplishing this are limited. Here we report, using malaria as a model, a method which elucidates the profile of antibodies that develop after natural or experimental infection or after vaccination with attenuated organisms, and which identifies immunoreactive antigens of interest for vaccine development or other applications. Expression vectors encoding 250 *Plasmodium falciparum* (Pf) proteins were generated by PCR/recombination cloning; the proteins were individually expressed with >90% efficiency in *Escherichia coli* cell-free in vitro transcription and translation reactions, and printed directly without purification onto microarray slides. The protein microarrays were probed with human sera from one of four groups which differed in immune status: sterile immunity or no immunity against experimental challenge following vaccination with radiation-attenuated Pf sporozoites, partial immunity acquired by natural exposure, and no previous exposure to Pf. Overall, 72 highly reactive Pf antigens were identified. Proteomic features associated with immunoreactivity were identified. Importantly, antibody profiles were distinct for each donor group. Information obtained from such analyses will facilitate identifying antigens for vaccine development, dissecting the molecular basis of immunity, monitoring the outcome of whole-organism vaccine trials, and identifying immune correlates of protection.

45: *Trans R Soc Trop Med Hyg*. 2008 Nov;102(11):1089-94

**Using malarial retinopathy to improve the classification of children with cerebral malaria.**

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The mechanisms leading to death in cerebral malaria (CM) remain unclear. We

compared clinical and laboratory data among children with CM, categorized by ocular fundus findings, to elucidate differences that suggest different underlying pathological processes. From 1999–2005, standard examinations, treatment and record keeping were used for children with a clinical diagnosis of CM. Children were divided into ocular subgroups: normal fundus (N), malarial retinopathy (R), or papilloedema alone (P) and appropriate statistical tests were used to compare clinical and laboratory findings among groups. Eight hundred and eighty children who had eye examinations within 6 h of admission were included in the analysis. The groups differed significantly in case-fatality rates: Group P, 44.4% (95% CI 25.3–63.2), Group R, 18.0% (95% CI 15.6–22.3) and Group N, 7.0% (95% CI 4.2–9.8). There were also significant differences among the groups in blood pressure, prevalence of deep breathing, haematocrit, parasite density, platelet concentration and, among survivors, hours taken to recover from coma. Differences among groups suggest that different underlying pathophysiological processes are operating in children with CM defined by existing criteria. Our proposed classification, by improving the specificity of diagnosis, would enhance consistency among different study sites and prove useful in future research studies.

46: *Trans R Soc Trop Med Hyg.* 2008 Nov;102(11):1081–8.

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

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The Anti Malaria Campaign distributed approximately 300,000 long-lasting impregnated nets (LLINs) to malaria-endemic areas in Sri Lanka during the years 2005 to 2007. We conducted a community-based cross-sectional survey among 2467 households distributed among the three major ethnic groups of Sri Lanka to study the perceptions and practices with regard to the use of LLINs in order to improve their use. In a majority of households the number of LLINs available was not sufficient for the number of people, although there was a small percentage of households that had excess nets. The information and advice given at the time of distribution regarding use of the nets differed amongst the three groups and was not consistent. Dissemination of this knowledge within the family was not observed. A relationship between knowledge regarding LLINs and reported practices on washing and drying of LLINs was found. It was noted that net shape may influence net use, with cone shaped nets being more popular. Efforts to increase knowledge on LLINs using behaviour change communication techniques would have more effectively contributed to achieve planned outcomes. Proper use of LLINs will undoubtedly contribute to further reduction of malaria in Sri Lanka.

47: *Trans R Soc Trop Med Hyg.* 2008 Nov;102(11):1064–6.

#### **Making malaria testing relevant: beyond test purchase.**

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Malaria rapid diagnostic tests (RDT) are being procured and used in increasing numbers. However, the resultant effect of RDT-based diagnosis on fever management has been limited by lack of confidence in RDT results or the inability to act on results appropriately. If the utilisation of malaria RDTs is going to achieve the significant public health and financial benefits anticipated, they must be

introduced in a carefully structured way and viewed as a tool for the management of febrile illness, not just malaria. If this is to occur, a re-think is required of the way many malaria programmes are funded and run.

48: *Trans R Soc Trop Med Hyg.* 2008 Nov;102(11):1095-101.

**The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for post-exposure prophylaxis of Plasmodium vivax malaria in the Southwest Pacific.**

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Tafenoquine is being developed for radical cure and post-exposure prophylaxis of Plasmodium vivax malaria. In an open-label study, 1512 Australian Defence Force personnel received one of three tafenoquine 3 d regimens [400 mg once daily (od), 200 mg twice daily (bid), 200 mg od] or daily primaquine (22.5 mg) plus doxycycline (100 mg) over 14 d in Bougainville and in Timor-Leste for post-exposure prophylaxis. The relapse rate of subjects treated in Bougainville with tafenoquine (n=173) was 1.2% (200 mg bid x 3 d) and 2.3% (400 mg od x 3 d), while primaquine plus doxycycline (n=175) was 3.4%. For subjects treated in Timor-Leste with tafenoquine (n=636), the relapse rate was 4.9% (200 mg od x 3 d), 5.3% (200 mg bid x 3 d) and 11.0% (400 mg od x 3d), while primaquine plus doxycycline (n=289) was 10.0%. The most frequent adverse events reported across all groups were nausea, abdominal distress and diarrhoea. There was a dose-dependent reduction in adverse events with a reduced dose of tafenoquine, with the lowest dose (total 600 mg over 3 d) producing rates of adverse events equivalent to that of primaquine plus doxycycline. The much shorter dosing regimen of tafenoquine should increase compliance, which is often suboptimal with primaquine after leaving an endemic area. [Australian New Zealand Clinical Trials Registry Number 12607000588493].

49: *Trans R Soc Trop Med Hyg.* 2008 Nov;102(11):1062-3.

**Improving quantitation of malaria parasite burden with digital image analysis.**

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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.

50: *Trans R Soc Trop Med Hyg.* 2008 Oct 25.

**Lessons learnt from the six decades of chloroquine use (1945-2005) to control malaria in Madagascar.**

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On the island of Madagascar, malaria was nearly eradicated in the highland areas and malaria transmission was significantly decreased in the coastal areas between the 1940s and 1960s. The success of the control programme was primarily achieved by chloroquine (CQ) use at the community level. CQ was administered to children weekly on a routine basis for malaria prevention in the period 1949-1971. Then, the Malagasy Government was unable to financially support the malaria control programme. The malarial situation worsened in the 1980s, partly due to the shortage of CQ. A malaria epidemic occurred. To deal with this epidemic, massive CQ use was urgently adopted. CQ has remained the first-line drug since 1945, but the prevalence of *Plasmodium falciparum* carrying the *pfcr* mutation associated with CQ resistance remains low (<3%). However, late CQ treatment failure has been reported and the prevalence may be as high as 35% during 14-day follow-up since 1982. In an effort to eliminate malaria as a public health problem, a shift from CQ to artemisinin-based combination therapy has been advocated by a new policy since December 2005. A change of this kind is complex and the lessons learnt from the six decades of CQ use are of the utmost importance to achieve malaria control.

51: *Trans R Soc Trop Med Hyg.* 2008 Oct 24.

**Mosquito abundance and behavior in the influence area of the hydroelectric complex on the Madeira River, Western Amazon, Brazil.**

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Malaria is currently highly prevalent and restricted to the north of Brazil, and its dynamics are severely affected by human environmental changes, such as the large dam construction recently approved by the Brazilian Government in Rondônia. We studied the mosquito fauna and behavior before hydroelectric construction. Mosquitoes were captured by human landing catches on the riversides of the Madeira River in Porto Velho, Rondônia. A total of 3121 mosquitoes from eight different genera were collected; only *Mansonia* and *Anopheles darlingi* were found in all 21 collection sites throughout the night. These results suggest that the riverines of the study area are exposed to malaria.

52: *Trans R Soc Trop Med Hyg.* 2008 Oct 22.

**Socioeconomic and environmental factors important for acquiring non-severe malaria in children in Yemen: a case-control study.**

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Little is known about the relative importance of environmental and socioeconomic

factors for acquiring malaria in Yemen. A case-control study was conducted to determine the importance of these factors for acquiring malaria among children in Yemen. Cases of non-severe malaria were recruited from health centres; community controls were from the neighbourhood of the cases. Data were collected by personal interview and direct inspection during home visits. In total, 320 cases and 308 controls were recruited. In the multivariate analysis, environmental factors (living near streams and freshwater marshes), earth roofs of houses and history of travel were all significantly and positively associated with the occurrence of malaria, whilst regular spraying with insecticides at home was a protective factor. There was no association with socioeconomic factors, including crowding, education and occupation of parents, and ownership of house assets. An index created based on a number of indicators of wealth showed a significant association with malaria in the univariate analysis but was not significant in the multivariate analysis. Control activities can be targeted on identifiable environmental factors such as stream and freshwater marshes, although this needs further investigation. Extra protective measures may be needed by all those who travel in Yemen.

53: *Trends Parasitol.* 2008 Oct 30.

**Keeping it simple: an easy method for manipulating the expression levels of malaria proteins.**

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The ability to genetically manipulate malaria parasites in recent times has contributed considerably to our understanding of the biology of this deadly pathogen. Epp et al. have now expanded the repertoire of molecular tools available for the transgenesis system for the human malaria parasite *Plasmodium falciparum* by developing a simple methodology to regulate malaria gene expression. In this article, we comment on this technique and discuss its potential applications in the study of the biology of malaria parasites.

54: *Trends Parasitol.* 2008 Oct 18.

**Molecular approaches to field studies of malaria.**

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The third 'Molecular Approaches to Malaria' conference was held in Lorne, Australia, in February 2008 and provided extensive information on the application of molecular tools in field studies on malaria. In recent years, technological advances and capacity building in malaria-endemic countries have permitted molecular tools to be applied much more frequently and successfully with exciting new findings. In this review, Hans-Peter Beck and Kevin Tetteh report on the most recent findings using molecular tools in field studies.

55: *Trends Parasitol.* 2008 Oct 18.

**Malaria vaccines: into a mirror, darkly?**

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Recent advances in adjuvant and delivery systems, in addition to a wealth of genomic and proteomic information on parasite composition, are being harnessed to develop a malaria vaccine. To do so effectively, it might be necessary to reassess the criteria by which formulations have been selected to progress to clinical trials. Specifically, better in vitro surrogates of protective immunity, better animal models and a more complete understanding of the unique canvas presented by the immune system of individuals who have experienced multiple malaria infections are needed.

56: *Trends Parasitol.* 2008 Oct 18.

**Comparative evolutionary genomics of human malaria parasites.**

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The parasites *Plasmodium falciparum* and *Plasmodium vivax* are responsible for the majority of human malaria cases worldwide. Despite many similarities in their biology, they frequently are studied in isolation. With the completion of the *P. vivax* genome and the generation of an initial *P. falciparum* genetic diversity map, attempts are being made to infer inter- and intra-species genome evolution. Here, we briefly review our current knowledge of comparative evolutionary genomics of the two species in the light of several presentations at the Molecular Approaches to Malaria 2008 meeting in Lorne, Australia and ask the question: can evolutionary genomics of one species inform the other?

57: *Trends Parasitol.* 2008 Oct 18.

**Recent highlights in antimalarial drug resistance and chemotherapy research.**

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This review summarizes recent investigations into antimalarial drug resistance and chemotherapy, including reports of some of the many exciting talks and posters on this topic that were presented at the third Molecular Approaches to Malaria meeting held in Lorne, Australia, in February 2008 (MAM 2008). After surveying this area of research, we focus on two important questions: what is the molecular contribution of *pfcr* to chloroquine resistance, and what is the mechanism of action of artemisinin? We conclude with thoughts about the current state of antimalarial chemotherapy and priorities moving forward.

58: *Trends Parasitol.* 2008 Oct 15.

***Plasmodium* gene regulation: far more to factor in.**

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Gene expression in the malaria parasite has received generous attention over the past several decades, predominantly because of the importance of *var* gene regulation, which is key to antigenic variation and host immune evasion. However, the role of transcriptional regulation in governing other genes expressed during

the various stages of development has remained less well characterized. This mostly has been due to the lack of defined transcriptional regulators in Plasmodium parasites. Here, we describe recent advances that have become possible by joining traditional biochemistry with new technological innovations. These studies have increased our understanding of the role of transcriptional regulation, not only in the control of gene expression for antigenic variation but also in the coordination of stage-specific parasite development.

59: *Trends Parasitol.* 2008 Oct 15.

**Plasmodium pre-erythrocytic stages: what's new?**

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The pre-erythrocytic (PE) phase of malaria infection, which extends from injection of sporozoites into the skin to the release of the first generation of merozoites, has traditionally been the 'black box' of the Plasmodium life cycle. However, since the advent of parasite transfection technology 13 years ago, our understanding of the PE phase in cellular and molecular terms has dramatically improved. Here, we review and comment on the major developments in the field in the past five years. Progress has been made in many diverse areas, including identifying and characterizing new proteins of interest, imaging parasites in vivo, understanding better the cell biology of hepatocyte infection and developing new vaccines against PE stages of the parasite.

60: *Trends Parasitol.* 2008 Oct 8.

**Severe malaria in children and pregnancy: an update and perspective.**

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This review summarizes progress in preventing and treating severe malaria, which has been accompanied by advances in our understanding of the pathogenesis of severe malaria complications. New drugs such as intravenous artesunate and oral artemisinin combinations, with increased access to insecticide-treated bed nets, are improving outcomes and decreasing malaria deaths. Several groups are beginning to identify characteristics of parasite var genes associated with cerebral malaria. Understanding of the interactions between malaria and other diseases in causing severe anaemia and cerebral malaria has increased substantially, and at the cellular level, the disturbances leading to coma or other complications are becoming clearer.

61: *Trends Parasitol.* 2008 Oct 8.

**Recent insights into humoral and cellular immune responses against malaria.**

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Effective immunity to malaria has been clearly demonstrated among individuals naturally exposed to malaria, and can be induced by experimental infections in animals and humans. The large number of malaria antigens has presented a major challenge to identifying protective responses and their targets, and it is likely

that robust immunity is mediated by responses to multiple antigens. These include merozoite surface antigens and invasion ligands, variant antigens on the surface of parasitized red blood cells, in addition to sporozoite and liver-stage antigens. Immunity seems to require humoral and cellular immune components, probably in co-operation, although the relative importance of each remains unclear. This review summarizes recent progress towards understanding the targets and mechanisms that are important for mediating immunity to malaria.

62: *Vaccine*. 2008 Oct 29.

**Antibody responses to a panel of Plasmodium falciparum malaria blood-stage antigens in relation to clinical disease outcome in Sudan.**

Iriemenam NC, Khirelsied AH, Nasr A, Elghazali G, Giha HA, A-Elgadir TM, Agab-Aldour AA, Montgomery SM, Anders RF, Theisen M, Troye-Blomberg M, Elbashir MI, Berzins K.

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Despite many intervention programmes aimed at curtailing the scourge, malaria remains a formidable problem of human health. Immunity to asexual blood-stage of Plasmodium falciparum malaria is thought to be associated with protective antibodies of certain immunoglobulin classes and subclasses. We have analysed immunoglobulin G profiles to six leading blood-stage antigens in relation to clinical malaria outcome in a hospital-based study in Sudan. Our results revealed a linear association with anti-AMA-1-IgG1 antibodies in children <5 years and reduced risk of severe malaria, while the responses of the IgG3 antibodies against MSP-2, MSP-3, GLURP in individuals above 5 years were bi-modal. A dominance of IgG3 antibodies in >5 years was also observed. In the final combined model, the highest levels of IgG1 antibodies to AMA-1, GLURP-R0, and the highest levels of IgG3 antibodies to 3D7 MSP-2 were independently associated with protection from clinical malaria. The study provides further support for the potential importance of the studied merozoite vaccine candidate antigens as targets for parasite neutralizing antibody responses of the IgG1 and IgG3 subclasses.

63: *Vaccine*. 2008 Oct 27.

**Reflections on early malaria vaccine studies, the first successful human malaria vaccination, and beyond.**

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Advances towards protective vaccines against malaria were made feasible by the development of a rodent model of mammalian malaria that allowed production of all stages of the malaria parasite for study. Investigations with sporozoites (the stage transmitted by mosquitoes in their saliva) demonstrated that immunization with radiation-attenuated sporozoites could produce a solid, sterile immunity, first shown in studies with mice and later with human volunteers. Protective immune mechanisms involve anti-sporozoite antibodies that immobilize sporozoites injected into the skin by mosquitoes, followed by CD4+ and CD8+ T-cells acting against liver stage parasites produced by sporozoites that have escaped antibody-based immunity and invaded hepatocytes. Two alternative approaches now being used in human trials are immunization with intact, attenuated sporozoites vs. immunization with "sub-unit" vaccines based on immunogenic components of

sporozoites or liver stage parasites. In addition to immunization against these pre-erythrocytic stages, encouraging progress is being made on immunization against blood stage parasites and on immunization for production of transmission-blocking antibodies. There is reason to be optimistic that one or more of the approaches will work on a large scale, and that a multi-stage vaccine may be able to combine several of these approaches in a sequential immunological assault against the malaria parasite as it progresses through its stages.

64: *Vaccine*. 2008 Oct 15.

**A Phase 1 trial of PfCP2.9: An AMA1/MSP1 chimeric recombinant protein vaccine for *Plasmodium falciparum* malaria.**

Malkin E, Hu J, Li Z, Chen Z, Bi X, Reed Z, Dubovsky F, Liu J, Wang Q, Pan X, Chen T, Giersing B, Xu Y, Kang X, Gu J, Shen Q, Tucker K, Tierney E, Pan W, Long C, Cao Z.

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Apical Membrane Antigen 1 (AMA1) and Merozoite Surface Protein 1 (MSP1) were produced as a recombinant fusion antigen and formulated with the adjuvant Montanide ISA 720 with the aim of replicating the structure present in the parasite protein. A previous trial with this construct demonstrated the vaccine was safe and immunogenic but was associated with injection site reactogenicity. This Phase 1a dose-escalating, double blind, randomized, controlled trial of PfCP2.9/Montanide ISA 720 was conducted to evaluate alternative dose levels and vaccination schedules, with a pre-formulated vaccine that had undergone more in-depth and frequent quality control and stability analysis. The trial was conducted in seventy healthy Chinese malaria-naïve volunteers between January 2006 and January 2007. The objective was to assess the safety, reactogenicity and immunogenicity of 5, 20 and 50µg of PfCP2.9/ISA 720 under 2 different schedules. The most common adverse event was injection site tenderness (53%). The frequency and severity of adverse events was similar in both vaccination schedules. Antibody responses were induced and remained elevated throughout the study in volunteers receiving vaccine ( $p < 0.001$ ). Although high antibody titers as measured by ELISA to the PfCP2.9 immunogen were observed, biological function of these antibodies was not reflected by the in vitro inhibition of parasite growth, and there was limited recognition of fixed parasites in an immunofluorescence assay. At all three dose levels and both schedules, this formulation of PfCP2.9/ISA 720 is well tolerated, safe and immunogenic; however no functional activity against the parasite was observed.