Abstracts

Parallel Sessions—Abstracts of Presentations

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1. Prevention of malaria

Monday 14 November 14:30–16:30—Bubinga Hall

Chairs: Robert Leke (Yaounde) and Stephen Rogerson (Melbourne)

O-1

Intermittent preventive treatment of malaria in pregnancy (Cameroon experience)

Robert Leke

University of Yaounde I, Yaounde, Cameroon

Introduction: Malaria is a major cause of morbidity and mortality in the developing countries especially in Africa. Most deaths from malaria occur in children under 5 years. About 24 million pregnancies in Africa are threatened by malaria each year, where less than 5% of pregnant women have access to adequate treatment. The socio-economic consequences of malaria are several folds: affects about 1/3 of the world’s population, about 1 million children under 5 years die from malaria, an increasing resistance to most drugs currently used for the treatment of malaria, 40% of the budgets of developing countries are spent on malaria and 30% of the family expenditure is on the treatment of malaria.

Methods: A literature review on malaria in pregnancy revealed that in high endemic regions, the consequences of malaria on the pregnant women are: anaemia (2.15%), small for dates (8–10%), neonatal mortality, abortion, placental malaria and maternal mortality. The community also suffers from malaria because affected persons are absent from work, thus loss of salary, absence from school, anaemia and its consequence on the growth and development of the child, the wastage of the minimal resources of the government for the purchase of drugs and consequently the increased risk of death for children and the pregnant women. However, for any country to change its treatment policy as regards prevention of malaria in pregnancy, the following criteria must be met: a proven pharmaco-resistance to chloroquine, non compliance of pregnant women to the preventive measures and the absence of a link between the programme for fight against malaria and the prenatal services. Besides the existing protocol should therefore be modified only when the following conditions are ascertained. Efficacy and innocuity of the new drugs proposed, potential adherence to treatment, possibility of preventing the development of drug resistance and the financial consequences of the change of the treatment policy: ”Direct cost and indirect expenditure”. Intermittent preventive treatment of malaria in pregnancy is therefore the regular intake of anti-malaria from the second trimester of pregnancy to prevent a malaria attack. This modality of treatment has as advantage, better compliance because it is easy, efficient and cost effective, and easy to be administered by service provider (single curative dose) at each time. The Cameroon government therefore adopted sulfadoxine-pyrimethamine in three doses in the second and third trimester and the last dose toward the end of the third trimester for the prevention of malaria in pregnancy. All malaria attacks in the first trimester are managed as severe malaria. Some other African countries like Malawi, Kenya, Botswana, Tanzania and the Democratic Republic of Congo have also adopted the same policy.

Results: The goal of the above programme is to render easy accessibility of the drug, better coverage of the rural communities where access to health services is difficult, distribution of Impregnated mosquito nets, involve the private sector in the above action, promote quality prenatal care services and encourage research in the areas of drug resistance, efficacy and acceptability. Measures for the prevention of malaria in pregnancy include intermittent preventive treatment, use of insecticides, insecticide impregnated mosquito nets, environmental hygiene, prevention of complications of malaria such as anaemia and the prevention of sexually transmitted infection (STI) example HIV. A period of at least one month should separate two doses of intermittent treatment. It should not be administered in the first trimester because it is teratogenic. Intermittent preventive treatment in pregnancy may be hindered by resistance to sulfadoxine-pyrimethamine, allergy to sulphonamides, or the high rates of delivery at home. Inaccessibility to mosquito nets or its improper use may also influence intermittent preventive treatment. Some side effects associated with sulfadoxine-pyrimethamine are gastro-intestinal disorders, allergy, lyell syndrome and hematologic disorders. Sulfadoxine-pyrimethamine is therefore contra indicated during the first trimester of pregnancy, existing allergy to sulphonamides, severe renal and liver disease.
Conclusion: The Cameroon government has adopted sulfadoxine–pyrimethamine for the intermittent preventive treatment of malaria in pregnancy; administered in three doses in the second and third trimester of pregnancy. Malaria attack in the first trimester is managed as severe malaria with salts of quinine.

O-2 The malaria-attributable fraction of fever episodes in health facilities in four African cities [MIM-CL-404690]
S. Wang, T. Smith, C. Lengeler
Swiss Tropical Institute, Basel, Switzerland

Introduction: Urbanization has transformed malaria epidemiology in urban areas in sub-Saharan Africa (SSA). One important feature is that endemicity levels tend to be lower than in surrounding rural areas. As a result, a higher proportion of fever episodes in children are not due to malaria but to other causes, and this has important implications for fever case management. We estimated the probabilities that individual febrile episodes were attributed to malaria in four urban centers in SSA.

Methods: We estimated the malaria-attributable fraction of fever (MAFF) as part of a multi-site assessment of urban malaria in SSA. The fieldwork took place in Abidjan, Ouagadougou, Cotonou and Dar es Salaam. We categorized each site into three to four zones (centre, intermediate, periphery and rural areas), and randomly chose one clinic in each zone. In each clinic, we recruited 200 fever cases and 200 non-fever controls. Outpatients with a history of fever or a measured temperature >37.5 °C were defined as cases. Controls were recruited from patients in the same clinic without current or recent fever, matched by age and residency. Each patient had an interview, body temperature check, and thin and thick blood films taken.

Results: The fever and control groups had a medium level of parasitaemia in Abidjan (34.7% versus 16.4%) and in Ouagadougou (22.0% versus 20.1%). The odds ratio of having parasitaemia in fever cases varied by different age groups: from 2.0 to 2.6 in Abidjan and from 0.8 to 2.1 in Ouagadougou. The estimated MAFF in infants, 1–5 years old, 5–15 years old and >15 years old were: 0.12, 0.22, 0.27, 0.13 in Abidjan, and −0.03, 0.13, 0.04, −0.02 in Ouagadougou. The overall prevalence of malaria was surprising low in both fever and control groups in Dar es Salaam (5.2% versus 2.8%) and Cotonou (2.0% versus 1.8%). Odds ratios for parasitaemia ranged from 0.58 to 2.3 and 0.45 to 2.53. Both sites had extremely low MAFF too: −0.01 to 0.04. According to the routine malaria weekly reports we collected from each municipal health department for the last 3 years, 30–40% of all out-patient consultations in the four cities had a final diagnosis of malaria. Given the very low MAFF, misdiagnosis must be high and over-treatment frequent.

Interpretation: Using a fever sign alone as a basis for malaria diagnosis leads to many unnecessary treatments, with consequences on cost, side-effects and the development of resistance. The clinical management of fevers in urban areas should be reviewed urgently.

O-3 Extended follow-up of intermittent preventive anti-malarial treatment in Tanzanian infants [MIM-JA-654]
(1) Unidad de Epidemiología. Centre for International Health, Hospital Clinic, Barcelona, Spain (J.J. Aponte MD, D. Schellenberg MRCP, C. Menendez MD, P. Alonso MD); (2) Ifakara Health Research and Development Centre, Kilombero, Tanzania (D. Schellenberg, E. Kahigwa MSc, H. Mshinda PhD); (3) Department of Infectious and Tropical Disease, London School of Hygiene and Tropical Medicine, London, UK (D. Schellenberg); (4) Swiss Tropical Institute, Basel, Switzerland (Prof. M. Tanner PhD)

Introduction: Antimalarial chemoprophylaxis can reduce malaria disease in African children. However, cessation of chemoprophylaxis may be followed by an increased risk of malaria, suggesting that it can interfere with the development of anti-malarial immunity. Intermittent Preventive Treatment in infants (IPTi) has been shown to reduce the incidence of clinical malaria. We assessed the possibility of an increased risk of malaria following IPTi using a randomised, placebo controlled, double blind trial.

Methods: Seven hundred and one children living in Ifakara, southern Tanzania, were randomly assigned to receive sulphadoxine–pyrimethamine (SP) or placebo
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at 2, 3, and 9 months of age, when attending routine vaccinations. Malaria episodes were documented using hospital-based passive case-detection. The primary outcome was the incidence of clinical malaria in the period from one month after dose three of IPTi up to 2 years of age. Results were expressed as the protective efficacy (PE = 100\(\times\) [1 – hazard ratio]) % in those children who received all three doses of IPTi/placebo.

**Results:** Five hundred and thirty-seven (76.6%) children received three doses of IPTi/placebo. The baseline characteristics, compliance and completeness of follow-up of IPTi and placebo recipients were similar. The rate of clinical malaria (events per person-year at risk) was 0.28 in the SP group versus 0.43 in the placebo group (PE = 35.7% [95% CI 11.4, 53.0]). Diverging Kaplan–Meier curves confirmed that IPTi-recipients remained at lower risk of clinical malaria than placebo-recipients.

**Interpretation:** Interpretation IPTi produced a sustained reduction in the risk of clinical malaria extending well beyond the duration of the pharmacological effects of SP. IPTi may facilitate the development of immunity against *Plasmodium falciparum* disease.

**O-4**

The impact of anaemia, falciparum malaria and malnutrition on psychomotor development of infants exposed to intense and perennial malaria transmission [MIM+{F-472601}] V. Fumado, L. Quinto, E. Kahiqwa, H. Mshinda, P. Alonso, C. Menendez

(1) Centre for International Health, Hospital Clinic, Barcelona University, Unitat Integrada Clinic-Sant Joan de Deu, Barcelona, Spain; (2) Ifakara Health Research Center, St Francis Designated District Hospital, Ifakara, Tanzania

**Introduction:** Effect of malaria and anaemia on psychomotor development (PD) has not been studied in children less than one year. It is unclear whether uncomplicated malaria is associated with neurological effects and what its effects are on PD during the first year of life. There is very little information about the impact of anaemia and undernutrition on PD. Objective of this study was to determine effects of malaria, anaemia and malnutrition on infants, who live in an area of intense malaria transmission.

**Methods:** The effects of malaria, anaemia and undernutrition during the first year of life on the PD was studied in 661 Tanzanian children at 2, 8, 12 and 18 months of age. The study was carried out within the frame of an intervention trial of malaria chemoprophylaxis and/or iron supplementation for the prevention of malaria and anaemia in infants. Children found to be severely anaemic were withdrawn from continuation into the study. The PD was assessed using a developmental test adapted for this population from the Bayley’s scale of infant development. Four different psychomotor development areas were evaluated: behaviour, hearing and speech, gross motor, and hand manipulation. The muscular tone and awareness were also examined.

**Results:** Malnutrition was found to have the greatest negative impact on PD, this effect was evident at all cross-sectional visits and started at two months of age. At 12 and 18 months of age anaemia had an effect on the motor area. Malaria episodes affected awareness at eight months, and the area of speech at 18 months of age. Prematurity and low weight birth negatively affected the muscular tone at two months. Our findings suggest that malaria and anaemia episodes may affect negatively the psychomotor development during the first 18 months of life, but malnutrition is the factor with the greatest on the psychomotor development. This study provides for the first time some insight into the potential neurodevelopment consequences of the dynamic interactions between poor nutrition, malaria infection and anaemia, all of them common in malaria endemic areas in infants and young children.

**Interpretation:** Effective control of malnutrition, anaemia and malaria during infancy, is essential to prevent a delay and/or abnormalities on psychomotor development.
Efficacy and safety of malaria intermittent preventive treatment administered through the EPI scheme on the prevention of malaria in Mozambican infants [MIM-EM-72603]

E. Macete, P. Aide, M. Espasa, I. Mandomando, S. Sanz, J. Aponte, S. Mabunda, C. Dobado, P. Alonso, C. Menéndez

(1) Center for International Health, Hospital Clinic, University of Barcelona, Spain; (2) Manhiça Health Research Center, Manhiça, Mozambique; (3) Ministry of Health of Mozambique, Mozambique

Introduction: Intermittent preventive treatment in infants (IPTi) consists in the administration of an antimalarial drug at specified intervals administered through the EPI scheme. This strategy has been found to be efficacious in preventing malaria and anaemia in infants in a previous trial in Tanzania. Further information on the efficacy and safety of this intervention in different malaria endemic areas is needed before widespread implementation can be considered.

Methods: We carried out a randomised, double blind, placebo-controlled trial of IPTi with sulfadoxine–pyrimethamine (SP) administered to Mozambican infants at 3, 4 and 9 months of age through the EPI scheme. The objective of the trial was to assess safety and efficacy of IPTi in the reduction of malaria and anaemia episodes. Participants were recruited when attending the EPI clinic to receive the second dose of DTP at which time the first dose of SP/placebo was given. The second and third doses were given coinciding with the administration of the third dose of DTP and measles, respectively. The intervention was given according to body weight. Outcomes were assessed through passive clinical surveillance. Haematology and biochemistry were assessed 1 month after dose 2 and two cross-sectional surveys were carried out at 12 and 24 months of age malaria and anaemia evaluation.

Results: 1503 children were recruited and received dose 1 (SP or placebo). 1398 received dose 2, 1269 dose 3 and 1245 (83%) completed the three doses. Up to date no adverse events associated with the intervention have been observed. No dermatological nor analytical abnormalities related to the intervention have been detected. Study children are continued to be followed up to 24 months of age. We will present the results of the main primary analysis up to 12 months of age.

Interpretation: It will be presented at the conference.

Combined Vitamin A and zinc supplementation on falciparum malaria among preschool children in West Burkina Faso: A randomised controlled trial [MIM-OJ-2927]

A. Zéba, H. Sorybo, I. Zongo, N. Mockhtar, N. Rouamba, C. Muller, T. Guiguemé, J. Ouédraogo

(1) Institut de Recherche en Sciences de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso; (2) Centre Muraz, Bobo-Dioulasso, Burkina Faso; (3) International Atomic Energy Agency (IAEA), Vienna, Austria; (4) Laboratoire National de Santé, Luxembourg

Introduction: In Burkina Faso, 150 per 1000 of children die by malaria. Iron deficiency, vitamin A and zinc deficiency are also major public health of concerns. Few studies have shown that Vitamin A or zinc supplements can reduce malaria infection. The objective of this study was to evaluate the impact of zinc and Vitamin A double supplementation on malaria infection in children during the high season of malaria transmission. The immunological and nutritional impact has also been addressed.

Methods: One hundred and forty-eight children aged from 6 months to 5 years were enrolled in a randomised double blind Vitamin A/zinc versus placebo supplementation trial. The children were randomly allocated in two groups: Vitamin A + zinc (n = 74) and placebo (n = 74) and were followed up, daily during 6 months. The supplemented group received a single oral dose of 200,000 IU of Vitamin A plus a daily 10 mg of zinc. Children’s body temperature was measured daily and malaria diagnosis was done in case of fever (temperature = 37.5 °C). In addition to anthropometric parameters measurement, total IgG and IgG subclasses (IgG1–4) against three different Plasmodium falciparum antigens were detected by ELISA (GLURP, MSP1–19, CSP).

Results: The supplemented group had less fever episodes than placebo group (p = 0.038). The number of fever manifestation was lower in the supplemented group than the placebo group with 121 versus 153 (p = 0.023). We observed the same situation with
malaria attacks; the number of malaria episodes was lower in the supplemented group with 78 versus 106 ($p = 0.026$). The time of the first malaria episode during the follow up estimated by Kaplan–Meier test was the same in the two groups. The prevalence rates of malnutrition and growth retardation were also the same in both groups. The mean antibody titres were globally higher in the supplemented group than the placebo group but statistically non significant. In the supplemented group the mean antibody titre was increase against CSP ($p < 0.001$) and remains unchanged against the others at the end of the study. Except MSP1, the reactivity against all antigens had increased ($p \leq 0.026$) in the placebo group at the end of the study. Considering isotypes, we found that cytophilic isotype (IgG1 and IgG3) antibody titres were higher in both groups at the end of the study and the supplemented group reactivity was higher than the other ($p < 0.001$). However, the isotypes IgG2 and IgG4 remains unchanged in both groups.

Interpretation: The combined supplementation of Vitamins A + Zinc seems to reduce the malaria and fever attacks risk among children. This supplementation appears to offer a protection by contributing to increase IgG subclass 1 and 3 titres but not with total IgG.

O-7 Intermittent preventive treatment of malaria with sulfadoxine–pyrimethamine in HIV seropositive pregnant Zambian women [MIM-MM-393346]

V Mwanakasale, L. Mwananyanda, V. Chalwe, D. Mukwamataba, C. Gill, R. Chilengi, M. Mulenga, D. Thea, W. Mcleod, D. Hummer

(1) Tropical Diseases Research Centre, Ndola, Zambia; (2) Center for International Health and Development, Boston University School of Public Health, Boston, MA, USA

Introduction: The WHO currently recommends the use of intermittent preventive treatment (IPT) to reduce the burden of disease due to malaria during pregnancy. Recent evidence suggests that more frequently monthly dosing may be more effective for HIV seropositive pregnant women. In Zambia, the recommended regimen is three doses of sulfadoxine–pyrimethamine (SP) during the second and third trimesters.

Methods: We conducted a randomized, double blind placebo-controlled trial in HIV seropositive pregnant women in Ndola, Zambia comparing two SP dosing regimens: monthly versus once/trimester. SP was administered under direct observation. The study was conducted in an urban locale of Ndola, Zambia with mesoendemic transmission. Participants were assessed monthly until delivery to detect malaria, anaemia and SP adverse effects. Primary outcomes included histological evidence of placental malaria and maternal peripheral parasitaemia by microscopy. Placental histology was graded as C1—acute infection; C2—acute on chronic infection; C3—past infection; and C4—uninfected. Secondary outcomes included third trimester anaemia, birth weight, and gestational age as assessed by modified Dubowitz criteria.

Results: Of 3030 pregnant women screened, the HIV seroprevalence was 23.3% (707). After randomization, 224 women were in treatment group 1 and 232 in treatment group 2 (preliminary blinded analysis). Baseline demographic and clinical characteristics of the treatment groups were comparable. Of 265 women who had placental biopsies performed, the proportion of women with any placental parasitemia (C1 + C2 + C3) was comparable between group 1: 33.9% (40/118) and group 2: 34.0% (50/147) (OR: 0.99; 95% CI: 0.60, 1.66). The proportion of women with peripheral parasitemia during pregnancy was also comparable between the two groups (data not shown). The frequency of maternal anaemia (Hb < 11.0 g/dL) was 44.7% (80/179) in group 1 and 38.0% (70/184) in group 2 (OR: 0.76; 95% CI: 0.50, 1.15). No significant difference was found in the proportion of babies with low birth weight (<2500 g) in group 1, 14.5% (27/186) compared to those 15.7% (31/197) in group 2 (OR: 0.91; [95% CI: 0.52, 1.59]). The proportion of babies who were born prematurely in group 1, 13.0% (22/169) was similar to that 12.7% (24/189) in group 2 (OR: 0.97; 95% CI: 0.52, 1.81).

Interpretation: In this urban setting of Zambia with high HIV seroprevalence, there was no evidence of a meaningful difference in pregnancy outcomes between monthly dosing versus a two dose regimen of SP for IPT.
2. Parasite biology
Monday 14 November 14:30–16:30—Ebony Hall
Chairs: Tom Egwang (Kampala) and Genevieve Milon (Paris)

O-8
Malaria under the microscope (The Masamichi Aikawa Memorial Lecture) [MIM-LB-239440]
L. Bannister
King's College London, London, UK

Introduction: From the 1960s, electron microscopy has transformed our view of malaria parasite biology. The work of Masamichi Aikawa has been critical to this effort, and will continue to inform our view of the genus Plasmodium. In this lecture, current knowledge of malaria microstructure will be reviewed, focusing on the opportunities for finding new antimalarial targets.

Methods: Recent developments in optics and computerised imaging are providing new tools for this exploration, with much future potential. Three-dimensional computerised reconstruction from serial and tomoscopic sections plus immunolocalization enable the complex molecular organization of the parasite blood stages to be visualised in detail.

Results: Recent research using light- and electron-microscopy is providing new data clarifying the molecular organization of malaria parasites through their life cycle. It has been shown that the pathways of protein synthesis and subsequent trafficking are similar throughout the life cycle, though with stage-specific modification. The pathways are centred on a minimal Golgi complex formed by vesicle fusion from the nuclear envelope, which itself is confluent with the endoplasmic reticulum. From the Golgi complex, different proteins are targeted to the parasite surface in ways depending on the parasite's stage. Some are sent directly into the host cell to modify its properties. Others are stored in secretory organelles and then transported to their sites of secretion for use during host cell invasion. The molecules using these routes can now be defined and followed by appropriate microscopic methods, which can be used to monitor attempts to disrupt their function. Microscopic analysis of other compartments within the parasite includes the apicoplast, pigment vacuole and secretory apparatus of the merozoite, all of them targets for antimalarial drugs or vaccine development.

Interpretation: A detailed microscopic knowledge of these and other components of the parasite will be an important factor in the development of new weapons against malaria, so urgently needed.

O-9
Apical regulated exocytosis in Plasmodium sporozoites is activated by pyrimidine nucleotides and mediated by cAMP/PKA/G-proteins [MIM-LC-59748]
L. Cabrita-santos, O. Diaz-pulido, M. Mota, A. Rodriguez
(1) Medical Parasitology, NYU School of Medicine, New York, USA; (2) Instituto de Medicina Molecular, Lisboa, Portugal

Introduction: Plasmodium sporozoites migrate through several hepatocytes, before infecting a final one. Migration activates sporozoites for infection because it induces apical regulated exocytosis in the parasite, which is necessary for the final infection of hepatocytes with formation of a vacuole. We characterized the signaling pathway leading to regulated exocytosis and investigated its regulation.

Methods: We isolated P. yoelii sporozoites from Anopheles infected mosquitoes salivary glands. Sporozoites where treated with activators (8-Br-cAMP, Forskolin) and inhibitors (MDL, H-89 and Cholera Toxin) of the Adenylate cyclase, Protein Kinase A and G-proteins pathways to study sporozoite exocytosis, sporozoite migration and infection. Exocytosis was measured as percentage of sporozoites with TRAP exposure in their apical end, migration as the percentage of wounded cells and infection as number of EEF's per coverslip. Sporozoites viability after drug treatment was confirmed by their ability to traverse cells.

Results: We found that P. yoelii exocytosis and infection of host cells is mediated by increases in cytosolic cyclic adenosine monophosphate (cAMP) and activation of Protein Kinase A (PKA) in the parasite. G-proteins are also required downstream of cAMP in the signaling cascade. By blocking any of these exocytosis mediators we observe a significant reduction both in sporozoites regulated exocytosis and in their infectivity of hepatocytes. We also found that
lysates and incubation medium from host cells induce exocytosis and increases in cAMP levels in *P. yoelii* sporozoites. Using size-fractionation, we have identified that certain pyrimidine nucleotides induce exocytosis in *P. yoelii* sporozoites. The free bases (uracil and thymine), the nucleosides (uridine and thymidine) and the mono and di-phosphate nucleotides (UMP, UDP, TMP, TDP) stimulate exocytosis, while tri-phosphate pyrimidines (UTP and TTP) had no effect. Cytosine derived nucleotides also failed to induce exocytosis.

**Interpretation:** When sporozoites migrate through the cytosol of host cells they encounter high concentrations of pyrimidine nucleotides that induce apical regulated exocytosis required for infection through a signaling cascade mediated by cAMP/PKA/G-proteins.

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**O-10 Invasion et cytoadhérence sur les cellules endothéliales et le placenta: rôle de RSP2, antigène des rhoptries transféré à la surface des hématies [MIM-JL-38212]**

J. Lekana-douki, J. Lekana-douki, Y. Sterkers, C. Lepolard, B. Traore, F. Costa, A. Scherf, J. Gysin
(1) Université de la Méditerranée (Aix-Marseille II), Marseille, France; (2) Institut Pasteur, Paris, France; (3) Université des Sciences de la Santé (USS), Libreville, Gabon

**Introduction:** Pendant longtemps il a été admis que la cytoadhérence des Hématies Parasitées (HP) par *P. falciparum* par les formes matures. Ce dogme devint caduc avec les résultats d’écritant la cytoadhérence des HP par les formes jeunes, ring (HPr). Ce nouveau phénomène concerne uniquement les HP cytoadhérent sur la CSA aux stades matures (HPCSA). Ici, nous caractérisons RSP2, antigène des rhoptries transféré à la surface des hématies pendant l’invasion.

**Methods:** Des sous populations d’HPCSA ont été sélectionnées à partir d’isolats périphériques et pla-centaires. Des souris ont été rendues tolérantes aux hématies saines (HS) puis immunisées avec des HP. Des anticorps monoclonaux (mAbs) spécifiques des HP ont été ainsi développés. Des western blots et des immunoprécipitations après marquages radioactifs ont permis d’identifier l’antigène cible de ces mAbs. L’implication de cet antigène dans la cytoadhérence des HS et HP a été analysée en conditions statiques et sous flux. Son rôle dans l’invasion des hématies par les mérozoites a été examiné.

**Results:** Trois mAbs reconnaissant spécifiquement des HP ont été sélectionnés. Ces mAbs reconnaissent RSP2 (Ring Surface Proteïn 2), antigène de 42 kDa. RSP2 interagit avec deux protéines de 72 et 79 kDa formant un complexe dans les rhoptries. Les mAbs anti-RSP2 inhibent spécifiquement la cytoadhérence des HS et HP; ils induisent le décrochage spécifique des HS et HP cytoadhérant, en conditions statiques et sous flux. Le rôle de RSP2 dans la cytoadhérence des HS et HP est exclusif des HPCSA. La cytoadhérence des HS et HP est maintenue aux forces de cisaillement physiologiques. Les anti-RSP2 inhibent également l’invasion des hématies par les mérozoites. La chronologie d’expression, montre qu’au stade trophozoïte RSP2 a une localisation intraparasi-taire. Lors de la différenciation des mérozoites, RSP2 est transporté à la surface des mérozoites via les rhoptries. Pendant le contact entre le mérozoïte et l’hématie, RSP2 est transféré à la surface de l’hématie indépendamment de la pénétration parasitaire. Ainsi, RSP2 est présent indéfiniment à la surface des HS et des HP pendant les 20 premières heures post-invasion. De 16 à 20 heures post-invasion la présence de RSP2 à la surface de l’HP est concomitante à PfEMP1: l’HP peut cysdomériter doublement.

**Interpretation:** RSP2 est un ligand polyvalent de la physiopathologie palustre: il intervient à la fois dans la cytoadhérence des HS et HP et dans l’invasion. Sa présence à la surface des HS suggère une implication dans la physiopathogénèse de l’anémie sèvère.

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**O-11 Malaria in CD36 knockout mice [MIM-MR-112948]**

M. Rodrigues, B. Franke-fayard, J. Ramesar, M. Febbraro, A. Waters, C. Janse, M. Mota
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**Introduction:** Certain plasmodial species acquired adhesive properties allowing them to adhere to...
endothelia. This sequestration is considered of great significance for the outcome of disease, including cerebral malaria. CD36 is considered the major receptor in sequestration and other adhesion phenotypes during infection. Evidences up now are based only on in vitro studies and there is still no clear evidence that CD36 mediated adherence has any phenotypic relevance for pathology in malaria infection.

**Methods:** We used C57Bl/6 CD36$^{-/-}$ mice to follow infection with four rodent Plasmodium strains that represent different features of disease (P. berghei ANKA, P. yoelii 17X, P. yoelii YM and P. chabaudi AS) as compared to C57Bl/6 CD36$^{+/+}$ mice. Infection was followed by counting parasitemia in Giemsa stained thin blood smears and observing mice general health status. For in vivo visualization of infected erythrocyte sequestration we used transgenic, luciferase expressing, P. berghei ANKA parasites (PbGFP-LUCSCH) and measured the luciferase activity in whole bodies of live animals and in dissected organs using a photon-counting I-CCD video camera (IVIS, Xenogen, USA), throughout the infection in both CD36$^{-/-}$ and CD36$^{+/+}$ mice.

**Results:** CD36$^{-/-}$ mice consistently had a slightly lower parasitemia throughout infection with all different rodent parasites. Both wild type, CD36$^{+/+}$, and CD36$^{-/-}$ mice infected with P. berghei ANKA developed neurological symptoms (ataxia, deviation of the head, paralysis, and coma) and died early in infection with cerebral malaria. Visualizing the luciferase activity throughout infection, until the onset of CM symptoms, confirmed the absence of sequestration in brain tissue in both wild type and CD36$^{-/-}$ mice. Furthermore, the results show no organ sequestration in CD36$^{-/-}$ mice and increased numbers of schizonts in the circulation and in the spleen of CD36$^{-/-}$ mice as compared to wild type mice.

**Interpretation:** Results show that CD36 is required for sequestration. Since CD36$^{-/-}$ mice show no signs of organ sequestration (only “trapping” in the spleen) but still develop cerebral malaria, we conclude that sequestration is not the basis of CM in murine models.

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**Abstracts / Acta Tropica 95S (2005) S1–S506**

**O-12**

*P. falciparum* in The Gambia: Persistence in the dry season and increased complexity in mosquitoes in the wet season [MIM-HB-81294]

D. Nwakanma, S. Dunyo, M. Jawara, P. Milligan, M. Pinder, D. Walliker, H. Babiker

(1) School of Biological Sciences, University of Edinburgh, UK; (2) The Medical Research Laboratories, The Gambia; (3) Biochemistry Department, Faculty of Medicine, Sultan Qaboos University, Oman

**Introduction:** Multiplicity of Plasmodium falciparum clones, in natural infections, their ability to produce gametocytes and consequent crossing and recombination are the driving evolutionary forces of this parasite. In this study, we have examined gametocytogenesis of individual *P. falciparum* clones during the dry season in villages in The Gambia, and their longevity and infectivity to *Anopheles gambiae*.

**Methods:** We recruited a cohort of 31 *P. falciparum*-infected individuals in the middle of the dry season (May 2003), then examined them again at the beginning (August) and the middle (October) of the transmission season. On each occasion we examined the diversity of genes of asexual forms and gametocytes using PCR and RT-PCR, respectively. At the same time we examined parasite infectivity to *Anopheles gambiae*.

**Results:** A large proportion of *P. falciparum* clones persisted as asymptomatic sub-patent infections during the dry season and produced gametocytes. Different gametocyte genotypes coexisted in the same infection in mosquitoes was sometimes greater than that seen in the individual from whom they were infected.

**Interpretation:** The proportion of clones producing gametocytes among the cohort was high in the dry season, and rose significantly late in the transmission season. Some *P. falciparum* clones which existed in the blood below PCR detection limits proved more infectious to mosquitoes than more abundant clones.
O-13  
Effects of blood meal acquisition method on prevalence and heterogeneity of *P. falciparum* malarial oocyst in *An. gambiae* [MIM-LG-227058]  
L. Gouagna, T. Tchuinkam, S. Bonnet, R. Sauerwein, C. Boudin  
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**Introduction:** In a compatible Plasmodium–Anopheles model, an infectious individual does not necessarily infect all mosquitoes that feed on him and, within infected mosquitoes that fed on the same gametocyte source, the aggregated parasite distribution is a well-established phenomenon. However, little is known about the underlying mechanisms. We assessed the influence of bloodmeal acquisition process on the distribution of *Plasmodium falciparum* oocyst population within infected *Anopheles gambiae* mosquitoes.  

**Methods:** Batches of inbred *An. gambiae* mosquitoes were simultaneously fed, either directly on *P. falciparum* gametocyte carriers (DSF) or through membrane feeders (DMF) containing blood from the same source. All infection trials within each group provided oocyst scores to which the analysis was applied. The outcomes examined included the proportion of mosquitoes with oocyst and the average numbers of oocysts per mosquito. Frequency distribution tables of oocyst loads were constructed and ability of each feeding method to reproduce oocyst aggregation was examined using Taylor’s power law.  

**Results:**  
Thirty eight paired infection trials were conducted. Negative binomial model was fitted to the number of infected mosquitoes per group and indicated a significant difference between DSF and DMF ($p = 0.0178$). Population sizes of oocysts varied between 1 and 150 oocysts per infected mosquitoes, though the chance of high quantum of oocyst production (>50 oocysts per mosquito) was low, and beard a strong relationship with initial gametocyte density ($r = 0.46, p = 0.004$). However, mean oocyst burdens did not differ significantly between DSF and DMF ($p$-value = 0.24), suggesting that the *P. falciparum* infection outcome may be determined by other factors than the mode of infection acquisition. In both methods, the Taylor’s power law gave a good fit to the data with a population index of aggregation varying from $1.48 ± 0.12$ in DSF, to $1.65 ± 0.08$ in DMF. The a non-linear model which gives the relationship between the proportion of infested mosquitoes ($P(I)$) and the mean oocyst intensity ($m$) yielded a good fit to the observed data ($r^2 = 0.94, p < 0.01$ for DSF, $r^2 = 0.83, p < 0.01$ for DMF), indicates similar dispersion for almost all individual infection experiments within each group.  

**Interpretation:** Oocyst aggregation within mosquitoes is a stable property of Plasmodium which probably has a genetic basis. Thus malaria parasites may adaptively adjust their population in the way that is less harmful to the vector.

3. Immuno-epidemiology  
Monday 14 November 14:30–16:30—Iroko Hall  
Chairs: Daniel Dodoo (Accra) and Haider Giha (Khartoum)  

O-14  
The immunoepidemiology of malaria [MIM-km-46728]  
K. Marsh  
KEMRI CGMRC, Kisii, Kenya  

**Introduction:** Our knowledge of the mechanisms and key targets of immunity to malaria is incomplete. We propose that there are three main reasons for this: (1) problems in defining the status of individuals with regard to protection or susceptibility; (2) problems in defining immune responsiveness and (3) failure to ade-
quately take account of antigenic polymorphism and antigenic variation.

**Methods:** Malaria is one of the few diseases where the introduction of an effective vaccine can be said to be genuinely essential to achieve a decisive change. Broadly, there have been two approaches to vaccine development: in one the aim is to mimic and enhance the mechanisms by which humans naturally develop protective immunity. In the second the aim is to identify key points in the parasites biology that are susceptible to immunological attack. A detailed understanding of the mechanisms of human immunity to malaria is important both in identifying targets and mechanisms and in monitoring human responses to candidate vaccines.

**Results:** Susceptibility: Prevalence of parasitaemia, clinical attacks and severe life threatening attacks all show evidence of acquisition of resistance with increasing age but the indicators have quite different kinetics. Thus it is essential to define an individuals status in terms of susceptibility over time to clearly defined levels of disease, and ideally to examine outcome in relation to severe disease. Measuring immune response: A standard approach is to measure a candidate immune response and relate it to disease incidence over time. However, this may be confounded by the fact that the ability to detect a given immune responses may be short lasting it may therefore be impossible to distinguish those who genuinely have not made a particular response from those who would be capable of making it if challenged. Polymorphism: The emerging evidence of protective responses being directed to polymorphic and variant epitopes implies that it is unlikely that immune status will ever be defined by any single response. Rather, it is likely that different antigens different responses will be more or less important and that any individual will rely on several different responses which are likely to be additive or synergistic.

**Interpretation:** Potential problems emerging from failure to adequately take account of these factors in immunoevidemiological studies will be reviewed and suggestions made to improve the design of field studies.

**O-15**

**Target antigen, age and duration of antigen exposure independently regulate IgG subclass switching in malaria [MIM-ET-59857]**


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**Introduction:** Antibodies play a crucial role in the protective immune response to malaria and seroepidemiological studies have characterised quantitative (prevalence, OD) and qualitative (subclass) aspects of antibody responses in diverse settings. Importantly, antibody subclass has been identified as a crucial variable affecting antibody function and protective capacity but little is known of antigen-specific factors which regulate Ig class switching.

**Methods:** The aim was to explore factors (such as age, duration of exposure, the nature of the antigen) which might influence class switching of anti-malarial antibodies. In this work, I describe the development of different IgG subclass (and IgG total) responses to a number of malaria antigens (MSP-119, MSP-2, AMA-1 and GPI) in individuals (n = 970) with ages ranging from 0 to 45 years living at varying altitudes (and thus varying levels of *P. falciparum* malaria transmission) in Northeast Tanzania. Optical densities were determined for single point dilutions (1:1000 for malaria antigens, 1:100 for GPI). Non-malaria exposed European sera (n = 10) were used to define a cut-off (mean + 3 S.D.) for antibody positive and negative sera.

**Results:** I demonstrate that different malaria antigens can simultaneously polarise the IgG response to different cytophilic subclasses (AMA-1 and MSP-119 drives IgG1 whereas MSP-2 and a lesser extent GPI skew towards IgG3) and that age and intensity of malaria
exposure independently influence the subclass of antibody responses. These observations provide insight into the effects of immune system maturity (i.e. age), duration and intensity of antigen exposure and inherent characteristics of individual antigens (IgG3 predominance correlated strikingly with polymorphic or repetitive sequences) on the process of class switching in human B cells.

Interpretation: Insights might be applied to the development of vaccines that incorporate factors (chimaeric antigens) that preferentially drive cytophilic antibody thereby enhancing vaccine-induced protective immune responses.

O-16
The fine specificities of natural IgG responses to Plasmodium falciparum MSP1–19, a malaria vaccine candidate in two Ugandan studies [MIM-BO-134732]
B. Okech, P. Corran, J. Todd, A. Joyson-hicks, C. Uthaipibull, T. Egwang, A. Holder, E. Riley
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Introduction: MSP1–19 is a leading asexual blood stage malaria vaccine candidate believed to elicit immune responses that block merozoite invasion of host erythrocytes. The present work assessed the fine specificities of natural antibodies to MSP1–19 using sera of children from different studies. Two sets of sera, one from a longitudinal study of semi-immune and clinically immune malaria in Uganda and another from a case-control study of severe malaria in infants in a Ugandan hospital were used.

Methods: The specificity of anti-MSP1–19 antibodies was investigated by two methods. Competition ELISA using a panel of mouse monoclonal antibodies of known specificity was used to assess varying MSP1–19 antibody specificity of individual sera. Specificity was further assessed by comparing antibody binding to wild type or mutated recombinant MSP1–19 proteins in direct ELISA. The mutated proteins used were made to prevent binding of non-protective, blocking anti-MSP1–19 antibodies that interfere with the protective action of ‘inhibitory type’ antibodies which are protective.

Results: A marked variation was observed between individuals in the fine specificity of naturally acquired antibodies to MSP1–19. Furthermore, although neither the prevalence nor the concentration of total anti-MSP1–19 IgG antibodies were associated with resistance to malaria in African children, significant associations were observed between antibody fine specificity and subsequent risk of infection and high density parasitaemia during a period of follow-up in the longitudinal study. Furthermore, individuals with sera rich in putative ‘inhibitory type MSP1–19 antibodies’, which would be expected to inhibit MSP1–42 processing and therefore parasite invasion of the red blood cell were correlated with protection from high density parasitaemia. Certain antibody specificities were associated with protection from severe malaria. Prevalence of one of the putative ‘inhibitory type MSP1–19 antibodies’ was higher in the low parasitaemia infants in the case-control study of severe malaria. Our studies suggest a role for MSP1–19 antibody specificity and quality in protection from high malaria parasitaemia in both children and infants.

Interpretation: The fine specificity of naturally acquired human anti-MSP1–19 antibodies is crucial. Future field studies, including the evaluation of MSP1–19 vaccine trials, should include assays that explore antibody fine specificity as well as titre.

O-17
Plasmodium falciparum antigenic variation: Differential expression of var gene subsets during clinical malaria [MIM-PI-406866]
(1) Wellcome Trust/KEMRI Collaborative Programme, Kisii, Kenya; (2) Wellcome Trust Sanger Institute, Cambridge, UK; (3) Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, UK
S13

Introduction: The variant surface antigens (VSA) expressed on Plasmodium falciparum infected erythrocytes are potentially important targets of immunity to malaria. VSA are encoded, at least in part, by a large family of parasite “var” genes. We have used a sequencing approach to compare var gene expression patterns between clinical parasite isolates and assess their relationship to the host immune response at the time of disease.

Methods: A total of 1754 short var gene sequence “DBLalpha tags” from genomic and cDNA were sequenced from 12 clinical isolates (called “genomic tags” and “cDNA tags”, respectively). Using semi-conserved regions of the sequences these were assigned to 364 different “core tag sequence” categories and 6 major “tag groups”.

Results: (1) for each isolate the cloning frequency of genomic tags from each tag group was close to that expected from the repertoire of var genes in the completely sequenced parasite isolate 3D7. However, (2) var gene expression apparently varies dramatically between isolates. Furthermore (3) expression of var genes belonging to one of the tag groups “tag group1” was negatively associated with the repertoire of VSA antibodies carried at the time of disease.

Interpretation: Differential expression of broadly distinct groups of var genes, assessed by sampling only a small stretch of sequence from each expressed var gene, may provide useful information about the host–parasite relationship during clinical infections.

O-18

In utero sensitization to Plasmodium falciparum antigens [MIM-AL-173628]


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Introduction: Placental Plasmodium falciparum (PF) infection is associated with increased susceptibility to malaria during the first year of life. While analyses of cord blood immune cell responsiveness have suggested the induction of suppressive pathways, there is currently no information concerning the mechanism(s) involved. Here I will present recent data suggesting that the induction, in utero, of parasite antigen-specific T cells with a regulatory phenotype may be pivotal in this context.

Methods: Health records and detection of placental PF infection at delivery were used to identify three distinct groups of mothers (infected, treated, negative) with differing histories of malaria during gestation. Corresponding cord blood samples from these groups were collected, the mononuclear cell populations (CBMC) isolated and stimulated for short periods in vitro with PF antigens. Flow cytometric methods were then used to determine the activation status and/or cytokine profile of monocytes, T and B cells. Depletion of specific T cell populations as well as addition of inhibitory antibodies or recombinant cytokines to in vitro cultures was used to determine the respective roles of components of the CBMC PF antigen (Ag)-specific immune response.

Results: PF Ag-driven IL-10 was derived primarily from CD25hiCTLA-4+ cells within the CD4+ T cell population, and such cells were present at a higher frequency in CBMC of those born to mothers with active PF infection at delivery. The median frequency of PF Ag-specific CD4+CD25+IL-10+ cells was inversely associated both with the frequency of activated PF Ag-specific CD4+ and CD8+ T cells, as measured by IFN-g production and CD25 expression, and of activated monocytes, as measured by MHC classes I and II expression. Depletion of CD4+CD25+ cells prior to co-culture with either PF Ag or aCD3 antibody resulted in a significant increase in the median frequency of activated, IFN-g-producing T cells and of monocyte surface MHC expression levels. The addition of rhIL-2 to co-cultures of CBMC from PF-exposed neonates led to increased frequencies of Ag-specific CD4+CD25+IL-10+ cells and to decreases both in the frequency of activated IFN-g+ T cells and of monocyte MHC expression. In contrast, addition of rhIL-2 led to activation of T cells and monocytes in co-cultures from unexposed neonates.

Interpretation: Placental PF infection induces, in utero, IL-2-sensitive PF Ag-specific CD4+CD25hiIL-10+ T
cells - the phenotype of inducible Tr1-type regulatory cells – that suppress the activity of potentially protective Pf Ag-specific IFN-γ+ T cells.

O-19 Characterization of the malarial antibody response of Cameroonian infants throughout the first year of life using a multiplex assay [MIM-GF-181010]

G. Fouda, R. Leke, G. Sama, C. Long, D. Taylor, A. Johnson

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Introduction: The presence of maternally transferred antibodies is believed to be important in the resistance of infants to clinical malaria, but the specificity of protective antibodies is unknown. Studies in neonates have been limited by the small amount of blood that can be collected. We have developed an assay to measure antibodies against 11 malarial antigens simultaneously using less than 1 μl of plasma and characterized the antibody response of Cameroonian children during the first year of life.

Methods: A total of 40 babies were recruited at birth in Yaoundé, the capital city of Cameroon (n = 18) and in Ngali, a nearby rural area (n = 22) and were followed through the first year of life. Peripheral blood was collected at nine scheduled time points (7 days, 6 weeks, 3, 4, 5, 6, 8, 10, and 12 months) and in cases of clinical disease. The presence of Plasmodium falciparum was assessed using both light microscopy and PCR. The levels of IgM, IgG, IgG1, IgG2, IgG3 and IgG4 to 11 malarial antigens were measured using a multiplex suspension array technology assay. The antigens included two variants of the MSP-142, two variants of MSP-2, two variants of AMA-1, MSP-3, and EBA-175 and a few had antibodies to RESA, CSP, and LSA-1. Some infants had anti-malarial IgG at birth. Infants from Ngali tended to have lower levels of anti-MSP-142 FVO (means: 2375 MFI versus 7275 MFI) and MSP-142 3D7 (means: 6220 MFI versus 10435 MFI) than those from Yaoundé. Upon first malarial infection, most infants had an increase in IgM and IgG, whereas in the absence of infection, maternal IgG persisted until 4–6 months of age. At 1 year, most infants had IgG to one or more antigens and infants from Ngali tended to have higher levels of anti-MSP-142 FVO (means: 5175 MFI versus 2525 MFI) and MSP-142 3D7 (means: 9485 MFI versus 3430 MFI) IgG than infants from Yaoundé. Throughout the first year of life, the antibody response was mostly IgG1 for all antigens. IgG2 and IgG4 were rare but, a high IgG3 response was observed against MSP-3.

Interpretation: Our results suggest possible differences in the distribution of anti-malarial antibodies at birth and throughout the first year of life in an urban and a rural area of Cameroon and ought to be confirmed using a larger sample.

O-20 Cellular immune responses to several pre-erythrocytic antigens correlate with protection from P. falciparum malaria in Gambians [MIM-FT-179394]

F. Thompson, S. Keating, T. Berthoud, B. Imoukhuede, V. Moorthy, S. Todryk, A. Hill

(1) Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Oxford, UK; (2) Medical Research Council Laboratories, Banjul, Gambia; (3) London School of Hygiene and Tropical Medicine, London, UK; (4) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
Introduction: The observation that protection against malaria infection can be induced by immunisation with radiation attenuated sporozoites has led to a focus on the liver stage of *Plasmodium falciparum*, in the hope of identifying the antigens involved. Residents of malaria endemic areas develop clinical immunity, resulting in a lower incidence of clinical malaria. Knowledge of the antigen specific immune response to the liver stages of individuals in endemic areas may help in the development of new malaria vaccines.

Methods: We identified samples from volunteers for two Gambian malaria vaccine trials. We selected those in control groups, who had received only rabies vaccine. PBMC samples were taken 7 days after vaccination, and volunteers were followed for 11 weeks. Smears were taken weekly, or if volunteers developed symptoms of malaria. Serum was taken 7 days after vaccination and volunteers were followed for 28 days, with daily finger-prick blood taken for parasite PCR, and smears from any with symptoms of malaria. Time to infection was recorded in weeks of the surveillance period, beginning 14 days after the final vaccination. Samples were used to measure T cell and antibody responses to various liver stage antigens including LSA3, STARP, Exp1, TRAP and LSA1.

Results: Individuals from the Gambia had evidence of T cell and antibody (by ELISA) responses to LSA3, STARP, Exp1, TRAP and LSA1. Those that did not develop malaria in the subsequent follow up period had significantly higher T cell responses (as measured by IFN gamma ELISPOT) than those who succumbed to infection during that period (LSA3 \( P = 0.04 \), STARP \( P = 0.018 \) calculated by Student’s t-test). The time to malaria infection was also significantly longer in those with higher T cell responses to the liver stage antigens including LSA3, STARP and LSA1 (correlated with longer time to infection \( P < 0.05 \), analysis by Kaplan–Meier method, probability calculated by log rank test).

Interpretation: T cell and antibody responses to these liver stage antigens are likely to play a role in clinical immunity; so induction of immune responses against them may lead to protection from disease. This supports their inclusion in a new polypeptide vaccine.

4. Bio-ecology, behaviour and transmission I

**Monday 14 November 14:30–16:30—Mahogany Hall**

Chairs: N’Fale Sagnon (Ouagadougou) and Willem Takken (Wageningen)

**O-21**

Mosquito-host interactions in a complex ecosystem:
The advantage of being anthropophilic

*Willem Takken*

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Mosquitoes use physical, visual and olfactory senses for orientation when foraging. Blood feeding is essential for survival and reproduction, and sensitive and selective receptors have evolved through which this process is accomplished. Because of their nocturnal behaviour, olfaction is the principal sensory modality through which these behaviours are regulated. Among the numerous olfactory cues emitted by potential blood hosts, it is essential that correct decisions are taken based on reliable information. Being anthropophilic is advantageous when strong associations between human hosts and the mosquito have evolved. Human hosts provide not only food, but also a refuge and adequate microclimate for egg development. In addition, human settlements are usually located in the vicinity of water and hence, breeding sites. The anthropophilic, endophagic and endophilic behaviours of several anopheline mosquitoes have rendered them highly efficient malaria vectors. Recent studies have elucidated the mechanisms of olfactory behaviour in mosquitoes, from receptor-neuron through the brain to upwind anemotaxis and host contact. Examples will be given how the *Anopheles gambiae* and *Drosophila melanogaster* gene projects and ‘classic’ behavioural and ecological field studies have each contributed to these developments, as an example of the interaction between molecular, physiological, behavioural and ecological studies for the elucidation of foraging behaviour of these important mosquitoes. Novel research tools allow appropriate understanding of the host-seeking mechanism of anthropophilic mosquitoes and make possible the development of novel intervention methods.
O-22 The neglected sex: Male anopheles ecology and its relevance to vector control [MIM-HF-4116]

H. Ferguson, B. John, K. N'Ghabi, B. Knols
(1) Ifakara Health Development and Research Centre, Ifakara, Tanzania and Wageningen University, Wageningen, Holland; (2) Ifakara Health Development and Research Centre, Ifakara, Tanzania and University of Dar es Salaam, Dar es Salaam, Tanzania; (3) International Atomic Energy Agency, Vienna, Austria

Introduction: The recent development of transgenic mosquitoes that are resistant to malaria infection is a promising new tool for control. However, results of large-scale field releases of alternatively modified mosquitoes carried out during the 1970s suggest that this approach could be difficult to implement in the field. These previous control attempts largely floundered as a result of our insufficient understanding of the behavioural ecology of released males.

Methods: Here, we analyze progress made in knowledge of male Anopheles ecology over the past 25 years. We analysed all citations arising from a Web of Science literature search under the term ‘Anopheles gambiae’ during the period from 1980 until 2004, and categorized them into seven distinct categories on the basis of their main methodology and research focus. We computed the proportion of research effort devoted to studies of male Anopheles ecology or mating during this period, and compared trends in research focus with two other insect pest species, the Mediterranean fruit fly and the Melon fly, who have both been successfully controlled by the release of sterile males.

Results: Since 1980, a mere 2.1% of all studies listed on Web of Science under ‘Anopheles gambiae’ had focused on male ecology and/or mating success. Of this 2.1%, at least half were conducted in the laboratory, leaving only a few providing information about free-living males. In contrast, study of male ecology and behaviour has featured prominently in research conducted on the successfully controlled Mediterranean fruit fly and the Melon fly, who have both been successfully controlled by the release of sterile males.

Discussion: Little progress has been made towards resolving key ecological uncertainties that impeded early vector control efforts based on male release. Study of male Anopheles ecology requires greater priority if failure with transgenics are to be avoided.

O-23 Natural swarming behaviour in the molecular M form of Anopheles gambiae from Burkina Faso [MIM-RD-207660]

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Introduction: Anopheles gambiae s.s. bionomic suggests a speciation process involving in West Africa, five incipient chromosomal forms. But a recent molecular analysis suggested the existence of two entities referred to as molecular forms M and S. In An. gambiae, as in most mosquito species, mating is initiated in flight. The males aggregate in aerial swarms and females fly to these swarms to couple with males. We investigated mating barriers between these two molecular forms M and S during swarming.

Methods: This study was conducted since 2003 in the rice field area of the Vallée du Kou in Burkina Faso where both molecular M and S forms of An. gambiae were found in sympatry. Indeed on 144 mosquitoes tested in PCR in October 2004, 40 were An. gambiae S form representing 28% versus 72% of M form. Two years observations were done to describe swarming characteristics and mating events which consisted to assess swarm numbers, height, composition (single or mixed swarm) occurrence of couples. Environmental parameters near the swarming places were also described.

Results: Swarms formed a few minutes after sunset in more than five places close to cow herds generally in open flat areas, 2–3 m above the ground. Overall, more than 9700 anopheline mosquitoes were collected from 45 swarms composed primarily of males. Coupling occurred about fifteen min after the beginning of the swarming and some couples were caught in copula. More than the half of 164 females caught was fertilised. On the 45 swarms collected only one mixed swarm was identified representing 2.2% versus 97.8% of monospecific form M swarms. A few specimens of Culex quinquefasciatus and An. pharoensis were collected from three An. gambiae swarms.

Discussion: Although both molecular M and S forms were found in sympatry in the village, swarms...
were composed mainly of the molecular M form. That raises the question of alternative swarming habits for both M and S molecular forms of *An. gambiae* in nature.

O-24

A method to assess the age and mating history of male *Anopheles gambiae* [MIM-BJ-246864]

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Introduction: Genetic manipulation of vector populations is emerging as a promising malaria control tool. Only male mosquitoes can be used to carry new genetic traits into wild populations, as releasing females could be potentially harmful. Thus for GM control to succeed, knowledge of the dynamics of male mosquito ecology must be acquired. We developed a statistical method for determining the age and reproductive history of male *Anopheles gambiae* and used it to calculate the fitness of wild-living males.

Methods: Laboratory-reared virgin and mated male *An. gambiae* in age groups ranging from 1 to 20 days old were dissected and their reproductive system excised. Changes in the morphology of reproductive organs such as the number of spermatocysts, proportion of the testes filled by the sperm reservoir, and the appearance of the accessory glands, were noted. As males aged and mated, several changes in their reproductive morphology were observed. Based on these changes, a statistical model was created to predict the age and mating history of males of unknown background. This model was used to determine the survival and reproductive capacity of both laboratory and field-collected males.

Results: Both the number of spermatocysts and the proportion of the testes filled by the sperm reservoir predicted male age. As males got older, the number of spermatocysts fell \((F_1, 366 = 256.25, P < 0.01)\) and the proportion of the testes filled by the sperm reservoir increased \((F_1, 366 = 289.58, P < 0.01)\). These relationships did not vary between virgins and those that had mated, indicating these morphological changes can be used to predict male age irrespective of mating history. The appearance of a clear area around accessory glands was linked to both male age (present in 97% of 1–2 day old males) and mating status. Male mosquitoes that had mated the night before were 7.2 times more likely to have a clear area than those that had not. A statistical model based on these morphological features predicted 59% of the variation in male age in our original data set \((r^2 = 0.59)\), and 18% of that in mating status \((r^2 = 0.18)\).

We conducted a blind trial to assess the accuracy of this model for predicting the age and mating history of males of unknown background. Post hoc comparison of real age with those predicted by the statistical model indicated it overestimated the age of males younger than 8 days.

Interpretation: A statistical model based on male *An. gambiae* morphology can be used to predict age with moderate accuracy. Male survival and the proportion that contribute to the gene pool can thus be determined using this method.

O-25

*Plasmodium falciparum* development in the midgut of *Anopheles gambiae* s.s. feeding on some predominant plants in Western Kenya [MIM-HM-137100]

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Introduction: Transmission of the malaria parasite *Plasmodium* is influenced by many different host, vector and parasite factors. Both male and female mosquitoes feed on sugars from plants to build up their energy reserves and in addition they also ingest plant tissue. Here we conducted a field-based study in Mbita, Western Kenya to investigate the possible effect of the ingested plant tissues or fluids on the development of *Plasmodium falciparum* in the midgut of *Anopheles gambiae* s.s.
Methods: Six plant species: *Hamelia patens* L., *Lantana camara* L., *Puthernium hysterophorus* L., *Ricinus communis* L., *Senna didymobotrya* F. and *Tecoma stans* L. were used. Seventy-five *P. falciparum* gametocyte carriers (5–10 years) were recruited for two studies. Laboratory-reared *An. gambiae* s.s. were experimentally infected using blood from carriers by membrane feeding. In one study (*n* = 24), mosquitoes were fed on plants prior and post infection. In the second (*n* = 51), plants found to have reducing effect on infection in the first study were tested. For each, mosquitoes were divided in three groups: one fed on plant prior to infection and on glucose after, one fed on glucose before infection then on plant after and one group fed on plant throughout.

Results: In the first study, the proportion of infection was high when mosquitoes fed on *H. patens* (20%), low when they fed on *R. communis* (7%) (P < 0.05) and nil when they fed on *P. hysterophorus*. Mean parasite densities were significantly low (P < 0.05) when mosquitoes fed on two plants species: *L. camara* and *S. didymobotrya* with densities of 1.6 and 1.2 oocysts per mosquito, respectively, and higher in mosquitoes fed on *T. stans* and on the control glucose: 3.3 and 2 oocysts per mosquito, respectively. In the second study, for both plants reducing parasite load, the mean parasite densities when mosquitoes fed on plant before infection only, after infection only and throughout were comparable (P > 0.05) and were, respectively: 2, 2.5, and 2 for *L. camara* and 2, 2.5 and 1.3 oocysts per mosquito for *S. didymobotrya*. These densities were significantly lower than the oocyst density (7.5) of mosquitoes fed only on glucose (P < 0.05). With low gametocyte densities (mean 56 gametocytes/μl of blood) (P = 0.03), the infection outcome was nil on mosquitoes fed on *P. hysterophorus*, but with high gametocyte densities (176), they were infected with a mean of 2.3, 3.2 and 1.7 oocysts per mosquito when fed on that plant before, after infection and both, respectively.

Interpretation: From our findings, mosquito plant diets can inhibit or block parasite development in their midgut. But blocking effect depends on the gametocyte densities infecting mosquitoes. The time of feeding on plants may not affect the infection outcome.

O-26
Speciation within the Anopheles nili group of malaria vectors in Cameroon and the bionomics of sibling species [MIM-AP-143599]

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Introduction: *Anopheles nili* transmits malaria to humans along streams and rivers in Africa. Morphological and bionomical heterogeneities were reported in natural populations, leading to the description of three species and one “variety” of uncertain taxonomic status. To further explore the extent of biological and genetic polymorphism in natural populations of *An. nili*, we conducted detailed field studies in Cameroon and, for the first time, explored genetic polymorphism using a variety of markers.

Methods: Mosquitoes were collected indoors and outdoors in suburban and rural localities along rivers in South Cameroon. Adult mosquitoes were collected through night-landing catches on volunteers and by indoor pyrethrum spraying. Larvae were picked from breeding sites. Morphological characters were finely recorded on the adults as well as larvae. Feeding and resting habits were monitored, and infection rates were determined through dissection of salivary glands and using ELISA techniques. Genetic polymorphism and differentiation between morphological types was assessed using multilocus enzyme electrophoresis and sequence variation in the ribosomal DNA cluster (ITS2 and D3 regions).

Results: New and old morphological variations were recorded in larvae and adult mosquitoes from the *An. nili* group collected from savannah and forest locations. The formerly described *An. nili* s.s., *An. carnevalei* and *An. somalicus* were subsequently found to be genetically distinct by both enzyme and rDNA sequence variations patterns, with fixed differences and overall significant levels of differentiation indices (Wright’s Fs). These results ascertain specific rank for these species. We describe a new species, named *An. ovengensis*, which appears both morphologically and genetically distinct from the other members of the group. Fixed nucleotide differences between these four species in ITS2 haplotypes were
used to develop a PCR-based diagnostic assay that allows rapid identification of members of the group. *An. nili* was widespread throughout our study sites, where it was responsible for 20–200 bites/man/night and 20–100 infected bites/man/year. *An. carnevalei* and *An. ovengensis* were captured only in forest sites, mostly outside houses. Their human biting rate reached 50–300 bites/man/night on the riversides, and sporozoite rate was 1.2% and 1.9%, respectively. *An. somalicus* was found only at larval stages, thriving together with *An. nili*.

**Interpretation:** Bionomical and genetic heterogeneities uncovered a new malaria vector within the *An. nili* group. We developed a convenient PCR based assay that will be useful to better understand human malaria transmission dynamics in forest areas of Central Africa.

**O-27**

**A new efficient and cheap trap for the collection of outdoor-biting mosquitoes [MIM-DC-2881]**

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**Introduction:** Although a variety of methods have been developed to sample the indoor-biting fraction of malaria vectors the only suitable sample to date for outdoor biting insects has been the use of landing collections by sentinel individuals, a technique that has a number of inherent disadvantages. A new collection method involving tents and modified CDC-traps has been developed and tested as part of the SIMA project in Mozambique.

**Methods:** The trap involves attaching a CDC trap (without light) horizontally to the partially open zip of a tent with a bulldog clip and string so that access is not impeded. The opening provides a concentrated source point of host odour. Insects are collected by the trap as they approach the opening. Thousands of insects have been caught on a single night, without risk to the sleeping host using such traps. They are now used for mapping of malaria vectors in the village of Massavase as part of the SIMA project in southern Mozambique.

**Results:** Maps of mosquito density throughout the village and in uninhabited areas have been produced. The technique has been shown to be uncritical in its installation and provides easily replicable samples at pre-determined geo-referenced sites.

**Interpretation:** Given that the only alternative to the presently described trap is by human bait catches, and given that malaria transmission occurs outside as well as indoors the development of this trap is welcome news.

**5. Drug discovery and development**

**O-28**

**In vitro antimalarial properties of two plants used by traditional herbal practitioners of Burkina Faso: Pavetta crassipes and Mitragyna inermis [MIM-SS-81397]**

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**Introduction:** In spite of huge progresses by modern medicine, malaria remains a disease with a high burden because of increasing Parasite to the most affordable drugs. It is urgent to look for new effective and affordable molecules for malaria treatment. The natural products isolated from plants, having an antiplasmodial activity in vitro, might be a source of new antimalarial drugs. This study aimed to assess the antimalarial activity of two local plants used by traditional healers for the malaria treatment.

**Methods:** Plants were identified by an ethnobotanic survey. During the screening with solvents of different polarity, promising chemical components of plants were revealed by TLC. The extracts and molecules were obtained by specific extraction and isolated by column chromatography. The molecules were
described by nuclear magnetic resonance and mass spectrometry. The antiplasmodial tests were carried out with a chloroquine-resistant strain of *Plasmodium falciparum* W2 using the Trager and Jensen culture technique. The IC50 was determined by the software Table Curve version 5.0.

**Results:** One hundred and eighty-five traditional healers responded to the ethnobotanic survey. The screening of the seven most quoted plants revealed that *Pavetta crassipes* and *Mitragyna inermis* were the most promising regarding their antiplasmodial activities. The tests on the *P. falciparum* resistant strain showed that the total alkaloids of the two plants had antiplasmodial activity in vitro at the IC50 of 1.23 µg/ml for *P. crassipes* and 14 µg/ml for *M. inermis*. The LC50 of these alkaloids was 66.85 µg/ml for *P. crassipes* and >250 µg/ml for *M. inermis* showing that they did not have any cytotoxic effect on human monocytes (line THP1). The genotoxicity tests on methyl-green DNA showed that the two extracts had no mutagenic effect. Some of the isolated molecules of *P. crassipes* (Elaeocarpidin, Rutin, Acanthospermolgalactosidopyranoside) had good antimalarial activity, with IC50 varying between 4 and 10 µg/ml. However, the OH-elaeocarpidin was identified as the new molecule presenting the best inhibitory activity, with an IC50 of 1.83 µg/ml. The isobolograms showed that *P. crassipes* and *M. inermis* had a synergic effect.

**Interpretation:** The total alkaloids of *P. crassipes* and the OH-elaeocarpidin were identified as having potential anti-malarial and anti-leishmanial activities in vitro and in vivo. A lead candidate against *P. falciparum* is being considered for pre-clinical trials. However, the mode of action of the chalcones remains unknown. Our aim is to find the target of the chalcone LC1462 in *P. falciparum*, using *E. coli* as a model.

**Methods:** A chalcone resistant *E. coli* strain, RLC1462 was selected using random mutagenesis. A RLC1462 genomic library was created and over-expressed in a LC1462 sensitive background so that regions of the RLC1462 genome conferring resistance could be isolated and identified. The individual genes, on the two commonly found RLC1462 genomic fragments conferring resistance, were subsequently investigated for transcriptional differences using quantitative real time PCR in the RLC1462 and wild type background, with and without drug treatment. The individual genes were also functionally expressed to determine if a single gene alone could confer resistance to LC1462. Drug inhibition assays will subsequently be carried out.

**Results:** RLC1462 was found to be resistant to at least two other chalcone analogues tested, suggesting a common mode of action. One of the predominantly found RLC1462 fragments conferring resistance, fragment A, encoded genes involved in the shikimate biosynthetic pathway. The shikimate biosynthetic pathway, absent in mammals, is essential in microorganisms and protozoans and is a validated drug target. The second fragment, B, contained genes coding for components of a multi-drug efflux pump. The quantitative real time PCR results showed no differences in transcriptional profiles for the genes tested between RLC1462 and DH5a but did indicate an increase in shikimate pathway biosynthesis in response to LC1462 treatment. Functional expression showed that over-expression of aroD (3-dehydroquinase) alone conferred resistance to LC1462.

**Interpretation:** Our results show that AroD, 3-dehydroquinase of the shikimate biosynthetic pathway is a putative target of LC1462. Identification and cloning of this enzyme in *P. falciparum* will define a novel drug target.
O-30
Bisthiazolium as dual molecules for new antimalarials: Performance and mechanism of action [MIM-HV-143280]

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Introduction: A new antimalarial pharmacological approach based on inhibition of the plasmodial phospholipid metabolism has been developed. The drugs mimic choline structure and inhibit de novo phosphatidylcholine biosynthesis. The third generation compounds are bis-thiazolium salts and their non-ionic precursors: prodrugs, which in vivo can lead to thiazolium drugs after enzymatic transformation.

Methods: These molecules exert a very rapid cytotoxic effect against malarial parasites in the very low nanomolar range. They are active in vivo against P. vinckei-infected mice, after ip, intra-rectal or oral administration. They are able to cure highly infected mice and retain full activity after a single injection. They also retain full activity against P. falciparum and P. cynomolgi in primate models with no recrudescence and at lower doses. The current leader compounds are accessible in few steps from commercial products. These crystalline molecules present a remarkable biological activity and low toxicity which is promising for the development of a new antimalarial drug.

Results: Compounds are accumulated in P. falciparum-infected erythrocyte, which ensures their potency and specificity. Recently, we discovered that compounds also interact with malarial pigment enhancing the antimalarial effect. It is quite likely that they are dual molecules, exerting their antimalarial activity via two simultaneous toxic effects on the intracellular intraerythrocytic parasites. Such properties should delay the appearance of resistance. Compounds are also accumulated inside Babesia-infected erythrocytes to levels 60-fold higher than in uninfected erythrocytes. Analysis of lipid metabolism revealed that T16 exerted an early and specific inhibition on the de novo biosynthesis of phosphatidylcholine in both Babesia and Plasmodium. Comparative studies using Babesia which, like Plasmodium belongs to the Apicomplexa phylum help to understand the molecular mechanisms by which they exert a potent and selective antiparasitic toxicity.

Interpretation: These compounds are at the forefront of antimalarial research and could be applicable for human infection; detailed toxicological studies are under way. We are also conducting studies to unravel the unique dual aspect of their mechanisms of action.

O-31
2,4-Dimanopteridin based compounds as inhibitors of folate pathways: New class of antifolate drugs falciparum [MIM-AN-247758]

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Introduction: We have hypothesized that malaria growth can be inhibited by small organic molecules that require metabolic conversion via the folate pathway to antimalarial entities. We have tested the hypothesis that 2,4-diamino hydroxymethyl pteridine (DAP), 2,4-diamino-6-methyl hydroxy-pteric acid (DAPA), and 2,4-diamino-A10-methyl-6-methyl hydroxy-pteric acid (DAMPA) can function as precursors for the de novo synthesis of aminopterin (from DAP and DAPA) and methotrexate (from DAMPA).

Methods: To address this hypothesis, we have tested the in vitro activity of these compounds alone or in combination with other antifolates against the multi-resistant isolate V1S and the fully antimalarial isolate sensitive M24. In vitro culture of malaria parasites was carried out in RPMI 1640 medium (GIBCO). Antimalarial activity was measured in the presence of varying concentrations of the compounds to test, using radioisotopic hypothanxine incorporation, and results
were expressed as the drug concentration required for 50% inhibition of parasite growth (IC50). We have also analysed the effect of these compounds on *Saccha-
romyces cerevisiae* dependent upon the *P. falciparum* DHFR. *S. cerevisiae* in YEPD medium.

**Results:** We first established the in vitro activity of DAP, DAPA and DAMPA in physiological folate. The result show that these compounds inhibit the parasite growth in the micromolar range; DAMPA was the most active with an IC50 value of 0.7 μM against the antifo-
late sensitive strain M24 and 2 μM against the highly resistant strain, V1S, in physiological folate condition. When experiments were carried out in minimum folate concentration, the activity of these compounds were increased by a least, a factor of 4 against M24, and a factor of 2 against V1S, a clear indication that DAP, DAPA and DAMPA target the folate pathway. Synergy analyses indicate that DAMPA potentiates the activity of the sulfone, dapsone, an inhibitor of DHPS, but not of chlorcycloguanil, a known inhibitor of dihydrofo-
late reductase (DHFR). These data indicate that DHFR is the primary target of DAMPA, explaining why this compound does synergise with dapsone but not with chlorcycloguanil. In addition, experiments with trans-
fected *S. cerevisiae* show that DHFR is a target of DAMPA in that system.

**Interpretation:** These data support the hypothesis that DAMPA is converted to methotrexate. This de novo synthesis will not occur in the host since it lacks the complete folate pathway. These compounds could serve as lead for the design of new antifolates.

**O-32**

**Pyronaridine artesunate (PA) oral combination: Phase I clinical and pharmacokinetic study results [MIM-SA-10485]**


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**Introduction:** Artemisinin-based combination thera-
pies are effective antimalarials and are considered to afford a lower propensity to resistance. A fixed dose combination oral therapy of pyronaridine (PP) and artesunate (AS) in the ratio of 3:1 is currently in development for the treatment of acute uncomplicated falciparum and vivax malaria. The results from a ran-
domised, blinded phase I clinical trial of the fixed dose combination of pyronaridine:artesunate are presented.

**Methods:** The clinical protocol of randomised, double-
blind, placebo controlled study combined single and multiple dose-rising and food-effect and drug-
interaction. Dose levels of PA were investigated 6.2;
9.3; 12.4; 15.5 mg/kg, PP:AS, respectively (SD/MD). The study recruited healthy male and female subjects aged 19–40 years: 9 per group (7 active, 2 placebo) in single- and multiple-rising dose; 10 per group (half female) in both the interaction and food effect stud-
ies; 8 per group (6 active, 2 placebo). Assessments of safety evaluation (vital signs, ECG, laboratory tests, adverse events) taken up to 14 days after dosing. Sam-
ples taken for PP, AS and DHA assay from blood drawn up to 240 h post drug administration.

**Results:** No iclinically significant sustained drug-
related changes were recorded in vital signs, ECGs and clinical laboratory tests in the single, multiple ris-
ing and food effect studies. The adverse event profile at all doses studied was minor or moderate in nature and resolved without sequelae. No severe adverse events have been reported. Blood PP was measured by HPLC and plasma AS and DHA were determined by LC/MS. Blood PP and plasma AS exhibited approxi-
mate linear dose proportionality over the dose range: 6.2–15.5 mg/kg (PP:AS, respectively) in the single dose study. Absorption was rapid, with a *T*<sub>max</sub> of 1.6–4.8 h for PP and 0.6–0.9 h for AS. The elimination half-life ranged from 6.6 to 9.7 days for PP, 0.44–0.53 h for AS, and 0.9–1.5 h for DHA. In the multiple dose study, the same dose range was studied with three daily doses. PP AUC and Cmax after the third dose increased in a linear fashion with dose. *T*<sub>max</sub> was 1.6–2.4 h and the elimination half-life ranged from 5.3 to 6.6 h. For AS and DHA, there was no accumulation with multi-
ple daily dosing and kinetic parameters were similar to those following a single dose.

**Interpretation:** Safety profile of PA from this study is consistent those reported in the literature for both PP and AS are predominantly GI in nature. The results justify development of PA as a fixed dose combination oral therapy in acute uncomplicated malaria.
O-33
No abstract received.

O-34
No abstract received.

6: Bio-ecology, behaviour and transmission 2
Tuesday 15 November 11:00–13:00—Ebony Hall
Chairs: Yeaya Toure (Bamako) and Didier Fontenille (Montpellier)

O-35
Genetic diversity in Anopheles funestus and An. gambiae in Africa: Same causes, same effects? [MIM-DF-217623]
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Introduction: Anopheles gambiae s.s. and An. funestus are major human malaria vectors continent-wise in Africa. Both species have a wide geographic distribution throughout sub-Saharan Africa, colonizing extremely diverse biotopes, ranging from the humid forested environments of Central Africa to dry savannas and rice fields in Sahelian areas. We discuss similarities in genetic polymorphism and chromosomal organisation between these two species, with special emphasis on ecotypic adaptation.

Methods: In depth genetics (molecular polymorphism, cytogenetics) and genomics investigations conducted together with fine-scale monitoring of population biology, precise description of mosquitoes physical and climatic environment – including larval development sites characterisation and assessment of adult resting sites preference – provide the basis for a better understanding of the adaptive mechanisms involved. We demonstrate how comparative genomics and genetics across species allow pinpointing the genetic basis of ecological adaptation.

Results: The high diversity of ecological settings colonized by An. gambiae and An. funestus in Africa is mirrored, at the genetic level, by high amounts of polymorphism in paracentric chromosomal inversions observed within both species. In adult mosquito populations, clines of inversion frequencies, especially involving inversions 2La in An. gambiae and 3Ra/b in An. funestus, was shown to correlate with ecological factors such as the degree of aridity of the environment, suggesting strong adaptive values for inversions. Uneven distribution of alternative chromosomal arrangements within and among villages in West Africa and significant deficit in heterozygotes observed within locale populations lead to the designation of five ‘chromosomal forms’ in An. gambiae and two in An. funestus. Microgeographic and temporal distribution of these chromosomal forms was shown to correlate with the nature and availability of larval development sites, suggesting differential breeding site preferences. Furthermore, an almost perfect conservation of synteny was observed at the arm level between An. gambiae and An. funestus and, several genes located within the An. gambiae 2La inversion correspond to genes located within An. funestus 3Ra and 3Rb inversions.

Interpretation: Remarkable similarities observed at the genetic level between An. gambiae and An. funestus provide insight into the evolutionary history of these major human malaria vector species, suggesting conserved mechanisms for ecotypic adaptation.

O-36
Micro-geographic larval habitat partitioning between molecular forms of Anopheles gambiae: Meta-analysis of a four-year survey in central Burkina Faso [MIM-CC-33480]
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Introduction: Burkina Faso lies in the West African sahel, an area prone to severe droughts. Many artificial lakes were created to cover the water needs of the population across the dry season. Other temporary sources of water are available at the onset of rains through the wet season. Two reproductively isolated molecular
forms of Anopheles gambiae share such larval habitats. We performed a long-term study to investigate whether such forms partition their larval habitat to reduce intertaxonomic competition.

Methods: Larval surveys were carried out in Goundri (12° 30′ N, 1° 20′ W), in the dry savanna of Burkina Faso, during four rainy seasons (July–October). Pre-imaginal stages of the An. gambiae complex were dipped from ten larval habitats, representing the full range of breeding sites available in this rural village: margins of an artificial lake, temporary shallow streams and their associated small pools, borrow pits, hoof prints, rain puddles, irrigation pits, ditches, seasonal rice fields, and a well. Larvae were molecularly identified by taxon (An. arabiensis versus An. gambiae, and An. gambiae molecular form M versus form S). Logistic regression, meta-analysis, and ordination techniques were applied to infer general patterns of habitat partitioning.

Results: Overall, 11,162 larvae and pupae of An. gambiae s.s. were collected and identified from 206 breeding sites across the 8 months of the surveys. Despite large temporal and spatial variation in the frequency of the two molecular forms between sampling sites and across the rainy season, the molecular form M consistently predominated in the artificial lake. No habitat partitioning consistent with larval habitat type could be detected between An. arabiensis and An. gambiae s.s.

Interpretation: The ecology of An. gambiae s.s. in the dry savanna of Burkina Faso is affected by the presence of artificial lakes, which represent a preferred larval habitat for the molecular form M of this species.

Introduction: The utility and success of transgenic Anopheles for the control of malaria depends on their mating competitiveness within wild populations. Current evidence suggests that transgenic mosquitoes have reduced fitness (Cattaccuccia, 2003; Moreira, 2004; Irvin, 2004). One means of compensating for the reduced fitness of transgenic mosquitoes would be to identify the ecological condition that increase their mating competitiveness, and incorporating them into laboratory rearing regimes.

Methods: First instar Anopheles gambiae larvae were assigned randomly to density treatments of 100, 200 and 300 larvae per tray. Each tray was filled with 1 L of water and supplied with fish food (Tetramin®). In each tray, 0.2 mg of tetramin® was added for each larva. Trays were inspected twice a day for pupae which, when observed, were collected and held individually in vials to allow for emergence. Males were marked with fluorescent dust to differentiate them on the basis of larval treatment. Mating experiments were conducted in which 30 males (10 from each larval treatment) were competed against one another for access to females. The mating success, energetic reserves, and survival of males from different larval crowding conditions were compared.

Results: A total of 1120 An. gambiae mosquitoes were used in 28 nights of mating experiments (280 females and 840 males). Restricting consideration to the first to mate, we found that males from low density rearing environments were much more likely to succeed ($\chi^2 = 13.61, p = 0.01$). Males from the low density treatment won 11 times often than those reared at higher density, while those from medium density rearing environments were much more likely than those reared at higher density, while those from medium density rearing environments were much more likely to succeed ($\chi^2 = 2.12, p = 0.13$). Males from the low-density treatment won $4 \times$ often than those from the higher larval density treatment. Analysis of all copulations in all nights (not just the first) showed no difference in mating frequency between males from different density treatments ($\chi^2 = 5.06, p = 0.08$). The mean wing lengths of males from all treatments were found to be similar ($F_2, 232 = 1.14, p = 0.32$), as were their levels of ten- eral reserves (lipids: $F_2, 66 = 1.56, p = 0.26$; sugars: $F_2, 66 = 2.16, p = 0.12$; glycogen: $F_2, 66 = 2.12, p = 0.13$). The survival rate of males from the low-density treatment was significantly shorter than those from medium (Log-rank = 7.14, d.f. = 1, $p = 0.007$) and higher density conditions (Log-rank 8.14, d.f. = 1, $p = 0.004$). Males from medium and high-density treatments had similar survival (Log-rank = 0.12, d.f. = 1, $p = 0.73$).
Interpretation: Larval crowding has a strong effect on the mating success of adult male An. gambiae, even when it does not change their body size or energy reserves.

O-38 Fitness consequences of Anopheles gambiae population hybridization [MIM-TG-261288]
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Introduction: The use of transgenic mosquitoes with parasite-inhibiting genes to control malaria transmission will bring along novel alleles with unknown effects on native mosquito populations hence the need to study the response of fitness traits to the introduction of novel alleles. Fitness and feeding behaviors of hybrids between the introduced and native mosquitoes should be evaluated since these hybrids may become an increased nuisance if they bite vigorously and survive longer than native mosquitoes.

Methods: Two Anopheles gambiae strains, Mbita from Kenya and Ifakara strain from Tanzania were crossbred and monitored for 20 generations. We measured fitness of mosquitoes sampled from cage populations of the parent strains and hybrids between the two at F1, F5, F10, F15 and F20 generations for fecundity, body size, blood meal size, larval survival, and adult longevity in two replicate experiments (n = 50). Reciprocal crosses of either Mbita male and Ifakara female and vice versa was done to confirm the direction and effect of heterosis and to control any assortative mating of mixed populations. Their F1 and F5 were also subjected to fitness measurements. The populations and filial generations used in this study were reared in the same manner.

Results: There was significant difference in fecundity (p < 0.001) between the Mbita and Ifakara strain, and between the parents and their progenies (p < 0.001). Inter-progeny significant difference was only found between F1 and F10 (p < 0.05). The mean wing size of Mbita (2.86 mm) and Ifakara (2.89 mm) was not significantly different, but was consistently different between either parent and their filial generations (p < 0.05). Inter-progeny difference was not significant except for between F1 and F5 (p > 0.05). The mean blood meal size was significantly higher (p > 0.001) in Mbita (3.15) than Ifakara (2.54) and also between the parents and F1 (3.96) (p < 0.05), with all the filial generations having significantly higher means than Ifakara (p < 0.001). Inter-progeny significant difference was observed except for between F1 and F5. The mean longevity of Mbita strain (22.31 days) was significantly higher than Ifakara (14.52 days). Progeny showed increased longevity from F1, F5, and F10, and a decline at F15 and F20. Hybrids showed higher values of fitness traits, with reciprocal crosses also showing significantly higher means of body size, fecundity and blood meal size both at F1 and F5 generations than either founder strain.

Interpretation: If exotic genetically modified mosquitoes are introduced, hybrids may live longer, with high fecundity, large body size, and will engorge more, which calls for need to release transgenes of same or very similar background to the native populations.

O-39 Identification of bacteria isolated from the midgut of field-caught Anopheles mosquitoes [MIM-JL-78021]
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Introduction: We have conducted a screen for cultured and uncultured midgut bacteria from Anopheles gambiae and An. funestus mosquitoes with the aim of identifying candidate bacteria for a paratransgenic Anopheles mosquito. Previous studies on midgut flora of Anopheles mosquitoes have exclusively used culture dependent methods; this is the first report including a culture independent method.

Methods: Mosquitoes were collected in Lwanda, Suba district, Western Kenya. Two different techniques were used for screening of the gut flora, a culture dependent and a culture independent. Culturing was performed using LA-plates. The culture independent method was based on PCR of the 16S rDNA gene. PCR prod-
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ucts were TA-cloned and after transformation vectors with correct insert were screened for using blue/white screening, antibiotics and PCR. Sequencing and phylogenetic analysis of the 16S rDNA genes have been used for identification of all the bacteria.

Results: Altogether 16 species were identified, eight by each method, they belong to 14 different genera representing 3 phyla. Since streaks on LA-plates and DNA isolation were performed on each midgut it is surprising that the PCR based method did not retrieve what was found with the culture dependent method. Interestingly, several of the bacteria identified are related to bacteria known as symbionts in other insects. Bacteria representing three intracellular genera were identified, among these the first identifications of Anaplasma species from mosquitoes and a new mosquito-Spiroplasma association. Moreover, one of the isolates is a novel species within gamma-proteobacteria that could not be phylogenetically placed within any of the known orders in this class. All of the isolated bacteria will be further evaluated for their suitability as paratransgenic tools. One step of this will be to determine if any of the bacteria are attractive for mosquitoes (see abstract by K. Eriksson-Gonzales).

Interpretation: From this study we can conclude that the two methods used complement each other. The majority of the bacteria genera identified have been identified in previous mosquito midgut screens, however, also novel bacteria-mosquito associations were found.

O-40
New evidence of potential importance of Anopheles pharoensis as a malaria vector in the Senegal River delta [MIM-LK-167112]

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Introduction: Environmental modifications can induce a more or less durable change in the vector system responsible for malaria transmission. In order to study the influence of rice growing on the local epidemiology of malaria, the vectorial role of anophelines was investigated in Kassack (Senegal River Basin, Sahelian region) and Medina Dianguette (Basin of Anambe, Sudanian region), two villages situated in rice-field areas.

Methods: Mosquitoes were collected by human-landing catches. For each anopheline species, a sample of specimens was dissected to determine the parity rate. The trophic preferences, anthropophilic rates as well as Plasmodium falciparum infection rates were studied by ELISA.

Results: The study showed that Anopheles gambiae s.l. and An. pharoensis were the major anopheline species, constituting, respectively, 98.1% and 1.8% in Medina Dianguette and 8.9% and 77.7% of human-landing catches in Kassack. In Medina Dianguette both An. gambiae s.l. and An. pharoensis were found to be infected by P. falciparum using monoclonal antibody against the circumsporozoite protein. The infection rate was 5.02% (877 tested) for An. gambiae s.l. and 6.25% (16 tested) for An. pharoensis. In Kassack, only An. pharoensis was found carrying P. falciparum. Its parity and infection rate were, respectively, 51.0% (n = 289) and 2.42% (289 An. pharoensis tested versus 33 An. gambiae s.l. tested). This contrasts with a previous study where no infected An. pharoensis were found among 7088 females tested including 5959 in Kassack (Faye et al., 1995). Even if this finding is not in agreement with this latter study, it confirms the first incrimination of An. pharoensis in Plasmodium falciparum transmission in the delta of the Senegal River (Carrara et al., 1990).

Interpretation: A relative increase in the proportion of An. gambiae s.l. and the parity rate of An. pharoensis were observed in this study. The increase of the number of gametocyte carriers and An. pharoensis longevity they would have induced could explain the observed change in An. pharoensis infection rate.
O-41
Biodiversity and dynamics of malaria transmission in a highland area of Western Cameroon [MIM-TT-39520]

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Introduction: Longitudinal entomological studies of malaria transmission were conducted in two localities of Western Cameroon: Santchou situated at 750 m above sea level in the flooded plain of Mbo, and Dschang located in the highland area at an elevation of 1400 m. These two localities are only 22 km apart but are separated by a sheer cliff, making climate and environmental conditions different in both sites. Mosquito collections revealed higher anopheline diversity in Dschang where 10 species were collected.

Methods: Anopheles gambiae, An. coustani, An. funestus, An. hancocki, An. moucheti, An. namibiensis, An. paludis, An. ziemanni, An. nili and An. wellcomei. In Santchou, eight of the above mentioned species were found except An. hancocki and An. moucheti. Among members of Anopheles gambiae complex, determined by cytogenetical analysis of chromosomal inversion in half-gravid ovaries and by PCR, only Anopheles gambiae sensus stricto of the Forest chromosomal and S molecular form, was represented. In both areas, An. gambiae was the major malaria vector across the year, with mean sporozoite rates determined by CSP-ELISA of 2.1% and 2.3% in Santchou and Dschang, respectively, followed by An. funestus with 1.0% and 4.2%.

Results: Population dynamics were very similar in both agglomerations, presenting two peaks, closely associated with rainfalls. At the beginning of the raining season (June), the overall Anopheline biting rate was 22.4 and 19.1 b/m/n in Santchou and Dschang, respectively, whereas these values were 29.8 and 7.8 b/m/n at the end (October). During the dry season, malaria transmission was maintained in Dschang and essentially due to An. funestus, with a sporozoite rate reaching 4.1%. An. funestus larvae were found to develop in permanent ponds and swampy lake sides, especially widespread around Dschang city. Absence of such breeding sites in Santchou resulted in a more seasonal pattern of malaria transmission, with a dramatic drop in density of all anopheline species during the December to February dry season period. A striking increase in biting rate of An. paludis and An. ziemanni was noted at the middle of raining period (September), however, they were all negative for CSP-ELISA. There was no difference in the feeding preference assessed by Blood meal ELISA, between An. gambiae from Dschang and Santchou; they were all highly anthropophilic. Population genetic structure analysis using microsatellite markers did not reveal any difference either.

Interpretation: Availability of suitable dry season breeding sites, seems to bridge the gap and compensate for lack of malaria transmission, which is normally induced by microclimate of highlands. Environmental management may likely result in efficient control.

7. Immunological host factors

Tuesday 15 November 11:00–13:00—Iroko Hall
Chairs: David Modiano (Rome) and Teresa Nkou-Akenji (Yaounde)

O-42
Influence of maternal malaria on neonatal immune status and the roles of antibodies and cytokines in the pathogenesis of severe malaria [MIM-EA-569734]

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Introduction: In endemic areas, exacerbation of malaria in pregnancy, has been reported. Newborns are protected from malaria mainly by transplacentally acquired antibodies. Some children develop severe forms of malaria after infection while others do not
probably partly due to specific antibodies and a balance in cytokine expression. We investigated the impact of maternal malaria on the immune status of neonates and the roles played by malaria specific antibodies and cytokines in severe disease pathogenesis.

**Methods:** Malaria parasites were detected by light microscopy in the blood samples of maternal, umbilical cord, placental biopsy, uncomplicated malaria (UM), severe malaria (severe malaria anaemia: SMA and cerebral malaria: CM) and healthy control (CT) children between the ages of 1–14 years. The numbers of IFN-gamma and IL-4 cells produced by maternal cord blood after in vitro stimulation were enumerated using the ELISPOT assay. In the children’s plasma samples, levels of MIF, IL-10, TNF-alpha, TGF-beta cytokines including IgE and glycosylphosphatidylinositol (GPI) antibodies were measured by indirect ELISA and compared among the patient groups.

**Results:** Malaria parasite rate of maternal, placental biopsy and cord blood was 32.8%, 33.7% and 7.8%, respectively. The mean number of IL-4 producing cells of neonates born of mothers who were malaria positive ($P < 0.05$) or from positive placentas ($P < 0.025$) was higher than from those who were malaria negative. Neonates born of malaria positive mothers or from parasitized placentas mounted predominantly Th2 type immune responses. Seropositivity for GPI, total IgE and malaria specific IgE in the study children was 15.3%, 45.6% and 5.1%, respectively. Levels of anti-GPI IgG and malaria specific IgE antibodies were significantly higher in UM and CT groups when compared with CM and SMA children. There was a positive correlation between malaria specific IgE ($P < 0.0001$; $n = 143$) and GPI ($P < 0.01$; $n = 144$) antibody levels and age of child. TGF-beta but not IL-10, TNF-alpha and MIF was significantly different amongst the study children. However, when grouped into severe and UM/CT groups the former demonstrated higher levels of TNF-alpha and lower levels of TGF-beta while the SMA group had the lowest IL-10 levels.

**Interpretation:** It appears from this study that neonates born from malaria infected mothers or placentas may relatively be more susceptible to malaria attack during the first years of life. Also raised levels of GPI and TGF-beta may protect against severe malaria.

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**O-43**

**Immunological host factors in human malaria**

E. Masoungou

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**Introduction:** *Plasmodium falciparum* malaria is a major cause of death in the tropics, particularly in Sub-Saharan Africa. The clinical outcome of malarial infection depends on many factors including parasite, host and geographic and social factors. Host factors consist of immunity, pro-inflammatory cytokines, genetics (sickle cell trait, thalassemia, ovalocytosis, Gerbich RBC, CD36, TNF-alpha, ICAM-1, MHC locus) age and pregnancy. Our talk aims to review recent advances in analysis of immunological host factors.

**Methods:** Immunological engineering were mainly used in studies exploring immunological host factors.

**Results:** Infection with *P. falciparum* can lead to a series of outcomes represented by asymptomatic infections, mild and severe malaria. Although chronic, asymptomatic infection does contribute to anaemia. The most common clinical presentation is uncomplicated or mild malaria. It is a serious but not immediately life-threatening illness. Severe malaria is a life-threatening condition with hospital case fatality rate of 15–40% and requires hospital treatment as a medical emergency. Increase susceptibility of women during pregnancy remains a disquieting point so far. It is unclear why the effects of *P. falciparum* infection vary so greatly. Important factors probably include innate and acquired immune responses. In malaria endemic areas the protective immunity takes place slowly. This may reflect the acquisition of specific immunity to many non-cross-reactive natural variant antigens. An alternative explanation for the poor acquisition of malaria immunity in naturally exposed populations is that the parasite actively modulates the immune system of the host, preventing the development of specific immune response. This review focuses on cellular immune responses to the pre-erythrocytic and to blood stage of parasite’s life cycle.

**Interpretation:** Despite the capacity of antibodies alpha/beta- and gamma/delta-T lymphocytes, monocyte/macrophage, dendritic cells and natural killer cells to control parasite growth, protective immunity to
malaria in areas of high endemicity takes several years to develop.

**O-44**

Regulatory T cells facilitate establishment of human blood stage *Plasmodium falciparum* infection [MIM-MW-23105]


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**Introduction:** Despite an explosion of interest in regulatory T lymphocytes their relevance to human disease has been uncertain. In the context of a longitudinal study of human sporozoite infection via the natural way to evaluate malaria vaccines, we have assessed immune responses during the pre-patent period of human *Plasmodium falciparum* infection, and provide evidence that regulatory T cells have modifying effects on blood-stage infection in vivo in humans.

**Methods:** Levels of IFN-gamma and TGF-β were assessed for 26 volunteers, using ELISA on samples collected on day of challenge, day 4, and 12 hourly from 6 days post infection until the first microscopic detection of parasitaemia (when subjects were treated). Parasite density was assessed using quantitative PCR. On cells collected from 10 subjects from day 0, 7, 10 and 35 post infection FOXP3 levels were determined using RT-PCR, and the proportion of CD3+ CD4+ CD69− CD25hi cells was measured by FACScan. To confirm the suppressive effect of T reg, *P. falciparum* schizont extract-induced IFN-gamma production and lymphocyte proliferation determined by [3H]-thymidine incorporation was assessed in PBMc with or without magnetic bead depletion of CD25hi cells.

**Results:** In 50% of donors we observed a transient but highly significant increase in bioactive TGF-beta at the time of parasite emergence from the liver. This was associated with significantly higher levels of parasitaemia and lower inflammatory cytokine responses. Plasma TGF-beta concentrations are positively correlated with levels of expression of the regulatory T cell (Treg)-specific transcription factor, FOXP3 at day 10 post infection, and with numbers of circulating CD4+ CD25hi Treg. The presence of CD4+ CD25hi FOXP3+ T cells is associated with higher rates of parasitic growth. Furthermore, in vitro depletion of CD25hi cells from the peripheral blood mononuclear cell population collected on day 10 post infection resulted in a significant increase in *P. falciparum*-induced proliferation and IFN-gamma production.

**Interpretation:** *P. falciparum* is able to induce/activate Tregs most likely in a mechanism involving TGF-β. Whilst initially of benefit to the parasite, *P. falciparum*-induced Treg activity may limit immunopathology during the course of disease.

**O-45**

The cerebral-malaria-associated expression of RANTES, CCR3 and CCR5 in post-mortem tissue samples [MIM-BS-163324]


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Introduction: Although the involvement of cytokines and adhesion molecules in malaria-induced brain inflammation has been established, the role of chemokines and chemokine receptors remain unclear. This unexplained component of cerebral malaria pathology may be responsible for the heterogeneity and the difficulty in characterization of the disease. RANTES, a chemokine involved in the generation of inflammatory infiltrates plays a special role in the modulation of inflammation. It is hypothesized that RANTES and corresponding receptors mediate cerebral malaria immunopathogenesis.

Methods: Autopsy samples were obtained with informed parental consent of children below the age of 9 who died from CM while on admission at the Korle-Bu Teaching Hospital, Accra, Ghana. Inclusion criteria for sample collection were WHO Blantyre coma score of 5 or less and the presence of malaria parasites. To determine which region of the brain expresses high levels of RANTES and receptors CCR1, CCR3 and CCR5, brain sections were taken from cerebellum, cerebrum, brain stem and hippocampus of confirmed human CM and non-malaria post-mortem brain tissue samples and stored in RNAlater (AmbionTM Inc., TX, USA). Studies were directed at evaluating retrospectively, the expression of RANTES and receptors, CCR1, CCR3 and CCR5 in these regions of the brain of post-mortem CM and non-malaria tissue samples using RT-PCR and Western Blot analyses.

Results: RANTES, CCR3 and CCR5 but not CCR1 mRNA were significantly upregulated in the cerebellum and cerebrum (P < 0.0001) in CM than NM samples. There were no changes in the expression of CCR1, CCR3 and CCR5 mRNA in brain stem and hippocampus of CM and NM. RANTES mRNA expression in cerebellum and cerebrum is highly significant (P < 0.0001) compared with the brain stem (P = 0.0018) and hippocampus (P = 0.0027) in CM group. CCR5 and RANTES proteins were significantly upregulated in cerebellum (P < 0.0013 for CCR5, P < 0.0001 for RANTES) and cerebrum (P < 0.0142 for CCR5, P < 0.0001 for RANTES) but not brain stem and hippocampus of CM than in NM.

Interpretation: The cerebellum and cerebrum in humans appeared to be the focal points for increased malaria-induced RANTES and CCR5 expression. Active sequestration of malaria-infected red blood cells and platelets in addition to leukocytes in these regions of the brain could exacerbate CM immunopathology.

O-46 Role of the NOS2 promoter polymorphism (G-954C) in protection from high-density Plasmodium falciparum malaria and malarial anaemia in Kenyan children [MIM-CO-481474]
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Introduction: Plasmodium falciparum is one of the leading causes of childhood morbidity and mortality of infectious origin throughout the world. Although the inflammatory profile associated with protection against malarial anemia (MA) is largely unknown, high levels of nitric oxide (NO) generated from nitric oxide synthase (NOS2), appears to limit parasitemia. Genetic polymorphisms in the NOS2 promoter, such as G-954C, have been associated with protection against malaria disease severity.

Methods: The association of NOS2 (G-954C) with protection against MA was investigated in a holoendemic P. falciparum transmission area. Children (n = 277) were enrolled at Siaya District Hospital in western Kenya. Complete hematological panels were obtained with a Beckman Coulter Counter, and Giemsa-stained slides were used to determine parasitemia. Children were divided into two groups based on anemia and parasitemia status; protected (Hb ≥ 8.0 g/dL, with or without parasitemia) and susceptible (Hb < 8.0 g/dL, with parasitemia). DNA was extracted from blood spotted on filter paper using the Chelex method. PCR was performed on a thermocycler with NOS2-specific primers. The resulting DNA fragment was digested with Bsa I and analyzed on a 3% agarose gel.

Results: The frequencies of polymorphic individuals in the cohort were; 79% (218/277) wild-type (GG), 16% (43/277) heterozygous (GC), and 5% (16/277) homozygous (CC). The allele frequencies were; p = 0.868 and q = 0.132. Logistic regression revealed that heterozygotes and homozygotes for the C allele were non-significantly protected from para-
sitemia (OR [95% CI]: 0.87 [0.41, 1.84], p = 0.71 and 0.65 [0.22, 1.97], p = 0.45, respectively). In the subset of children with parasitemia, heterozygotes and polymorphic homozygotes were not protected from high-density (>10,000 parasites/mL) parasitemia (0.90 [0.41, 2.01], p = 0.81 and 0.83 [0.23, 2.95], p = 0.77, respectively). To assess the association of the G-954C polymorphism with MA, children were categorized into protected and susceptible groups. The genotype frequencies in the protected group (n = 138) were: 75.4% (104/138) GG, 17.4% (24/138) GC, and 7.2% (10/138) CC, and the frequencies in the susceptible group (n = 139) were: 82% (114/139) GG, 13.7% (19/139) GC, and 4% (6/139) CC. Heterozygotes and homozygotes were non-significantly protected from MA (0.72 [0.37, 1.39], p = 0.33 and 0.55 [0.19, 1.56], p = 0.26, respectively).

Interpretation: Preliminary analyses of the association between the NOS2 G-954C polymorphism and malaria disease severity in Kenyan children suggests a slight protective effect of C allele against parasitemia and a stronger protective effect against MA.

O-47 Antibodies to malaria vaccine candidates and chloroquine or sulfadoxine/pyrimethamine treatment efficacy in an endemic area of Burkina Faso [MIM-DA-39094]

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Introduction: During the assessments of malaria treatment efficacy, cure rates were reported to increase with age. In endemic areas, spontaneous clearance of Plasmodium falciparum is likely to be the result of specific immunity. In order to evaluate whether the therapeutic response was associated with any specific immune response, antibodies to malaria vaccine candidates were measured before a longitudinal assessment of in vivo chloroquine and sulfadoxine/pyrimethamine efficacy in rural area.

Methods: The target population of this surveys were children aged 0.5–15 years living in the village of Balongueng. Prior to malaria transmission season, 5 ml venous blood were taken from each target child and plasma used for antibodies measurement. The longitudinal assessment of chloroquine efficacy of the study protocol was based on the 2001 WHO guidelines for monitoring the efficacy of antimalarial drugs. The level of antibodies (IgG, IgM and IgG subclasses) to the selected antigens (MSP1, GLURP and MSP119) were determined by ELISA.

Results: One hundred and ninety-five children were treated with chloroquine and 53 were treated with sulfadoxine/pyrimethamine. Adequate clinical and parasitological response rates were 8.2% and 37.7%, respectively, for chloroquine and sulfadoxine/pyrimethamine. Treatment failure rates including early treatment failure and late treatment failure were 91.8% for chloroquine and 62.3% for sulfadoxine/pyrimethamine. The treatment failure rate was higher in younger children compared to the older children. In both treated group, the antibodies level were high in Adequate clinical and parasitological response group except for IgM to MSP1 and IgG4 to GLURP in chloroquine treated sample and IgG1 to MSP1 to GLURP in Sulfafoxine/pyrimethamine treated group. However, the difference was statistically significant for IgG, IgG1, IgG2 and IgG4 to MSP3, IgM and IgG to GLURP for chloroquine treated patients and IgG3 to MSP1 in sulfadoxine treated patients. The ratio of cytophilic to non-cytophilic subclasses ([IgG1 + IgG3]: [IgG2 + IgG4]) for the three antigens were higher in children who were able to clear their parasites but no significant difference was observed.

Interpretation: Our data suggest that the efficacy of the therapeutic response seem to result from the combined effects of treatment and the individual immune status of the patients at the time of drug cure.

O-48 Cord blood cytokine levels predict malaria morbidity during infancy [MIM-EK-157608]

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Introduction: Inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and anti-inflammatory
cytokines like interleukin (IL-10) have been implicated in the pathogenesis of disease due to *Plasmodium falciparum*. Because sensitization to some parasitic infections in utero may influence long-term immunity, we hypothesized that the cytokine profile at birth may predict malaria morbidity throughout infancy.

**Methods:** We recruited mother–infant pairs in a longitudinal cohort study in northeastern Tanzania where malaria transmission is intense. Four hundred and seventy-five cord blood plasma samples were assayed for TNF-α, TNF receptor I (RI), TNF R II, interferon-γ (IFN-γ) and IL-10 by Bioplex multiplex assay using antibody-conjugated fluorescent microspheres and flow cytometry. Infants were actively followed up every 2 weeks and for any illness or hospitalization. Malaria morbidity in this cohort was analysed in relation to cord blood levels of the cytokines measured at birth.

**Results:** TNF-α, TNF R I and TNF R II were detected in nearly all cord blood samples (97–100%), and IFN-γ and IL-10 in 9.4% and 89.3%, respectively. After excluding for twin birth, HIV, or early neonatal death (n = 22), we examined outcomes of 453 infants, including 57 born to women with placental malaria. By Mann–Whitney test, placental malaria significantly increased cord TNF RII (p = 0.03) and TNF RII:IL10 ratio (p = 0.03) among offspring of multigravid but not primigravid women. Other cord cytokines did not vary based on placental malaria. By Cox regression, cord TNF RI:IL10 ratio (b = −0.0002, p = 0.047) was inversely related to age at first malaria hospitalization during infancy, after accounting for birth weight, birth season, maternal parity, and placental malaria status. By logistic regression, cord TNF RI:IL10 ratio (b = −0.0001, p = 0.07) was inversely related to risk of any malaria hospitalization during infancy, after accounting for birth weight, birth season, maternal parity, placental malaria status, and duration of followup.

**Interpretation:** Cord TNF RI:IL10 ratios predict reduced risk of malaria morbidity during infancy. Cytokine responses established at birth may have prolonged effects on the malaria immunity in exposed populations.

8. Malaria epidemiology

**Tuesday 15 November 11:00–13:00—Mahogany Hall**

Chairs: Ogobara Doumbo (Bamako) and Charles Mbogo (Kilifi)

**O-49 Opportunities for integrated malaria control in African cities [MIM-GK-3080]**


(1) Ifakara Health Research and Development Centre, Ifakara, Tanzania; (2) Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland; (3) Department of Geography, University of South Carolina, Columbia, SC, USA; (4) City Medical Office of Health, Dar es Salaam City Council, Dar es Salaam, Tanzania; (5) Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania; (6) School of Biological and Biomedical Science, University of Durham, Durham, UK; (7) Office of Population Studies, Princeton University, Princeton, NJ, USA

**Introduction:** The vast bulk of malaria research and control in Africa has been conducted in rural settings. Soon most Africans will live in poorly understood urban setting where new strategies are required but the potential for impact is huge.

**Methods:** Literature review and reanalysis of existing datasets are used to compare the existing situation and prospects for improvements of malaria control in urban versus rural Africa. We particularly focus on Tanzania and the Dar es Salaam Urban Malaria Control Programme as examples.

**Results:** Urban areas generally have higher human population densities with better access to facilities and services, notably healthcare and sanitation. Vector densities are lower, more focally distributed, and easier to suppress in urban settings. We show that cities have higher effective coverage of diagnosis, treatment, insecticide-treated nets, house screening and personal protection measures. We also outline how additional interventions such as larvicides and environmental management are feasible in cities within the context of decentralization and local government reform.
Interpretation: We demonstrate that African cities are already experiencing better malaria control than rural areas and have great potential for implementing effective and integrated malaria control in the immediate future.

O-50
No abstract received.

O-51
Monitoring malaria trends in Southern Africa: Use of geographical information system [MIM-SK-409610]


Introduction: Malaria control programmes collect a lot of information on the malaria situation in their countries. Usually there is limited analysis and use of most of the data collected. Monitoring malaria trends across borders is important for evidence based regional malaria control initiative. Monitoring malaria trends and spatial variation of malaria transmission is important in guiding implementation of effective targeted malaria control interventions.

Methods: Malaria control programmes in Southern Africa collect and malaria morbidity and mortality data through vertical malaria information systems, integrated surveillance systems and through the routine HMIS. In a number of countries databases are maintained on coverage of spraying operation by catchments area and ITN distribution by district. Surveillance and routine malaria data and data on coverage of ITNs and IRS coverage is managed in the Healthmapper Program at the country level for analysis and monitoring of trends and the spatial distribution of morbidity, mortality and coverage of interventions. The same data is forward to the WHO Office and subsequently to the regional office for monitoring the malaria situation in the region.

Results: Surveillance and routine malaria data is currently being maintained and analysed by malaria control programmes in a number of countries in the region. Surveillance data is monitored weekly to detect and monitor malaria epidemics including monitoring effect of epidemic in neighbouring districts. In a number of countries data collected from routine information system by month and district is available. Malaria maps can be produced for periods from 2000 to date. The effect of the successful distribution of ITNs in Malawi can be shown by the reduction in incidence rates in districts where nets were distributed. In the same way the effects of heavy rains in Angola and subsequent flooding in Namibia and Botswana on malaria situation in two countries and the control of malaria in Swaziland to the borders with Mozambique can be shown. Spatial distribution of malaria incidence and case fatality rates in Zambia over the past 5 years and the extent of malaria epidemics in Zimbabwe and effects of subsequent response can be clearly shown. The data available is used in cross border collaboration among countries in the region. Plans are underway to build capacity in Tanzania, Madagascar and Angola.

Interpretation: Use of geographical information systems allows for monitoring of malaria trends by sub-national level in countries and is useful in cross border collaboration by countries in Southern Africa.

O-52
Schistosoma haematobium infection is associated with protection incidence of clinical malaria [MIM-AD-453061]

A. Dahi, K. Sissoko, L. Sangaré, S. Touré, I. Saye, P. Druilhe, O. Doumbo

(1) Department of Epidemiology of Parasitic Diseases, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Mali; (2) Institut Pasteur de Paris, France

Introduction: In many endemic areas, both malaria and schistosomiasis are prevalent in all over the countries, but few studies have addressed the impact of
their co-infection on the clinical malaria in the human host. Available findings from previous studies were obtained in mice models resulting to contradictory results. These findings therefore deserved to be investigated in humans infected by schistosome and exposed to malaria.

Methods: After stool and urine examination at baseline for schistosome infection, we enrolled 172 pairs of school children (344 subjects) 4–14 years old. Schistosoma haematobium-infected children group were matched to schistosome-uninfected group by age, sex, residence and preventive protection tools against mosquito bites. Parasitemia, anemia and the incidence of clinical malaria were recorded in the two study groups during malaria transmission season from July to December 2003. The protocol was submitted and approved by the IRB of the faculty of medicine.

Results: Malaria infection and parasitemia were comparable in the two schistosome-infected and -uninfected groups (p > 0.05). The spleen index was significantly higher in S. haematobium-infected children than in schistosome-uninfected group in September (p = 0.009). Clinical incidence of Plasmodium falciparum malaria was 0.76 times higher in S. haematobium-non-infected individuals than in S. haematobium-infected subjects (p = 0.02; RR = 0.76; IC 95% [0.59–0.97]). This finding suggests that the management as well as the evaluation of malaria vaccine design in the field should take into account schistosome infections in areas where the two diseases are co-endemic.

Interpretation: Our finding support that underlying schistosomiasis has a beneficial effect in protection from clinical falciparum malaria. However, there is an urgent need to further investigate malaria and helminthic co-infections in endemic areas.

O-53 Social, economic, ecological, and behavioral determinants of urban malaria transmission: A case study for Dar es Salaam, Tanzania [MIM-MD-11554]

M. Castro, K. Kennedy, U. Fillinger, D. Mtasiwa, S. Lindsay, G. Killeen, B. Singer, M. Tanner

(1) Department of Geography, University of South Carolina, Columbia, SC, USA; (2) Office of Population Research, Princeton University, Princeton, NJ, USA; (3) City Medical Office of Health, Dar es Salaam City Council, Dar es Salaam, Tanzania; (4) School of Biological and Biomedical Sciences, University of Durham, Durham, UK; (5) Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland; (6) Ifakara Health Research and Development Center, Ifakara, Tanzania

Introduction: Unprecedented urban growth in Africa has important implications for understanding and controlling malaria. In such diverse urban settings, it is expected that social, economic, ecological, and behavioral factors are likely to assume a critical role in determining the patterns of malaria transmission. In this study we carry out a spatial integrative analysis characterizing the linkages among those factors, and the manner in which they influence transmission.

Methods: We use data from household the Urban Malaria Control Programme (UMCP) to collect information on habitat (natural and man-made), personal behavior, knowledge about malaria transmission, use of protective measures, and income, among other variables. The survey has a follow-up design, which allows the evaluation of seasonal variability in transmission, and all data is geo-referenced. We analyze the data using local indicators of spatial association, spatial econometrics, and grade of membership models. We expect to identify the main determinants of malaria transmission, and to suggest targeted social policies that could mitigate the malaria burden.

Results: This is an ongoing research (interviews started on April 2004, and currently the first follow-up round is being conducted). An initial exploratory analysis was carried out last October, and a thorough investigation will be performed next June and July. Building upon previous research in Dar es Salaam, we show that (1) malaria transmission in an urban space has
a strong spatial component determined by different levels of urbanization; (2) ecological factors are the most important determinants of malaria transmission in areas of early and/or unplanned expansion (peri-urban), while social, economic, and behavioral factors assume a much important role in older and/or planned urban settings. A comprehensive understanding of this distinction facilitates the implementation of spatially targeted public initiatives aiming to address social, economic, ecological, and behavioral matters.

Interpretation: The sustainability of integrated malaria control strategies (larviciding, environmental management, ITNs, rapid diagnosis and treatment) can be better accomplished if personal, household, and community aspects are joint targets of public programs.

O-54 Screening of blood donations for malaria parasites by microscopy: RDTs and quantitative nucleic acid sequence based amplification assay—Sudan [MIM-BN-50982]

B. Nour, H. Schallig, G. Schoone, O. Saeed, A. Mohamadani

(1) Blue Nile Research and Training Institute/Faculty of Medicine, University of Gezira, P.O. Box 20, Wad Medani, Sudan; (2) Royal Tropical Institute, (KIT) Biomedical Research, Meibergdreef 39, 1105 AZ, Amsterdam, The Netherlands

Introduction: The safety of the blood supply is critical to many parts of modern medicine and it is essential that transfusion services globally ensure the safety of the blood supply. The problem now is the increased risk of blood transmitted malaria in highly endemic countries, and occurring also in non-malaria area. In Sudan, the prevalence of infected donors was 6.5% as reported in Khartoum, indicating that exclusion of infected donors minimizes the risk of transfusion-induced malaria.

Methods: At the blood bank of Wad Medani Teaching Hospital in central Sudan, 100 samples were randomly collected from blood donations and analysed for the presence of malaria parasites. These samples were examined by the standard microscopy, rapid diagnostic tests (RDTs=Paracheck test (HRPII) optiMAL test (pfLDH) and quantitative nucleic acid sequence based amplification (QT-NASBA).

Results: In these 100 blood donations, no parasites were found by the microscopy, and all RDTs were negative within the limited reading time (10–15 min) that described by the manufacturers. But after a prolonged time (20–30 min), 18 of 100 showed positive by Paracheck test (HRPII) while they remained negative by optiMAL test (pfLDH). The blood samples were further analysed by QT-NASBA (lower detection limit for quantification 0.1 parasite/μl of blood). Three samples were lost due to transport and 17 gave insufficient amount of RNA after extraction. Out of the 100 samples, 80 were analysed by QT-NASBA, 55 of them were found Plasmodium falciparum negative, i.e. <0.001 parasite/μl of blood, four samples gave a result that was in the cut-off area of the test, i.e. parasite count between >0.001 and 0.1 parasite/μl of blood. Twenty-one of the 80 samples (26.3%) found QT-NASBA positive, i.e parasites count >0.1/μl of blood. The mean parasite count of the positive samples was 1.73 parasite/μl of blood (ranging between 0.13 and 18.6).

Interpretation: Microscopy and RDTs are not sensitive to detect low parasitemia in apparently healthy donors. Improved screening of blood banks for malaria to reduce the risk of transfusion transmitted malaria is necessary, and QT-NASBA is a powerful tool.

9. Treatment of malaria/rational drug use 1

Tuesday 15 November 14:30–16:30—Bubinga Hall

Chairs: Theonest Mutabingwa (Muheza) and David Ofotobi-Adjei (Accra)

O-55 Management of severe malaria

B.R. Ogutu

Walter Reed Project, Centre for Clinical Research, Kenya Medical Research Institute, Kenya

About 5% of the world population is affected by malaria with majority being in Africa. Severe malaria is a medical emergency which carries a high mortality if appropriate effective treatment is not accorded promptly. Reported deaths do not take into account those who die at home and after discharge from hospital thus representing the tip of the iceberg. It has become evident that an effective antimalarial alone will
not reduce mortality in severe and complicated malaria even if administered promptly. This may have contributed to lack of superiority of the use artemisinin derivatives over quinine in treatment of severe malaria despite the former being most rapidly acting antimalarial. Therefore, identification of children at risk of death (cerebral malaria, severe anaemia and respiratory distress), provision of supportive treatment targeting the complications of severe malaria such as acidosis, severe anaemia, hypoglycaemia, convulsions, pulmonary oedema, coma and electrolyte imbalance remain the most important strategy in reducing deaths in severe malaria. Therefore, there is need for increased effort in the evaluation of the use of fluids, blood transfusion, anticonvulsants, glucose and pharmacological products that may prevent neuronal and other cellular damage and correct acidosis. However, the ultimate way to avert deaths by reducing the number of patients developing severe malaria is a functional health infrastructure and an effective, accessible and available first line treatment for malaria. Therefore, there is greater need to attain functional health infrastructure and availability of an effective first line antimalarial treatment in malaria endemic areas.

**O-56**

Is pharmacovigilance regarding antimalarials in Africa an illusion? [MIM-AT-14101]

A. Talisuna

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**Introduction:** Prior to product registration and marketing, data about safety and efficacy are limited to observations in preclinical and clinical trials. However, such trials utilize a small numbers of subjects because of the strict criteria and requirements to rigorously follow up the subjects. Therefore, the conditions in clinical trials do not necessarily reflect what will happen in the general practice in the “real world” post marketing. Consequently, data from clinical trials alone, though useful for product registration, might not be adequate in the documentation of adverse drug effects. Pharmacovigilance involves the monitoring of pharmaceutical products as they are used in the “real world”. The purpose is to identify previously unrecognized patterns or changes of adverse effects; assessing the risks/benefits of medicines in order to improve their safe use; providing information to clients to optimize safe and effective use of the medicines; and monitoring the impact of actions taken about the specific warnings in product information, which allow safe and effective use of the products.

**Methods:** In this paper, we present the rationale for pharmacovigilance of antimalarial drugs in Africa and review the different models/approaches that could be used for its implementation.

**Results:** Rationale for pharmacovigilance in Africa: Presently, antimalaria drug pharmacovigilance is a topical issue in Africa, largely because of the high prevalence of parasite resistance to safer drugs such as chloroquine and sulphadoxine–pyrimethamine, which has compelled several African countries to adopt combination therapy, most preferably artemisinin combination therapy (ACT). Although ACTs have good safety profiles in clinical trials, there is little data about their safety post-marketing outside south-east Asia. Pharmacovigilance for ACTs and other newer antimalarials is therefore important because these medicines are relatively new in Africa and are being adopted simultaneously in several African populations which offers an opportunity to identify rare or unexpected adverse effects not previously documented during the pre-registration clinical trials. Secondly, the safety for these medicines is a concern because of the high prevalence of co-morbidity of malaria with HIV/AIDS, tuberculosis, malnutrition and thirdly, the safety profile of ACTs in pregnancy is yet to be established. Despite the keen interest for pharmacovigilance in Africa, the approaches to use are problematic. Passive reporting of adverse effects is notoriously poor in developed countries and largely non existent Africa.

**Interpretation:** Pharmacovigilance is likely to be problematic in Africa, but we contend that it is not an illusion. What is required is a combination of models and a multidisciplinary approach. In order to “kick start” pharmacovigilance in Africa, we propose that pharmaceutical industry should pledge at registration to support pharmacovigilance in the first few years post marketing.
O-57
Therapeutic efficacy of amodiaquine (AQ), sulfadoxine–pyrimethamine (S-P) and AQ + S-P combination for treating uncomplicated malaria in Cameroon [MIM-AI-150510]

(1) Biotechnology Centre, University of Yaounde I, Cameroon; (2) Gates Malaria Partnership, London School of Hygiene and Tropical Medicine, London, UK

Introduction: High mortality from malaria has been attributed to the emergence and spread of drug resistant parasites. Due to high levels of chloroquine resistance, Cameroon opted for an interim policy of amodiaquine. Between 1999 and 2002, the efficacy of alternatives to chloroquine showed rising levels of resistance to anti-folates (6–14%) and amodiaquine (0–6%). We investigated the efficacy of amodiaquine (AQ), sulfadoxine–pyrimethamine (S-P), and (AQ + S-P) in different ecozones in Cameroon in 2004.

Methods: A randomised double-blinded efficacy and safety study was conducted between January and December 2004 on children between 6 and 59 months. After informed consent from parents or guardians patients were clinically examined and supervised for the administration of AQ, SP or AQ + SP. Quinine was the rescue drug. Patients were followed up for 14 days and subsequently for 28 days and scored using the WHO 2002 protocol for assessing clinical and parasitological outcome. Biochemical indices were determined on days 0 and 7. Day 3 blood filter paper samples were analysed for whole blood drug concentrations. Molecular markers are being investigated by PCR-RFLP for mutations on genes dhfr (51, 59, 108), dhps (437, 540), pfcrt (76) and pfmdr (86).

Results: The prevalence of fever at enrolment was 99%, 97% and 77% in Limbe, Yaounde and Garoua, respectively. Loss of appetite was observed in 48%, 49% and 9% of patients in Limbe, Yaounde and Garoua. Nausea/vomiting was common in all the sites. Diarrhoeal cases occurred highest in Garoua (31%), than in Limbe (12%) and Yaounde (4%). The mean haemoglobin concentration increased from days 0 to 7 for all the drug groups in Limbe and Yaounde. No patient developed side effects due to drug administration during the trial.

The adequate clinical and parasitological responses on day 14 for S-P, AQ and S-P + AQ were 59.5%, 82.2%, 85.5% in Limbe, 60.0%, 86.4%, 80.0% in Yaounde and 69.6%, 71.8%, 82.6%, in Garoua, respectively. In all three sites, the development of gametocytes was observed in patients treated with S-P more than in any other group (Limbe (13.9%, 5.0% and 2.4%), Yaounde (14.5%, 7.1% and 8.9%), Garoua (2.9%, 0.0% and 1.4%), respectively, for SP, AQ and S-P + AQ at day 7 post-therapy). The molecular markers for determination of resistance are still being assessed and will be presented.

Interpretation: Our findings demonstrate that clinical efficacy to S-P is deteriorating and has policy implications and the need to adopt a strategy that includes artemisinine-based drugs in the combination.

O-58
Efficacy of artether/lumefantrine for the treatment of moderately severe malaria in children [MIM-PS-206376]

P. Sasi, M. English, S. Muchohi, M. Makanga, A. Nzila, B. Lowe, G. Kokwaro
KEMRI Center for Geographic Medicine Research, Coast, Kilifi, Kenya

Introduction: Hospitalised children with malaria, who have no features of severe disease, are at risk of clinical deterioration. Most do not vomit everything and oral medication can be given. Treatment with artether/lumefantrine is an attractive option. But adequate lumefantrine bioavailability determines efficacy and co-administration of food determines this bioavailability. Thus, poor food intake may prevent adequate bioavailability. We have examined efficacy of this combination in these children.

Methods: An open-label randomised controlled trial of artether/lumefantrine (Coartem®) and sulphadoxine/pyrimethamine (Fansidar®), the standard treatment at the time of the study. Children were randomly allocated to receive either a six-dose regimen of Coartem® or a single dose of Fansidar® and observed in hospital for 3 days. Active follow-up was on day 7, 14 and 28. Adequate clinical response (ACR) on day 14 was the principal outcome measure, and plasma lumefantrine concentration above 280 ng/ml on day 7 was defined as adequate bioavailability.
Results: A policy statement to change from sulphadoxine/pyrimethamine to artemether/lumefantrine as the first line drug for treatment of uncomplicated malaria was made halfway through the planned sample size, and randomisation was stopped. Sixty-five and 55 eligible children had been randomised to the sulphadoxine/pyrimethamine and artemether/lumefantrine, respectively. In an intention-to-treat analysis (ITT), the PCR-adjusted adequate clinical response on day 14 was 54/55 (98%) in artemether/lumefantrine treated children compared with 56/65 (86%) in the sulphadoxine/pyrimethamine arm ($P = 0.01$). By 28 days after start of treatment PCR-adjusted adequate clinical response was 53/55 (96%) in artemether/lumefantrine treated children and 55/65 (85%) in children treated with sulphadoxine/pyrimethamine ($P = 0.142$). No serious adverse events were observed in any of the treatment arm. Lumefantrine bioavailability was adequate (>280 ng/ml on day 7) in 19/48 (40%) of the artemether/lumefantrine treated children.

Interpretation: Despite inadequate lumefantrine bioavailability in most children in this study, the six-dose regimen of artemether/lumefantrine is efficacious, and can be used in children with moderately severe malaria with good results.

O-59 Amodiaquine-artesunate versus artemether-lumefantrine: Efficacy and safety for single or repeat episodes of uncomplicated malaria in Ghanaian children [MIM-GA-200658]

G. Adjei, B. Goka, O. Rodrigues, E. Kitcher, E. Badoe, M. Alifrangis, J. Kurtzhals

(1) Department of Child Health, Korle Bu Teaching Hospital, Accra, Ghana; (2) Department of Surgery, Korle Bu Teaching Hospital, Accra, Ghana; (3) Institute of Medical Microbiology and Immunology, Denmark; (4) Department of Clinical Microbiology, Centre for Medical Parasitology, Copenhagen University Hospital, Copenhagen, Denmark

Introduction: Amodiaquine-artesunate (A-A) is the current first-line antimalarial in Ghana. Audiometry is recommended as part of evaluating artemisinins, and it is important for new antimalarial regimens to be assessed for cardioxicity. No such reports are available for A-A, and previous audiometric studies of other artemisinin regimens are inconclusive. We evaluated audiometric and electrocardiographic parameters for children treated with A-A, artemether-lumefantrine (A-L), or amodiaquine-alone (controls).

Methods: In an on-going study, we enrolled children aged 0.5–12 years with slide-positive uncomplicated malaria (UM), presenting to two primary health facilities in Accra, Ghana. The patients received A-A ($n=40$), or A-L ($n=33$), in a single-blind randomised controlled trial. A control group ($n=17$) with UM, received amodiaquine alone. All treatments were directly observed, and subjects were followed up on days 3, 7, 14, 28, and then actively every month, plus whenever they needed medical attention. Audiometry, electrocardiography (ECG), haematology, and neurological examinations were conducted at each scheduled follow up visit, and during any episode of UM occurring after day 28. Repeat episodes were treated with the same regimen as at randomisation.

Results: Admission parameters were similar between the groups. All treatments were well tolerated. Day 14 cure rates were 93% [37/40], 94% [31/33], and 88% [15/17] ($p=0.77$), in the A-A, A-L, and control groups, respectively. The corresponding rates at day 28 were 88% [35/40], 85% [28/33], and 88% [15/17], ($p=0.92$). Symptoms had subsided 24 h after initiation of treatment in 71%, 44%, and 40% in the A-A, A-L, and control groups, respectively (A-A versus control, $p=0.02$, A-L versus control, $p=0.59$). The only case of early treatment failure was in the control group. Parasitological cure at 24 h was 40%, 45%, and 15% in the A-A, A-L, and control groups, respectively. There were no differences in audiometric thresholds, Q-T and other ECG intervals, haemoglobin levels or neutrophil counts between groups-before or after treatment. Three subjects (two in the A-A, and one in the A-L groups) exhibited nystagmus after initiating treatment. These were transient, and had resolved by day 14 in all the three cases. The mean heart rate was slower on day 3 than on day 0 in all subjects—which is consistent with changes associated with recovery from fever. Eighteen patients were retreated uneventfully for symptomatic parasitaemia, 7 before day 28, and 11 after day 28.

Interpretation: Cure rates were similar for the two combination regimens at days 14 and 28, but symptomatic cure was more rapid after treatment with amodiaquine-artesunate. No changes in audiometric thresholds, ECG intervals or laboratory parameters were observed.
O-60
HIV infection increases the risk of retreatment for uncomplicated malaria in Uganda [MIM-AG-346150]

(1) Makerere University, Kampala, Uganda; (2) Ministry of Health, Uganda; (3) University of California, San Francisco, USA

Introduction: Malaria and HIV are each responsible for staggering morbidity and mortality in sub-Saharan Africa. HIV may amplify the burden of malaria by increasing susceptibility to infection or by diminishing response to antimalarial treatment. We investigated the effect of HIV on malaria treatment in patients with uncomplicated falciparum malaria in Uganda.

Methods: Study subjects, aged 18 months and above, were participants in antimalarial trials conducted between December 2002 and July 2004 at 7 Uganda sites. Patients were randomized to receive sulfadoxine–pyrimethamine (SP) + chloroquine, SP + amodiaquine (AQ) or AQ + Artesunate and classified as treatment success or failure at 28 days according to WHO guidelines. Molecular genotyping was used to differentiate recrudescence from re-infection. HIV testing was performed on stored dried blood spots using two enzyme-linked immunosorbent assays in parallel (Vironostika HIV-1 Plus O Microelisa System, and Genetic Systems rLA V EIA). Discordant specimens were tested with Western blot (Genetic Systems HIV-1 Western Blot).

Results: Of 1965 study subjects, 95 (5%) were HIV-infected. HIV prevalence varied with age with lowest prevalence in patients aged less than 5 years (2%) and highest in those 18 years and above (31%). HIV was associated with increased risk of treatment failure in patients >18 years (HR 3.28, CI 1.25–8.59). Failure was increased due to reinfection (HR 6.35, CI 1.64–24.5), not recrudescence. The risk of treatment failure was similar among HIV-infected and uninfected persons less than 18 years old.

Interpretation: HIV seroprevalence is very high in adults presenting with malaria supporting HIV counseling and testing in this subpopulation. In adults, HIV increases the risk of reinfection and thus retreatment but does not increase the risk of recrudescence.

O-61
Albumin reduces the risk of death in children with severe malaria and acidosis in a Phase II randomised controlled trial of volume expansion [MIM-KM-16564]

(1) The Centre for Geographic Medicine Research, Coast, KEMRI, Kenya, PO Box 230, Kilifi, Kenya; (2) Department of Paediatrics, Faculty of Medicine and the Wellcome Trust Centre for Clinical Tropical Medicine, Imperial College, London, UK

Introduction: Metabolic acidosis is the best predictor of death in children with severe falciparum malaria however, its treatment presents a therapeutic dilemma as acidosis may co-exist with coma, which maybe complicated by cerebral oedema. We postulated that volume resuscitation with albumin might correct acidosis and hypovolemia with a lower risk of precipitating cerebral oedema than crystalloid. In a RCT we compared the safety of resuscitation with albumin to saline in Kenyan children with severe malaria.

Methods: We randomly assigned children with severe malaria and metabolic acidosis (base deficit (BD) >8) to intravenous volume resuscitation with either human albumin solution or normal saline. Those with moderate acidosis (MA) (BD 8–15) received 20 ml/kg; and those with severe acidosis (BD > 15) received 40 ml/kg of the intervention fluid. A control ‘no-bolus’ group was included for those with moderate acidosis as it was considered unethical to withhold volume expansion from the severe acidosis group. In all other respects treatment was identical. Endpoints of the study were the reduction in base deficit at 8 h, requirement for alternate therapy, in hospital mortality and adverse events and neurological sequelae at discharge.

Results: We evaluated 151 children: In the MA group 36 received normal saline, 33 albumin and 33 no bolus control. In the SA group 26 received saline and 23 albumin. There was no difference in the rate of resolution of acidosis between the treatment arms, however rescue therapy was more common in the control group (p = 0.004). Mortality was significantly lower in patients receiving albumin (256: 3.6%) than those treated with saline (1262: 19%) (p = 0.08); relative risk 5.5; [95% CI 1.2–24.8] P = 0.013. The improved mor-
Tality was entirely confined to the children admitted in deep coma (a group predicted in our hypothesis to benefit from the use of albumin) only 1/21 (5%) albumin recipients died compared with 11/24 (46%) receiving saline ($P=0.002$). Mortality in the saline group was similar previous reports from comparable patient groups (28–41%), but our study design did not enable us to determine whether saline boluses are preferable to fluid restriction, or are actually hazardous. For non-comatose children receiving either saline or albumin, death was rare (1/70.1%), lending crucial support for the value and safety of volume expansion in this group. One case developed pulmonary oedema (1%) and only 10/135 (7%) survivors had neurological sequelae.

**Interpretation:** In high-risk children with severe malaria and acidosis, fluid resuscitation with albumin may reduce mortality. Further studies are needed to confirm our findings before definitive treatment recommendations can be made.

10. Capacity building

**Tuesday 15 November 14:30–16:30—Ebony Hall**

**Chairs:** Lars Gustafsson (Stockholm) and Olumide Ogundahunsi (Geneva)

**O-62** No abstract received.

**O-63** No abstract received.

**O-64** Use of community resource persons effective in malaria control [MIM-DO-3105]

**D. Owuor, P. Odiambó øchola, L. Tsoma, P. Mentangmo**

**Introduction:** Plan International in Kenya implemented a 3 year Child Survival program in two divisions of Kwale District in Coast Province of Kenya, from 2001. The program covered 196 villages with a population of 134,141 persons with 21,523 children below 1 year of age and 24,383 women of reproductive age. The program objectives included improvement of malaria control it focused on building the capacity of community resource persons (CORPs) and linking them to dispensaries strengthened to provide quality care.

**Methods:** The strategy was to create demand for quality care by empowering the community and improve access to services by building capacity of frontline staff. Households organized to form village health committees (VHCs) to oversee CORPs. The VHCs were linked to the formal health system via Dispensary Health Committees. Strengthening of dispensaries included staff training on IMCI, enhancement of drug supply, and supportive supervision. CORPs trained on community IMCI were used to raise mothers’ awareness of malaria and counsel them on appropriate care and referral of children; promote use of ITNs by pregnant women and children below 2 years; and to promote use of IPT and iron/folate supplements by pregnant women.

**Results:** The percent of mothers aware of malaria danger signs using IMCI protocols rose from 17% at baseline to 76% at close of the program while the percentage of children with fever that were taken to an IMCI trained health provider rose from 38% to 77.7%. The use of insecticide treated bed-nets during pregnancy increased from 14% to 32.5%. More children slept under ITNs at the end of the program (31%) as compared to the baseline (19%). The percent of mothers who reported receiving iron/folic acid during their last pregnancy went from 76% at baseline, to 100% at final. The proportion of mothers attending antenatal clinics that received malaria prophylaxis increased from 7.5% at baseline to 100% at close of the program. At the close of the program 92% of CHWs practiced IMCI as per their training, and 75% still stocked of anti-malarial drugs. The percent of DHCs conducting community outreaches increased form 0% at baseline to 85% at the close of the program. Only 61.5% of dispensary staff trained in IMCI protocols for malaria still practiced them. Terminal Focus group discussions indicated that the community awareness on malaria had increased. The community also stated that ready availability of trained CORPs meant fewer of their children dying.

**Interpretation:** The results show that use of trained community resource persons extends the reach of malaria control interventions and is an effective strategy in achieving malaria control in children below 2 years and antenatal women even in remote areas.
Supporting national community health schemes: Initial lessons from the Uganda Malaria Partnership Programme [MIM-CW-45415]

C. White, A. Kilian, J. Rwakimari, A. Bell, V. Mukasa, R. South

(1) African Medical and Research Foundation (AMREF), Nairobi, Kenya; (2) USAID/CDC, Kampala, Uganda; (3) National Malaria Control Programme, Ministry of Health, Kampala, Uganda; (4) Malaria Consortium, Kampala, Uganda; (5) African Medical and Research Foundation (AMREF), Kampala, Uganda; (6) GlaxoSmithKline, London, UK

Introduction: In Uganda, home-based management of fever (HBMF) is constrained by performance and retention of community drug distributors (CDD) and community acceptance of services. Supported by the GlaxoSmithKline African Malaria Partnership, four NGOs (AMREF, Red Cross, Africare, and CDFU) used behaviour change communication and the drug distributor network to improve utilisation of Insecticide Treated Nets (ITN), Intermittent Preventive Treatment among pregnant women (IPT) and HBMF.

Methods: From three districts, 12 sub-counties (population 230,000) were identified as project sites. A baseline study of knowledge and practices regarding malaria control and an assessment of existing coverage with ITN, IPT and HBMF was conducted. Drug distributors were trained, equipped and supervised. Various media were used for intensive behaviour change communication. After 18 months, an assessment of progress and impact to-date was conducted. Monthly reports from drug distributors, malaria caseload data from health facilities, a rapid assessment of ITN coverage using a primary school survey and data from the 2004 re-treatment campaign were utilised.

Results: HBMF has resulted in a dramatic increase in the number of children receiving correct treatment within 24h. This is observed both in project sub-counties (pSC) and non-project sub-counties (npSC), the proportion of treatment by community drug distributors within 24h being approximately 75%. The most notable differences observed between pSC and npSC relate to the motivation, performance, and retention of drug distributors, with a drop out rate of 1–2% in pSC of Kumi district, compared with 22–33% in npSC. In Kiboga district, 88.1% of CDD in pSC submitted timely and accurate reports compared to 75.8% in npSC. Coverage with IPT 2 has increased over time in all three districts, with all but one set of pSC performing better than npSC (Kanungu 42% versus 27%; Kumi 51% versus 37%; and Kiboga 37% versus 47%; respectively). Regarding ITN, school surveys in Kanungu revealed at least 37% of pSC households having one net compared with 11% of npSC households. The proportion of households with a child using an ITN was 14% and 1.4%; respectively. In Kumi, the 2004 re-treatment campaign showed higher availability and treatment of ITN in pSC (96.8 treated nets per 100 households, versus 72.3 among npSC households).

Interpretation: Differences in CDD performance and retention and community use of interventions are observed between project sites and other areas. This is due to the provision of a modest support package and a simple health communication exercise.

Development of a malaria field research station in Zambia [MIM-SM-188350]

S. Mharakurwa, G. Stam, P. Thuma, C. Shiff

(1) The Malaria Institute at Macha, Choma, Zambia; (2) Johns Hopkins Bloomberg School of Medicine, Baltimore, USA

Introduction: The continued upsurge in malaria burden underscores the need for endemic country research capacity building to improve control. In the Macha chiefdom, Southern Zambia, ravaged by malaria for many years, a local mission hospital signed an MOU with the Ministry of Health, Brethren in Christ Church, Johns Hopkins University and a US-based NGO called Macha Malaria Research Institute (Mmri), giving birth to the Malaria Institute at Macha (MIAM) in 2003.

Methods: Principally sponsored by the Johns Hopkins Malaria Research Institute (JHMRI) and the MMRI, MIAM was officially opened in January 2005, with the principal goals of becoming a regional centre of excellence in malaria research and training, and of eventually actualizing the dream that malaria will no longer be a problem in Macha. The genesis of MIAM is based on the potent approach of building a critical
mass of research personnel and material resources in the stronghold of malaria itself. Furthermore, through its close institutional affiliations, MIAM affords the unique opportunity for direct interface between basic and applied researchers, with the aim of strengthening malaria control.

**Results:** In her early formative years MIAM already has a functional molecular biology/parasitology lab, with access to clinical/haematology/biochemistry laboratories through the mission hospital (Macha Mission Hospital) stake holder. A full-fledged MIAM laboratory, which will include an insectary, immunology and parasite culture divisions is in advanced stages of construction. MIAM now has seven ongoing clinical and epidemiological research programmes and 22 publications emanating from her early stages of creation. The entire working vicinity around MIAM and Macha Mission Hospital has been divided into 25 km² geographical grids, of which a representative 32 have been mapped to household level, on a one headman area per grid basis, commencing a GPS/GIS data base. In this rural setting biomedical literature access and internet communication have been set up via a VSAT dish. The research campus includes staff houses, guest flats, library/auditorium and warehouse facilities, with wireless LAN connectivity. The institute is reachable by gravel roads, and flights are now available to and from the nearby ABFA-Macha airstrip, opened as of 2004, thus facilitating visits from international collaborators.

**Interpretation:** A strategic field research station has been set up that will afford substantial leverage and resources for malaria research and training in Southern Africa.

**O-67 Building multidisciplinary teams for malaria control in Sub-Sahara Africa [MIM-SA-92833]**

I. Agyepong, S. Al-hussein, M. Awuah, K. Opoku-mensah, M. Pappoe, I. Quakyi

(1) School of Public Health, College of Health Sciences, University of Ghana, Legon; (2) National Malaria Control Programme, Ghana Health Services, Ghana; (3) Gates Malaria Partnerships, London School of Hygiene and Tropical Medicine, London, UK; (4) Partnerships for Social Sciences in Malaria Control

**Introduction:** Staff leading the design of malaria control programmes in Africa are mainly biomedical rather than social scientists. Social science has been limited mainly to developing behaviour change communication programmes (IE&C) but has more to contribute to effective malaria control programmes. A programme designed to strengthen social sciences input into malaria control policy and programme implementation commences in August 2005.

**Methods:** The basic premise on which the training design is based is that if people who currently work with malaria control and social scientists are brought together they will better understand each other’s roles and be able to work together to improve control policy, programme development, and implementation. A series of discussions, working sessions and on-line reviews involving biomedical and social scientists and Managers of Health systems were organised to develop the training package. Reference materials were collected from libraries and on-line to develop the package.

**Results:** The course is designed to develop capacity to improve social science input into malaria control and programme implementation. The intention is to build multidisciplinary teams within malaria endemic countries in sub-Saharan Africa. The teams are expected to consist of national malaria control personnel, district and regional level officers, social scientists, policy makers and planners. The teams will be helped to finalise project proposals and develop implementation plans that will be put into practice with support and supervision from their countries. This forms part of the practical component of the programme. The training curriculum consists of five modules. Experiential learner-centred focus is given to the modules, which consist of the following: Module 1: Team Building and Working in Groups; Module 2: The Basic Sciences; Module 3: Principles of malaria control and the role of the Social Sciences; Module 4: Malaria Control Policy Development and Implementation, Advocacy for malaria control; Module 5: Group Project: Planning, Implementation and Management of an intervention to improve malaria control.

**Interpretation:** The course will enhance social science input to malaria control programme planning, implementation, monitoring and evaluation by strengthening multidisciplinary approach in malaria control in Africa thus meeting crucial capacity needs in Africa.
**O-68**

**Computational chemistry as part of capacity building in malaria research in the African continent**

[MIM-LM-1840]

L. Mammino

University of Venda, Thohoyandou, South Africa

**Introduction:** Computational chemistry studies of biologically active molecules provide information relevant to the understanding of their activity and to the design of improved molecules. Computational studies on malaria-related molecules constitute an ideal option to expand chemists’ involvement in malaria research in African universities. The investigation of substances derived from traditional remedies favours the search for new lead compounds and interfaces advanced research with traditional medicine.

**Methods:** Two directions were pursued, one with a mainly pedagogical objective, the other aimed at a concrete evaluation of feasibility. The former involved an overview of computational chemistry studies on malaria-related molecules from available literature, in order to design and evaluate the best ways for the presentation, to students, of the nature and objectives of this type of studies. The latter (still in progress) involved the proposition of the computational study of biologically active molecules from traditional remedies as postgraduate students’ projects, and an evaluation of the level of results obtainable within existing (or reasonably easily attainable) facilities.

**Results:** The pedagogical-oriented study highlighted the significance of the presentation of examples of malaria-related computational works. The relevance for the local communities attracts students’ interest and attention. In order to encourage and facilitate students’ familiarisation with the different research questions (e.g. the various features of the relationships between molecular structure and biological activity), an outline discussing examples apt to illustrate the major aspects, in a way that is easily understandable at introductory level, has been prepared. The experiment proposing computational studies of biologically active molecules from traditional remedies highlights several relevant features: the feasibility of this type of project (at least at its initial and simplest levels) within already existing or easily attainable facilities (e.g. freely-downloadable software); the interest of chemistry students towards medically-focussed studies targeting issues relevant for their communities; the possibility of developing simpler-level studies into more complete ones, if more advanced computational facilities are made available; the relevance of interactions with, and support from, centres with long-standing expertise.

**Interpretation:** The enhancement of computational chemistry studies on malaria-related molecules in African universities is a realistic option to the purpose of expanding the scope of malaria research in the continent and chemists’ involvement in this research.

**11. Plasmodium/human/Anopheles genomics 1**

Tuesday 15 November 14:30–16:30—Iroko Hall

Chairs: Ingrid Faye (Stockholm) and Christian Happi (Lagos)

**O-69**

No abstract received.

**O-70**

**VectorBase: A bioinformatics resource center for invertebrate vectors of human pathogens**

[MIM-CL-6190]


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**Introduction:** Since acquisition of the Anopheles gambiae whole genome sequence, more insect disease vectors have become the focus of such projects. This sort of biological information offers a novel toolbox that can be used to develop strategies for vector control. The large amount of information directly or indirectly linked to the genome sequence though, makes it necessary to develop specific resources that can manage the data and make them accessible to the research community in a convenient form.

**Methods:** VectorBase (http://www.vectorbase.org) is a centralized relational database and web interface focused on invertebrate vectors of human pathogens. Its purpose is to manage, display, and analyze data for
all vectors for which genome level data sets are developed (genome sequences, extensive EST sequence sets, other large scale genome-derived data sets, or data sets based on functional analysis of the genome). These will be supplemented with additional information on the biology of the invertebrate disease vectors. Data will be stored using public domain software including the database schema Chado, recently developed by the open source Genome Model Organism Database Construction set, GMOD.

Results: Browsing of VectorBase’s genomic data will be via the Ensembl Genome Browser. VectorBase will be guided largely by features and goals embraced by two of its core partners, FlyBase and Ensemb. Initially, VectorBase will provide a bioinformatics resource for the display and analysis of genome data that relate to arthropod disease vectors. We expect that this will include data related to complete draft genome sequences for *An. gambiae*, *Ae. aegypti*, *Cx. pipiens quinquefasciatus*, *Ix. scapularis*, *An. funestus*, and *Glu. morsitans*, and possibly more limited data sets for additional vector species. In addition to the genome components modules of VectorBase will be provided by the EMBL in Heidelberg, Germany, the Institute of Molecular Biology and Biotechnology in Heraklion, Crete, the FlyBase group at Harvard University, and the Center for Tropical Disease Research and Training at the University of Notre Dame. These institutions will also represent a major conduit to the scientific community that will facilitate use of VectorBase, also to include specific training. The VectorBase partners will also help develop the level of coordinated scientific interest in a particular vector that could lead to a genome project.

Interpretation: VectorBase will help analyze genes involved in immune responses, host seeking behavior and insecticide resistance; together with pathogen genome data, VectorBase will lead to a better understanding of the interplay between vector and pathogens.

**O-71**

**Systems biology of the *Anopheles gambiae* innate immunity [MIM-GC-22590]**


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**Introduction:** Our work aims at understanding of the mosquito immune system and its interactions with the malaria parasite *Plasmodium*. We have shown that innate immunity has a key role in mosquito’s vectorial capacity: it accounts for extensive parasite losses during invasion of the midgut, but also allows immune evasion. We have elucidated key reactions, including parasitic lysis and melanisation, at the molecular and cellular level, and begun to dissect regulatory networks underlying these immune reactions.

**Methods:** Comparative bioinformatic analysis of insect genomes identified putative components of the mosquito innate immune system. Three *A. gambiae* cDNA microarray platforms, 4 K (ca. 2500 EST contigs), MMC1 (ca. 9000 EST contigs) and MMC2 (ca. 12,500 predicted genes), were constructed and utilized to study the transcriptional responses of the mosquito genome following bacterial and *Plasmodium* infections. Genes of interest were silenced in adult mosquitoes and cell lines as previously described (Blandin et al., 2002). Knockdown (KD) mosquitoes were assayed for compromised immunity against bacteria, and for parasite survival to the oocyst stage. Combina
tion of RNAi and genome-wide expression profiles were used to detect regulatory immune networks.

**Results:** The *A. gambiae* REL2 gene, which is orthologous to Drosophila Relish, produces two isoforms through alternative splicing. REL2-S structurally resembles the Drosophila Dif, which is absent from the mosquito, and mediates responses to Gram-negative bacteria, while REL2-F and IMD are involved in defense against Gram-positive bacteria, together illus-
trating a surprising switch in pathway function between mosquitoes and flies. Interestingly, the IMD/REL2-F pathway also regulates the intensity of mosquito infection with Plasmodium. Microarray analysis identified several genes regulated by REL2, including Cecropin 3, Gambicin and LRIM1. The latter is also a strong antagonist of parasite survival in the mosquito, as its KD leads to three to five-fold increase of oocyst numbers. In addition, LRIM1 mediates parasite melanisation in mosquitoes deficient for CTL4, a protein that together with other immune molecules support parasite survival. LRIM1 encodes a haemolymph protein that is strongly induced after bacterial and malaria infections. Its expression throughout mosquito development correlates with that of other immune genes, and its KD affects the expression of numerous other genes, all of which may belong to a regulatory immune network.

**Interpretation:** Mosquito innate immunity plays a central role in malaria transmission efficiency, interacting both positively and negatively with the parasite. Understanding the nature of these interactions could open new possibilities to disease control.

O72  
**Genetic polymorphism in cacophony and period genes within the M and S molecular form of African malaria vector Anopheles gambiae [MIM:CB-285285]**

C. Brengues, A. Diabaté, K. Dabire, F. Simard, D. Fontenelle, P. Kengne  
(1) Institut de Recherche pour le Développement (IRD), Montpellier, France; (2) Institut de Recherche en Science de la Santé (IRSS), Bobo-Dioulasso, Burkina Faso; (3) Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroon

**Introduction:** Among others mating behaviour factors, acoustic signals are known to be very important in the reproductive isolation of closely related species in insects. At pre-copulatory level, genes like cacophony would play important role in the reproductive isolation of the two molecular forms of *Anopheles gambiae*. In this study, we investigated the extent of genetic divergence in cacophony and period genes between wild populations of the two forms captured in the swarms.

**Methods:** Entomological survey was conducted from July to December 2004 in the Vallée du Kou of Burkina Faso, a rice cultivation area where the M and S molecular forms of *A. gambiae* were found sympatric. Mosquitoes were collected, using insect net, from the swarms formed in the evening, 5–10 min after the sunset. Mosquitoes were sorted using morphological key and *A. gambiae* genomic DNA was extracted from whole individual or legs. Species and molecular forms were determined using standard PCR-based diagnostic tools. Target genes (cacophony and period) were amplified and sequenced using primers selected from the Pest strain genome database. Sequences were aligned with Clustal X software and statistical analysis conducted using MEGA 2.1 software.

**Results:** *A. gambiae* M and S molecular forms were found in sympatry with a near-absence of hybrids (1%) in the Vallée de Kou. Although swarms were composed exclusively of males of the same form, a number of mated anopheles was found after fine-scale observation of swarms. All paired mosquitoes belonged to the same form. As preliminary results, sequence comparison of cacophony IVS6 intron performed on a limited number of the two molecular forms found some variations. Mean genetic distance between M and S molecular form was two-fold the mean genetic distance (*d = 0.0118 ± 0.008*) within form. Eight of 10 registered polymorphic sites were parsimony-informative. Although no fixed differences were observed in a molecular form, the number of polymorphic sites shared (*n = 2*) is smaller than the number of exclusive polymorphic sites (*n = 6*). These results are consistent with the process of incipient speciation. Period, another gene involved in the production of acoustic signals in drosophila was also analysed. Sequence analysis revealed less (4) polymorphic sites that were not partitioned by molecular form.

**Interpretation:** Preliminary results of this study revealed differences in frequencies of polymorphic loci of love song genes but failed to find fixed differences between forms. More data would allowed to address this question of sexual isolation using acoustic gene.
O-73
Host–parasite interactions involved in erythrocyte invasion by malaria parasites: Role of the erythrocyte binding protein family
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Introduction: Red cell invasion by malaria parasites is mediated by a family of erythrocyte binding proteins (EBP) including Plasmodium vivax and P. knowlesi Duffy binding proteins (PvDBP and PkDBP) and P. falciparum EBA-175. The EBP family share conserved cysteine-rich receptor-binding domains called Duffy-binding-like (DBL) domains. We have used genetic, molecular and biochemical approaches to study the functional role of the EBP family of proteins in red cell invasion.

Methods: We used transfection to delete the α gene encoding PkDBP. P. knowlesi was transfected with a construct designed to knockout the α gene by recombination upon selection for pyrimethamine resistance. The invasion phenotype of cloned P. knowlesi knockout (PkKO) parasites was analyzed using in vitro invasion assays and electron microscopy. Receptor-binding DBL domains of PvDBP and PkDBP were expressed as recombinant proteins in mammalian cells as well as Escherichia coli and used in receptor binding functional assays. Site-directed mutagenesis and proteolytic cleavage of recombinant DBL domains were used to map binding sites within DBL domains. Antisera raised against DBL domains were tested for inhibition of invasion in vitro.

Results: PkKO parasites do not invade human erythrocytes following loss of the Duffy binding protein although they invade chimp monkey erythrocytes by other pathways. Electron microscopy demonstrated that PkKO parasites are unable to form a junction with human erythrocytes leading to abortion of invasion at this step. The receptor-binding DBL domains of PvDBP and PkDBP contain ~330 amino acids with 12 conserved cysteines, whereas, the receptor binding DBL domain of EBA-175 contains 14 conserved cysteines. Truncated DBL domains containing cysteines 5 (C5)-C8 of PvDBP and C5-C10 of EBA-175 bind red cells with the correct specificity. Site-directed mutagenesis of residues in the central C5-C8 region of PvDBP followed by quantitative binding assays demonstrated that hydrophobic residues play a key role in interaction with the Duffy antigen. Whether hydrophobic residues play an important role in binding of other DBL domains remains to be determined. Given the critical functional role of EBPs in invasion, we tested the ability of antisera raised against the receptor-binding DBL domains to block invasion. Antisera directed against binding domain of EBA-175 blocked invasion by P. knowlesi and P. falciparum in vitro.

Interpretation: The EBP family mediates junction formation during invasion. Hydrophobic residues in central region of DBL domain of PvDBP make contact with Duffy antigen. Antisera against DBL domains of EBPs block invasion, providing support for their inclusion in malaria vaccines.

O-74
Use of plasma proteomics to discriminate between malaria infected mice from non-infected control mice [MIM-EG-111840]
E. Gitau, G. Kokwaro, C. Newton, S. Ward
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Introduction: Animal models of neurological involvement have been developed to provide insight into the pathogenesis of cerebral malaria (CM) in humans. Such a model can be induced in susceptible strains of mice by Plasmodium berghei ANKA strain. We have used a global proteomic strategy to identify differentially expressed proteins in this murine model of CM in the hope of facilitating future development of novel diagnostic, disease monitoring and treatment strategies.

Methods: Mice (4-week-old CD1 male mice) were infected with P. berghei ANKA strain, and infection allowed to establish until a mean parasitaemia of 30% was attained. Total plasma and albumin depleted plasma samples from infected and control (non-infected) mice were separated by two-dimensional gel electrophoresis (2-DE). After staining, the gels were imaged and differential protein expression patterns were interrogated using image analysis software. Spots of interest were then digested using trypsin and the...
proteins identified using matrix-assisted laser desorption and ionisation-time of flight (MALDI-TOF) mass spectrometry (MS) and peptide mass fingerprinting software.

**Results:** Master gels of control and infected mice, and the corresponding albumin depleted fractions had exhibited distinctly different 2D patterns between control and infected plasma, respectively. Differentially expressed proteins identified included common circulating proteins such as albumin and apolipoproteins, blood transporters and binding proteins, protease inhibitors, enzymes, cytokines and hormones, and channel and receptor-derived proteins.

**Interpretation:** Plasma proteomics can be used to discriminate malaria-infected mice from non-infected control mice. We are exploring the utility of this technique in discriminating between plasma from infected and healthy human subjects.

12. Priorities in social and economic aspects of malaria research and control

**Tuesday 15 November 14:30–16:30—Mahogany Hall**

Chairs: Martin Alilio (Washington, DC) and Anne Mills (London)

**O-75 The challenges of scaling up malaria control [MIM-AM-376420]**

A Mills

Health Economics and Financing Programme, London School of Hygiene and Tropical Medicine, UK

**Introduction:** Achieving the MDGs is focusing attention on the need to greatly scale-up access to malaria prevention and treatment interventions. It is well recognised that weak health systems hamper scaling up efforts, but the solutions are unclear, disputed, and weakly supported by evidence. Competing solutions encompass a broad health systems strengthening approach; focusing efforts on a limited package of highly cost-effective interventions; and highly targeted, disease specific efforts.

**Methods:** This presentation draws on the work of the Disease Control Priorities Project (DCPP), which is producing evidence-based analyses and materials to inform policy-making in developing countries. DCP-2, available early 2006, reviews and summarises the evidence on the cost-effectiveness of interventions, service packages and levels of care against a variety of diseases and conditions, and addresses how the health system can be strengthened in order to deliver interventions effectively, efficiently and equitably. This presentation draws on the chapter on malaria, and especially on the overview chapter on strengthening health systems which draws on the evidence in the health systems-related chapters of the volume and on other published evidence.

**Results:** The presentation reviews the historical evidence on how to improve health outcomes through health sector action, in particular addressing the vertical/horizontal debate and its implications. Recent literature is used to summarise the main constraints to scaling up malaria control. The presentation then evaluates the evidence on what changes might need to be made in health systems to increase their capacity, drawing on DCP-2 chapters to identify key functions and areas that need strengthening, including general management, human resources, and quality assurance. Evidence is reviewed on the instruments of policy available to shape service delivery towards health system goals of efficiency and equity, including action at both systems and service delivery levels. Research priorities are identified. Conclusions emphasise that while health systems face numerous constraints in low income countries, they represent the long term future of sustained health improvements. Where capacity constraints are such that a focused disease or programme specific effort is desirable to address an urgent problem such as malaria, such a programme should be designed in such a way that it contributes to long term system strengthening.

**Interpretation:** Reforms affecting organisational structures and human resources are especially important. Capacity strengthening efforts must encompass action at all levels, from leadership roles at national level, through to strengthening support to the periphery.
O-76
Priorities in economic aspects of malaria research and control [MIM-OO-294570]

O. Onwujekwe
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Introduction: The economic priorities of malaria should be targeted at interventions chosen by African heads of States as well as those with proven effectiveness that are important for achieving the millennium development goals and significantly decrease the burden of malaria in short to medium term, as well as in the long-term. Hence, this presentation describes the priorities in economic aspects of malaria research and control.

Methods: Review of literature, consultation with key researchers and programme managers as well as personal experiences were the study tools.

Results: The priorities in research include economic and equity analyses of malaria control tools and of access and availability of high priority effective control tools in different settings, research on how to strengthen the health systems for sustainable provision of malaria control strategies. The priorities also include the establishment of the costs, budgetary requirements and short to long-term funding sources, strategies for sustainable and equitable financing of malaria control, analysis of environmental management and associated multi-sectoral collaboration, as well as economics of public–private partnerships for malaria control and economics of improving diagnosis and treatment at the PHC level. Within the sphere of control, the priorities are costing and budgeting for the resources that are needed for control activities. There should be continuous monitoring and evaluation of costs and cost-effectiveness ratios, benefit incidence analysis and financing strategies of the control interventions during and after implementation. The cost of getting Research into Policy and Practise (GRIPP) and scaling-up of high priority interventions of proven effectiveness or cost-effectiveness that would enhance the achievement of MDG.

Interpretation: Economic analyses of scaling-up of interventions, strengthening case management at the PHC level, GRIPP and multi-sectoral approach including environmental management are priorities in economics aspects of malaria research and control.

O-77
Community’s perceptions and use of antimalarial drugs in the home management of malaria in rural Tanzania [MIM-JM-107371]

J. Msechu, M. Hetzel, B. Obrist, A. Makemba, C. Lengeler, H. Mponda, K. Sono, H. Mshinda
(1) Ifakara Health Research & Development Centre, Ifakara, Tanzania; (2) Swiss Tropical Institute, P.O. Box, 4002 Basel, Switzerland

Introduction: Malaria remains the leading cause of mortality and morbidity in Tanzania and home management of fevers is a crucial part of any effective malaria control intervention. We carried out an in-depth assessment of people’s perceptions and treatment seeking for malaria in 2004. Our assessment is part of a social marketing based project aiming to improve people’s access to prompt and effective malaria treatment in a rural and highly endemic area.

Methods: A total of 500 households were sampled for the assessment. 300 households were selected from 20 villages under full demographic surveillance, while a further 200 households were randomly sampled from a semi-urban area. In total, 156 households met the inclusion criteria: having at least one child under 5 years and having a family member who had fever within the past 2 weeks. Detailed data on perceived causes, patterns of distress and treatment for malaria were collected using a locally adapted exploratory model interview catalogue (EMIC) administered to caretakers.

Results: A total of 80 interviews of children fever episodes were carried out. Sixty-three (78.8%) cases were detected while family members were in their main home, of which 94% reportedly received an antimalarial drug. The remaining 17 cases (21.2%) were detected while the family was in a secondary home in the fields and there, only 59% were reported to have received an antimalarial drug. A considerable number of caretakers (58%) said malaria was not a serious illness while only 22% thought it is serious and can be fatal. With regard to antimalarial drugs, 48.8% and
15.0% originated from private health facilities and drug shops, respectively, while the remaining (36.2%) were obtained from neighbors and relatives. Quinine was used first in 51% of the cases that received an antimalarial, while the rest received SP, Amodiaquine and Chloroquine (30.0%, 17.5% and 1.5%, respectively).

**Interpretation:** Use of antimalarial drugs at home is very popular but there is lack of access to recommended drugs (SP). Caretakers' knowledge, place of recognition and perceived severity are crucial determining factors of treatment seeking and need to be improved.

**O-78**

L’observance des traitements antipaludiques au Sénégal: Le rôle des différents dispensateurs de traitements [MIM-TN-156048]

T. Ndoye

Institut de Recherche pour le Développement (IRD)-Ecole des Hautes Etudes en Sciences Sociales (EHESS-SHADYC), Dakar, Marseille

**Introduction:** Au Sénégal, les stratégies de prise en charge du paludisme prolifèrent dans le temps. On note un changement graduel des molécules, surtout dans les traitements de première ligne: on est ainsi passé de la chloroquine à l’association amodiaquine/sulfadoxine pyriméthamine (SP). L’annonce est par ailleurs faite qu’un autre passage va bientôt s’opérer, de la formule amodiaquine + SP aux dérivés de l’artémisinine.

**Methods:** Nous avons mené des enquêtes qualitatives de 2002 à 2004 durant les périodes hivernales, moments d’endémicité du paludisme au Sénégal, en milieu urbain: enquêtes poursuivies d’une incursion en milieu rural lors de la saison des pluies 2004. Elles ont consisté en l’observation des interactions entre soignants et soignés au sein des structures de santé et à des intervenants en différents acteurs intervenant dans la lutte contre le paludisme (soignants, patients, responsables de programmes, acteurs des comités de santé, etc.). Les enquêtes ont porté sur 6 structures de santé à raison de 2 mois par structure et ont concerné plus d’une centaine de personnes.

**Results:** Ces enquêtes nous apprennent qu’à-delà de l’importance du médicament dans la prise en charge du paludisme, d’autres facteurs intervennent dans l’observance des traitements et conséquemment dans le rétablissement des patients. Les perceptions populaires voient dans des signes - médicalement liés à des formes de complexifications du paludisme - les symptômes d’une autre maladie: le pays (entité nosologique de la fièvre jaune). Ce qui oriente les patients vers les guérisseurs. L’incapacité de la médecine moderne - selon les conceptions populaires - à venir à bout de la maladie ainsi désignée fausse les directives en faveur d’un recours précoce en cas d’accès palustre. De leur côté, les guérisseurs consultés - souvent en premier recours - pour déterminer la nature de l’affection et la traiter élaborent un discours suffisamment persuasif pour retenir leur clientèle. Le travail des guérisseurs, celui des délégués médicaux et des vendeurs ambulants de médicaments permettent de comprendre certains mécanismes d’observance et surtout de non observance. En l’absence d’un vaccin efficace, la prise en compte de ces facteurs - essentiellement socioculturels - peut contribuer de façon décisive à une bonne maîtrise de la maladie.

**Interpretation:** Il y a différentes étapes, différentes offres de soins qui chacune peuvent influer sur l’observance du traitement et donc une approche du déroulé du parcours du malade est indispensable pour une approche de santé publique adaptée.

**O-79**

How local community knowledge about malaria affects insecticide treated net use in northern Ghana [MIM-PA-238950]

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**Introduction:** Large-scale trials of insecticide treated nets (ITNs) throughout sub-Saharan Africa demonstrated that they reduce child mortality in malaria endemic countries. However, regular utilization of ITNs under routine or non-project conditions has been beset with several problems.
Methods: This paper explores how local community knowledge about malaria act as a barrier to the use of ITNs in three different settings. This study utilized both qualitative and quantitative methods including participant observation, structured formal observation and a range of interviewing techniques which included informal interviews, focus group discussions, semi-structured in-depth interviews, and structured survey interviewing.

Results: People recognize the term ‘malaria’ but have limited biomedical knowledge of the disease, including its aetiology, the role of the vector, and host response. Also, severe forms of malaria (convulsions and anaemia) are rarely linked to it.

Interpretation: The people acknowledged a role for ITNs in nuisance reduction, but not for malaria prevention.

O-80 Public health campaigns’ dilemma: Field experience about malaria control in a rural setting, northwestern Tanzania [MIM-SN-36540]
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(1) National Institute for Medical Research, Mwanza, Tanzania; (2) London School of Hygiene and Tropical Medicine, UK; (3) DBL Institute for Health Research and Development, Denmark; (4) Institute of Anthropology, University of Copenhagen, Denmark

Introduction: We explored the dilemma in implementation of public health anti malaria campaigns, caused by ambivalence towards mosquito insecticides and anti-malarial drugs.

Methods: This study is based on 17 months’ fieldwork that took place from August 2003 to December 2004. The fieldwork took place in a rural ward in Sengerema district, near Lake Victoria in North-western Tanzania. It relied on multiple data collection methods, participant observation being the main technique that was employed throughout the fieldwork.

Results: Despite ongoing national public health campaigns, with their emphasis on the importance of ITN for malaria control, re-treatment of bed-nets in rural settings remains at a very low level. In this study most survey respondents reported to have heard about mosquito insecticides, and also thought malaria a major health problem. However, the majority of those who knew about insecticide (NGAO) did not retreat their bed-nets. Low re-treatment rates were often due to the cost, and/or inaccessibility of insecticide. But, even those who could afford the price, and who knew where to obtain insecticides, did not buy them. Apart from cost and accessibility factors, other critical concerns which inhibited re-treatments were (i) a fear that the insecticides were toxic; (ii) doubts about the efficacy (“insecticide had no strength to kill mosquitoes”), (iii) conspiracy between politicians, and scientists for personal gains, (iv) little motivation (‘don’t have good reason’). Similar concerns were expressed about sulphadoxine–pyrimethamine (SP).

Interpretation: The paper will address these concerns, and their implications for the promotion of pharmaceutical products through public health campaigns.

13. Clinical presentation and diagnosis of malaria in children
Wednesday 16 November 11:00–13:00—Bubinga Hall
Chairs: Brian Greenwood (London) and Zul Premji (Nairobi)

O-81 Progress and challenges in the diagnosis of severe malaria
K.A. Bojang
MRC Laboratories, Banjul, The Gambia

Prompt and accurate diagnosis of malaria is an important part of effective case management. Traditionally, diagnosis of severe malaria has been made on the basis of clinical symptoms and microscopic detection of malaria parasites. Unfortunately, neither method is entirely satisfactory. In malaria-endemic areas, a proportion of the population infected with *P. falciparum* parasites remain asymptomatic. Thus, it is often difficult to determine if the parasites in the peripheral blood of a symptomatic individual are causing the symptoms or are merely incidental. Moreover, severe malaria in particular cerebral malaria is not a homogenous condition, but rather a collection of syndromes presenting with a range of clinical features which varies in different age groups.

Recently, it has been shown that ocular fundus findings associated with malaria retinopathy, distinguished
those with histological evidence of parasite sequestration from those without. This finding and other recent developments in diagnosis of severe malaria will be discussed.

O-82

Severe malaria in African children (SMAC): An experiment in multi-center studies [MIM-TT-3960]

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Introduction: The first clinical trials network dedicated to African children with severe malaria was established in 1999. Initial activities included a surveillance study of all parasitemic children admitted to each hospital, an evaluation of malaria pigment as a prognostic feature, and a pilot study of pentoxifylline as adjunct treatment. Future activities include validating the malarial retinopathy and mortality-based studies of new interventions for severe malaria.

Methods: SMAC research activities are developed by the group as a whole. Administrative activities are coordinated through a core facility at Michigan State University, and include disbursing funds (via subcontracts) to each site, developing protocols and tracking their progress, maintaining compliance with ethical review committees, arranging travel, and shipping supplies and equipment. The data management core is based at one site; data managers at each site supervise local data collection and entry, and files are submitted on a monthly basis to the core. The network data coordinator is responsible for developing databases, cleaning the data, and coordinating analyses.

Results: Between December 2000 and December 2003, 20,333 patients were enrolled. The frequency of severe malaria syndromes (cerebral malaria, severe malarial anemia and acidosis) differed between sites, as did the syndrome-specific mortality rates. Handheld glucose and lactate analyzers provided reliable data. Blood gas analyzers were expensive and difficult to sustain; nearly equivalent prognostic information was available using clinical observations and blood glucose concentration. Incidental parasitemias contributed to false positive diagnoses of cerebral malaria; including ocular fundus findings may improve the specificity and positive predictive value of the assessment. An intervention study, using a two-sided 0.05 level test, powered at 80% and addressing studying patients with hyperlactatemia would need to enroll 5250 patients to detect a 20% decrease in mortality. If patients with cerebral malaria were targeted, 2958 patients would need to be enrolled to detect a 20% decrease in mortality. For a network-wide intervention involving one of these target groups, 3–4 years would probably be required to enroll a sufficient number of patients.

Interpretation: Standardized data will improve descriptions of the patterns of severe malaria and estimates of disease burden, support the generation of hypotheses about pathogenesis and treatment, and permit more precise sample size calculations.

O-83

A multicenter, prospective study of intraleukocytic and intraerythrocytic pigment as prognostic features in African children with falciparum malaria [MIM-AM-33336]


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Introduction: Life-threatening malaria can develop rapidly in children. Quick and simple identification of potentially severe cases is important in case management. Malaria pigment in white cells is easily identifiable and may be associated with outcome by reflecting total parasite load or by interfering with immunological response of leukocytes. We examined intraleukocytic and intraerythrocytic pigment for their role as prognostic markers of severity in African children with malaria.

Methods: Children with asexual *Plasmodium falciparum* parasitemia admitted to the pediatrics ward in six participating hospitals of the Severe Malaria in African Children network (The Gambia, Ghana, Gabon, Kenya, and Malawi) were included. On admission we collected demographic, clinical, medical history and laboratory data. Malaria pigment was assessed in a Giemsa stained thick blood smear. Two hundred polymorphonuclear leukocytes, 200 mononuclear leukocytes and 200 parasitized red cells were counted, and, in the process, the number of pigment globules in each cell type was noted. The primary endpoint was clinical outcome (died versus survived). We assessed whether presence or absence of pigmented cells or the percentages of cells with pigment predicted death.

Results: Between December 2000 and December 2003 we enrolled 20,262 patients, 892 of whom died (4.4% mortality rate). Of the 20,262, 67% had at least one pigmented cell (polymorphonuclear leukocytes, mononuclear leukocytes, or red blood cells). The proportion of patients with at least one pigmented cell of any type was significantly different across sites (P < 0.001, chi-squared test), with the lowest rates in Kenya and Malawi (57% in each) and the highest in Libreville, Gabon (94%). Overall, 492 out of 13,422 patients (4.7%) with pigmented cells on admission died, versus 262 out of 6580 patients (4.0%) without pigmented cells on admission. However, there was significant heterogeneity in the association between presence of pigmented cells and mortality across sites (P = 0.003, Mantel-Haenszel test of homogeneity), with odds ratios ranging from 2.4 (95% CI, 1.5-3.7) in Malawi to 0.6 (95% CI, 0.3-1.3) in Libreville, Gabon.

Interpretation: This preliminary analysis suggests that proportion of subjects with pigmented cells and the ability of pigmented cells to predict death varies for different sites across Africa. Further analyses will determine if pigmented cells could predict death.

O-84
The effectiveness of rapid test compared to blood slide diagnosis in guiding prescription of antimalarial treatment [MIM-HM-7064]

H. Mbukisho, H. Reyburn, O. Mwerinde, R. Msangi, S. Shilcutt, A. Mills, C. White, C. Drakeley
(1) Joint Malaria Programme, Moshi, Tanzania; (2) London School of Hygiene and Tropical Medicine, London, UK

Introduction: The introduction of relatively expensive and scarce artemisinin combination therapies for malaria in Africa raises the need to review how malaria is diagnosed in order to more effectively target treatment. There have been suggestions to make rapid diagnostic tests (RDTs) more widely available to achieve this but there is evidence that blood slide diagnosis often does not guide treatment and there are no studies on whether this will also apply to diagnoses made using RDTs.

Methods: We are conducting a study in three outpatient clinics at low, moderate and high levels of *P. falciparum* transmission. Patients who were suspected of having malaria were randomised to be tested by either Paracheck™ or by routine hospital blood slide. Reference slides were taken on all. We aimed to recruit 800 patients to the trial in each site, the timing aimed to capture peak transmission. Data on presenting symptoms and socio-economic characteristics were collected on participants and their willingness to pay for tests was assessed. The primary outcome measure was the prescription of an antimalarial with a negative test result, the secondary outcome was the cost of diagnosis by each method and treatment prescribed.

Results: To date (April 2005) we have completed the study in the low transmission site. Over a 2-month period 824 patients who had been sent for a malaria test were recruited to the study. Four hundred and eighteen were reported as negative by blood slide of whom 227 (54.4%) were treated with an antimalarial compared to 403 patients tested negative by RDT of whom 168 (41.7%) were treated with an antimalarial (p = 0.3). Overall only four patients were reported as positive by either test. In a multiple logistic regression model only being under the age of 15 years was predictive of being treated for malaria with a negative test result (p = 0.01).
An additional 13 patients were given antimalarial treatment with no test request.

**Interpretation:** Our data suggest the introduction of RDTs to improve targeting of antimalarials needs to be accompanied by a focus on prescribing behaviour. Full results of the study will be available by July 2005.

**O-85**

Morbidity and co-morbidity in children with signs of severe malaria [MIM-JB-166260]


Centre for Geographic Medicine Research (coast), Kenya Medical Research Institute (KEMRI), PO Box 230, Kilifi, Kenya

**Introduction:** Attributing disease to falciparum malaria can be difficult because the clinical features of malaria overlap with other illnesses, microscopy may be unavailable or unreliable and children may have asymptomatic parasitemia. We aimed to determine the frequency and types of morbidity and co-morbidity amongst hospitalised children with clinical signs of severe malaria and to examine the effect of co-morbidity on community-based estimates of the burden of malaria.

**Methods:** From 1998 to 2002, we prospectively investigated all children age >59 days admitted to Kilifi district hospital with fever plus one or more of impaired consciousness, respiratory distress or severe anaemia. Blood was drawn for culture, malaria slide and full blood count. Lumbar puncture was guided by a clinical protocol. To describe co-morbidity, we first identified those with meningitis or bacteremia, then those severely underweight or kwashiorkor then other significant clinical diagnoses other than malaria, recorded at death or discharge. We calculated the incidence of admission with the signs of severe malaria, using data from admissions resulting in the hospital catchment area, under a continuous demographic surveillance system (DSS).

**Results:** 3362/17,301 (19%) admissions had signs compatible with severe malaria. In 294 (8.7%), the blood culture was contaminated or missing—these data were excluded. Of 3068 admissions analysed, 2048 (67%) had malaria parasitemia: 1986 seen on the first slide, 35 on the second and 28 on the third. A co-morbidity was identified in 498/2048 (24%) children (13% at high parasite density to 46% at low parasite density \( p < 0.001 \)). Co-morbidities included 145 (7.1%) with an invasive bacterial infection 211 (10%) with severe malnutrition and clinically diagnosed co-morbidities including LRTI and gastroenteritis in 142 (6.9%) children. Amongst those with co-morbidity, 90/744 (12%) died compared with 83/1304 (6.4%) without (age adjusted odds ratio 2.03 [95%CI 1.48–2.77]). Between 37% and 71% of the deaths in admissions with signs of severe malaria and parasitemia were associated with a significant co-morbidity depending on parasitemia \( (p=0.004) \). The community-based incidence of admission with severe malaria per 100,000 per year in children <10 years was 974 for admission with clinical signs of severe malaria, 664 for clinical signs plus parasitemia and 425 for clinical signs plus parasitemia without significant co-morbidity.

**Interpretation:** Our results highlight the complexity of severe disease in children in sub-Saharan Africa, and support approaches to clinical management and measurement of the burden of disease that recognise multiple risks rather than single diagnoses.

**O-86**

Diagnostic présomptif d’accès palustre et positivité de la épaisse dans un centre goutte de santé périphérique d’Abidjan (Côte d’Ivoire) [MIM-AT-46896]

A. Offuman, L. Penali, D. Khali, G. Opportune, O. Makilala, G. Beugre

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**Introduction:** En zone d’endémie, le traitement du paludisme est donné à la suite d’un diagnostic clinique basé essentiellement sur la fièvre. La conséquence de cette stratégie de lutte antipaludique est le traitement comme accès palustres de certaines fièvres d’autres origines avec des molécules plus onéreuses et moins bien tolérées. Le but de la présente étude est de comparer le diagnostic clinique présomptif et le diagnostic biologique du paludisme dans un centre de santé périphérique.

**Methods:** 4393 patients venus en consultation du 1er février 2003 au 31 janvier 2004 pour fièvre, avec
ou sans signes d’accompagnement, chez qui le diagnostic présomptif d’accès palustre était posé par le médecin après interrogatoire, examen physique et recherche négative des points d’appel infectieux ont bénéficié d’une goutte épaisse et d’un frottis sanguin. Les patients ayant reçu dans les 30 jours précédents ou entrain de recevoir un traitement antipaludique ont été exclus. Une lame était positive si au moins un trophozoïte était présent, et négative si aucun trophozoïte n’était décelé après examen de 50 à 100 champs microscopiques.

**Results:** Au total 2312 sur 4393 patients recrutés présentaient une goutte épaisse positive soit un taux de 52,63%. Le taux d’erreur par excès du diagnostic présomptif du paludisme de notre étude est donc de 47,37%. 80% des antipaludiques prescrits en première intention étaient représentés par les dérivés de l’artémisinine seuls ou en association en particulier. Ce taux d’erreur reste élevé si on considère que nous n’avons pas fixé de seuil pyrogène. La fiabilité du diagnostic uniquement clinique de l’accès palustre, même par un médecin expérimenté est limitée. Une goutte épaisse doit être obtenue chaque fois que possible. Sa positivité est un critère de confirmation nécessaire. Mais il peut s’agir de la survenue d’une fièvre non palustre chez un porteur asymptomatique de Plasmodium où l’intérêt d’un seuil pyrogène.

**Interpretation:** Le traitement du paludisme doit être basé sur un diagnostic de certitude afin de protéger les nouvelles molécules. En zone d’endémie où les méthodes de diagnostic ne sont pas toujours disponibles le traitement présomptif des fièvres reste approprié.

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**O-87**

**Lack of utility of clinical algorithms in malaria diagnosis among people of different age groups [MIM-TM-253344]**

T. Mwangi, M. Mohammed, H. Dayo, R. Snow, K. Marsh

**Introduction:** In the absence of malaria diagnostic facilities, the Integrated Management of Childhood Illnesses guidelines attempt to ensure that all young children at greatest risk (<5 years), receive appropriate treatment. Among older children and adults there are no accepted treatment guidelines and though at much lower risk of serious morbidity, they often account for higher total drug use. The study seeks to investigate whether clinical algorithms would target anti-malarial treatments to those in need.

**Methods:** A total of 1602 people of all age groups were recruited into this study for a period of 2 years. Smears were taken and clinical signs and symptoms (prompted or spontaneous) recorded among all those presenting to the study clinic with a history of fever. A malaria case was defined as a person presenting to the clinic with a history of fever. A malaria case was defined as a person presenting to the clinic with a history of fever and an accompanying parasitaemia. A set of clinical signs and symptoms (algorithms) with the highest sensitivity and specificity for diagnosing a malaria case was selected for the age groups ≤5 years, 6–14 years and ≥15 years.

**Results:** These age-optimised derived algorithms were able to identify about 66% of the cases among those <15 years old but only 23% of cases among adults. Were these algorithms to be used as a basis for a decision on treatment among those presenting to the clinic, 16% of children ≤5 years, 44% of those 6–14 years of age and 66% of the adults who had a history of fever and parasitaemia ≥5000 parasites/µl of blood would be send home without treatment.

**Interpretation:** Clinical algorithms appear to have little utility in malaria diagnosis, performing even worse in the older age groups where avoiding unnecessary anti-malarial use would ensure more drugs were available to the needy population of children under 5.
14. Vaccine development

Wednesday 16 November 11:00–13:00—Ebony Hall

Chairs: Alioune Dieye (Dakar) and Ricardo Thompson (Maputo)

O-88
Polymorphic malaria antigens and polyvalent malaria vaccines—A challenge for translational research and development [MIM-DC-167760]

D. Conway
(1) MRC Laboratories, Fajara, P.O. Box 273, Banjul, The Gambia; (2) Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK

Introduction: A vaccine to protect against malaria is still awaited after decades of effort. Repeatedly infected individuals acquire partial but life saving immunity to blood stage malaria parasites, which is largely antibody mediated. Many antigens of the antibody-accessible stages (merozoite and infected erythrocyte) are polymorphic, and there is evidence that some of the polymorphisms are maintained by natural selection from acquired immune responses that effectively kill the parasite.

Methods: Molecular population genetic and evolutionary analyses, combined with immunological and epidemiological studies, can identify which malaria parasite protein antigens are under the strongest natural immune selection. For some of these, it is then possible to incorporate the necessary polymorphic epitopes into polyvalent immunogens that can prime immune responses to most or all of the existing allelic forms. Different approaches and technical platforms are being tried, foremost of which are mixtures of recombinant proteins representing different serotypes or variants, and recombinant proteins that express designed synthetic sequences.

Results: Examples of malaria parasite antigens for which these approaches are showing promise will be briefly reviewed, with a focus on most recent results. An increasing number of studies are giving concordant results that identify the principal targets of antibody-mediated immunity, a substantial proportion of which are polymorphic targets. Recent vaccine design and development efforts that incorporate these findings are showing some promise. Such candidates are relatively new in malaria vaccine development, compared to longstanding candidates that were purposely based on conserved protein sequences. The demonstrated efficacy of multiple-serotype pneumococcal and meningococcal vaccines, and innovative approaches to develop vaccines against polymorphic viruses, provide further encouragement for these approaches.

Interpretation: The evidence suggests it would be expedient to focus more efforts on the design and production of polyvalent vaccines that induce immune responses to different allelic forms of blood stage proteins that are targets of naturally acquired immunity.

O-89
Development of an anti-toxic vaccine against malaria [MIM-LS-150738]

L. Schofield
The Walter and Eliza Hall Institute of Medical Research, Australia

Introduction: A toxic basis for malaria pathogenesis and the existence of anti-toxic immunity have gained firmer footing with the elucidation of glycosylphosphatidylinositol (GPI) as a bioactive toxin of Plasmodium falciparum origin. This molecule regulates the expression of multiple host loci implicated in acute and severe malaria pathogenesis, e.g. TNF, IL-1, IL-6, IL-12, iNOS, ICAM-1, E-selectin, etc. We have sought to develop a vaccine against this target with a view to prevention of fatalities in humans.

Methods: The non-toxic oligosaccharide of the core glycan of GPI was synthesized through two routes using semi-automated solid phase chemistry. Various linker chemistries were utilized to couple the synthetic lead compound to generic carriers in specific orientation. Formulation was undertaken in different adjuvants. Dose ranging and immunogenicity studies were undertaken in small animals, and efficacy testing was determined using P. berghei ANKA, the best available experimental model of severe malarial pathogenesis.

Results: GPI was shown to be immunologically non-self in mice and humans. The synthetic conjugate oligosaccharide vaccine displayed convincing efficacy against diverse disease syndromes in pre-clinical models. Histological and biochemical correlates of disease
were also abrogated. Microarray analyses of disease states were undertaken. Interpretation: The data provide proof-of-principle for the development of an anti-toxic vaccine against malaria. A route to the production of this lead to cost and scale is now being developed in a joint venture between academia, biotech, Big Pharma and government.

O-90 Immune responses of Ugandans and inhibitory properties of rabbit antiserum to a fragment of the interspecies conserved P. falciparum antigen (Pfp70) [MIM-PV-88704]

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Introduction: Immune responses of Ugandans to Plasmodium falciparum infection and the in vitro inhibitory properties of rabbit antiserum to a 35 kDa recombinant antigen from the interspecies conserved antigen (Pfp70) of P. falciparum were investigated. The P. falciparum antigen Pfp70 (Genebank Accession no. X91661) is a novel 70 kDa interspecies conserved merozoite apical antigen expressed predominantly in the schizont and trophozoite stages of the parasite (Ma et al., 1996).

Methods: We amplified by PCR genomic P. falciparum DNA encoding a 35 kDa fragment of Pfp70, ligated the DNA in pGEX-4T-1 vector and expressed a 63 kDa recombinant fusion protein designated (p35-GST) comprising 35 kDa polypeptide from Pfp70 and 28 kDa glutathione S-transferase (GST) from Schistosoma japonicum. We tested in ELISA, the reactivity of human serum antibodies from residents of malaria endemic area in Uganda to determine whether they react to or recognize p35-GST. We also assessed IgG subclass reactivity of the endemic sera to p35-GST and the correlation of immune reactivity to age. We further tested the inhibitory properties of the rabbit antiserum to p35-GST in abrogating parasite invasion and intra-erythrocytic growth in vitro.

Results: Of the 160 malaria endemic area sera tested, more than 65%, showed positive reactivity to the recombinant antigen (p35). The immune reactivity positively correlated to age of the serum donors ($r = 0.20$), indicating that the immunity develops with age. Immunoglobulin subclass reactivity showed a predominance of the cytophilic isotypes (IgG1 and IgG3), suggesting that they are preferentially induced. Serum antibodies of rabbits immunized with the recombinant antigen, showed reciprocal antibody titres between 100,000 and 1,000,000. The antiserum inhibited parasite invasion in vitro in a dose dependent manner ending in a maximum effective concentration, suggesting that at higher concentrations, they may become close to 100% effective in inhibiting parasite invasion. Interpretation: The over 65% sero-reactivity found in this study suggests that antibodies to p35 are part of the natural immune response to P. falciparum infection. Inhibition of parasite invasion demonstrates the functional importance of this antigen.

O-91 Antibodies to the cleavage site of Plasmodium falciparum merozoite surface protein 1–42 (MSP1–42) inhibit merozoite invasion [MIM-SA-312030]

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Introduction: The blood stage of Plasmodium falciparum is characterized by highly specific recognition/invasion of erythrocytes by merozoites. An essential prerequisite for invasion is the processing of the major surface protein, MSP-1. Processing involves two cleavage steps in which the 200 kDa protein is first cleaved into four fragments. Then, cleavage of the C-terminal 42 kDa product into a 33 kDa and MSP1–19 kDa fragment occurs. The latter segment remains attached to the merozoite and is carried into the new erythrocyte upon invasion. Antibodies that inhibit processing of MSP1–42 inhibit invasion.

Methods: The amino acid sequence for the cleavage site of MSP1–42 is characterized by highly specific recognition/invasion of erythrocytes by merozoites. An essential prerequisite for invasion is the processing of the major surface protein, MSP-1. Processing involves two cleavage steps in which the 200 kDa protein is first cleaved into four fragments. Then, cleavage of the C-terminal 42 kDa product into a 33 kDa and MSP1–19 kDa fragment occurs. The latter segment remains attached to the merozoite and is carried into the new erythrocyte upon invasion. Antibodies that inhibit processing of MSP1–42 inhibit invasion.
obtained. 16mer peptides corresponding to amino acid sequences around the cleavage site were synthesized. Peptides were coupled to a carrier and used to immunize rabbits. The ability of the elicited antibodies to prevent the processing of the MSP1–42 and inhibited parasite growth in vitro was assessed.

**Results:** Sequence alignment of the cleavage site of MSP1–42 revealed that there are only two variants of the cleavage site. Antisera to the synthetic peptides of the cleavage site recognized recombinant MSP1–42 (under denaturing and non-denaturing conditions). They also prevented the processing of MSP1–42 and merozoite invasion. Additional data suggest that the MSP1–42 cleavage site is only transiently exposed on the surface of merozoite upon release, a result consistent with the low level of peptide-recognizing antibodies present in the serum of individuals living in endemic areas.

**Interpretation:** Our results suggest that antibodies to the cleavage site of MSP1–42 of *P. falciparum* may help control parasitemia and reduce infection.

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**O-92 Improving the immunogenicity of MSP1.42 Alhydrogel vaccines by the addition of CPG 7909 [MIM-LM-72314]**


(1) Malaria Vaccine Development Branch, National Institutes of Health, Rockville, MD, USA; (2) Malaria Vaccine Initiative, PATH, Bethesda, MD, USA

**Introduction:** MSP1.42 proteins from FVO and 3D7 *Plasmodium falciparum* parasites are the most divergent in nature and have been chosen for clinical development ultimately as a combination vaccine, MSP1.42-C1. Alhydrogel, selected based on established safety with licensed vaccines, however, may not be the optimal adjuvant for inducing protective immune responses to MSP1.42 in humans. To augment the immunogenicity of MSP1.42 Alhydrogel vaccines, the addition of the immunostimulator CPG 7909 was evaluated.

**Methods:** cGMP produced MSP1.42-FVO and MSP1.42-3D7 proteins were used to formulate MSP1.42 Alhydrogel based vaccines. MSP1.42-FVO/Alhydrogel and MSP1.42-3D7/Alhydrogel were administered at 0 and 1 month. Antibody titers, IgG isotypes and parasite growth inhibition assays were evaluated.

**Results:** Phase 1 study (ongoing): Both MSP1.42-FVO/Alhydrogel and MSP1.42-3D7/Alhydrogel were safe and immunogenic in that at least a proportion of the volunteers in all dose groups produced anti-MSP1.42-FVO and anti-MSP1.42-3D7 specific antibodies. For each vaccine, the number of individuals with a rise in antibody units after vaccination was greater with increasing dose. To date, the recorded unsolicited or solicited adverse events were minimal and all were mild to moderate in severity. Preclinical studies with CPG 7909: The immunogenicity of the MSP1.42-FVO, MSP1.42-3D7 and MSP1.42-C1 Alhydrogel formulations was significantly augmented in rats and mice by the addition of CPG 7909. The elevated antibody levels correlated with higher parasite growth inhibition in vitro, demonstrating biologic activity of the antibodies. Analysis of antigen specific IgG isotypes revealed that CPG 7909 added to the Alhydrogel formulation shifted the antibody response from predominately IgG1 (Th2-like) to IgG1 and IgG2a (indicative of a balanced Th1–Th2 response). The addition of CPG 7909 to MSP1.42-C1/Alhydrogel increased the magnitude and altered the quality of the specific antibody response in a manner that is likely to enhance cell mediated immunity.

**Interpretation:** The applicability of these observations in experimental animal studies to humans will be evaluated in a Phase 1 trial in which MSP1.42-C1/Alhydrogel with and without the addition of CPG 7909 will be compared in healthy US adults.
O-93 Rapid and robust induction of anti-PfEMP1 CIDR1-alpha antibodies protects against Plasmodium falciparum-FVO challenge in Aotus monkeys [MIM-MM-267168]

M. Makobongo, S. Gratepanche, C. Long, L. Miller
(1) Malaria Vaccine Development Branch/NIAID/NIH, Bethesda, USA; (2) Pasteur Institute, Paris, France

Introduction: PfEMP1 is a multi-domain molecule expressed on parasitized erythrocyte surface. It is involved in both severe malaria and parasite survival strategies. PfEMP1 is thus an important target of naturally acquired immunity. CIDR1 is the PfEMP1 domain that is responsible for cytoadherence via CD36. Anti-CIDR1 antibodies have previously been shown to protect Aotus monkeys against homologous challenge with the less virulent Malayan Camp. We investigated the protective efficacy of CIDR1 against Plasmodium falciparum-Vietnam Oak Knoll (FVO), the most virulent strain in Aotus models.

Methods: Aotus monkeys were immunized with either FVO-CIDR1 or a combination of three recombinant proteins (FVO-, A4tres- and MC-CIDR1) in Freund’s adjuvant. Immunization using Pfs25, a sexual stage antigen, was used as control. The monkeys were first challenged with FVO parasites (FVO1) and monitored for parasitemia. To mimic endemicity and compare the effect of vaccination and infection versus infection alone on protection against different antigenic variants of FVO, the monkeys were re-challenged with parasites collected from one of the delayed infections (FVO2). Sera were collected and ELISA titers determined. Antibody binding to CIDR1-PfEMP1 and/or variant antigens on the surface of FVO-infected erythrocytes was assessed by flow cytometry.

Results: In the first challenge, compared to control monkeys immunized with Pfs25, CIDR1-immunized animals showed a 5-day delay in patency and a 5-day delay in peak parasitemia that correlated with the level of anti-FVO1 surface antibodies. Despite the delay, the rise in parasitemia was similar in all groups, once patent, and all animals were treated for high parasitemia (>5%). Characterization of the parasitized erythrocytes (PE) collected before treatment and assayed for expression of FVO1-CIDR1 showed that PE from controls were all FVO1 variant whereas those from CIDR1 immunized animals had switched to new PfEMP1 variants, a classical phenomenon of parasites under immune pressure. In the second challenge with new variant, FVO2, four of seven control monkeys required treatment for parasitemia or anemia whereas none of the CIDR1-vaccinated animals were treated showing the capacity of anti-FVO CIDR1 antibodies to mediate anti-parasite and anti-disease immunity. Two of the vaccinated monkeys had no patent parasitemia; seven of nine had recrudescence compared to one of seven control monkeys. Protection against anemia and fulminating parasitemia during second challenge correlated with an induction of robust and rapid anti-FVO2 antibodies.

Interpretation: Our data on protection from anemia, reduced parasitemia and more rapid antibody responses may support the use of a PfEMP1-based vaccine to protect children from malarial disease by accelerating the acquisition of immunity to new CIDR1 variants.

15. Operational research
Wednesday 16 November 11:00–13:00—Iroko Hall
Chairs: Martin Allilio (Washington, DC) and Carol Baume (Washington, DC)

O-94 The challenges of operational research in malaria control [MIM-MT-92184]

M. Tanner
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Introduction: Malaria control efforts have gained a new momentum through a higher degree of international awareness and commitment towards the diseases of poverty. Besides the development of new tools such as drugs, vaccines and intervention packages, there are nearly unprecedented funding opportunities to develop and evaluate malaria control strategies for different endemic settings. This calls for a new look at operational research issues that will assure that efficacious tools become part of effective control strategies and public health practice.

Methods: The paper reviews the key issues and questions of operational research in relation to the priority areas of malaria control: (i) drug policies and the decisions to change in relation to the development of
resistance and economic factors, (ii) going to scale of established efficacious and effective control tools such as insecticide treated nets or intermittent preventive treatment, (iii) strategies for surveillance and for early diagnosis and treatment, (iv) vector and environmental control and (v) design of integrated control strategies.

Results: The comprehensive analysis of the key areas of malaria control in Africa reveals three key issues. First, operational research is of crucial importance in understanding on how proof of principle established in small-scale intervention studies and efficacy trials can be assessed for effectiveness at district or national level, a prerequisite for policy formulation and routine public health practice. Second, operational research is too often confounded with evaluation and monitoring. Research is based on research questions with their underlying hypotheses while evaluation is based on terms of references to be pursued. Third, operational research not only acts as a transmission belt between the field and laboratory/bench research, but also helps to reconcile the public health and ecosystemic approaches in malaria control and, thus, harmonically allows the design of integrated malaria control packages and strategies for different endemic settings. Effective evaluations will be described with particular reference to an evaluation of Intermittent Preventive Treatment for malaria and anaemia control in infants in southern Tanzania.

Interpretation: The three conclusions reached will be illustrated through relevant case studies. Their potential for generalization for ongoing malaria control efforts and for the process of policy formulation at global and national level, will be discussed.

O-95 Evaluating the effectiveness of malaria control tools: The example of Intermittent Preventive Treatment in infants (IPTi)

D. Schellenberg

(1) Ifakara Health Research & Development Centre, Dar Es Salaam, Tanzania; (2) London School of Hygiene and Tropical Medicine, London, UK

Most clinical trials concentrate on evaluating safety and efficacy in optimal conditions. There are many examples of interventions with proven safety and efficacy that are not widely implemented. Translating efficacy results into public health action requires an understanding of the practical issues surrounding implementation in real-life conditions. Evaluation of the effects of new interventions, delivered as they would be after completion of the evaluation, generates useful experience on operational aspects of implementation and permits the assessment of feasibility, cost and cost-effectiveness, as well as the investigation of intervention-specific issues. Effective evaluations can therefore help to optimise implementation of new interventions and generate valuable information for prioritisation of health services and advocacy. Different approaches to effectiveness evaluations will be described with particular reference to an evaluation of Intermittent Preventive Treatment for malaria and anaemia control in infants in southern Tanzania.

O-96 Compliance to Coartem® for the treatment of uncomplicated Plasmodium falciparum infection in Zambia [MIM-PW-302880]

N. Sipilanyambe, P. Chanda, P. Wamulume

National Malaria Control Centre, Lusaka, Zambia

Introduction: As a result of high chloroquine treatment failure rates for uncomplicated Plasmodium falciparum malaria, Zambia has adopted Coartem® an artemisinin-based combination. Although Coartem® is known to be highly efficacious, concerns have arisen regarding patient compliance.

Methods: A descriptive study was conducted in January 2004, to measure the level of compliance to the six dose regimen in malaria patients seeking treatment at health facilities and to determine whether Coartem® was being correctly administered by health workers. Compliance was firstly measured as follows: Total Compliance: Verbal confirmation of completion of all doses in the presence of an empty blister pack and a correct description of how the doses were taken. Probable Compliance: Confirmation of completion of all doses in the absence of a blister pack and correct description of how the doses were taken. Non-Compliance: Presence of tablets on the blister pack on day 3 and or inability to explain how the doses were supposed to be taken.

Results: All patients suspected of having uncomplicated falciparum malaria visiting health centres in the month of January in Chongwe, Kabwe, Chibombo, Kalomo and Livingstone, were eligible. Of the 568 patients enrolled, 536 completed the study. Full compliance was observed in 64–82%, 18% were probably
non-compliant and 18% were definitely not compliant. Patient age, education level, sex and distance to the health centre were potential risk factors. The setting of the health centre had no effect on the compliance of the population. Of the 85 health workers observed at the dispensing point, 23.5% \((n = 20)\) were rated as excellent, 29.4% \((n = 25)\) were good, 24.7% \((n = 21)\) performed fairly and 22.5% \((n = 19)\) were bad at giving the key messages needed to encourage the correct use of Coartem®.

Interpretation: The patients who did not take Coartem® appropriately were at risk of treatment failure and this contributes to the emergence of parasite resistance. Implementation of best strategies to improve adherence and maintain efficacy of Coartem®.

O-97
Medical informatics in medical research: The Severe Malaria in African Children (SMAC) Network’s Experience [MIM-CO-454800]

(1) Kenya Medical Research Institute-CGMRC, Kilifi, Kenya; (2) Albert Schweitzer Hospital, Lambarene, Gabon; (3) Komfo Anokye Teaching Hospital, Kumasi, Ghana; (4) Royal Victoria Teaching Hospital, Banjul, Gambia; (5) Queen Elizabeth central Hospital, Blantyre, Malawi; (6) Children’s Hospital, Boston, USA

Introduction: Computers are widely used in developed countries for data management. Dependable systems are vital for data generation, improving quality, and medical decisions making in clinical research. Monitoring and evaluation of data management is critical. We investigated systems structures and procedures used to implement, coordinate, and sustain data management in five sites in Africa. We outline lessons, challenges and successes, and recommendations for informatics application in biomedical research.

Methods: A consortium of research units at five sites in Africa, each with experience in studying children with severe malaria, formed a new clinical trials network, Severe Malaria in African Children (SMAC). In December 2000, the network introduced an observational study involving sites in Gabon, Gambia, Ghana, Kenya and Malawi. After thorough prototyping, a relational database management system (DBMS) for double data entry and verification, data submission deadline and quality control/assurance monitoring systems were implemented.

Results: Between December 2000 and January 2005, the study enrolled 25,858 patients, with sites starting at different time points. The overall missing data rate was 0.5%. The systems functioned well, with minimal troubleshooting. Failure to meet data submission deadline (lateness) was experienced from the fourth quarter of second year of the study and the situation remained perennial thereafter. There were no significant differences in lateness rates in the pooled annual data \((R^2 = 0.147)\) and across the years \((R^2 = 0.245)\). Similar patterns were observed in data entry error rates in the pooled annual data and across the years \((R^2 = 0.026, R^2 = 0.012, \text{respectively})\). However, error rates remained perennial throughout the study period. Both lateness and data entry errors rates were more prevalent during the first and last quarter of each year, with more data errors occurring in the third and fourth year of the study. Both lateness and data errors rates correlated inversely with hospital admissions, and were more prevalent during the time when there were lower hospital admissions.

Interpretation: Developing and sustaining dependable DBMS, ongoing modifications to optimize data management is crucial for clinical studies. Monitoring and communication systems are vital in a multicentre network for the smooth data management process.

O-98
Home based management of fever improves treatment practices in Uganda [MIM-JN-388671]

J. Naungwa-Sabititi, G. Tomson, G. Pariyo, J. Ogwal-Okeng, S. Peterson
(1) Department of Pharmacology and Therapeutics, Makerere University, Kampala, Uganda; (2) Institute of Public Health, Makerere University, Kampala, Uganda; (3) Child Health Division, Ministry of Health, Kampala, Uganda; (4) Department of Public Health Sciences, International Health (IFICAR), Karolinska Institutet, Stockholm, Sweden; (5) Medical Management Center (MMC), Karolinska Institutet, Stockholm, Sweden; (6) Faculty of Medicine, Gulu University, Uganda
Introduction: Effective treatment of malaria remains a challenge. To reach the Abuja target of treating 60% of all cases of malaria promptly with appropriate anti-malarial treatment, countries have adopted Home Based Management of Fever/malaria (HBMF). Little is however known about community effectiveness of large-scale implementation of HBMF. In rural western Uganda we evaluated the effect of the Home Based Management of Fever approach in improving treatment of fever (presumed malaria) among under-fives.

Methods: Two households surveys were conducted before (n = 500), and 18 months into implementation (n = 587) of HBMF in intervention and control areas in Kasese district, western Uganda. HBMF consisted of trained volunteer community drug distributors (CDD) distributing free pre-packaged combination chloroquine (CQ) and sulfadoxine pyrimethamine (SP) to fever cases. Access to and utilisation of CDDs, timeliness, appropriateness and compliance with treatment of fevers in under-fives in the preceding two weeks was assessed by interviewing the child’s caretaker.

Results: Intervention and comparison areas were comparable at baseline. At follow-up in the intervention areas 36% of households had a CDD as nearest source of treatment. Forty percent of children had been febrile in the last two weeks. In intervention areas 26% had consulted a CDD for treatment versus 4% in the control area. Any treatment was initiated within 24h of fever onset for 74% versus 65% (NS). In the intervention areas 32% treated the child with first-line drug CQ-SP versus 7% in the control area (p < 0.001). Correct dose was reported given in 40% versus 26% (p = 0.06) and correct duration of treatment in 33% versus 21% (p = 0.2). The cumulative compliance with all treatment steps above was 13% in intervention areas and 0% in control areas (p = 0.002). Marked improvements were noted for choice and dose of drugs. However, with this sample size no significant improvement were noted for promptness and giving drugs for the recommended duration.

Interpretation: In a rural setting in Uganda HBMF improved malaria treatment practices for children. Efforts should be made to determine and address causes of low CDD utilization. Further efforts should be made to ensure prompt initiation of correct treatment.

O-99 Caregivers’ perceptions and acceptance of artesunate suppositories as treatment for malaria in children [MIM-RH-86380]

R. Hinton, J. Reeder, O. Ou, A. Auwun, T. Davis, H. Karunajeewa
(1) Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea; (2) University of Western Australia, Perth, Australia

Introduction: Malaria continues to be a major disease burden in the Pacific Island nation of Papua New Guinea (PNG). Local research has shown artemisinin suppositories (Plasmodium Rectocaps®, Mepha, Aesch-Basel, Switzerland) to be highly efficacious for the treatment of childhood malaria in PNG. This intervention has the potential to reduce malaria-related mortality in rural areas. However, it was not known whether rectal administration of antimalarials would be culturally and socially acceptable in PNG.

Methods: We conducted a program of qualitative and quantitative social research in a rural area of PNG to examine community perceptions and acceptability of the rectal administration of antimalarials, and to identify possible impediments to the deployment of artesunate suppositories at a primary health care and community level. Preliminary formative research was used to formulate a standard questionnaire for caregivers who presented to a primary health-care facility with children aged 6 months to 7 years with confirmed P. falciparum malaria. The questionnaire was administered to the time caregivers were offered treatment with suppositories and again on completion of therapy.

Results: Preliminary findings show a moderately high willingness by caregivers to accept suppositories and to administer the treatment themselves. Shame or embarrassment with the treatment route was not identified as a significant issue. The results show that even without medical training, suppositories are easy to use. However, there were, however, some anxieties concerning the merits of suppositories over other treatment forms and also of practical aspects of their administration. Preliminary findings show a moderately high willingness by caregivers to accept suppositories and to administer the treatment themselves. Shame or embarrassment with the treatment route was not identified as a significant issue.

Interpretation: Introduction at a primary health-care level is unlikely to be problematic, however deployment of artesunate suppositories should be combined with appropriate health education activities to overcome anxieties and concerns surrounding their use.
O-100
‘To help them is to educate them.’ Power and pedagogy in the prevention and treatment of malaria in Tanzania [MIM-CM-424410]
C. Montgomery, M. Kongongo, W. Mwengee, R. Pool
(1) London School of Hygiene and Tropical Medicine, UK; (2) Tanzanian Ministry of Health, Tanga, Tanzania

Introduction: Acknowledging that mothers are often the primary caregivers at the household level, malaria control efforts have emphasised educating women in its early recognition. This fails to consider the context in which knowledge will be transformed into action, as women are often denied decision-making responsibility and financial resources. We examine the knowledge and power dynamics of provider–patient interactions and the implications for malaria treatment of educating mothers during consultations.

Methods: In-depth interviews were conducted with 309 household participants over a 2-year period to explore knowledge and perceptions of febrile illness, its treatment and prevention. In addition, in-depth interviews were conducted with 59 medical practitioners at both government and private health care facilities. Informal observation was conducted on 33 occasions as members of the public presented at a health centre or dispensary with febrile illness.

Results: The data indicated that most informants were well informed on the aetiology of malaria. Signs and symptoms of malaria were well recognised and first-, second- and third-line treatments known. Doctors reported that mothers were able to give them sufficient information about their child for them to make an accurate diagnosis. Mothers were said to recognise the severity of the illness and know when and what action should be taken. However, health staff continued to see mothers who present ‘late’ as undereducated, intellectually incapable and even lazy. Whilst there is evidence showing that decisions about treatment are often not in the hands of mothers, but rather of male family members, it is women who continue to be blamed and targeted with health education in the consultation room. The often aggressive didactic teaching methods used by health staff may be disempowering those already equipped with the necessary knowledge, yet unable to control treatment decisions within the household. Maintaining and reinforcing disabling power structures through the commodification of knowledge and the labelling of women as uneducated is likely to alienate women and lead to further delays in presentation at a health care facility.

Interpretation: Health education should be context-sensitive, acknowledging gendered power relations. It must target not only women, usually primary caregivers, but also men, who may have ultimate responsibility for decision-making or access to financial resources.

16. Insecticide resistance
Wednesday 16 November 11:00–13:00—Mahogany Hall
Chairs: Maureen Coetzee (Johannesburg) and Etienne Fondjo (Yaoundé)

O-101
No abstract received.

O-102
Genetic diversity of insecticide resistance in Anopheles gambiae s.l. from Cameroon: A key issue for selective vector control [MIM-JE-16392]
J. Etang, F. Chandre, J. Hougard, M. Akogbeto, L. Manga, E. Fondjo, P. Nwane, M. Chouaibou, F. Simard
(1) Institut de medical research and studies of medical plants, Yaoundé, Cameroon; Organisation de coordination pour la lutte contre les endémies en Afrique centrale, Yaoundé, Cameroon; (2) Centre de recherches entomologiques de Cotonou, Benin; (3) Institut de recherche pour le développement, Cotonou, Benin; (4) Centre de recherches entomologiques de Cotonou, Benin; (5) World Health Organization, Harare, Zimbabwe; (6) National malaria control program, Yaoundé, Cameroon; (7) Organisation de coordination pour la lutte contre les endémies en Afrique centrale, Yaoundé, Cameroon; (8) Organisation de coordination pour la lutte contre les endémies en Afrique centrale, Yaoundé, Cameroon; (9) Organisation de coordination pour la lutte contre les endémies en Afrique centrale, Yaoundé, Cameroon

Introduction: Cameroon is commonly considered as Africa in miniature, according to its ecological and climatic variability. In addition to the subsequent com-
plexity in malaria transmission, the current report is addressing diversity in insecticide resistance mechanisms in Anopheles gambiae populations as one of the key issues for selective vector control.

**Methods:** A screening of insecticide resistance was conducted in An. gambiae s.l. populations from 32 sites located all over the country. It included (1) WHO susceptibility tests with DDT, permethrin and deltamethrin, (2) molecular diagnosis of species and forms, as well as kdr and acetylcholinesterase (AChE) genotypes, (3) biochemical assays to measure esterase, oxidase and glutathione S-transferase (GST) activity. Knockdown and killing effects of permethrin-treated nets (PTN) against a laboratory strain of An. gambiae s.s. with oxidase-based pyrethroid resistance were assessed using WHO cone test. Plasmodic index and malaria morbidity were scored in children using PTN in an area of An. gambiae s.l. displaying metabolic-based pyrethroid resistance.

**Results:** In the northern cotton and rice fields, An. gambiae s.s. and An. arabiensis showed metabolic-based resistance to pyrethroids, with elevated activity of esterases or oxidases compared with the Kisumu susceptible reference strain (P < 0.001). In the western tropical gardens (Foumbot), DDT and pyrethroid resistance in An. gambiae s.s. was mainly attributed to kdr mutation, all specimens belonged to S molecular form. In the coastal and inland equatorial areas, most of tested populations were more or less resistant to DDT and pyrethroids, with elevated activity of esterases (P < 0.02) or GST (P < 0.001), sometimes coupled with kdr mutation in M or S molecular form. Carbosulfan resistance was only seen in south west, but was not associated with insensitive AChE. Bioassays revealed a significant decrease of mortality rates using PTN against a metabolic-based tolerant strain of An. gambiae (P < 0.001). In the field, PTN reduced parasite density (more than 10-fold). Plasmodic index (18%) and malaria morbidity (16.7%) were also decreased but not significantly (P > 0.05) compared with untreated nets (26.2% plasmodic index and 23.4% morbidity).

**Interpretation:** Pyrethroid resistance is widespread in An. gambiae from Cameroon, with a range of mechanisms likely having an impact on treated net efficacy. Management strategies are now to be explored, especially where kdr mutation and detoxification are coupled.

O-103 Malaria vector control and insecticide resistance: Complex interactions [MIM-FC-20280]


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**Introduction:** Despite the recent findings on insecticide resistance mechanisms in major malaria vectors from Africa, some critical questions remain such as the impact of resistance on the efficacy of vector control operations and the evolution of resistance in mosquito populations exposed to insecticides from Public Health Programmes.

**Methods:** Here are presented some results obtained in West Africa that can partly respond to these questions. These studies were based on small scale field trials using impregnated treated nets (ITNs) in experimental huts with various insecticides, either alone or in combination. Two large scale trials were also implemented with pyrethroids in Côte d’Ivoire, in an area where An. gambiae was strongly resistant to pyrethroids (F(kdr) > 0.85) and slightly resistant (F(kdr) < 0.15). Evolution of resistance mechanisms were followed using PCR and biochemical assays.

**Results:** The main resistance mechanisms in An. gambiae from West Africa are target site mutations, (kdr, Ace-1R) sometimes present at high frequencies in natural populations. Most studies done in experimental huts did not show any significant decrease of ITNs efficacy in resistant areas compared to susceptible ones. This was confirmed by a large scale trial done in Côte d’Ivoire, an area where An. gambiae was strongly resistant to pyrethroids, showing that reduction of malaria morbidity were similar to that observed in susceptible areas. The selection pressure induced by impregnated nets on mosquito population is also a key point difficult to predict. In experimental huts, bed-
nets impregnated with alpha-cypermethrin selected for kdr mutation while permethrin did not. At operational level, selection of resistance by vector control operations depends from several factors including insecticide itself, external selection pressure, environment, mosquito behaviour, etc. In areas where there is a high level of kdr resistance ITNs did not have any significant impact on resistance pattern. Conversely, in area of low resistance level, ITNs or indoor residual spraying can increase the frequency of resistant mosquitoes.

Interpretation: Because such results could be different if metabolic resistance is associated or not to target site mutations, it is now essential to characterise and design diagnostic tools for genes involved in metabolic resistance in malaria vectors.

O-104

Inheritance of resistance and relative fitness of pyrethroid resistant Anopheles funestus (Diptera: Culicidae) [MIM-NO-9861]

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Introduction: The discovery of insecticide resistance in Anopheles funestus in South Africa has prompted an urgent enquiry into the necessity of incorporating insecticide resistance management strategies into formal malaria control policy in affected regions. The aim of this study is to establish the genetic mode of inheritance, relative fitness of insecticide resistance genes as well as the stability of the resistance phenotype in the absence of selection pressure in this malaria vector.

Methods: We examined the inheritance of pyrethroid resistance by crossing a permethrin resistant strain of An. funestus to a susceptible strain of the same species. Adult mosquitoes from the crosses were exposed to 0.75% permethrin and knockdown and mortality was recorded. Adult mosquitoes from a cohort of the resistant strain kept without insecticide selection were exposed to 0.75% permethrin to determine level of resistance. The effect of resistance on fitness in An. funestus is currently being evaluated in terms of adult longevity, fecundity, fertility, development time, survivorship, and sex ratios. The age-specific life table of resistant and susceptible strains will be compared to determine relative fitness based on population trend indices.

Results: The heterozygous offspring (F1) resulting from reciprocal crosses between the resistant colony and its susceptible counterpart responded alike to bioassay tests. This suggests that there was no maternal influence on inheritance of resistance. The time mortality curve of the reciprocal crosses was close to that of the resistant parental colony and significantly different from that of the susceptible colony. Data on insecticide susceptibility of the F1 progeny and F2 progeny following backcrosses to their respective susceptible and resistant parental strains were consistent with a monofactorial and autosomal mode of inheritance in which the resistant genes presented as incompletely dominant. In addition, bioassay results from resistant colonies maintained over several generations without insecticide selection showed that the resistance gene was stable. Preliminary data is currently being generated on the fitness cost of insecticide resistance in this malaria vector.

Interpretation: Results show that resistance is inherited in an autosomal, monofactorial and incompletely dominant manner. These findings and those on the fitness cost of insecticide resistance are of value to malaria control authorities in southern Africa.

O-105

Characterization of permethrin tolerance in Anopheles arabiensis in Tanzania using a novel high-throughput method to detect knockdown resistance (kdr) [MIM-MK-93757]

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(1) McGill University, Montreal, Canada; (2) Joint Malaria Programme, Moshi, Tanzania; (3) London School of Hygiene and Tropical Medicine, UK; (4) University of Copenhagen, Copenhagen, Denmark

Introduction: Insecticide-treated nets (ITN) are an important component of the global strategy for malaria control; however, the spread of pyrethroid resistance in African malaria vector populations may jeopardize the sustainability of this method. The current insecticide resistance status of malaria vectors in Tanzania is not...
known. This study aims to assess the pyrethroid resistance status of malaria vectors in northern Tanzania using bioassay and molecular methods.

Methods: WHO susceptibility testing of *Anopheles arabiensis* from larval and indoor-resting collections from agricultural sites in the Kilimanjaro Region of Tanzania provided longitudinal data on pyrethroid and DDT susceptibility, 2004–2005. Mosquitoes were tested for the knockdown resistance (kdr) mutation that confers resistance to pyrethroids using a multiplex PCR method. To enable detection of kdr mutations at low frequencies, a novel high-throughput method, employing sequence-specific oligonucleotide probes (SSOP) in an enzyme-linked immunosorbent assay (ELISA) format, is being developed and established locally for large-scale screening of mosquitoes.

Results: Bioassays revealed seasonal fluctuation in the permethrin susceptibility of *An. arabiensis* in Lower Moshi (24 h mortality: range 74–92%). Selection of a permethrin-resistant strain is underway to aid in characterization of resistance mechanisms. Metabolically based resistance will be identified by synergist bioassay. The leucine-serine kdr mutation was absent in 418 specimens tested by multiplex PCR. Additional high-throughput screening will permit accurate assessment of target-site resistance. Both the East and West African kdr mutations can be detected in pooled samples by the novel SSOP-ELISA method thus enabling rapid and cost-effective testing of large numbers of mosquitoes. This method will be applied to mosquitoes from regional and national resistance surveys, local longitudinal collections and experimental hut work. A training workshop will be held to strengthen local capacity for resistance monitoring. Application of the SSOP-ELISA technique for detection of other point mutations in malaria vectors and non-vector mosquito species is envisaged, e.g. the ace-1 insensitive acetylcholinesterase mutation in organophosphate-resistant *An. gambiae* and kdr and ace-1 mutations in *Culex quinquefasciatus*.

Interpretation: Temporal variation in permethrin susceptibility of *An. arabiensis* from northern Tanzania was seen. Characterization is underway to determine the resistance basis. A high-throughput method was developed for kdr screening of local vector populations.

**O-106**

**Dynamics of permethrin resistance in field population of Anopheles gambiae s.s. in southwestern Nigeria [MIM-AS-335002]**

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Introduction: In West Africa, pyrethroid and DDT resistance in *Anopheles gambiae* s.s. is widespread. We investigated this at Ipokia in the suburbs of Lagos in 1999, when the mosquito was shown to be fully (100%) susceptible to permethrin and DDT. From 2001, populations of *An. gambiae* s.s. in the area have become resistant to permethrin and DDT. In the present study, we determined the dynamics of the knock down resistance (kdr) gene in field populations from 2001 to 2004.

Methods: Aliquots of genomic DNA of adults *An. gambiae* collected from human dwellings quarterly from 2001 to 2004 were subjected to PCR assays for: (i) species identification within the *An. gambiae* complex (ii) identification of the molecular M and S forms and (iii) detection of the West African leucine-phenylalanine kdr gene. During the period, 2–3 days old adult *An. gambiae* reared from larval collection were also assayed using the WHO insecticide susceptibility test kits to assess their permethrin and DDT resistance status which was compared with a reference susceptible laboratory colony of *An. gambiae* named “AGIB” established in 2001 from mosquito collected in Ibadan in southwestern Nigeria.

Results: A total of 2850 adult *An. gambiae* s.s. were identified and characterized for the kdr gene. The molecular S form was predominant (>62%) and the proportions of both forms from 2001 to 2004 were not statistically different. Both forms also occurred throughout the year with no apparent relationship to...
wet or dry season. The kdr gene was found only in the molecular S form with an overall frequency of 6.6, 6.5, 5.7 and 7.9% for 2001, 2002, 2003 and 2004, respectively. Homozygous and heterozygous resistant mosquitoes were detected in samples from each year collections subjected to the kdr PCR assay. However, the frequency of the homozygous resistant mosquito diminished from 2001 to 2004. Results of the bioassay showed that the level of permethrin and DDT resistance has not increased significantly from 2001 to 2004.

Interpretation: Both molecular M and S forms were found but the kdr gene was absent in the M form over a 4 year period. The kdr frequency did not change significantly from 2001 to 2004. This result is in support of restriction in gene flow in these sympatric taxa.

O-107
Microarray analysis of genes mediating metabolic insecticide resistance in a pyrethroid resistant Anopheles gambiae s.s. strain from Ghana [MIM-PM-10428]

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Introduction: The development of pyrethroid resistance in Anopheles gambiae populations threatens malaria control programmes. While three enzyme families (cytochrome P450s, glutathione S-transferases and carboxylesterases) have been associated with metabolic resistance, the role of individual enzymes in insecticide detoxification remains elusive. We investigated expression levels of the genes putatively involved in conferring pyrethroid resistance in an A. gambiae strain from Ghana using DNA microarrays.

Methods: The insecticide resistant A. gambiae s.s. strain was collected in the field from a site in Odumase, Ghana, and maintained under regular selection pressure by exposure to filter paper treated with 0.75% permethrin. The susceptible reference strain originates from Kisumu, Western Kenya, and is susceptible to permethrin. mRNA was extracted from 1-day-old mosquitoes, reverse transcribed and amplified in vitro. Amplified copy messenger RNA from each strain was labelled with either Cy3 or Cy5 and hybridised to custom-made DNA chips containing 230 A. gambiae gene fragments from enzyme families associated with insecticide resistance. After hybridisation, Cy3- and Cy5-signal intensities were measured and compared gene by gene.

Results: The Odumase strain showed high levels of resistance to permethrin. The observed survival rate for an exposure to 0.75% permethrin for 1 h following WHO recommendations was 70%. Hybridisations of the arrays with the two A. gambiae strains revealed that in females of the resistant strain the cytochrome P450s CYP6Z2 and CYP6M2 were highly over-expressed along with a member of the superoxide dismutase (SOD) gene family. Gene expression levels for these three gene products were similar in both females and males.

Interpretation: The candidate genes associated with pyrethroid resistance, identified in this study, differ from those found in East African strains of A. gambiae s.s. The implications for the management of pyrethroid resistance will be discussed.

17. Drug resistance 1

Wednesday 16 November 14:30–16:30—Bubinga Hall
Chairs: Robert Guiguemde (Bobo-Dioulasso) and Fred Kironde (Kampala)

O-108
Drug resistance surveillance in Africa [MIM-WM-130350]

W. Mfou-ham
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Introduction: Drug resistance and an increase in world population account for more Plasmodium biomass and the rise of malaria-attributable deaths today. Chloroquine resistance has spread to Africa from SE Asia. Efficacy studies show an increasing east-to-west gradient in resistance to chloroquine and Fansidar with similar mutations and markers to those in SE Asia. To monitor the spread from different foci molecular markers still need identification and validation.

Methods: A literature search was conducted by entering key words into PubMed or Medline search engines. Information on resistance from 1996 to 2005, was assembled for in vivo efficacy of symptomatic patients
with uncomplicated malaria, in vitro drug susceptibility assays and molecular markers of resistance on genes pfmdr, pfcrt, dhfr, dhps and microsatellites. Therapeutic response was judged and reviewed by the WHO 1996 and 2002 protocols. Pooled information on median tendencies for countries were regrouped into East, South, Central and West. The course of drug resistance in SE Asia was reviewed as the alarm signal to Africa’s drug resistance.

Results: In vivo efficacy to anti-malaria drugs are as disparate and difficult to interpret due to different reporting methods. The range of median for chloroquine treatment failure Africa show for the East [28–79%], Central [26–64%], West [5–27%] and South [21–44%] compared to SE Asia (66–100%). Failure rates for Fansidar are: East [8–35%], Central [0–16%], West [0–30%] and South [4–18%] compared to SE Asia [9–35%]. Parasite failure rates were higher than the in vivo rates but show same trend. Mefloquine and quinine show 6–20% failure rates in Asia compared with Africa (0–12%). Amodiaquine resistance is 83–100% in China but remains relatively efficacious in Africa (90–100%). The pfct 76T has a prevalence of 61–87% in West and Central Africa and 76–100% in East and Southern Africa. The pfmdr1 86Y mutation is at 33–36% in East Africa. There is a paucity of data on Fansidar resistance markers dhfr and dhps across Africa. Microsatellite makers to flanking regions of the dhfr show the double and triple mutants as having independent origins with the latter spreading from SE Asia. Accruing evidence show Pfcrt and pfmdr1 as pivotal in altering sensitivities to diverse antimalarials and may serve as markers for these drugs.

Interpretation: There is an east-to-west spread of resistance to anti-malarial in Africa with origin from SE Asia. Molecular markers need to be developed for efficient monitoring of other antimalarial drugs for effective evidence-based policy change.

O-109
Are mutations in Plasmodium falciparum dihydrofolate reductase (DHFR) and dihydroypteroate synthase (DHPS) genes from southern Mozambique increasing? [MIM: jr-11832]
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Introduction: The Lubombo Malaria Control Initiative, a cross border collaboration between Mozambique, South Africa and Swaziland, has established a contiguous malaria controlled area of over 100 000 km². The control consisted of two arms, vector control by IRS and parasite control by effective malaria treatment. Drug efficacy monitoring is essential for effective malaria treatment. We describe DHFR and DHPS mutational data from southern Mozambican Plasmodium falciparum isolates over spatial and temporal dimensions.

Methods: Fingerprick blood spots from malaria positive children aged between 2 and 15 years of age were collected between 1999 and 2004 at sentinel sites in southern Mozambique. Parasite DNA was Chelex extracted and then subjected to mutational analysis which consisted of nested PCR and restriction endonuclease cleavage. Point mutations at sites 51, 59 and 108 of the DHFR gene as well as sites 436, 437, 540 and 581 of the DHPS gene were investigated. Mutational data were classified as pure wild, pure mutant or mixed (where both mutant and resistant genotypes were present in a single sample). Statistical inference took account of within-site correlations of resistance markers.

Results: In 1999 prior to the commencement of the malaria control programme, fewer southern Mozambican P. falciparum parasites carried the triple DHFR mutations (30%) associated with pyrimethamine (P) resistance and the double DHPS mutations (10%) associated with sulphadoxine (S) resistance compared to those from KwaZulu-Natal, South Africa (42% and 62%), respectively, where SP clinical failure exceeded 70% in 2000. However, since 1999, the prevalence of the triple DHFR mutations in southern Mozambique
has increased annually across all zones to a prevalence of 92% [95% CI 87–96%] in 2004. In contrast isolates with the double DHPS mutations associated with sulphadoxine resistance peaked in 2001 relative to 1999 (P < 0.0001) but in 2004 dropped to levels similar to those present in 1999 (P = 0.85). Similarly parasites carrying the quintuple mutations associated with SP failure peaked in 2001 (26% 95% CI 21–32%) declining to 11% (95% CI 5%–25%) in 2004. The peak in quintuple mutations in 2001 is most likely associated with excessive SP usage in 2000. There is no evidence of an increase in quintuple mutations in 2004 compared to 1999 (P = 0.28). Ongoing monitoring will evaluate parasite resistant profiles following the introduction of ACT in April 2004.

**Interpretation:** Our findings show a temporal increase in DHFR triple mutation approaching a prevalence of 100%. On the other hand DHPS double mutation prevalence displayed a temporal variation peaking in 2001 but declining in subsequent years.

**O-110**

Point mutations in dhfr, dhps, pfcrt and pfmdr1 *P. falciparum* genes within a clinical trial of Intermittent Preventive Treatment (IPT) in Infants [MIM-AM-75096]

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**Introduction:** Point mutations in codons 51, 59, 108, and 164 of *Plasmodium falciparum* dihydrofolate reductase (dhfr) and 437, 540 and 581 of dihydropertenate synthase (dhps) cause in vitro resistance to pyrimethamine (P) and sulphadoxine (S). Certain combinations of these mutations are necessary for survival of parasite after SP treatment and are thought to be associated with higher use of SP. It is a priority to determine if IPT interventions increase the carriage or spread of drug resistant *P. falciparum* malaria.

**Methods:** DNA was extracted from 180 dried filter paper blood samples randomly selected from *P. falciparum* parasitemic children attending the outpatient clinic in the context of a double-blind, placebo controlled trial of IPT with SP in Manhiça. Nested-PCR followed by restriction endonuclease digestion was used to detect polymorphisms in codon 76 (pfcrt), codon 86 (pfmdr), codons 51, 59, 108, and 164 (dhfr), and codons 437, 540 and 581 (dhps). Conality of infection was estimated by PCR typing of the *P. falciparum* merozoite surface proteins 1 and 2. We used Chi-2 test to assess the significance of the association between mutations and the intervention in a univariate analysis. Fisher’s exact test was used to correct the Chi-2 test when necessary.

**Results:** Of the 180 samples randomly selected, 171 were successfully amplified for all the loci studied. Eighty-two of the samples corresponded to placebo recipients, and 89 to SP recipients. Mean multiplicity of infection was 2, and no difference was found between the placebo and the SP recipient groups. Prevalence of mutant type infections are high and comparable to previous studies in the country: 81% for codon 51 (6% mixed), 93% for 59 (2% mixed), 95% for 108 (1.7% mixed), 0% for 164, 54% for 437 (8% mixed), 52% for 540 (13% mixed), 0% for 581, 92% for 76 (4% mixed) and 60% for 86 (13% mixed). A statistically significant association between codons 51, 59 and 108; and 437 and 540 was found. Frequency of triple mutant parasites for dhfr was 80%, and 45% for dhfr/dhps quintuple mutants. Polymorphism of codon 86 was associated with dhps haplotype (p = 0.011). Frequency of dhps double mutant and quintuple mutants tended to be higher in placebo recipients (p = 0.052, p = 0.086, respectively).

**Interpretation:** High prevalence of mutations in the genes studied is shown. An association between dhps-437/540 codons, and pfmdr1-86 codon is suggested. Preliminary data show that IPT in infants is not associated with an increase in the prevalence of the mutations analysed.
O-111
High prevalence of the molecular chloroquine resistance marker pfcrt K76T and evidence for cross-resistance to amodiaquine in Ghanaian infants [MIM-SA-47502]
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Introduction: Current WHO recommendations on malaria treatment involve use of artemisinin-based combination therapies. However, the efficacy of the partner drug should be high. To determine drug resistance levels against chloroquine (CQ) we assessed background prevalence of pfcrt K76T in treatment naive infants presenting with Plasmodium falciparum parasitaemia at the age of 3 months. In addition we genotyped parasites that appeared after amodiaquine (AQ) treatment to assess cross-resistance between CQ and AQ.

Methods: P. falciparum positive samples of 3-month-old children attending routine vaccination sessions under the Expanded Program on Immunizations were enrolled in the study. Smears were analysed microscopically and a genus- and species PCR were performed. DNA was isolated from filter papers. Samples were analysed for pfcrt-genotype using a nested-PCR followed by APO-1 digestion and electrophoresis on agarose-gel. Children presenting with uncomplicated P. falciparum malaria were treated with full-dose amodiaquine and reassessed on day 28 follow-up for parasitaemia. Pre- and post-treatment isolates were genotyped for pfcrt and compared. Merozoite surface protein 2 (msp-2) was genotyped for differentiation of recrudescence from reinfection.

Results: The pfcrt K76T mutation was identified in 73/116 (63%) isolates of three month old infants representing the general background resistance pattern to chloroquine in this area. 54.4% of isolates contained mutated parasites only and 8.6% were composed from mixed parasites populations carrying mutation and wild-type. The multiplicity of P. falciparum infection (MOI) was 3.7 assessed through msp-genotyping. In the amodiaquine group 90% of post-treatment compared to 70% of pre-treatment isolates carried the K76T mutant gene.

Interpretation: Our data show high background resistance to chloroquine indicated by high pfcrt K76T prevalence and indicate cross-resistance between CQ and AQ, the drug chosen for ACT in this area. Close monitoring of resistance to this drug is indicated.

O-112
Pfcrt-based 76T mutation for evaluating chloroquine efficacy in malaria from ecologically distinct regions of Cameroon [MIM-ME-28938]
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Introduction: Genes responsible for transport and/or metabolism of drugs in Plasmodium falciparum serve as markers for resistance due to the acquisition of mutations. Since the observation of chloroquine resistance in the early 1980s in several towns in Cameroon, the efficacy of chloroquine and other anti-malaria drugs has dropped considerably. With the suggestion of the Pfcrt gene as a marker of resistance, an investigation was undertaken to assess its usefulness in determining the extent of resistance in Cameroon. The prevalence of the 76T mutation on the Pfcrt gene and the efficacy to chloroquine is useful in defining a genotype failure index.

Methods: Sixty six patients aged 6 months to 10 years after parental consent were administered chloroquine in Yaounde and followed up for 14 days according to the WHO 2002 protocol for clinical evaluation of anti-malaria drug efficacy. Blood samples further collected from symptomatic patients consulting at outpatient clinics in four ecologically distinct regions; Limbe, Fontem, Dschang and Nkambe, between October 2000 and October 2003, were used for the extraction of par-
asite DNA and for the performance of PCR of the Pfct K76T mutation. The cg2-w was used to assess recrudescence.

Results: The age geometric mean of the clinical study population was 5.6 years. Recruited non-anaemic patients had a mean packed cell volume (PCV) of 67%. Chloroquine treatment failure in Yaounde-South (rural forest) was 12% (8/66) for ETF and 25.7% for LTPF. This gives an adequate clinical and parasitological response to chloroquine to be 62.3%. The cg2 marker revealed two allelic variants at the cg2-E locus, the most preponderant of which was the 570 bp. The msp1 and cg2w genes were used to control for recrudescence and results revealed that only two out of eight had similar alleles before and after treatment. A very high frequency of mixed infection reveals that seven out of eight of those who failed therapy early share the same resistance genotype before and after treatment with chloroquine (87.5% of ETF). Mutation specific-PCR performed on pfcrt revealed the prevalence of the 76T mutation to be: Dschang (89%), Nkambe (83%), Limbe (81%) and Yaounde (89%), with mixed infections of both alleles (K76T), respectively, at 69%, 13%, 50% and 48%.

Interpretation: A high proportion of day 3 failure samples were not re-infection but recrudescing low parasite populations. High mutation frequency at the 76T locus reveals the potential for chloroquine resistance.

O-113 Polymorphisms in Plasmodium falciparum dhfr and dhps genes and in vivo response to sulfadoxine–pyrimethamine treatment in children from Nigeria [MIM-TE-0]

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(3) Special Program for Research and Training in Tropical Diseases (WHO/TDR), Geneva, Switzerland

Introduction: Mutations in Plasmodium falciparum dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) genes have been used as means to predict treatment failure to sulfadoxine–pyrimethamine (SP) and for monitoring/surveillance of resistance to the drug in many areas where malaria is endemic. However, patients responses to treatment is significantly dependent on factors like host immunity profile of treated patients. This study was designed to investigate the relationship between molecular markers of SP resistance, host immunity and clinical outcome.

Methods: The association between pre-treatment dhfr and dhps genotypes, age and treatment outcomes was also evaluated in 109 children treated with SP for acute uncomplicated falciparum malaria in Ibadan, Nigeria. Patients were enrolled, treated with SP, and followed-up for 28 days. Filter papers samples were collected at enrollment and during follow-up for determination of parasites genotypes and molecular markers of SP, using PCR and RFLP approaches. Mutant alleles of various dhfr and dhps codons of P. falciparum gene in patient samples collected before and after treatment with sulfadoxine–pyrimethamine were collated and compared with patients treatment outcome.

Results: Seventy-seven percent (73%) of the children were cured with the drug, while 27% failed treatment after 28 days of follow-up. All children infected with parasites harboring less than two dhfr/dhps mutations were cured with SP. Analysis of association between dhfr and dhps mutations and patient treatment outcome showed that apart from dhfrAsn-108, any other dhfr or dhps mutations and the dhfr triple mutant (Asn-108/Ile-51/Arg-59) or the dhps double mutant (Gly-437/Glu-540) were independently associated with SP treatment failure in children aged less than 5 years, but not in older children. The dhfr/dhps quintuple mutant (dhfr triple mutant + dhps double mutant) genotype was strongly associated with SP treatment failure (OR = 24.72; 95% CI = 8.24–74.15) in both younger and older children. This study showed that age had a major influence on the relationship between polymorphism in target enzymes and SP treatment outcome. Also older patients, presumably with greater partial acquired immunity, had superior treatment outcome compared to younger patients. Age is a major confounding factor for the association between molecular markers of SP.

Interpretation: This study showed that the dhfr/dhps quintuple mutant is the most reliable predictive marker of SP resistance in Southwest Nigeria and should be
optimized as molecular markers of SP to predict treatment outcome in different malaria settings.

18. Severe malaria in children

Wednesday 16 November 14:30–16:30—Ebony Hall

Chairs: Richard Idro (Kilifi) and Vivian Tchinda (Yaounde)

O-114
When is ‘malaria’ malaria? [MIM-MM-9592]

M. Molyneux
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Introduction: In endemic areas many people tolerate Plasmodium falciparum parasitaemia without symptoms. The presence of parasitaemia cannot therefore be taken as proof that an illness is due to malaria. This problem affects clinicians aiming to diagnose and treat, controllers defining management policies, epidemiologists wishing to define the impact of malaria, and clinical researchers who must identify malarial illness for enrolment criteria or for trial endpoints.

Methods: It is not surprising that malaria shares with many diseases, especially infections, syndromes that are clinically similar, since they share pathogenetic pathways in common. This diagnostic problem posed by malaria is relevant for uncomplicated malaria, for syndromes that define ‘severe malaria’, and for fatal disease. A number of approaches are used to cope with this dilemma. Uncertainty may be partially quantified by applying local statistics, as in the use of parasite density to calculate a malaria-attributable fraction for febrile illness in a community. This approach is of value to epidemiologists but not to clinicians.

Results: Rapid diagnostic tests, while making it easier to identify parasitaemias in a community or clinic without laboratory facilities, do not resolve the diagnostic difficulty concerning the relevance of parasites to illness. In the case of severe disease, several recent studies have shown that a diagnosis based on a clinical syndrome and parasitaemia may mistakenly attribute illness to malaria. This can be dangerous if it deflects the clinician from seeking an alternative treatable diagnosis. A recently described malarial retinopathy may help in many cases to strengthen a positive diagnosis of malarial coma. Although highly specific, the sensitivity of this sign remains to be defined, especially when ascertained by clinicians without ophthalmological training. For documenting the importance of malaria as a cause of death, autopsies provide information about tissue parasites—a supra-orbital needle sample allows simpler assessment of cerebral sequestration. Even cerebral sequestration does not, however, prove a malarial cause of a patient’s fatal illness.

Interpretation: New tools identifying microbe-specific host responses may one day enable us to distinguish disease from parasitosis. Until then we must combine clinical discernment with the awareness that parasites are sometimes pathogens and sometimes passengers.

O-115
No abstract received.

O-116
Immunological mechanisms determining why some African children develop severe malarial anaemia (SMA) and others cerebral malaria (CM) in response to P. falciparum infection [MIM-WM-392094]

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Introduction: The reasons why some African children develop SMA whilst others CM in response to Plasmodium falciparum infection are unclear. Acquisition of P. falciparum-specific antibody parallels immunity development but does not explain differential timing of SMA and CM, nor why CM patients already have P. falciparum antibody. CM is associated with high levels of pyrogenic cytokines like IL-12. Could CM be due to prior priming of memory T cells causing immunopathology and SMA be an immunologically naive response?

Methods: Study participants were children aged 6–60 months presenting at QECH, Blantyre, with SMA and CM, control groups with uncomplicated malaria and healthy children. Eligibility for each group was
determined by Blantyre Coma Score, malaria parasite slide positivity and haematocrit. After parental consent, a 3.5 ml blood sample was collected in EDTA tubes and 1 ml in Eppendorf tubes. The former was used for full blood count, lymphocyte subpopulation quantification, lymphocyte subpopulation activation state (CD69, CD25, CD38) and naïve:memory (CD45RA:CD45RO) status. Serum was extracted from the Eppendorf tubes for intracellular cytokine staining to identify the subpopulations of lymphocytes producing significant cytokine levels.

Results: Children with SMA had more circulating B cells than the healthy controls with a higher proportion of these being naïve. Children with SMA and CM had variable numbers of T cells, in a similar range to those of the controls. However, those with SMA and CM had higher proportions of activated (CD38+ and CD69+) T cells and NK cells than healthy controls. High level activation could therefore be attributed to the infection. The children in all three study groups had variable Th1 and Th2 cells and these did not differ from those of the healthy controls. Most of the CD4+ T (Th1) cells in children with SMA were naïve (CD45RA+) with only a small percentage of memory (CD45RO+) Th1 cells. The few samples from children with CM had either similar proportions of naïve and memory Th1 cells or more of the memory Th1 cells. More children will be recruited and intracellular cytokine staining will be done on the serum that we are collecting.

Interpretation: The high activation of T, B and NK cells in SMA patients suggest that the immunological response to SMA involves all these cell types. These findings do confirm that CM is a result of immunopathological response resulting from the primed T cells.

O-117
Risk factors for persisting neuro-cognitive impairments in children following cerebral malaria [MIM-RI-55680]
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Introduction: Recent studies show that persisting neuro-cognitive impairments are common after childhood cerebral malaria (CM). Although risk factors (RF) for gross neurological deficits on discharge have been described, few studies have examined those associated with persistent neuro-cognitive impairments, which may give insight into the mechanisms of neuronal damage.

Methods: We examined the hospital records of 143 children aged 6–9 years who were previously admitted with cerebral malaria and assessed at least 20 months later for motor (spasticity, ataxia and fine motor abnormalities), speech and language (fluency, comprehension, syntax, morphology, pragmatics, word finding, semantics, higher order language), and other cognitive (memory, attention [visual scan] and non-verbal functioning [construction task]) impairments to determine risk factors for persisting impairments.

Results: The median age on admission was 30 months (IQR 19–42) and the median time from discharge to assessment was 64 months (IQR 40–78). Thirty-four children (23.8%) had impairments: 14 (9.8%) in motor skills, 16 (11.2%) in speech and language and 20 (14.0%) in other areas of cognition in particular, memory. Previous seizures (adjusted OR 5.6 [95% CI 2.0–16.0], p = 0.001); profound coma on admission (adjusted OR 28.8 [95% CI 3.0–280], p = 0.004); focal neurological signs observed during admission,
(adjusted OR 4.6 [95% CI 1.1–19.6], p = 0.037) and neurological deficits on discharge, (adjusted OR 4.5 [95% CI 1.4–13.8], p = 0.01) were independently associated with persistent neuro-cognitive impairments. In addition, multiple seizures were specifically associated with motor impairment, while coma longer than 24 h after admission was associated with impairment of other cognitive and motor skills. Age <3 years, hypoglycemia, severe malnutrition and features of intracranial hypertension were associated with speech and language impairment.

Interpretation: Risk factors for persisting impairments after CM include multiple seizures, profound/prolonged coma, hypoglycemia and RICP. Mechanisms of neuronal damage for each domain may be different. These RF could form the basis of future preventive strategies.

O-118
A new and simple method to quantify erythrophagocytosis in patients with severe malarial anaemia [MIM-RF-1826400]

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Introduction: Anaemia caused by Plasmodium falciparum malaria is one of the major health problems in endemic areas, especially for young children. Very few is known about the pathogenesis of this severe form of the disease. Anaemia is caused by three major factors: decreased erythropoesis in the bone marrow, enhanced lysis of red blood cells (RBCs) or increased phagocytosis of erythrocytes in peripheral blood and spleen. We developed and validated a new fluorescence based ex vivo technique to measure phagocytosis of autologous and heterologous RBCs in children suffering from severe malarial anaemia (SMA).

Methods: Eight Gabonese children aged 1–6 years with SMA defined as a haemoglobin level below 5 g/dl were included into the study. Blood was taken during the acute phase of the disease and after a disease-free interval of 2 months. Monocytes were immunopurified by anti-CD14 coupled beads. Subsequently, monocytes were incubated for 4h in RPMI at 37 °C/5% CO2 with three RBC samples: an autologous RBC sample, a positive and a negative RBC control. All RBCs were labelled with CFDA-SE, a fluorescein-conjugated dye. For positive control, theus positive (Rh+) RBCs from a healthy donor were opsonised with anti-D antibody; for negative control, the same Rh+ RBCs were left non-opsonised. Following the incubation period, non-phagocytosed erythrocytes were lysed by osmotic shock and the sample was analysed for phagocytosis by flow cytometry.

Results: Using a definite gating strategy, only live monocytes were included in the flow cytometry analysis. The phagocytic activity of monocytes was quantified by measuring the rate of positive monocytes (having phagocytosed at least one RBC) to overall monocytes. The monocytes from children with SMA revealed a phagocytosis rate with autologous RBCs of 19.0% (CI: 3.1–34.9%). Positive control Rh+ RBCs were phagocytosed by 15.5% (7.5–23.6), whereas negative control RBCs were phagocytosed by 3.4% (1.2–5.8) of the monocytes. After a two-month disease-free interval, phagocytosis rate was reassessed in all participants. Monocytes showed phagocytic activity of 7.1% (–3.1–17.5) in autologous samples, whereas phagocytosis activity in positive and negative controls was 17.6% (2.2–33) and 3.6% (–0.3–7.6), respectively. Validity of the assay was assured by the significant differences between positive and negative controls both during the acute and the reconvalescent phase (p < 0.002 and p < 0.001, respectively, paired t-test). When admission and reconvalescence samples were compared, we detected a highly significant increase of phagocytosis of autologous RBCs (p < 0.001) during the acute phase of the disease, whereas the uptake of heterologous opsonised and non-opsonised RBCs was not affected.

Interpretation: We validated a new technique to measure phagocytosis of RBCs. Phagocytosis of autologous RBCs is increased in children with SMA, whereas no effect of malaria on the uptake of heterologous control-RBCs was detected. Enhanced phagocytosis might be a determining factor for the development of SMA.
Blood transfusion practices and outcome in the management of severe malarial anemia at Kisii Hospital in the fringe highlands of western Kenya

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Introduction: Plasmodium falciparum malaria is the main cause of anemia in Kisii District of western Kenya and other highland fringe areas of sub-Saharan Africa. Case management of severe malaria-associated anemia is complicated by a substantial risk of HIV transmission associated with blood transfusion. We address the question: how clinicians should balance the risks of short-term mortality from severe malarial anemia and the long-term mortality from HIV/AIDS associated with blood transfusions?

Methods: The study was conducted at Kisii District Hospital, a 300-bed hospital in western Kenya and the principal referral center for a population of 800,000 residing in a highland area. In 1998–1999, malaria epidemics occurred in this area resulting in high blood transfusion (43%) and case fatality rates (CFR, 6%) among in-patients. In Kenya, the policy is to transfuse children with hemoglobin (Hb) <5 g/dl and respiratory distress and adults with Hb >5 g/dl. We randomly selected 804 cases from 3428 cases of patients admitted with a diagnosis of severe anemia during January to December 2001 to evaluate the effectiveness, defined as in-hospital survival, of clinical practices including diagnosis, anti-malarial therapy and blood transfusions.

Results: Few patients had hemoglobin (Hb) tested (38%) or blood slides for malaria parasitemia (21%), however, 81% (654/804) received blood transfusions and an equal proportion received anti-malarial therapy. The malaria slide positive rates differed among age groups (P < 0.001) and were highest in females >15 years old (80%) and children <5 years (78%). The blood transfusion rates also differed among age groups (P < 0.01) and were highest in children <5 years (87%). A total of 91 hospital deaths occurred among the 804 cases (CFR, 11%), 60% within 48 h of hospitalization. The CFR increased with age and was highest in adults >15 years (23%) (P < 0.01). A total of 306 patients had Hb checked. Of those, 38% (96/306) with Hb levels >5 g/dL received blood transfusions contrary to the national guidelines while 8% (13/306) were not transfused although their Hb >5 g/dL. Among children <5 years with Hb >5 g/dL, the CFR was higher among the non-transfused (33%) than the transfused (6%) group (P < 0.01). Among children <5 years with Hb > 5 g/dL, CFR was not significantly different between the two groups, but was significantly lower (P < 0.001) among those treated with antimalarials (4%) compared to those without (36%) regardless of performance of a blood smear.

Interpretation: Only severely anemic children benefited from blood transfusion. Hb measurements should avoid unnecessary transfusions where blood safety remains a concern. Antimalarials to anemic children without slide examination are justified where malaria is the predominant cause of anemia.

Cross-reactivity among DBL1, CIDR and C2 domains of Plasmodium falciparum erythrocyte membrane protein 1 (PIEMP1)

(1) Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark; (2) National Institute for Medical Research, Amani Centre, Amani, Tanzania

Introduction: PIEMP1 are responsible for antigenic variation and the pathogenesis of severe malaria. The var gene family comprising about 60 highly diverse genes per haploide genome encodes PIEMP1 molecules. Each PIEMP1 contains three domain types DBL (a, b, g, d and e), CIDR (a, b and g), C2 and according to, e.g. domain structure group into five distinct subgroups. Group A PIEMP1 and B/A molecules.

Cross-reactivity among DBL1, CIDR and C2 domains of Plasmodium falciparum erythrocyte membrane protein 1 (PIEMP1) [MIM-AJ-182622]

(1) Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark; (2) National Institute for Medical Research, Amani Centre, Amani, Tanzania

Introduction: PIEMP1 are responsible for antigenic variation and the pathogenesis of severe malaria. The var gene family comprising about 60 highly diverse genes per haploide genome encodes PIEMP1 molecules. Each PIEMP1 contains three domain types DBL (a, b, g, d and e), CIDR (a, b and g), C2 and according to, e.g. domain structure group into five distinct subgroups. Group A PIEMP1 and B/A molecules.
believed to be associated with severe malaria seem more conserved than PfEMP1 molecules belonging to Groups B, C, or B/C.

**Methods:** Recombinant DBL, CIDR, C2 domains representing group A–C and B/A PfEMP1 molecules were expressed in *Escherichia coli* or in baculovirus. Using a pool of high-titer plasma from Tanzanian adults cross-reactivity ELISA was done to analyse cross-reactivity among the different PfEMP1 domains. In indirect ELISA, antibody reactivity was measured to each of the domains using plasma samples from children (aged 2–4 years) living in a high (Mgome) and moderate (Ubiri) transmission area of Tanzania.

**Results:** CIDR domains of PF110008 (CIDR2b), PFD1235w (CIDR2b), PF130003 (CIDR1g) and PF110107 (CIDR1g) were found to cross-react and to be well recognised by plasma antibodies from Tanzanian children 2–4 years of age. By contrast DBL domains from any of the major Groups A–C or B/A only showed slight cross-reactivity and were only weakly recognised by plasma antibodies from the same children. Interestingly, C2 domains, which differentiate Group A PfEMP1 from other groups of PfEMP1 did not show any cross-reactivity.

**Interpretation:** CIDR domains were found to be well recognised by antibodies from children and to cross-react independent of subtype (a, b and g) or grouping (A or C).

**19. Health systems research**

**Wednesday 16 November 14:30–16:30—Iroko Hall**

**Chairs:** Don de Savigny (Basel) and Martin Sama (Yaounde)

**O-121**

**A health systems strengthening approach to malaria control in South Africa**

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Malaria is a leading cause of ill health and disease in many parts of the world and is responsible for the largest burden of disease in Africa. Malaria is endemic in three of the nine (9) provinces of South Africa namely the eastern parts of Limpopo province, Mpmalanga province and the north-east parts of KwaZulu-Natal province. Annual reported malaria cases varied between 2 000 and 13 000 during the 1975 to 1995 period. However, in the late 1990s, reported infections increased significantly to a peak of 64 222 cases and 458 deaths in 2000. The incidence rates and case fatality rates have improved significantly over the last 3 years. This paper outlines the importance of strengthening health systems and a comprehensive approach to the delivery of malaria interventions to reduce morbidity, mortality and improve malaria indicators. In particular it gives insight into South Africa’s comprehensive approach to malaria control which combined health systems strengthening, a renewed and concerted effort to vector control, prompt treatment and the use of geographic information systems for improved programme management interventions, including the use of combination drug therapy in KwaZulu-Natal and Mpmalanga, the reintroduction of DDT as an effective insecticide for indoor residual house spraying following a suspension in the use of DDT in 1996, and collaborative malaria control efforts with neighbouring countries. The paper focuses on the health systems approach and illustrates how this approach has contributed to improved malaria rates. Lessons are drawn from the South African Comprehensive, Management and Care and Treatment Programme for HIV and AIDS to further illustrate how strengthening of health systems as driven health improvements with a particular focus on health improvements in malaria control strategy.

**O-122**

**Les retards a la prise en charge precoce des accès palustres chez les enfants (Province de l’Extreme Nord – Cameroun) [MIM-GM-240671]**

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**Introduction:** La stratégie de lutte contre la morbidité des accès palustres est fondée désormais sur une prise
en charge précoce et adéquate des épisodes. Cependant, le principal symptôme du paludisme, la fièvre, surtout chez l’enfant, est loin d’être toujours perçu comme un signe d’alerte, et, si l’on ajoute à cela le manque de réactivité des systèmes de santé au niveau local, on est alors souvent confronté à un contexte peu favorable à la mise en œuvre de la stratégie recommandée.

Methods: Les résultats dont nous faisons état procèdent d’entretiens réalisés auprès de mères d’enfants hospitalisés dans le service de pédiatrie d’un hôpital de référence de la partie septentrionale du Cameroun, ainsi qu’auprès de personnels de structures de soins situées à différents niveaux de la pyramide sanitaire.

Results: Dans la partie septentrionale du Cameroun, zone à transmission saisonnière, la période de transmission maximale du paludisme, et le terme "palu" sont bien connus des populations, même si le lien est loin d’être toujours fait entre moustique et paludisme. En même temps la "fièvre" apparaît souvent comme un événement inéluctable dans le cycle de développement de l’enfant, et ce n’est qu’en cas d’aggravation de l’état général de la personne qu’une décision de traitement sera prise. Pour cette pathologie bien connue, des personnels de santé et des populations, les itinéraires des patients sont aussi longs que pour toute autre pathologie. De la longueur de ces itinéraires qui aboutissent à une hospitalisation dans un service de référence, on peut en conclure l’inadéquation de la prise en charge au niveau périphérique. Ces délais à la prise en charge sont la conséquence d’une méconnaissance de la gravité des conséquences possibles du paludisme au niveau périphérique, par les parents et par les personnels de santé, d’une prise en charge médicale inadéquate au niveau périphérique (absence de médicaments, erreur de diagnostic, erreur de prescription, etc.), et des limites financières des ménages.

Interpretation: La stratégie préconisée de prise en charge précoce fondée se heurte à des perceptions non stabilisées de la gravité des conséquences du paludisme, tant parmi les personnels de santé que parmi les individus les plus vulnérables.

**O-123**

Inequities in drugs prescription, costs and quality of malaria treatment in Nigeria: Implications for use of artemisinin-based combination therapy [MIM-OO-245336]

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Introduction: There is paucity of knowledge about inequities in drugs’ prescription, quality and actual expenditures on malaria treatment from various healthcare providers. The information is vital for improving malaria treatment especially using artemisinin-based combination therapy (ACT). The study examined the levels of socio-economic and geographic inequities in choice of providers, drugs prescribed, expenditures as well as the quality of malaria treatment services from different providers.

Methods: Exit polls using a pre-tested questionnaire were used to interview a total of 750 people (500 from an urban community and 250 from a rural community) from Anambra state, southeast Nigeria who had just received treatment for fever from a range of public and private providers. Data was collected on the treatment received (diagnostic processes, drugs prescribed and dispensed, cost of treatment, advice given and satisfaction with quality of care) and household’s socioeconomic status. A socio-economic status (SES) index, divided into SES quartiles (poorest, poor, average, below average) was used to examine socio-economic inequities, whilst comparison of data from urban and rural areas was used to explore geographic inequities.

Results: There was low availability, low prescriptions but high cost of ACT. Chloroquine and sulfadoxine-pyrimethamine (SP) tablets were the most commonly prescribed drugs. The average cost of drugs was lowest for SP ($1.51) and highest for ACT ($5.06). The people were least satisfied with quality of services of patent medicine dealers (PMD) and pharmacy shops (PS) and were mostly satisfied with services ren-
dered by specialist and general hospitals. There were inequities in the use of different providers, drugs prescribed, cost and quality of services. The better-off SES groups used more of private hospitals/clinics and specialist hospital, whilst the poorest and poor SES groups used more of drug sellers. The poorest SES and rural dwellers were prescribed the cheaper drugs whilst the better-off SES and urbanites were prescribed the more costly drugs. The cost of treatment was lowest with PMD and PS ($1.62 and $2.17, respectively) and highest with specialist ($20.46) and private hospitals ($8.69). The better-off SES and urbanites were more satisfied with quality of services than the rural dwellers and worse-off SES groups.

Interpretation: Inequities in malaria treatment are tilted against the poorest and rural people. The quality of services offered by the commonly used drug sellers should be improved so as to decrease inappropriate drug prescribing, use, costs and resistance to ACT.

O-124
A randomized controlled trial of improved financial access to health care on morbidity due to malaria among children 6–59 months of age in Ghana

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Introduction: Financial barriers to effective treatment for malaria can be reduced but there is currently no direct evidence that doing so leads to reduced morbidity from malaria. This study set out to determine by means of a randomized trial the impact of reducing financial barriers to health care on morbidity due to malaria among children. The setting is a rural district in Ghana. Malaria accounts for over 50% of reported cases to outpatients. Prior to this study chloroquine was the first-line treatment.

Methods: Preliminary studies included qualitative studies and an assessment of the therapeutic efficacy of chloroquine. 2151 households with 2523 children 6–59 months were randomized into two groups for the randomized controlled trial. A baseline survey involving the collection of parasitological, anthropometric, haematological and household socio-economic data was carried out in April 2004. Passive morbidity monitoring of study subjects for 24 weeks was followed by a final cross-sectional survey. The intervention group were enrolled in a pre-payment scheme which allowed them free access to primary care including diagnosis and drugs whenever they are ill, with limited access to secondary health care. The control group still paid user fees.

Results: An initial assessment of chloroquine showed only 39.6% (n=42) of the patients showed adequate clinical and parasitological response. As a result of this the first line drug for the treatment of malaria in the study district was changed to the proposed national policy of amodiaquine + artesunate. Qualitative studies showed that causes of delay in seeking care include cost of care, trying other sources nearer home and less expensive, transport costs, distance from facility, and perception of poor quality of care. For the main trial, at baseline the cross-sectional survey showed that several indicators of socioeconomic status and median haemoglobin level in the two randomized groups were very similar. The group who had chosen to enrol in the existing pre-payment scheme themselves were however significantly better off socio-economically and were also significantly different in terms of their median haemoglobin level at baseline (p 0.02). The final cross-sectional survey has been completed and data on health outcome measures including haemoglobin, parasite prevalence and healthcare utilisation will be available within a few months.

Interpretation: Qualitative data demonstrates that financial barriers are important reasons for delay in seeking treatment for malaria. Those who buy health insurance are significantly different from those who do not. The main trial outcome data is being analyzed.

O-125
Resilience of community and health systems under conflict for responding to malaria: A multi-country study, Congo case study

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Introduction: Individuals, communities and health systems respond to the disruption caused by conflicts. Some evidences on the responses are available for communities with much less documented on the reaction of health systems. The notions of vulnerability and the local responses have been explored to some degree in others sectors like food security but remains unclear or largely unexplored in infectious diseases like malaria. This highlights an important gap in current research.

Methods: Exploratory multi-country study using grounded theory approach in generating hypothesis. Data were gathered using key informants interview focus group discussion with community’s leaders, heal- ers, religious, women, NGO’s, local government health workers, services delivery providers, projects managers, policy makers, and aid agencies. Secondary data collected from published and gray literature. Participants and sites were purposively selected. Data were first audio taped before being transcribed verbatim. Categories and themes and were identified and continually compare to the ground. Triangulation of data and restitution were used for validation of data.

Results: Community vulnerability and responses to malaria: displacement leading to lack of shelter and host community, poverty and lack of food, poor mental health by lacking some family members, poor access to services and difficult to access to humanitarian aid, absolute lack of services and increase in self-medication, consultation of traditional and or faith healers. Factors that account to vulnerability and response in malaria: ethnicity in which all stakehold- ers in malaria programme are involved. Health sys- tems vulnerability and response to infectious disease: chronic shortage of input, health infrastructure among many targets, insecurity. Construction of new infras- tructures closed to population, alternatives sources of income from NGO’s, community’s resources. Effect of conflict on utilization and access of healthcare: utilization very low in formal health care system, poor quality of care, financial and cultural barriers, preventive care not widely provided by the public service. Determinants of resilience for communities and the Congolese health system: citizen and civil society response to the conflict, mobilization of external funds, adaptation in gender role, solidarity at family and community level, strong and innovative civil society and NGO commu- nity, trust in alternatives medicines.

Interpretation: Although population had developed many coping strategies in this context, malaria remains a big public health issues. Planners and policy makers need to learn from the local strategies to rebuild the health system in malaria program.

O-126 Application of medical informatics to investigate etiologies of pediatric severe malarial anemia in a Plasmodium falciparum holoendemic area [MIM-ZL-68012]


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Introduction: Severe malarial anemia (SMA) is a primary manifestation of severe childhood malaria in holoendemic Plasmodium falciparum transmis- sion areas. Since reduced hemoglobin levels in SMA (Hb < 5.0 g/dL) is exacerbated by co-pathogens, nutri- tional deficiencies, and hemoglobinopathies, a multi- disciplinary study was designed to characterize the genetic and immunological basis of SMA. To enhance complex data capture and clinical management a Patient Management Information System (PMIS) was developed.

Methods: The PMIS was designed for a rural hospit- al in Siaya, western Kenya and built on a framework developed by Baobab Health Partnership (BHP) in Lilongwe, Malawi. The PMIS employs three technolo- gies to foster robustness in a resource-poor setting: fault-tolerant hardware utilizes redundancy to protect data during power failure, hard disk crash, or power supply malfunction; compact touch-screen worksta- tions allow clinical staff to manage data with minimal training while consuming only 20% of the power of desktop computers; and barcode printers and scan-ners facilitate rapid access to patient information. The PMIS interface validates data collected in real-time to promote data accuracy and completeness, and tracks patient movement through the facility.
Abstracts / Acta Tropica 95S (2005) S1–S506

O-127

Routine delivery of antimalarial combination therapy with sulfadoxine/pyrimethamine (SP) plus artesunate in rural Tanzania: Coverage and adherence [MIM-JM-179448]


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Introduction: Artemisinin-containing combination therapies (ACT) for malaria are widely recommended to delay drug resistance and reduce malaria transmission. Evidence to support these claims is limited and experience delivering ACT in African settings is minimal. In particular, very little is known about how to achieve high levels of coverage and adherence with ACT through resource-constrained public-sector health care delivery systems.

Methods: In February 2003 the Rufiji District Council Health Management Team introduced ACT with sulfadoxine/pyrimethamine (SP) plus artesunate (AS) as routine first-line treatment for uncomplicated malaria at all 56 registered health facilities in the district. The ACT was introduced alongside health worker training and information, education and communication materials intended to optimize appropriate dosing and adherence. In the first 24 months, more than 500,000 patients received SP + AS at health facilities in the district. We assessed coverage through random audits of dispensing records at health facilities and through a survey of 4988 randomly selected residents of the district. We observed 453 patient-provider encounters to evaluate dispensing practices at health facilities and completed a follow-back study of adherence with 253 patients who received SP + AS.

Results: According to audits of dispensing records at all health facilities in the district, ACT with SP + AS accounted for 86% of all malaria-specific treatments dispensed. SP + AS doses outnumbered episodes of SP monotherapy 25:1. Coverage was highest at peripheral health facilities and relatively lower at hospitals and health centres where a greater proportion of patients with severe or complicated malaria were seen. SP + AS accounted for 86% of all malaria-specific treatments dispensed. SP + AS accounted for 61% of all antimalarial treatments obtained from any source as reported by randomly selected district residents in the household survey. The proportion of care-seeking visits made to health facilities increased from 32% to 55%. Health workers dispensed the correct number of tablets in 96% of observed encounters and administered the first dose under direct observation 99.7% of the time. Based on a conservative estimate that included reported behaviour and counting remaining tablets 48 h after the initial treatment, 75% of patients participating in the follow-up study took the remaining two doses exactly as directed. This is nearly twice the level of adherence observed elsewhere and was achieved under routine programmatic conditions.
We documented high coverage and adherence with ACT delivered through the existing health care system. Further efforts are underway to establish the impact of this intervention on malaria transmission, drug resistance, and public health outcomes.

Interpretation: We documented high coverage and adherence with ACT delivered through the existing health care system. Further efforts are underway to establish the impact of this intervention on malaria transmission, drug resistance, and public health outcomes.

Results: The analysis of data from Ethiopia has shown that monitoring minimum temperature may be useful in epidemic early warning, as abnormal increase in this variable was associated with major epidemic events during the late 1980s and early 1990s. Results also indicate that a dynamic immunity mechanism will be required in epidemic prediction models, in addition to meteorological variables. Robust forecasting systems are still required for use in the highlands, especially for decisions involving expensive preventive measures such as indoor residual spraying of insecticides. A new algorithm has been developed for detection of abnormal incidence based on de-trended baseline data. Computer-based weekly surveillance at district levels has proved to be feasible and has helped in epidemic detection within 1–2 weeks of onset in Kenya and Uganda. Response to epidemics has not always been as rapid and effective as it should be due to resource constraints and insufficient human, organizational and logistics capacity. Using effective interventions such as mass fever treatment during epidemics has become problematic due to parasite resistance against cheaper drugs and the much higher cost of the recommended artemisinin-based combination therapy.

Interpretation: Epidemic forecast is still a challenge, although early detection is feasible in the short-term. Response should be backed by a decision support system linked to dynamic risk mapping, rational use of interventions and selective targeting of areas.
for their prompt control. Ethiopia, Madagascar and Sudan host almost the half of the population at risk of epidemics in Africa. Over the last 5 years, each of them has used available tools to design and pilot Early Detection Systems (EDS). While using distinct strategies and objectives, common challenges and opportunities can be identified.

Methods: Between December 2003 and February 2005, the authors undertook an on-site functional analysis and data review of national malaria epidemic detection and surveillance systems in three African countries: Ethiopia, Madagascar and Sudan.

Results: The original reasons for improving EDS differed per country. Ethiopia was in the aftermath of the large 2003 epidemic in the highlands, Madagascar wanted to optimize the targeting of spraying operations on the plateau, which had been resumed since 1993, and Sudan was facing the impact of urban malaria and irrigation in the arid north. At the design stage, different spatial resolution of data collection, level of centralization of management and integration with surveillance of other diseases had been opted for. The study identified and analysed (1) challenges and methodological options to select, collect and analyse historical data to define abnormal incidence; (2) different choices to plan resources and guide procedures, analysis and decisions; and (3) opportunities to further improve capacities for malaria epidemic control in Africa.

Interpretation: Obstacles are numerous to timely detect malaria epidemics but valuable experience gathered from diverse initiatives can be profitable elsewhere.

O-130
Malaria epidemics in Tanzania: Retrospective assessment, early warning and detection in nine epidemic prone districts [MIM-FM-340949]
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Introduction: Malaria transmission is stable, perennial or seasonal, in 80% of in Tanzania and unstable, highly seasonal, in the remaining 20%. About 7 m people are living in malaria epidemics prone areas. Assessment of epidemic risk and impact, establishment of an early detection system in nine epidemic prone districts are illustrated and discussed.

Methods: Retrospective epidemiological information from 1996 to 2004 were collected in over 500 health facilities of nine districts that either have reported epidemics in the last 10 years or are classified as epidemic prone according to geo-climatic criteria. At least five years retrospective malaria morbidity and mortality indicators collected in the peripheral health facilities have been used to establish a profile for each area at risk of malaria epidemics. Frequency of malaria abnormal transmission has been used for calculation of epidemic risk indexes at different altitude and at different administrative and health facility levels. Severe malaria indicators for admitted patients were collected to assess the impact of previous epidemics.

Results: Malaria epidemic risk indexes have been estimated at sub-district level to map epidemic hot-spots. A considerable variation in the epidemic risk index has been observed within district (0.6–0.23). Significant higher epidemic risk indexes have been recorded between 1500 and 1750 masl, compared with higher and lower altitudes. The retrospective assessment of the impact of malaria epidemics in the nine districts shows that the number of admissions, blood transfusions and deaths during malaria outbreaks were, respectively, 4.1-, 4.6- and 4.0-fold higher than in non-epidemics years. Retrospective epidemiological information from clinic and hospitals were used to establish a “normal” pattern of malaria transmission for each given area. Suitable epidemic thresholds were empirically calculated and plotted into weekly and monthly malaria monitoring charts and distributed to all health facilities. If an unusual increase in the number of malaria cases is observed compared to the established profile, the possibility of an epidemic should be considered, the event notified and investigations started. The predictive value of regular collection of meteorological data for providing early warning is currently being investigated.

Interpretation: Our findings show that simple methods for mapping epidemic risk and early detection at the lowest health levels are of paramount importance in order to deploy effective preparedness plans at the right time and in the right place.
O-131
Weekly surveillance for decision making: An experience from Zimbabwe's malaria control pro-
gramme [MIM-VT-95168]

V. Teverdzi, S. Mudzi, S. Matinhure, J. Pasipamire, M. Netsa

Introduction: Frequent outbreaks have been a feature of malaria epidemiology in Zimbabwe from 1996. Diffi-
culties in deciding whether a district or sub-district was in an outbreak situation could sometimes be encoun-
tered. However, a system of monitoring epidemic prone diseases on a weekly basis using carefully chosen sen-
tinel sites has been in place since 1994. This system has been improved upon over the years and now includes
the use of thresholds to detect malaria outbreaks.

Methods: In 2004, 701 out of a total national of 1460 health facilities were selected as sentinel sites for
weekly surveillance. The criteria for selection included the presence of a functional radio or telephone, the
presence of a qualified nurse or environmental health technician and geographical representation in the dis-
trict. The week begins on Monday and ends Sunday. Rural health centres submit data to districts by Monday,
districts to provincial centres by Tuesday and provinces and main city hospitals to national level by Wednesday.
Completeness and timeliness are the two performance indicators measured when all data reaches national
level.

Results: At least seven different malaria outbreaks were detected using this system in 2004. The lower the level of
health care delivery the more sensitive the system was in picking outbreaks. Where district health
management was weak, the system failed to elicit the responses expected from disease control managers.

Interpretation: The weekly disease surveillance system provides early warning for preparedness and response
to outbreaks. It is an evidence based tool for declaration of epidemics.

O-132
Malaria risk prediction in holoendemic area: The potential of small scale mathematical modeling
[MIM-YY-27867]

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Introduction: Malaria transmission is driven by weather conditions, which influence the mosquito pop-
ulation dynamics. Further understanding of the transmission dynamics can facilitate the decision making
process for malaria control. A key tool for this understand-
ing is the use of models. This study aims to
enhance our understanding of the transmission dynam-
ics of malaria in the dry season in a holoendemic region,
Nouna district, Burkina Faso using process-based mod-
eling.

Methods: The study took place in Nouna health dis-
trict, Northwest Burkina Faso. Eight hundred and
sixty-seven children (6–60 months) were followed up
(December 03 to May 04) for Plasmodium falciparum
(Pf) detection. Mosquito population abundance was
monitored monthly by standard CDC Light Trap and
Human Landing Trap. A mathematical model devel-
oped was based on standard division into susceptible,
infected and infectious groups for both human and
mosquito. The model fitted was used to predict monthly
incidence case of Pf infection and compared to the
observed. The goodness of fit was assessed by the sum
of monthly square difference between observed and
model incidence. The best fit of the model parame-
ters was achieved by determining successively the best
value for each parameter.

Results: The overall Pf incidence was 313 cases among
867 participants involving 255 participants. The inci-
dence of Pf infection consistently decreased from
December to January in all sites followed by a sec-
ond peak ending with a decrease towards the end of the dry season. In the four sites Culex is the most prominent genus. In total, the Anopheles-Culex ratio is 1/6. The semi-urban site has the most number of Culex compared to the rural sites (622 versus 174). Gambiae was the most prominent among the Anopheles species (65%), funestus (33.3%) and nili (1.6%). The number of mosquitoes drops from December to January and rises again in February. There is a close match between the model prediction and observed cases for all months except March and May. The variance between the observed and the predicted monthly incidence was 835. The good fit of the mathematical model output to the observed P. falciparum infection incidence suggests that the model is indeed a good representation of the transmission dynamics. The discrepancies in May and March may be random variation due to the small number of cases. For both months, though the observed case counts are not significantly different from the previous month (Chi test; \( p = 0.09 \) and \( p = 0.12 \), respectively).

O-133 Managing and responding to malaria outbreaks in Mpumalanga, South Africa during the 2003/2004 season [MIM-AM-86913]
A. Mabuza
Department of Health, Mpumalanga Province, South Africa

Introduction: Malaria in Mpumalanga is unstable with localised outbreaks and epidemics in towns in the highest risk area in the eastern part bordering Mozambique and Swaziland. Malaria transmission is seasonal. The majority of the population at risk is non-immune. The main control measure in place is indoor residual spraying with DDT and a synthetic pyrethroid. In the 2003/2004 season outbreaks occurred in both high and low risk areas in the Ehlanzeni district.

Methods: Before the onset of the transmission season, the programme ensures that sufficient funds are available for equipment, transport, insecticides and maintenance. Feedback about the previous season is provided to health professionals. All health facilities are visited to check the availability of drugs and malaria rapid diagnostic tests and also sensitizing pharmaceutical services as to expected severity of malaria so that adequate anti-malarial drugs are procured. Malaria is a notifiable disease and the control programme collects notifications from health facilities weekly. The weekly data is monitored and a GIS used to monitor spatial distribution of cases and deaths.

Results: The first occurred in a low risk area during the beginning of the season between October and March. Seventy-seven cases were notified with six deaths. A team from the malaria control programme was deployed to the area. Surveillance visits to health facilities increased to daily visits. Malaria drug was changed from SP to SP and Artesunate. The area was sprayed, ITNs and repellants were distributed to community and awareness campaigns increased. The second occurred at one locality in the high risk area between January and March. 281 cases were notified with no deaths. A team was deployed to the area to stay for 2 days doing active surveillance in community. RDTs were used to detect parasites in people and treatment administered to positive cases.

Interpretation: The malaria outbreaks in Mpumalanga were detected in time and the situation was brought under control. A good surveillance system and readiness to respond to outbreaks is importance to prevent loss of lives in epidemic prone areas.

21. Vaccine trials
Thursday 17 November 11:00–13:00—Bubinga Hall
Chair: David Diemert (Rockville) and Kalifa Bojang (Fajara)

O-134 No abstract received.

O-135 No abstract received.
O-136
Randomized double-blind placebo-controlled Phase IIa trial to test efficacy of the malaria vaccine PCS102 to protect against falciparum challenge [MIM-BG-492462]
(1) Medical Outpatient Clinic, University of Lausanne, Swiss Tropical Institute, Basel; (2) Division of Immunology and Allergy, University Hospital, Lausanne; (3) Department of Medical Microbiology, Radboud University Nijmegen, Medical Centre, The Netherlands; (4) RMF Dictagene S.A., Lausanne, Switzerland

Introduction: The sporozoite stage of Plasmodium falciparum is a potential target for malaria vaccine development. The results of two phase I trials using the long synthetic peptide PCS282–383 (PCS102) formulated in different adjuvants showed good safety and immunogenicity profiles. The objective of the present study was to test whether a vaccination with PCS102 provided protection against malaria parasitaemia after infective bites in non-immune volunteers.

Methods: Sixteen non-immune volunteers were randomized to receive two doses of either 30 μg of PCS102 formulated in Montanide ISA 720 (verum) or ISA 720 alone (placebo), 60 days apart (double-blind). Fourteen days after the second immunization, 14 volunteers were challenged with bites of five infected mosquitoes using the NF54 strain of P. falciparum.

Results: Malaria parasitaemia developed in all study subjects, i.e. 8/8 in the verum group and 6/6 in the placebo group. In 12/14 volunteers, treatment was started with artemether/lumefantrine (Riamet®) on day 9, 1/14 on day 10 and 1/14 on day 11. The length of time from infection to the onset of malaria was similar in the verum and placebo group (median pre-patent period of 214 h in the verum group [197–240] and 216 in the placebo one [200–247]).

Interpretation: The vaccine candidate PCS102 provided no protection against artificial challenge in its current formulation with Montanide ISA 720, and in the conditions of the test (five mosquitoes infected with NF54 strain of P. falciparum).

O-137
Safety, immunogenicity and efficacy studies of candidate malaria vaccines FP9 and MVA encoding ME-TRAP in Kenyan children [MIM-PB-224908]
(1) Kenya Medical Research Institute (KEMRI), CGMRC, Kilifi, Kenya; (2) Nuffield Department of Clinical Medicine, University of Oxford, Oxford OX3 7BN, UK

Introduction: Vaccines that induce T lymphocyte responses against the pre-erythrocytic malaria antigen construct ME-TRAP (thrombospondin-related adhesion protein) and multiple T and B cell epitopes (ME), have protected non-immune volunteers in sporozoite challenges. Studies immunising adults in Kenya were conducted prior to immunising children.

Methods: Twenty-two 1–6-year-old children were immunised with two sequential priming immunisations of the attenuated fowlpox virus FP9 followed by modified vaccinia virus Ankara (MVA), both recombinant for ME-TRAP. Different doses were compared; either 5 × 10⁷ or 1 × 10⁸ plaque forming units (pfu) of FP9 before either 7.5 × 10⁷ or 1.5 × 10⁸ pfu of MVA ME-TRAP. Antigen specific interferon gamma producing T cells were counted by ELISpot.

Results: Reactogenicity was limited to local, self-limiting skin reactions at the site of intra-dermal injection, and mild febrile systemic reactions. All regimes were immunogenic, but low dose priming followed by high dose boosting induced the highest frequency of antigen specific T cells.

Interpretation: It is an ongoing study still enrolling 410 more children in a phase Ib trial to further observe immunogenicity, safety, and efficacy against febrile malaria during 9 months of follow up. These data will be analysed in February 2006.
O-138 Safety and immunogenicity of the WRAIR and GSK Biologicals’ malaria vaccine candidate AMA-1/AS02A versus rabies vaccine in adults in Bandiagara, Mali [MIM-MT-9315]


(1) Malaria Research and Training Center, University of Bamako, Bamako, Mali; (2) Walter Reed Army Institute of Research, Silver Spring, MD, USA; (3) USAID, Washington, DC, USA; (4) GlaxoSmithKline Biologicals, Rixensart, Belgium; (5) Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, USA

Introduction: Co-developed by WRAIR and GSK, the malaria vaccine FMP2.1/AS02A is comprised of lyophilized recombinant AMA-1 protein derived from the 3D7 Plasmodium falciparum clone, produced in Escherichia coli bacteria and formulated with the adjuvant AS02A. In a clinical study in malaria-naïve adults in the U.S. the FMP2.1/AS02A vaccine candidate was safe and highly immunogenic. Here we report the first study designed to evaluate the safety, reactogenicity and immunogenicity of the FMP2.1/AS02A in a malaria-endemic area.

Methods: We undertook a phase 1 double blind dose escalation randomized controlled trial beginning in December 2004, in Bandiagara, Mali. Sixty healthy adults aged 18–55 years were randomized to receive either one of two doses (25 mcg or 50 mcg of FMP2.1) of the malaria vaccine or rabies vaccine at 0, 1 and 2 months, and are being followed for one year. The primary analysis will be conducted on data collected through 30 days after the third immunization.

Results: As of March 2005, the vaccine has been shown to be safe and well tolerated by volunteers. Blinded safety analyses following the first two of three 25 mcg immunizations and the first of three 50 mcg immunizations revealed no significant safety or reactogenicity problems. Titers of anti-AMA-1 antibodies will be measured by ELISA and P. falciparum growth inhibition assays will be performed on sera collected at pre- and post-vaccination time points. Safety and immunogenicity data will be reported.

Interpretation: The FMP2.1/AS02A is a promising malaria vaccine candidate. The preliminary safety profile shown allows envisioning further clinical development of the candidate vaccine in pediatric population.

O-139 Phase 1 study of the safety and immunogenicity of the AMA1-C1/Alhydrogel® Vaccine for Plasmodium falciparum malaria in semi-immune malian adults [MIM-DD-109020]


(1) Malaria Research and Training Center, University of Bamako, Bamako, Mali; (2) Malaria Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, Rockville, USA

Introduction: Apical membrane antigen-1 (AMA1) is a surface protein expressed during the asexual blood stage of Plasmodium falciparum that has been implicated in parasite invasion of erythrocytes. AMA1-C1/Alhydrogel® consists of an equal mixture of recombinant AMA1 from the FVO and 3D7 clones of P. falciparum that is adsorbed onto Alhydrogel®. A Phase 1 trial in malaria-unexposed US adults demonstrated that this vaccine is safe and induces anti-AMA1 antibodies that partially inhibit parasite growth in vitro.

Methods: A Phase 1 study of 54 healthy adults was started in May 2004 in Doneguebougou, Mali. Participants were enrolled into 1 of 3 dose cohorts (n = 18/cohort) and randomized 2:1 to receive either AMA1-C1/Alhydrogel® or Recombivax® Hepatitis B vaccine. The first, second, and third cohorts were vaccinated successively at 3-week intervals, and received 5, 20 and 80 mcg of AMA1-C1, respectively. Vaccinations were administered on days 0, 28, and 360. Study participants were examined on days 1, 2, 3, 7, and 14 after each immunization. After the second and third immunizations, participants were seen monthly during the rainy season. The study will remain double-blinded until day 180, after which the randomization code will be revealed to the investigators.

Results: Fifty-three of 54 volunteers received both of the first two vaccinations, with the third vaccination scheduled for May–June 2005. As of study day 180, no vaccine-related serious or grade 3 adverse events have been observed. All injection site reactions
have been mild in severity, whereas systemic reactions have been mild to moderate in severity, with headache being the most commonly reported vaccine-related systemic adverse event. There does not appear to be any consistent pattern in local or systemic reactogenicity with respect to increasing dose of vaccine or increasing number of immunizations. No clinically-significant laboratory abnormalities have been observed, to date. Anti-AMA1 antibody responses are being measured by ELISA on sera collected at pre- and post-vaccination time-points, and biologic activity of these antibodies will be tested by the in vitro P. falciparum growth inhibition assay. After unblinding of the study code, safety and immunogenicity data will be compared between the AMA1-C1/Alhydrogel® and Recombivax groups.

**Interpretation:** The data so far demonstrate that AMA1-C1/Alhydrogel® has an excellent safety profile. Full unblinded safety and immunogenicity results obtained after all three immunizations have been administered will be presented.

**22. Drug resistance 2**

**Thursday 17 November 11:00–13:00—Ebony Hall**

**Chairs:** Alexis Nzila (Nairobi) and Collen Masimirembwa (Harare)

**O-140**

The interaction between malaria and HIV associated immune suppression among adults in Blantyre, Malawi [MIM-CP-906063]


(1) Center for Vaccine Development/University of Maryland School of Medicine, Baltimore, USA; (2) Department of Medicine/University of Malawi College of Medicine, Blantyre, Malawi; (3) Blantyre Malaria Project/University of Malawi College of Medicine, Blantyre, Malawi

**Introduction:** HIV and *Plasmodium falciparum* malaria are two of the greatest health threats in sub-Saharan Africa. These infections coexist in large populations in Africa so even a small interaction between the two may have important public health consequences. Although the interaction between HIV and malaria has been investigated in a variety of settings, no consistent relationship has emerged.

**Methods:** We conducted an observational cohort study of adults living with HIV in Blantyre, Malawi. Participants who volunteered for the study were evaluated on the day of enrollment and every month thereafter and were instructed to return to the study clinic for evaluation any time they were ill. CD4 cell counts were measured on enrollment and every 4 months. Malaria smears were obtained at every visit. A case of clinical malaria was defined as signs or symptoms of malaria in the presence of parasitemia. Clinical malaria with significant parasitemia used a parasite density cut-off, based on the distribution of parasite density among participants with asymptomatic parasitemia. Severe malaria was defined according to WHO criteria.

**Results:** We enrolled 660 adults from September 2002 through December 2003 and followed them through August 2004, accruing 599 person years of follow up. The mean age was 32 years and 66% were female. At enrollment, the mean CD4 cell count was 268 cells/μm³. The overall incidence of new parasitemia was 0.46 episodes/person year of observation (pyo). The rate of clinical malaria was 0.32 episodes/pyo and the rate of clinical malaria with a significant parasite density was 0.18/pyo. There were three cases of severe malaria. The incidence of new episodes of parasitemia and clinical malaria was not significantly different in CD4 count strata (<200, 200–499, ≥500), once we controlled for the non-independence of observations within person \((p = .69 \text{ and } p = .22)\). However, there was a trend towards decreased rates of clinical malaria with significant parasitemia \((p = .07)\) among individuals with higher CD4 counts, particularly over 500 cells/μm³. There was no difference in the threshold parasitemia that caused fever by CD4 count. Although the more immunosuppressed groups always had a higher incidence of fever, the probability of having fever with rising parasite density increased proportionately in all CD4 strata.

**Interpretation:** In this comprehensive community based study, we did not find a strong interaction between HIV immunosuppression and malaria. Clin-
O-141
Association of PfNHE polymorphisms and quinine usage in Mali [MIM-AK-15020]
A. Kone, A. Djimde, J. Ma, C. Plowe, O. Doumbo, T. Wellems
(1) Molecular Epidemiology and Drug Resistance Unit, Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases, Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali; (2) Malaria Section, Center for Vaccine Development, University of Maryland, USA; (3) Laboratory of Malaria and Vector Research, NIAID, NIH, Bethesda, USA

Introduction: The mechanism of Plasmodium falciparum resistance to quinine is not known. Recent QTL mapping studies indicate that genetic loci on several chromosomes may be involved, including polymorphisms in a P. falciparum Sodium-Hydrogen Exchanger (PfNHE) on chromosome 13.

Methods: To investigate the role of those polymorphisms in malaria endemic settings we conducted an association study between the prevalence of polymorphisms of the PfNHE microsatellite MS4760.1 and the rate of quinine usage in Kolle, Bandiagara and Faladié, Mali, using retrospective data. Our unpublished observations suggest increasing rates of quinine usage in these settings, respectively. Size polymorphisms of MS4760.1 were determined by nested PCR of DNA extracted from blood blotted onto filter papers.

Results: We found that the prevalence of the MS4760.1 polymorphism associated with decreased quinine sensitivity was 18.3, 26 and 30.2% in Kolle, Bandiagara and Faladié, respectively. The rates of these polymorphisms were significantly associated with quinine usage in the respective sites (Spearman correlation, p < 0.05).

Interpretation: These results indicate an association between the prevalence of quinine-resistance-associated PfNHE polymorphisms and usage of quinine in the field. Additional studies will improve our understanding of the molecular mechanism of quinine resistance.

O-142
Community-based approach to assess drug resistance in malaria: Establishment of resistance genogram by microarray technique [MIM-KM-185725]
K. Mugittu, A. Crameri, I. Felger, H. Mshinda, B. Genton, T. Beck
(1) Ifakara Health Research and Development Centre, Ifakara Tanzania; (2) Swiss Tropical Institute, Basel, Switzerland

Introduction: Monitoring drug resistance is essential and mutations in resistance conferring genes could be used for this purpose because the mode of action and mechanism of resistance of some antimalarials are well characterized. However, the association between these makers and treatment failure at health facilities has not been consistent. We assessed the relevance of community cross-sectional surveys versus clinical studies in monitoring resistance to antimalarials.

Methods: Between 2003 and 2004 we conducted two in vivo studies in three sites in Tanzania to assess sulfadoxine–pyrimethamine (SP) efficacy. A total of 455 patients aged 6–59 months with uncomplicated falciparum malaria were treated with SP and response assessed for 28 days. Concurrently, we collected blood samples from 1200 individuals living in proximity to the health facilities. By using a high throughput microarray-based technique we simultaneously determined single nucleotide polymorphisms (SNPs) in P. falciparum genes associated with antimalarials drug resistance (dhfr and dhps, mdr1, crt and ATPase).

Results: In 2003, of 228 patients 85% showed an adequate clinical and parasitological response on day 14 and only 51% on day 28. Similarly, in 2004, of 227 patients 83% and 56% showed an adequate response on days 14 and 28, respectively. A total of 628 (50%) out of 1200 individuals in the community had asymptomatic infections. The frequency of quintuple mutant haplotypes reflected SP failure rates at the health facilities. We also evaluated the potentials of a new microarray-based high throughput technique in prediction of resistance and assessment of dynamics of drug resistance. Therefore, we also determined the prevalence of 22 additional drug resistance associated SNPs in mdr1, crt and ATPase. The approach to assess the frequency
of molecular markers in communities using this new technology might replace tedious in vivo studies, provided the correlation between prevalence of molecular markers and treatment failure holds true in different epidemiological settings.

**Interpretation:** The application of microarray based tools to monitor drug resistance facilitates the assessment of the dynamic of drug resistance over time, and rapidly generates data on parasite’s resistance profile circulating in the community.

**O-143**

**Evolution of drug-resistance genes in *Plasmodium falciparum* in an area of seasonal malaria transmission in Eastern Sudan [MIM-AA-123782]**


(1) Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan; (2) Institute of Cell Animal and Population Biology, University of Edinburgh, UK; (3) Sudan Atomic Energy Commission, Sudan; (4) Department of Biochemistry, Faculty of Medicine, University of Khartoum, Sudan

**Introduction:** In this study we have examined genes implicated as determinants of resistance of *Plasmodium falciparum* to the two commonly used anti-malarial drug in Sudan, chloroquine and Fansidar as well as putative microsatellite markers in Asar village, eastern Sudan using isolates collected over a period of 12 years. We have identified a progressive increase in frequencies of drug resistance genes during this period, and a cyclical fluctuation in these frequencies between the wet and dry seasons.

**Methods:** The frequencies of alleles of four genes implicated as drug resistance determinants (chloroquine resistance transporter, pfcrt, dihydrofolate reductase, dhfr, dihydropteroate synthase, dhps, and multi-drug resistance, pfmdr1) were monitored over a period of 12 years between 1990 and 2001. In addition, between wet and dry seasons changes in frequencies of drug resistance genes were monitored between 1998 and 2000. Parasites were also typed for three putative neutral microsatellite loci (Poloy, Pfg377 and TA81).

Regression analysis was performed to test the pattern of increase in allele frequencies over time. Wet to dry season fluctuations were assessed by applying a standard population genetics index, FST using the computer package FSTAT.

**Results:** No significant variation in frequencies were observed for the microsatellite loci over the whole period of the study or between seasons. However, genes involved in resistance to chloroquine (pfcrt and pfmdr1) showed consistent increases in frequencies over time. The rate of annual increase was significant, 0.027 and 0.018 per year for pfcrt and pfmdr1, respectively. Genes involved in resistance to the second line drug in the area (Fansidar) remained at low frequencies between 1990 and 1993. However, they increased dramatically between 1998 and 2000 consistent with the shift to Fansidar usage during this period. Higher frequencies during the dry season than in the wet season were seen for mutant alleles of the primary drug resistance targets, pfcrt and dhfr, for chloroquine and pyrimethamine, respectively.

**Interpretation:** Increased frequencies of genes involved in resistance to Fansidar are consistent with the shift to its usage. Fluctuation in drug resistance genes reflects seasonal variation in drug pressure and in fitness of resistant and sensitive parasites.

**O-144**

**Acquisition of pfcrtT76 and pfmdr1Y86 alleles by Pf isolates is associated with reduced efficacy of cq-cp in Nigerian children with acute malaria [MIM-YO-193312]**

Y. Olukosi, B. Iwalokun, E. Magbagbeola, A. Akinwande, S. Awoyola, P. Agomo

(1) Department of Biochemistry, Nigerian Institute of Medical Research, Nigeria; (2) Department of Biochemistry, Lagos State University, Nigeria; (3) Department of Biochemistry, University of Lagos, Nigeria; (4) Department of Public Health, Nigerian Institute of Medical Research, Lagos, Nigeria

**Introduction:** Chlorpheniramine (cp) has been found effective in reversing chloroquine (cq) resistance in treatment failures due to either pfcrtT76 or pfmdr1Y86
in children with acute uncomplicated malaria. Effect of these mutant genes when jointly acquired on the pharmacokinetics of cq and efficacy of cq-cp in children with acute uncomplicated falciparum malaria is poorly understood and has compromised strategies that may support optimization of cq as an antimalarial or its replacement by alternative drugs.

Methods: A total 95 consented children aged 6 months–15 years presenting with 24–48 h old fever (body temperature >37.5 °C) due to Plasmodium falciparum parasitaemia (2350–42800 parasites/μL) at Massey Street, children Hospital and Ijede Primary Health Care Centre, Nigeria were consecutively enrolled into the longitudinal study. 3.8% citrated blood samples were collected on days 0 (before, and 6 h post-treatment with CQ (10 mg/kg) and CP (4 or 8 mg/kg dose), 3, 7 and 14. 50 mL blood aliquots were spotted on filter paper for P. falciparum pfcrtK76T and pfmdr-1N86Y genotyping and recrudescent determination by PCR. Pretreatment and post-treatment plasma CQ concentrations were determined by HPLC.

Results: Sixty-three (66.3%) of the 95 blood stage P. falciparum isolate harboured pfcrtK76T/pfmdr-1N86Y alleles in children with pretreatment mean CQ concentration of 318.4 ± 16.2 mmol/L and 6 h post treatment level of 560–710 nmol/L. 22.1% and 11.6% of cases were due to pfcrtK76T and pfmdr-1N86Y mutations in children with plasma CQ pre-and post-treatment profiles of 285.2 + 9.6 nmol/L; 580.4–870 nmol/L and 218.2 + 17.3 nmol/L; 572–910 nmol/L (P < 0.05), respectively. On day 14, CQ-CP cure rates in children infected with double, pfcrtK76T and pfmdr-1N86Y mutant strains were of 15.8%, 71.4% and 72.7%, respectively. Msp2 and glurp analysis showed that treatment failures were due to days 3–7 recrudescence and greater parasite diversity in double mutant infections compared to day 14 recrudescence and less diverse parasite clonality in pfcrtK76T or pfmdr-1N86Y infections in these children.

Interpretation: Pf infection due to pfcrtK76T and pfmdr-1N86Y parasitaemia is associated with greater clonal diversity and early recrudescence-mediated cq-cp treatment failure in Nigerian children with acute uncomplicated falciparum malaria.

O-145 Occurrence of the Pfcrt SVMNT haplotype in Plasmodium falciparum-infected individuals living in two villages in Korogwe district, Tanzania [MIM-MA-8844]


(1) Centre for Medical Parasitology, Institute of Medical Microbiology and Immunology and Institute of Public Health, University of Copenhagen, Copenhagen, Denmark; (2) National Institute for Medical Research, Arusha Centre, Arusha, Tanzania

Introduction: The wildtype at codon 72–76 of the Plasmodium falciparum Pfcrt gene is CVMNK, while two haplotypes may be associated with in vivo resistance to antimalarial drugs: the CVIET haplotype seems to be associated with resistance to chloroquine (CQ) and the SVMNT haplotype has been linked to resistance to amodiaquine (AQ). However, although AQ in vivo resistance has been confirmed at several sites in Africa, the SVMNT haplotype has so far only been identified outside the African continent.

Methods: As part of on-going immunoenpidemiological studies of malaria, cross-sectional surveys were conducted in July 2003 and in March 2004 in two villages, Mkokola and Kwamasimba, in Korogwe district in northern Tanzania. Finger prick blood samples were collected from underfives with confirmed P. falciparum infection and used to estimate the frequency of the main Pfcrt haplotypes. A high throughput method based on PCR and ELISA using sequence specific oligonucleotide probes enabled simultaneous detection of the haplotypes.

Results: The frequency of the Pfcrt CVIET haplotype in Plasmodium falciparum-infected individuals was approximately 95% in both villages in 2003, while the remaining samples were of the CVIET haplotype. In the following survey in 2004, the frequency of the CVIET haplotype had decreased to 89% in Mkokola (P = 0.278) and significantly to 69% in Kwamasimba (P = 0.001). Surprisingly, this was not only due to an increased frequency of the CQ and AQ sen-
sitive CVMNK haplotype, but due to the fact that also the SVMNT haplotype was detected in both villages in 2004 (frequencies of 6% in Mkokola and 13% in Kwamasimba, respectively). These findings were confirmed by DNA sequencing of a subset of samples with the SVMNT haplotype.

Interpretation: If the SVMNT haplotype is associated with AQ resistance, its occurrence on the African continent is of great concern to the future use of AQ, including in artemisinin-based combination.

O-146

A mechanism of malaria protection by hemoglobin C

T. Wellems, D. Diallo, A. Guindo, R. Fairhurst, O. Doumbo

Introduction: Many polymorphisms that protect against severe Plasmodium falciparum malaria are related to erythrocytes, reflecting the importance of these cells as the niche of parasites during infection and disease. Among these are hemoglobin C (HbC), sickle cell hemoglobin (HbS), and glucose-6-phosphate dehydrogenase (G6PD) deficiency. The mechanisms by which these mutations exert their influence are largely unclear.

Methods: HbC contains a lysine-for-glutamate substitution at the position 6 of the beta globin chain, the same position as the valine-for-glutamate substitution in HbS. HbC occurs mostly in West Africa where its prevalence in some regions is >20%. We and others have demonstrated substantial protection against severe malaria by both the AC and CC phenotypes in the Dogon and Mossi ethnic populations of Mali and Burkina Faso. Our research since that time has focused on the molecular mechanisms by which HbC confers this protection. Although P. falciparum multiplication is reduced in CC erythrocytes in vitro, parasite multiplication rates are the same in AC and AA erythrocytes and substantial parasite densities occur in both AC and CC malaria patients.

Results: Our observations suggest that a mechanism of protection other than inefficient erythrocyte invasion or impaired parasite growth must operate in vivo. P. falciparum parasites remodel the surface of their host erythrocytes with knob-like protrusions. These knobs carry PfEMP 1 adherence ligands and appear to be abnormally large and dispersed on HbC relative to normal (AA) erythrocytes. Since PfEMP 1 enables parasitized erythrocytes to sequester in the microvasculature and avoid clearance from the blood stream by the spleen, the aberrant display of PfEMP 1 ligands on knobs may impair the ability of parasites to sequester and cause inflammation in critical tissues such as the brain. Recently we have examined parasitized HbC erythrocytes from laboratory and clinical samples for evidence of impaired adherence.

Interpretation: Our results confirm reduced PfEMP-1-mediated adherence of parasitized HbC erythrocytes and suggest that HbC protects against malaria by mitigating the pathogenic effects of their sequestration in the microvasculature.

O-147

The role of malaria infection in morbidity and mortality in sickle cell disease in east Africa: Preliminary evidence from a cohort study

J. Makani, E. Meda, S. Rwezaula, F. Kalokola, S. Thein, T. Williams, K. Marsh

Introduction: One of the important genetic disorders associated with malaria infection is sickle cell disease (SCD). Individuals with sickle cell trait are protected from malaria deaths but it is considered to the leading causes of mortality in SS patients. Although protection has been confirmed by in-vitro and epidemiological studies, compelling evidence of an excess malaria-related mortality in individuals with SCD, even in the
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face of increasing chloroquine resistance and clinical studies is lacking.

Methods: This work attempts to determine the role of Plasmodium falciparum infection in SCD patients. A prospective, cohort study of all SCD patients attending Muhimbili National Hospital (MNH) was started on 25th March 2004. Surveillance involves three-monthly clinic visits where clinical and laboratory data (FBC, Malaria film, malaria genotyping) is collected. During admission, events leading to hospitalisation are documented and similar investigations performed. The aim is determine whether, and to what degree, malaria infection is associated with SCD morbidity and mortality.

Results: During the period starting April 2004 to January 2005, 800 patients were recruited into the cohort, with a median age of 10 years \(\pm\) 7.42, with 40% of patients between 10 and 19 years. There have been 142 admissions (70% children) corresponding to 80 individual patients. We present preliminary data comparing the age specific prevalence of infection and density of parasitaemia, using blood slides and \(P. falciparum\)-specific PCR, in outpatients and during admission and attempt to look at the association between \(P. falciparum\) infection and specific clinical syndromes at presentation.

Interpretation: Preliminary analysis shows a lower than expected overall prevalence and density of parasitaemia in both outpatient population and during admission. We propose various reasons to explain this.

O-148

Negative epistasis between the malaria-protective effects of alpha (+) thalassaemia and the sickle cell trait [MIM-TW-395505]

T. Williams, T. Mwangi, S. Wambua, S. Uyoga, D. Weatherall, S. Gupta, M. Recker, A. Macharia, R. Snow, K. Marsh

(1) KEMRI Centre for Geographic Medicine Research-Coast, Kenya; (2) Weatherall Institute for Molecular Medicine, Oxford, UK; (3) Department of Zoology, University of Oxford, Oxford, UK

Introduction: The haemoglobinopathies are common in many tropical populations because they confer a survival advantage against death from malaria. In sub-Saharan Africa, both haemoglobin S (HbS) and a+ thalassaemia occur at particularly high frequencies. While individually, each is protective against severe Plasmodium falciparum malaria, little is known about their malaria protective effects when inherited in combination.

Methods: We investigated two cohorts of children resident on the coast of Kenya, with a view to determining the relative incidence of malaria in normal children compared to those with either heterozygous or homozygous a+ thalassaemia, sickle cell trait (HbAS), or combinations of the two haemoglobinopathies. The first was an age cohort of 370 children \(\geq 8\) years old, which we followed by active surveillance for febrile events, while the second was a birth cohort of 2104 children, which we followed by passive surveillance for hospital admission with malaria and other diseases.

Results: When inherited alone, each condition was associated with protection from all forms of clinical malaria. For example, compared to the base-line group [children with both a normal a+ thalassaemia (aa/aa) and HbS (HbAA) genotype], the incidence rate ratios (IRRs) for severe \(P. falciparum\) malaria in HbAA children with heterozygous (−a/aa) and homozygous (−a/−a) a+ thalassaemia were 0.60 (0.39–0.90; 0.015) and 0.54 (0.30, 0.99; 0.045), respectively, and no episodes occurred in children of the two-locus genotype (HbAS/aa/aa). Moreover, parasite densities during incident malaria infections were significantly reduced by HbAS, but not by a+ thalassaemia. However, in the case of each outcome, protection was lost when both HbAS and a+ thalassaemia were inherited together, to such a degree that the incidence of all forms of clinical \(P. falciparum\) malaria was close to base-line in children with the two-locus genotype [HbAS/homozygous a+ thalassaemia (−a/−a)]. On modelling these effects, we conclude that current population frequencies of both conditions may reflect this negative interaction.

Interpretation: The interaction between a+ thalassaemia and HbAS is example of negative epistasis. Such interactions have implications for identifying new malaria protective genes, but potentially offer valuable opportunities for investigating their mechanisms.
O-149
Association of HLA alleles with P. falciparum severity in Malian children with severe malaria matched to uncomplicated malaria or healthy controls [MIM-KL-90160]
(1) Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland, USA; (2) C.W. Bill Young DoD Marrow Program, Naval Medical Research Center, Georgetown University, Kensington, MD, USA; (3) Malaria Research and Training Center, University of Bamako, Bamako, Mali; (*) Contributed equally to the study

Introduction: Genetically regulated immune responses to malaria may be influenced, in part, by gene polymorphisms in class I and class II major histocompatibility complex (MHC) antigens. Pre-erythrocytic immunity may be mediated by T cell recognition of malaria epitopes presented on infected host cells via MHC antigens. Previous studies have shown B*5301 and DRB1*1302 to be associated with protection to severe malaria.

Methods: Malian children aged 3 months–14 years (n = 253), with severe malaria as determined by modified WHO criteria, were enrolled and matched by age and residence to children with uncomplicated malaria and to healthy controls. Venous blood was drawn prior to therapy and peripheral blood mononuclear cells (PBMC) preserved in the field. Class I and II HLA-haplotypes were determined by polymerase chain reaction and sequence-specific oligonucleotide probes hybridization (PCR-SSOP) and the distributions of alleles of HLA-A, -B, -C and DRB1 loci were compared between different clinical groups (n = 726).

Results: We observed a new association between the A*3001, A*3301 and DRB1*080401 alleles and susceptibility to cerebral malaria. A higher proportion of subjects with cerebral malaria carried the A*3001 allele (38.7%) than children without cerebral involvement (22.9%) (Chi2 = 5.84, P < 0.05). Moreover, the A*2902 allelic frequency was higher in children with severe malaria and severe anemia (hb < 5 g/dl) (17.7%) than in those without severe anemia (3.2%) or in healthy controls (7.8%) (Chi2 = 7.1 and 5.36, P < 0.05, respectively). The A*3001 and A*3301 alleles share some sequence motifs and A*3001 appears to have a unique peptide binding repertoire compared to other A*30 group alleles. The increased frequency of DRB1*080401 appears to result from linkage disequilibrium with A*3001. The B*5301 and DRB1*1302 alleles were most frequent in healthy controls, followed by uncomplicated malaria controls with the lowest frequencies in subjects with cerebral or hyperparasitemic malaria. The differences in frequencies of these alleles were small and none of these comparisons reached statistical significance, suggesting that the protection to develop severe malaria conferred by B*5301 and by DRB1*1302 has a small or negligible impact in the Malian population.

Interpretation: High resolution HLA typing reveals A*3001 and A*3301 as newly identified potential susceptibility factors for cerebral malaria providing further evidence that polymorphism of MHC genes results in altered malaria susceptibility.

O-150
Genetic diversity in Plasmodium falciparum using comparative genomic sequence analysis [MIM-SV-104438]
S. Volkman, P. Sabeti, C. Kidgell, J. Daily, Ouaaz, D. Ndiaye, S. Mboup, E. Winzeler, D. Hartl, D. Wirth
(1) Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA; (2) Broad Institute/MIT, Cambridge, MA, USA; (3) Department of Cell Biology, The Scripps Research Institute, La Jolla, CA, USA; (4) Faculty of Medicine and Pharmacy, Cheikh Anta Diop University, Dakar, Senegal; (5) Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA

Introduction: Genetic diversity in Plasmodium falciparum is responsible for drug resistance and immune evasion in the human host. Genetic diversity in natural isolates, especially from Africa, is poorly understood. We have begun a genome-wide study of polymorphism to identify genetic loci under natural selection, which will allow development of intervention strategies to combat this important human pathogen.

Methods: DNA was derived from isolates obtained through MR4, or from natural isolates obtained from Senegalese patients presenting with mild malaria who
consented to this study. Parasites from these samples were culture adapted, and DNA was isolated using Qiagen isolation methods. Select regions were amplified using a polymerase chain reaction strategy for sequencing. For whole genome analysis, a custom-made oligonucleotide array based on the *P. falciparum* 3D7 sequence was used to probe genomic DNA, and the match only integral distribution algorithm (MOID) was used to assess the hybridization intensities for each and determine single feature polymorphisms (SFPs). Genomic and population genetic analyses were performed on the data.

**Results:** Comparative genomic sequence analysis of eight geographically diverse laboratory isolates reveals a nonrandom distribution of polymorphisms across the *P. falciparum* genome. Analysis of noncoding regions across 36,229 base pairs revealed 307 polymorphisms including 248 polymorphic microsatellites and 39 SNPs. There was an excess of polymorphic microsatellites and SNPs across chromosome 3 sequences, compared with chromosome 2 \((P = 0.0001)\). Using whole genome approaches these polymorphisms are concentrated in molecules associated with the parasite surface and in known or probable targets of natural immunity. Furthermore, these comparative studies have identified several novel gene families characterized by high levels of genetic diversity as putative targets of natural selection. This analysis is now being extended more broadly across the genome to natural isolates from Senegal. We will present genome-wide diversity data from natural populations of parasites and apply these data to the development of intervention strategies.

**Interpretation:** Comparative genomics using recent patient isolates, especially from Africa, can facilitate the identification of targets of natural selection and aid in the development of intervention strategies.

**O-151**  
**Structured African *Plasmodium falciparum* populations from urban and rural areas [MIM-CR-5130]**

H. Bogreau, F. Renaud, M. Henry, B. Pradines, T. Fauz, M. Kamal, O. Puijalon, C. Rogier  
(1) URBEP, IMTSSA, Marseille, FRANCE; (2) IRD-CNRS, Montpellier, France; (3) Centre Muraz, Bobo-Dioulasso, Burkina Faso; (4) Ministère de la Santé, Djibouti; (5) Institut Pasteur, Paris, France

**Introduction:** The genetic diversity and population structure of *Plasmodium falciparum* in Africa give rise to controversy and depend on a variety of situations. Multilocus genotyping studies did not show population structure in high malaria transmission areas but none addressed this issue at the continent scale. Moreover, the specificity of urban malaria epidemiology must be considered to adapt the strategies of control.

**Methods:** Our objectives were to assess the genetic diversity, structuration and differentiation of *P. falciparum* populations according to geographical distances and level of malaria transmission in urban and rural sites. We have assessed genetic diversity (expected heterozygosity), population structure (Fst) and linkage disequilibrium (LD) of *P. falciparum* using 22 microsatellite loci (16 of them used for the first time in a population genetic study) in four urban and rural sites (Dakar, Niamey, Djibouti and rural area of Zouan-Hounien, Côte d’Ivoire; \(n = 240\) isolates).

**Results:** Our results show a *P. falciparum* population structure stronger than it has been reported to date. Results support dramatic discrepancies between Djibouti city and other sites (Fst ranging from 0.17 to 0.25), and significant Fst between the other sites (Fst ranging from 0.04 to 0.12). The percentage of isolates that were multi-infected differed significantly only between the cities and the rural area \((p < 0.003)\). The unbiased expected heterozygosity \((He)\) estimated per locus differed significantly between Djibouti \((He = 0.55)\) and the other sites \((He > 0.73)\). It did not differ significantly between Dakar, Niamey and the rural area of Zouan Hounien. In Djibouti, the genetic diversity was significantly lower and the *P. falciparum* population exhibited significant LD.

**Interpretation:** Results support the view of structured African *P. falciparum* populations and suggest that malaria epidemiology in urban areas depends on local transmission, geographical isolation and parasite flow between the cities and the surrounding rural areas.
O-152
Ribosomal and mitochondrial DNA variation in the Anopheles moucheti group of malaria vectors [MIM-PK-205958]

P. Kengne, C. Antonio-nkondjio, P. Awono-ambene, Z. Kénon, F. Simard, D. Fontenille
(1) Institut de Recherche pour le Développement (IRD), Montpellier, France; (2) Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroun

Introduction: Anopheles moucheti is a major human malaria vector in villages situated along large rivers or slow moving streams in the equatorial rain forests of Africa. An. moucheti is described in literature as a group of morphological ‘sub-species’: An. m. moucheti, An. m. nigeriensis and An. m. bervoetsi. To assess relevance of morphological characters as accurate means for taxonomic classification within the group, we examined genetic differentiation within An. moucheti s.l. populations.

Methods: Mosquitoes from the Anopheles moucheti group were collected from three villages in Cameroon (Simbock, Olama and Nyabessan), two villages in the Democratic Republic of Congo, DRC (Tsakalakuku and Kasombo) and one village in Uganda (Bumfima). After morphological identification, genomic DNA was extracted from 48 female specimens (37 An. m. moucheti and 11 An. m. bervoetsi). Three DNA regions in the ribosomal DNA cluster (ITS1, ITS2 and D3) and the Cytb gene on the mitochondrial DNA were sequenced from each specimen. Sequences were aligned with CLUSTAL X software, and alignments were adjusted visually if needed. Statistical analysis was conducted using MEGA 2.1 software.

Results: Sequence comparison of the four markers (ITS1, ITS2, D3 and Cytb) between forms revealed two distinct patterns of sequence variation. Pairwise genetic distances between haplotypes were low (0.060, 0.20, 0.047 for ITS2, D3 and Cytb, respectively) and high (0.150 for ITS1). This latest value of genetic distance was higher than expected, and generally observed, between conspecific mosquito populations. These results are consistent with the splitting off An. moucheti in at least two species. Phylogenetic and diversity analyses of the nuclear and mitochondrial markers confirmed a monophyletic origin for all populations, separated in two main clades corresponding to An. m. moucheti (from Cameroon, Uganda and DRC) and An. m. bervoetsi (from DRC). Fixed nucleotide differences in the ITS1 region provided the basis for development of a sensitive allele specific PCR assay for rapid and reliable identification of each species within the An. moucheti group. Species-specific PCR primers were designed and the size of the diagnostic bands obtained were 300 bp for An. m. moucheti and 400 bp for An. m. bervoetsi. A total of 392 specimens of An. moucheti s.l. collected in Cameroon, DRC and Uganda were tested and the expected sizes were obtained.

Interpretation: Based on sequence analysis of nuclear and mitochondrial markers, we provide support for splitting An. moucheti s.l. into at least two species. We developed a convenient PCR-based assay for reliable species diagnostic.

24. Socio-economic realities of malaria
Thursday 17 November 11:00–13:00—Mahogany Hall
Chairs: Margaret Gyapong (Accra) and June Msechu (Tzarakara)

O-153
Malaria and its control—A glimpse at the interplay between people’s behaviors, economic situations, and the environment [MIM-JA-96912]

Alaii
Kenya Medical Research Institute, CVBR, Kisumu, Kenya

Introduction: Morbidity and mortality from malaria is rising despite existing simple effective curative and preventive interventions. Poor populations, particularly in rural sub-Saharan Africa bear the brunt of disease. Current control policy options include prompt effective treatment and prevention through vector control, with connotations for community participation as prerequisite for effectiveness. Cognizance of the significant influences of human behavior and political economic issues is crucial.

Methods: This paper draws from socio-behavioral components of three independent multi-disciplinary community-based malaria studies – an ITN trial, a needs assessment for larval ecology and control, and a home-case management program – undertaken in sep-
Abstracts / Acta Tropica 95S (2005) S1–S506

S95

Objectives: To determine the social and cultural context that shapes the uptake of malaria interventions in poor rural communities in sub-Saharan Africa, where malaria has been an endemic disease for thousands of years.

Methods: Qualitative and quantitative tools were applied to culturally define malaria and characterize environmental and structural variables impacting on people’s practices. Focus group discussion findings are pooled to revisit questions regarding the vital interplay between people’s behaviors, economic situations, and the environment as important antecedents to the uptake of malaria interventions in poor rural communities, as in much of sub-Saharan Africa.

Results: At the micro level, issues ranging from concepts of disease causation, to self-efficacy perceptions including behavioral and financial control affect people’s response to malaria and interventions for its control. Malaria is generally perceived to have multiple causes, including threats over which ITNs have no effect. Mosquito larval breeding control, also, is perceived unattainable—people’s livelihoods evolve around the offending water bodies while control is deemed government rather than community responsibility. Consequently, anti-malarial drugs are lay people’s preferred control strategy, though not without its peculiar challenges, including questions of access and adherence. The dilemmas participants face in malaria health-seeking and decision-making also highlight pertinent issues at the macro level including characteristics of health systems and predominant attitudes of health personnel.

Interpretation: Current impetus for malaria control emphasizes biological parameters and coverage issues. Uptake remains elusive, with people’s livelihoods implicated. This paper revisits key issues required to underpin malaria control in rural sub-Saharan Africa.

O-154

The social reality of malaria

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Introduction: The social reality of malaria can be assessed in several ways. The approach of this paper is to consider how social reality at the individual, household, community, national and global levels relates to perceptions of malaria and influences people’s willingness and ability to prevent, treat and control the disease. The paper critically examines the ways in which the social, cultural, political and economic contexts interact with disease to create various ‘social realities’ of malaria.

Methods: Over the past 10 years numerous implementation research projects aimed at reducing the burden of malaria have been undertaken. Most of these projects have involved a social science component to assess the acceptability of the intervention and to identify barriers to uptake and sustainability. A summary overview of the findings from such studies, together with data and experiences gathered while working as a social scientist in applied malaria research form the basis for this paper. Findings and experiences are extrapolated beyond their impact on specific intervention strategies into a discussion about the broader social cultural and political realities of people living with malaria and the consequences for malaria control interventions.

Results: In the areas of sub-Saharan Africa where malaria has been an endemic disease for thousands of years the social reality is that uncomplicated, non-severe malaria is considered ‘normal’. It is perceived as a non-threatening illness that carries no social stigma. Should the disease progress, dying of malaria is a socially acceptable death. Unlike tuberculosis or HIV/AIDS, malaria is not viewed as a threat to social or cultural norms, and the risks and consequences associated with the disease are perceived to operate at individual and household, rather than community level. On the other hand, susceptibility and ability to respond to malaria disease episodes are influenced by underlying social, cultural, political, historic and economic realities that are often beyond the capacity of an individual to influence. The social reality of malaria for an adult male suffering from the disease is different to that of a single mother with a sick child. Those who are the most biologically vulnerable are often also socially vulnerable and reality is that the voices of the vulnerable, pregnant women and women who care for children under 5 years, rarely count. They have little or no power to change their social reality.

Interpretation: Leaders at global and national levels must acknowledge the social reality of those who suffer most from malaria. Instead of using them as scapegoats for the failure of intervention programmes, those with the power to affect change should commit to capacity development and system strengthening to help create a new reality.
Abstracts / Acta Tropica 95S (2005) S1–S506

O-155

The costs of malaria among the poor and the vulnerable: Identifying households at risk and potential areas to strengthen resilience [MIM-JC-3525500]

J. Chuma, C. Molyneux

Kenya Medical Research Institute, Kenya

Introduction: We have explored the impact of illness costs on household livelihoods. Our focus has been on malaria costs because it is an important cause of morbidity and mortality, but little is known about the implications of these costs for household livelihoods. Our mixed methodology study aimed to: identify factors that increase vulnerability to catastrophic costs; explore the coping strategies; and suggest points of potential intervention to reduce household vulnerability and strengthen resilience.

Methods: The study used multiple methods including focus group discussions (n = 18), two cross-sectional surveys covering the wet and dry seasons (wet season n = 294 and dry season n = 285) and longitudinal case studies following households for eight months (n = 30). We will present findings on the two cross-sectional surveys and the longitudinal case studies.

Results: The results from this study show that malaria is a major concern for livelihoods. Household spend large amounts of money in their attempts to seek treatments. In most case the poor and vulnerable households are the most affected. In our study the findings indicate that households in the poorest income quintile incurred cost burdens of over 18% of their monthly income in both the dry and wet season. In contrast, the least poor households (wealthy) spent less than 5% of their monthly income on treatment. Mean costs burdens were higher in the wet season than in the dry season. This has important implications for livelihoods among the rural agricultural community who make a great proportion of their annual income in the wet season. Being ill during this season leads to high income losses. The case study data revealed that households that were highly vulnerable when the research started incurred the highest mean cost burden (10%) as compared to the least vulnerable (1.0%). Households that spent incurred high cost burdens on malaria treatment had recorded a decline in their livelihoods over the research period.

Interpretation: These findings show that the costs of malaria can be detrimental to livelihoods and if not protected these costs can push households towards poverty.

O-156

Market share of treatment sources before and after introducing community-based health workers in rural Nigeria [MIM-ND-52520]

N. Dike, O. Owojejewe, E. Shu, B. Uzochukwu

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Introduction: This presentation seeks to contribute to knowledge about the market share of different malaria treatment sources, before and after the implementation of a community-based health worker (CBHW) strategy in rural Nigeria with hyper-endemic malaria. It is important that the quality of treatment sources with large market shares is improved for timely and appropriate treatment of malaria. However, there is paucity of information on the market share of different treatment sources for malaria.

Methods: The study was undertaken in four malaria holo-endemic villages in Oji-River local government area (LGA) of Enugu state, southeast Nigeria. The CBHW strategy was implemented in two of the villages, while the other two served as controls. The study was conducted in three phases: (1) the first survey which was used to collect baseline data in the four villages; (2) the implementation of the CBHW strategy in the two intervention villages; and (3) a second survey to evaluate the intervention at the end of the second phase, in both the intervention and control villages.

Results: The market shares for different providers before the introduction of the CBHW strategy were 8.8, 6.6, 30.8, 6.6, 11.0% for treated at home, attended a clinic, patent medicine dealer, community-based health worker, and hospital in the two intervention villages combined. With the introduction of the CBHW strategy, the market shares became 0, 23.3, 17.8, 24.4, 7.8% (p < 0.05). The pattern of health seeking in the control villages remained more or less the same. The market share of CBHWs increased when they were formally...
introduced in the two intervention villages and became the second most frequently used source of treatment of childhood malaria.

Interpretation: Exploring how to scale-up the use of CBHW strategy for timely and appropriate malaria treatment could prove very useful in reducing disease burden and improving health amongst disadvantaged groups.

O-157
What’s really being spent on malaria research and development? [MIM-CH-29101]

M. Alliance

Project managed by: Sarah Esart, Malaria Vaccine Initiative, PATH (MVI), Anna Wang, Medicines for Malaria Venture (MMV), Andreas Heddini Multilateral Initiative on Malaria (MIM)

Introduction: The Malaria Research and Development Alliance, with APCO Worldwide and the Chartis Group, is conducting a study to quantify how much money is currently dedicated to developing new tools to fight malaria. Currently, no one knows how much money is being spent on malaria R&D, and estimates vary widely.

Methods: This study seeks to collect four years (2002–2005) of funding data from all major organizations involved in malaria R&D, including donors, funders, public–private partnerships, research entities, and industry. Respondents to the study will report annual figures for spending in major categories of malaria research and development. Organizations playing the role of both grantee and funder will provide figures for each role they play, and heavy emphasis will be placed on tracing the flow of funds to avoid double counting.

Results: Total funding numbers and disaggregated figures for malaria R&D during multiple years will be announced. Spending trends across six categories will be detailed. Those categories are: basic research, antimarial drug discovery and development, vaccine development and vaccine trials, vector control research, development of malaria diagnostics, and implementation research. The study establishes a credible baseline for community-wide use, and informs the design of a web-based system to track funding in the future. The results of this study will also be published in a credible policy report to inform policymakers, the scientific community, and donors about how much funding is really being spent on malaria R&D.

Interpretation: Even with resources on the rise, current funding of malaria R&D is likely insufficient to drive the innovation required to tackle this killer disease. This data will help advocates make an effective case for sustained and appropriate investment and help donors decide where to apply additional resources.

O-158
Le coût de traitement du paludisme sévère dans les hôpitaux de référence de Kinshasa est inaccessible à la majorité de la population [MIM-MM-249977]

M. Mulumba, I. Ilunga, A. Bankoto, D. Vanga (1) Université de Kinshasa, Dpt Biologie médicale, Service de parasitologie, R.D. Congo, (2) Institut Supérieur de Techniques Médicales, Kinshasa, R.D. Congo; (3) Hôpital Pédiatrique de Kalembe-Lembe, Kinshasa, R.D. Congo; (4) Hôpital Saint Joseph, Kinshasa, R.D. Congo

Introduction: Très peu de choses sont connues sur le coût direct, encore moins sur le coût indirect, du paludisme sévère en Afrique et particulièrement en R.D. Congo. C’est pour apporter un début de réponse à cette question cruciale que la présente étude a été entreprise.

Methods: Cette étude a eu pour cadre deux établissements sanitaires de référence de la ville de Kinshasa, l’un public, l’Hôpital Pédiatrique de Kalembe-Lembe (HPK) et l’autre privé, l’Hôpital Saint Joseph (HSJ). Dans chacun de ces sites, un échantillon de 100 patients a été sélectionné au fur et à mesure de leur hospitalisation. Était inclus dans l’étude, tout sujet âgé de moins de 12 ans, hospitalisé et traité uniquement pour paludisme grave et dont la famille a payé elle-même les soins. Étaient exclus, ceux qui ne remplissaient pas un des critères ci-dessus énumérés.

Results: La répartition des tableaux cliniques était homogène entre les deux hôpitaux (p = 0,284). La durée moyenne de l’hospitalisation était plus longue à HSJ (6,5 j vs 5,1 j; p = 0,002), et celle des comateux plus longue que celle des non-comateux (7,1 j vs 4,5 j; p = 0,001) et n’était pas influencée par les autres formes cliniques. Après ajustement pour une durée moyenne d’hospitalisation de 4,84 jours, le CDM global était de 90,5 $ US. Toute chose égale, il était plus élevé à HPK comparativement à HSJ.
(105.2 $ US vs 75.9 $ US; p < 0.001). Le traitement des comateux coûtait plus cher que celui des non comateux (103.2 vs 77.9 $ US; p < 0.001), ainsi que celui des transfusés par rapport aux non transfusés (97.9 vs 83.2 $ US; p < 0.001). La présence des autres syndromes cliniques n’avait aucune influence significative sur les CDM. Les frais de séjour et des médicaments représentaient 86.3% de la facture.

**Interpretation:**

Etant donné que le CDM du paludisme s’évère est équivalent au revenu annuel per capita en R.D. Congo, il est donc illusoire d’espérer combattre cette pathologie uniquement en comptant sur des ressources des populations pauvres à l’extrême. La majorité des patients risque ainsi de ne pas accéder aux soins de qualité, si l’État ne prend pas totalement en charge les frais de traitement des accès pernicieux.

**O-159**

**Sociocultural determinants of treatment delay for childhood malaria [MIM-CA-0]**

C. Ahorlu, K. Koram, C. Ahorlu, D. Savigny, M. Weiss

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**Introduction:**

Sociocultural factors influencing awareness of serious illness, help seeking, and treatment adherence may substantially influence disease outcomes and mortality. This study in two villages of Ghana examined determinants of timely, appropriate help seeking for children under 5 years of age with malaria or an illness with a similar clinical presentation, considering effects of reported illness-related experience, meaning and behaviour.

**Methods:**

Rooted in a cultural epidemiological framework the study used EMIC interviews to assess community views of childhood malaria. These EMIC interviews are locally created instruments to study the distribution of representations of illness reported by affected persons and their families. Caretakers of children with suspected malaria were interviewed to elicit illness narratives, from which categories of distress, perceived causes, and health-seeking behaviour were coded with particular attention to timely, appropriate treatment.

**Results:**

Only 11% of children suspected of having malaria-related illness received timely, appropriate treatment consistent with the Abuja target of treating malaria within 24 h of illness onset, and 33% of the children within 48 h. A multivariate logistic regression model identified “phlegm” as a perceived cause predicting timely, appropriate treatment within 24 h of illness onset (p = 0.04), and “playing on the ground” within 48 h (p < 0.01). Two categories of distress, “pallor or shortage of blood” (p = 0.05), and “sweating profusely” (p = 0.03) also predicted timely appropriate treatment within 24 h. Knowing that mosquitoes transmit malaria was not associated with timely, appropriate help seeking for the children, even though such knowledge may promote personal protective measures, especially use of bednets.

**Interpretation:**

Patterns of distress and perceived causes were related to timely help seeking, but not as expected. Effects on health seeking of illness-related experience and meaning are complex and explaining their role may strengthen interventions for malaria.

**25. Treatment of malaria/rational drug use 2**

Thursday 17 November 14:30–16:30—Bubinga Hall

Chairs: Oumar Gaye (Dakar) and Bernhards Ogutu (Kilifi)

**O-160**

**Prescription des medicaments antipaludiques en zones urbaines et rurales du Cameroun [MIM-SA-18722]**

A. Same-ekobo, A. Mfoulou, J. Meli, T. Nkoa

(1) Laboratoire de Parasitologie Mycologie, CHU Yaoundé, Cameroun; (2) Laboratoire de Parasitologie, FMSB Université, Yaoundé I, Cameroun; (3) Département de Santé Publique, FMSB Université, Yaoundé I, Cameroun

**Introduction:**

Face à l’émergence des souches plasmodiales multirésistantes aux antimalariques et dans le but de coordonner la prise en charge du paludisme au Cameroun, le PNLP a établi des normes de traitement. Le but de cette étude est de connaître les modalités de prescription des médicaments antipaludiques par le personnel soignant au Cameroun par rapport aux normes du PNLP.

**Methods:**

Il s’agit d’une étude transversale descriptive qui se déroule de 2003 à 2004 dans 3 sites urbains...
Des efforts supplémentaires doivent être déployés à l’encontre des prescrip- 
sion irrationnelles des méri- 
caments antimalariques, afin de 
se conformer aux recomman- 
dations du programme 
national de lutte antipaludique (PNLP).

O-161 
Malaria combination therapy use and monitoring in the African region [MIM-TS-22104]

T. Sukwa

(1) World Health Organization, Regional Office for 
Africa, Harare, Zimbabwe

Introduction: The emergence and spread of Plasmod- 
ium falciparum resistance to antimalarial medicines is 
a major challenge to treatment of malaria in Africa 
today. As a result, the WHO in 2001 recommended that 
countries with high levels of resistance to monother- 
apies should adopt combination therapy, particularly 
artemisinin-based combination therapy (ACT). The 
recommendation also short-listed four combination 
therapies deemed fit for use in Africa mainly based on 
efficacy and safety profiles. It is due to the above that 
countries in the region are updating treatment policies 
to ACTs. This paper highlights the use of ACTs in the 
African Region and its implementation challenges.

Methods: This is a review of status of antimalarial 
treatment policies in the African Region based on data 
available at WHO/AFRO.

Results: In the period 1993–2000, countries of the 
Region faced with high levels of chloroquine resis- 
tance were switching to sulfadoxine–pyrimethamine 
(SP). However, due to increasing resistance to SP, coun-
tries are now opting for ACTs. As of 15 March 2005, 
23 countries have adopted ACTs as first line treatment 
of which 5 are currently deploying such treatments 
in health facilities. Since 1995, AFRO has supported 
countries to develop capacity for monitoring antimalar- 
ial drug efficacy within National Malaria Control Pro-
grammes. The in vivo monitoring of therapeutic effi-
cacy now covers 41 of the 42 endemic countries of 
the Region with a total of 188 sentinel sites. Currently, 
15 of the 23 countries have baseline data on efficacy 
and safety of ACTs. In addition, 4 of the 15 coun-
tries have started routine surveillance for therapeutic 
efficacy of ACTs. Financial resources, availability and 
supply of ACTs are key global challenges to the imple-
mentation of treatment policies in Africa today. The 
ACTs cost about 10 times more than chloroquine and 
sulfadoxine–pyrimethamine. There is only one pre-
qualified fixed formulation of ACT registered with 
WHO and has only one supplier. In 2005, the demand 
for artemether-lumefantrine has already exceeded sup-
ply. The Global Fund to Fight AIDS, Tuberculosis 
and Malaria is the major financier for purchase of 
ACTs outside resources from governments of indivi-
dual endemic countries themselves. As ACTs lack phase 
four data, establishment of pharmaco-vigilance sys-
tems to capture adverse drug reactions is essential. This 
culture does not exist in most countries of the continent. 
WHO, therefore, has identified this area as a priority 
for its support to countries.

Interpretation: The implementation of ACT policies in 
the African Region has BEGUN. Monitoring of effi-
cacy is well established. We anticipate that as coverage 
of ACTs increases, the high morbidity and mortality in 
children under five years will begin to be reversed.
O-162
No abstract received.

O-163
Cost-efficacy of managing severe malaria in children aged 6–59 months following the WHO guidelines in two district hospitals in Cameroon [MIM-EF-29260]

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Introduction: Since efforts to eradicate malaria failed in the 1970s, sub-Saharan African countries have learned to live with malaria, which remains the main cause of morbidity and mortality in children less than 5 years old. There is increasing concern about the cost of health care, because of increasing poverty in the population. Our main aim was to determine the direct cost and efficaciy of case-management of severe malaria following the current WHO guidelines in children aged 6–59 months.

Methods: From January 1st to August 31st 2000, 148 children (aged 6–59 months, and who presented with at least one feature of severe malaria) were recruited by consecutive sampling, at Djoungolo and Mfou District Hospitals. Treatment according to WHO guidelines was implemented and there was rigorous in-patient monitoring and outpatient follow-up.

Results: There were 72 girls and 76 boys; the mean age was 23.1 ± 13.1 months and the commonest clinical forms of severe malaria were: generalised convulsions (54.7%), prostration (43.2%) and severe anaemia (14.9%). Most children (95.9%) were completely cured, 2.0% died and there were no neurological deficits over one month follow-up. We estimate the cost of hospital management of each episode of severe malaria at 26,000–36,000 FCFA and the overall direct costs (before and during hospitalisation) at 27,000–39,000 FCFA.

Interpretation: We conclude that the current WHO guidelines are efficacious, but expensive as compared to the standard of living in Cameroon.

O-164
Prevalence of malaria parasitemia among clients obtaining treatment for fever or malaria at drug stores in rural Tanzania, 2004 [MIM-SK-110123]

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Introduction: Specialist drug stores could play a role in expanding coverage of effective malaria treatment to households in highly endemic areas. No published studies have compared the prevalence of malaria parasitemia among drug store clients and patients at health facilities. We conducted a follow-back study to determine the prevalence of malaria parasitemia and other common illnesses among drug shop clients in one rural community.

Methods: We observed 2466 client visits selected from all 10 drug stores operating in the town of Ikwiriri between May 30 and August 31, 2004. Of these, 521 (21.2%) were made by or on behalf of persons ill with fever or malaria. Two hundred and ninety-three were eligible as residents of the surrounding nine villages and agreed to participate in the study. Each patient was evaluated by a clinical officer and provided a blood sample for malaria on the day of the shop visit, either at the shop or at home. Parasitemia prevalence was compared to findings from contemporaneous studies of healthy community members and fever or malaria patients at formal health facilities in the same district.

Results: Only 50 (17.1%) visits by or on behalf of febrile patients resulted in the purchase of an antimalarial drug, while an antipyretic medication was obtained...
at a sizeable majority, 77.1% (n = 226) of encounters. Clinicians assigned a clinical diagnosis of malaria to over half (63.8%) of the patients. Malaria parasites were identified in blood film samples from 24.2% (95% CI: 19.6, 29.5). This is double the parasite prevalence rate of 10.7% (95% CI: 8.6, 13.1) obtained from a household survey of 1004 healthy individuals selected from these villages at the same time. It is slightly lower, although not significantly, than the prevalence observed among 880 clients presenting with fever at health facilities in the district: 29.7% (95% CI: 23.0, 37.3). The prevalence of malaria parasitemia among children under 5 years whose families sought fever treatment from drug stores (42.1%; 95% CI: 31.4, 53.5) was equivalent to that of children presenting with fever at health facilities (42.5%; 95% CI: 25.0, 62.2).

Methods: We conducted a survey in 2004 including 758 potentially drug selling outlets in two rural Tanzanian districts. The aim was to assess the availability of antimalarials in all types of commercial outlets and the retailer’s knowledge of malaria treatment after sulphadoxine-pyrimethamine (SP) had been introduced as first-line antimalarial. We then compared the situation with data from a survey done in the same area in 2000 during the chloroquine era. In addition, “mystery shoppers” visited outlets with fixed malaria case scenarios for children and adults to investigate retailer’s drug selling practices.

Results: Four hundred and eighty-seven outlets were found stocking drugs in the study area, 152 of them in the semi-urban area of Ifakara. Of the total, 97% stocked anti-pyretics, only 12% stocked antimalarials (AM). SP was available in only 7% of the outlets. These percentages were even lower when only general shops were analyzed (6% stocked any AM, 2% stocked SP). While all drug shops stocked an antimalarial, SP was available in only 82% of them. Shop keepers reported paracetamol as the drug most frequently sold for fever/malaria. Of 54 “mystery shoppers” who bought drugs in a shop, 8 received SP and 13 another antimalarial (AM). SP was available in only 82% of them. Shop keepers reported paracetamol as the drug most frequently sold for fever/malaria. Of 54 “mystery shoppers” who bought drugs in a shop, 8 received SP and 13 another antimalarial (AM). SP was available in only 82% of them. Shop keepers reported paracetamol as the drug most frequently sold for fever/malaria. 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**O-166**
Using patterns of malaria care seeking to develop interventions aimed at improving the delivery of anti-malarial medicines in Kenya [MIM-TA-414834]

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Introduction: Treatment seeking takes place within a pluralistic health system including private medicine retailers (PMR). The strategic role of PMR as partners in malaria control is well recognized. However, malaria treatments obtained through PMR are often inappropriate or wrongly used. We undertook large-scale surveys in three districts in Kenya to describe malaria care-seeking patterns and inform the development of interventions to improve over the counter (OTC) malaria medicine use.

Methods: Household surveys were conducted on care-seeking patterns for recent febrile illnesses in children under five and acute illnesses in adults. We assessed large-scale surveys in three districts in Kenya to describe malaria care-seeking patterns and inform the development of interventions to improve over the counter (OTC) malaria medicine use.

Results: Results indicate that 55.3% (95% CI 53.7–56.8) of all fevers and 63.4% (95% CI 61.8–64.9) of all adult illnesses were first treated with OTC medicines across the three districts (p < 0.01). 4.8% (95% CI 2.7–8.0) of febrile children were taken to a trained provider within 24 h of onset of illness in all the three districts. Sulphadoxine pyrimethamine (SP) and amodiaquine medicines accounted for 80.1% (95% CI 76.4–83.3) of all OTC antimalarials used for fevers. Among children, 46.4% (95% CI 34.4–59.9) SP and 12.1% (95% CI 7.6–17.4) of all adult illnesses were first treated with OTC medicines across the three districts (p < 0.01). 4.8% (95% CI 2.7–8.0) of febrile children were taken to a trained provider within 24 h of onset of illness in all the three districts. Sulphadoxine pyrimethamine (SP) and amodiaquine medicines accounted for 80.1% (95% CI 76.4–83.3) of all OTC antimalarials used for fevers. Among children, 46.4% (95% CI 34.4–59.9) SP and 12.1% (95% CI 7.6–17.4) amodiaquine drugs were used correctly. SP use was better in adults than children (p < 0.001), but this was reversed for amodiaquine. Outlets in Kwale and Busia districts were more likely to stock amodiaquine than SP (p < 0.001). The cost of a full adult course of SP drugs in the retail outlets, ranged from USD 0.29 to 0.37 while that of amodiaquine was USD 0.24–0.74.

Interpretation: Frequent use of OTC medicines and inappropriate dosing confirm the importance of interventions targeting PMR. Low adherence for multidose OTC antimalarials is critical in the context of debates for delivery of artemisinin-combination therapies.

**26. Pregnancy-associated malaria**

**O-167**
*Plasmodium falciparum* malaria in pregnant Cameroonian women [MIM-DW-71775]

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Introduction: In African countries, including Cameroon, malaria during pregnancy increases the risk of maternal anemia, premature deliveries and low birthweight babies. The goal of our studies is to gain a better understanding of changes in antimalarial immune responses during the course of pregnancy and how immunologically-mediated changes in the placenta leads to poor pregnancy outcomes.

Methods: Cross-sectional and longitudinal studies of malaria in pregnant women were conducted in Yaounde and in the rural villages of Ngali II and Etoa. Prevalence was determined by microscopy and PCR using species-specific primers. Placental tissue samples were collected from over 1500 women and used to evaluate the affect of malarial parasites on placental histopathology, chemokine and cytokine profiles and number of parasite genotypes. Changes in the placenta have been correlated with pregnancy outcomes. Women were also enrolled during the first trimester of pregnancy and changes in T cell responses to classes I and II conserved malarial epitopes have been determined by ELISPOT and cytokine analysis as well as antibodies to nine malarial antigens.
Results: PCR-based detection of *Plasmodium falciparum* demonstrated that 82.4% of pregnant women were positive at delivery, with 27% being blood-smear positive and 54.9% having submicroscopic infections. The prevalence of *P. malariae* and *P. ovale* was 7.6% and 2.5% in these women, respectively. The number of parasite genotypes in the peripheral blood and placenta did not differ significantly between primi- and multigravidae. The major risk factor for placental malaria was age <25 years. Placental malaria was associated with anemia regardless of parity or age. However, the mean birthweight was lower and the percent pre-term deliveries and LBW babies higher in primigravidae and women <20 years who had placent al malaria. Women with placental malaria had an increase in β-chemokines, factors that are chemotactic for monocytes/macrophages. Elevated levels of TNFα and IL-10 were associated with an increased risk of premature deliveries. Antibodies inhibiting the binding of infected erythrocytes to chondroitin sulfate A were associated with a reduction in placental parasitemias, whereas antibodies to MSP1–19 were associated with the absence of placental infections. Antibodies to C5b-9 were not associated with a reduction of placental pathology.

Interpretation: Results will be presented on how changes in antimalarial immune responses correlate with increased susceptibility to infection and how immunologically-mediated changes in the placenta are associated with preterm deliveries.

O-168 The burden of malaria in primi- and secundigravidae in Boromo health district, Burkina Faso [MIM: sg-222015]

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Introduction: Malaria during pregnancy is a major public health problem. Though sulfadoxine–pyrimethamine intermittent preventive treatment (SPIPT) has been shown to reduce its burden, a good coverage is still difficult to achieve. The effectiveness of SPIPT supported by an educational campaign specifically targeted to pregnant women is investigated. Information on the burden of malaria during pregnancy was collected during one baseline year prior implementing the intervention.

Methods: Within a total population of 75,000, pregnant women (all parities) have been identified by trained women field assistants; primi and secundi gravidae were recruited for follow-up. Gestational age was estimated at time of recruitment by measuring the fun- dal height. Women were visited at home around their 32nd week of gestation and a blood sample (finger prick) for parasitaemia and PCV was collected. An additional blood sample was collected on filter paper for later PCR analysis. Women were then visited at delivery or as soon as possible after and an additional blood sample (finger prick) for parasitaemia and PCV was taken. When possible a placental smear was also taken.

Results: From March 2004 to February 2005 a total of 3448 pregnant women were identified and 1465 primi- and secundi gravidae recruited for follow-up. Thick blood film results are available for 789 women at 32 weeks of gestation and 722 at delivery. PCV results are available for 751 women at 32 weeks of gestation and 683 at delivery. Moreover, 504 placental smears were collected and read. Malaria prevalence varied according to season for peripheral parasitaemia at 32 weeks of gestation (ranged from 9.3% in May to 42.4% in October) as well as for parasitaemia at deliver- ery (ranged from 10% in May to 50% in August) and placental parasitaemia (ranged from 6.3% in June to 48.5% in August). Anaemia prevalence (PCV <33%) was 53% at 32 weeks of gestation and 45% at deliver- ery, and varied also with season, peaking in August/September.

Interpretation: Malaria during pregnancy represents a huge burden for pregnant women, despite good antenatal clinic attendance. Indeed, the majority of women attends at least once during their pregnancy, most of them during the second and third trimester.
O-169
In utero mother-to-child transmission (MTCT) of the human immuno deficiency virus type 1 (HIV-1) in relation to placental malaria in Yaounde-Cameroon [MIM-AK-312032]

Centre Pasteur du Cameroun, Laboratoires de Virologie, Cameroun; d’Epidémiologie, Yaoundé, Cameroun; Centre d’Animation Sociale et Sanitaire (CASS) Nkoldongo Yaoundé, Cameroun; Institut Pasteur Paris, Unité de Biologie des Rétrovirus, Cameroun; Unité d’Immunologie Moléculaire des Parasites, Cameroun

Introduction: Malaria and HIV-1 are both endemic in Cameroon and co-infections are common. We previously observed a three months periodicity between HIV-1 MTCT and peak of local rainfalls. We therefore hypothesised that malaria could be linked to the increase risk of in utero HIV-1 MTCT by interfering with the placental environment through the cytokine network.

Methods: Term placentas from 50 HIV-1 negative women and 81 HIV-1 positive women with or without peripheral malaria were collected for this study. *Plasmodium falciparum* was diagnosed in the placentas by microscopically examining placental blood. Placental explants were prepared to analyse the expression of cytokines by real time PCR and ELISA. In parallel, we used a recently developed method of placental tissue culture, histoculture, to study the regulation of infection with cell free HIV-1 pseudotypes by pro-inflammatory cytokines and *P. falciparum* strains.

Results: Our results show individual variations in the expression of cytokines namely; IL7, IL8, IL10, IL12, IL15, IL16, TNF-a, and RANTES. In parallel, we used a recently developed method of placental tissue culture, histoculture, to study the regulation of infection with cell free HIV-1 pseudotypes by pro-inflammatory cytokines and *P. falciparum* strains.

Interpretation: Our results highlights the fact that placental malaria, through the placental cytokine network could play an important role in the in utero transmission of HIV-1 that has been underestimated so far.

O-170
Placental malaria: Diversity of var gene expression and of humoral immune responses to different CSA adherent and placental isolates [MIM-SR-110968]


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Introduction: To new insights into the disease. The var2csa gene is clearly important in adhesion to CSA, but little is known about var2csa diversity among placental isolates. Other placental receptors and other var genes may also contribute. The evolution of immunity to placental malaria is still poorly understood, with diversity and conservation of antigenic epitopes within var2csa and between different var genes being important and unresolved issues.

Methods: We examined placental isolates from Malawi, and CSA-selected laboratory isolates, for var gene expression, using RT-PCR and real time PCR. DBL, gamma and DBL3X domains from var genes identified from placental and children’s isolates were compared to existing sequences, and quantitated by real time PCR to determine dominant transcripts. Sera from Malawian women were used to explore the diversity and conservation of responses among Malawian
women to CSA binding isolates with defined var gene expression, using flow cytometry.

**Results:** By real time PCR, var2csa sequences were dominant in CSA binding isolates and some placental isolates. Var2CSA sequences were obtained from most but not all placental isolates. By reverse transcription, DBL3X sequences varied from the 3D7 sequence by up to 23%, and were not expressed by children’s isolates. Sequences similar to varCS2 and to FCR3varCSA were obtained in a small number of cases. By flow cytometry and adhesion inhibition assays with two CSA adherent lines, there was an overall correlation between antibody reactivity by sera to the isolates, but sera showed differential activity in a number of instances. In cross agglutination experiments few mixed agglutinates were seen, suggesting diverse rather than conserved antigens.

**Interpretation:** Var2csa sequences of placental isolates vary, and other genes also encode adhesion to CSA. Cross-reactive antibodies agglutinating different CSA binding lines are uncommon, so a diverse range of antibody responses may be important in protection.

**O-171**

*Plasmodium falciparum* isolated from placenta transcribes high levels of var2csa and display variable adhesive abilities to placental CSPG [MIM-NT-338988]

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**Introduction:** Pregnancy-associated malaria is precipitated by placental sequestration of distinct *Plasmodium falciparum* parasite populations expressing unique variant surface antigens (VSAPAM) with affinity for binding to placental chondroitin sulphate proteoglycan (CSPG). We analyzed the ability of placental isolates to bind purified human placental CSPG and the transcription rates of var1csa and var2csa genes in parasites from pregnant and non pregnant women as well as anti-VAR2CSA IgG response in infected women.

**Methods:** Fifty delivering and 26 non-pregnant women presenting with an infection were enrolled. The ability of placental isolates to bind to CSPG and their serological properties were assayed in vitro. Parasite msp-2 genotypes were defined and the level of var1csa and var2csa transcription was measured by real time rt-PCR in all isolates from which DNA free RNA was successfully extracted. Anti-VAR2CSA IgG plasma levels were also measured in each infected woman by ELISA using three different VAR2CSA recombinant domains.

**Results:** A form of clonal restriction was observed at the level of msp-2 genotyping for pregnancy-associated malaria parasites. The binding levels to CSPG varied between isolates and negatively correlated with low birth weight of the offspring (OR = 5.2 [1.1–25.1]). Parasites with high binding ability transcribed higher levels of var2csa than parasites with low binding (P < 0.05). The level of anti-VAR2CSA specific IgG in pregnant women correlated to the level of parasite var2csa transcription (Spearman r between −0.3 and −0.5, P < 0.05).

**Interpretation:** As parasite ability to bind CSPG play an important role in the pathogenesis and clinical consequences of placental malaria, the results strengthen the rationale for developing VAR2CSA based vaccines to prevent pregnancy-associated malaria.

**O-172**

Antigenic variation in *Plasmodium falciparum* parasites infecting pregnant women [MIM-MO-7744]

M. Ofotu, T. Staalsoe, R. Megnekou, B. Akamori, L. Hviid

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Introduction: Variant surface antigens (VSA) mediate adhesion of infected erythrocytes (IE) to a range of host receptors. Chondroitin sulphate A (CSA)-specific IE sequestration in the placenta is mediated by antigenically distinct VSA (VSA-PAM) and may lead to pregnancy-associated malaria (PAM). It has been suggested that VSA-PAM may be a conserved antigen. We present evidence that PAM involves several antigenically distinct VSA-PAM that appear to be immunologically selected similarly to other VSA.

Methods: We isolated IE from the peripheral blood of Ghanaian primigravidae who were parasitemic at different time-points during pregnancy as described previously (Ofori, et al., 2004. Infect. Immun.). We used a panel of plasma samples from Ghanaian women of different parity and gestational age to type the VSA-PAM antigens expressed by flow cytometry (Ofori, et al., 2004. Infect. Immun.). We tested the host receptor specificity of the IE in adhesion assays using a range of cell lines expressing CSA or the non-pregnancy-related receptors CD54 (platelet glycoprotein IV) and CD54 (ICAM-1) (Megnekou et al., unpublished data).

Results: The majority of peripheral blood IE isolated from the pregnant women displayed the characteristic sex-specific and parity-dependent plasma IgG recognition phenotype of VSA-PAM-expressing parasites involved in the pathogenesis of PAM (Ricke, et al., 2000. J. Immunol. 165, 3309). Only VSA-PAM-expressing parasites were studied in detail. Typing of the VSA-PAM expressed by those isolates revealed a marked heterogeneity in recognition pattern between IE isolates. Importantly, the VSA-PAM expressed depended on the gestational age of the parasite donor. Parasites obtained early in pregnancy expressed VSA-PAM that were recognized both more often and at higher titers than those expressed by parasites obtained late in pregnancy. IE from all VSA-PAM-expressing isolates adhered significantly to CSA, and did not adhere to the non-placental receptors CD36 and CD54.

Interpretation: Individual parasite clones each possess several antigenically distinct VSA-PAM molecules, and the expression of these antigens is controlled by host immunity in a manner similar to that previously demonstrated for VSA in non-pregnant individuals.

O-173
Functional changes in fetal syncytiotrophoblast cells induced by specific binding of cytoadherent Plasmodium falciparum [MIM-JM-10375]
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Introduction: Placenta malaria (PM) is characterized by the accumulation of malarial parasite-infected red blood cells (iRBCs) in the placental intervillous spaces (IVS). This accumulation has been shown to be mediated by the binding of iRBCs to molecules in the IVS and expressed on the surface of syncytiotrophoblast (ST; fetal cells in direct contact with maternal blood). Little is known about how this binding influences ST function.

Methods: In this study a placental choriocarcinoma cell line, BeWo, and primary ST cells isolated from fresh placental tissue were induced to form ST and used to assess the biochemical and immunological changes found in ST following specific binding of BeWo (ST)-adherent iRBCs (iRBCST). iRBCs (laboratory isolate, 3D7) were selected for binding to ST by sequential rounds of panning. Changes in tyrosine phosphorylation of ST proteins and NF-kB translocation to the nucleus were assessed by immunoblotting following incubation with iRBCST and a positive control, LPS. Gene expression was evaluated by real-time, reverse transcription polymerase chain reaction.

Results: Modest translocation of NF-kB to the nucleus and tyrosine phosphorylation of 150 kDa and 85 kDa proteins, but not 140 kDa proteins, were observed following iRBCST binding. Stimulation of TNF-a (at 4 h post-binding), TGF-b (at 4 h), IL-10 (at 8 h) and IL-8 (at 8 h), but not MCP-1, were observed.

Interpretation: iRBC binding to ST stimulates intracellular signaling and changes in gene expression in the ST, thereby making these cells active immuno-
logic players which may influence maternal immune responses to PM in the IVS.

27. Vector/parasite relationships and population genetics

Thursday 17 November 14:30–16:30—Iroko Hall

Chairs: Bob Sinden (London) and George Dimopoulos (Baltimore)

O-174

Molecular evidence for selection acting on the Defensin gene in the Anopheles gambiae complex [MIM-FS-115040]

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Introduction: Defensin is a peptide involved in the immune response of insects against bacteria and parasites. It is strongly induced following infection. In mosquito vectors of malaria and lymphatic filariasis, immune response genes may be under parasite mediated selection. We have studied variability in the gene coding for defensin to evaluate whether human parasites (Plasmodium falciparum and Wuchereria bancrofti) are likely to exert selective pressures on this gene in their major anophelines vectors.

Methods: Genetic variation in the Defensin gene was analyzed within and between populations of three members of the Anopheles gambiae complex, naturally exposed to different levels of infection by human pathogens: the two anthropophilic species An. gambiae and An. arabiensis, and the zoophilic An. quadriannulatus. The first species was represented by four populations spanning the extremes of its genetic and geographic ranges. The other species were represented by a single population each. Mosquitoes were collected in Senegal, Kenya, Nigeria and Zimbabwe. DNA fragments were cloned prior to sequencing. Sequences were analyzed using GCG, MEGA and DnaSP packages. Additional calculations were carried out using programs written in SAS language.

Results: At total of 69 sequences of 1.2–1.4 kb in length were successfully aligned. To avoid sampling bias, a single allele was arbitrarily selected from each specimen. Analyses were conducted across the whole DNA region and partitioned between functional regions of the Defensin gene. We found (i) markedly reduced rate of nonsynonymous diversity compared to synonymous variation in the mature peptide and highly conserved amino-acid sequence of the mature defensin across species and populations, (ii) reduced polymorphism in the coding region with significant deficits in low-frequency sites compared to non coding regions, and (iii) increased divergence between species in the mature peptide together with reduced differentiation between populations of An. gambiae in this region. These patterns suggest strong purifying selection acting on the mature peptide and probably the whole coding region. Because An. quadriannulatus is not exposed to human pathogens, similar pattern of polymorphism across species implies that human pathogens played no role as selective agents driving molecular evolution of Defensin. It is more likely that structural constraints for a wide-spectrum anti-microbial effect of the mature peptide limit variation in its gene.

Interpretation: We demonstrated strong purifying selection acting on the Defensin gene within the An. gambiae complex, reflecting higher order structure conservation constraints rather than human pathogens selective pressure to escape their vector’s immune response.

O-175

No abstract received.

O-176

Variability of immune-related genes in the human malaria vector An. gambiae [MIM-AC-6988]

A. Cohuet, S. Krishnakumar, I. Morlais, A. Koutsos, M. Mindrinos, F. Kafatos

(1) LIN-IRD, Montpellier, France; (2) Stanford Genome Technology Center, Palo Alto, USA; (3) OCEAC, Yaounde, Cameroon; (4) EMBL, Heidelberg, Germany

Introduction: Sequencing of Anopheles gambiae genome has opened up the potential to discover of thousands of DNA sequences variants. Most of these are single nucleotide polymorphisms (SNPs), a dense set of which could allow the study of the genetic basis of complex traits by population approaches. One such challenge is to understand the genetic factors involved in
the susceptibility/refractoriness of An. gambiae populations to malaria parasites, with immune-related genes being the primary candidates.

**Methods:** We have examined nucleotide variations at coding regions of An. gambiae immune-related genes by sequence comparison of individual mosquitoes from laboratory strains and natural populations from Cameroon. An. gambiae is subdivided in two molecular forms, M and S, with limited gene flow between those forms. The laboratory strains consisted of the G3 strain (a mixture of M and S), L3–5 strain (a genetically selected refractory strain belonging to M form) and the Yaounde strain (M form), whereas wild mosquitoes belonged to sympatric populations of either M or S form.

**Results:** Preliminary results from more than 700,000 nucleotide pairs (32 individuals sequenced for 48 loci) revealed 938 SNPs. The overall average nucleotide diversity, \( \pi \), was \( 10.6 \times 10^{-3} \), ranging from \( 1.8 \times 10^{-3} \) to \( 50.5 \times 10^{-3} \). We particularly found high nucleotide diversity in mosquito immunity-related genes involved in recognition mechanisms (TEP1, TEP10, GNBPB3, FBN18). Hence, genetic diversity could be maintained by selective advantage for polymorphism (balancing selection). Natural populations revealed the higher intra-population variability (M: \( \pi = 9.2 \times 10^{-3} \); S: \( \pi = 8.4 \times 10^{-3} \)). In contrast, the L3-5 drastically reduced its genetic heterogeneity (\( p < 0.001 \), probably due to selection procedures.

**Interpretation:** The detection of those SNPs in An. gambiae immune-related genes should initiate association studies between genotypes and phenotypes that will facilitate the discovery of genetic factors linked to complex traits such as permissiveness to Plasmodium.

**O-177**

Genotype by genotype interactions underlying the resistance of Anopheles gambiae to Plasmodium falciparum [MIM-LL-8205]

J. Lambrecht, J. Halbert, P. Durand, L. Gouagna, J. Koella

(1) Université Pierre et Marie Curie, Paris, France; (2) IRD, Montpellier, France; (3) CIPE, Mbila, Kenya

**Introduction:** Most studies on the resistance of mosquitoes to their malaria parasites focus on the response of a mosquito line or colony against a single parasite genotype. In natural situations, however, it may be expected that mosquito–malaria relationships are based, as are many other host–parasite systems, on host genotype by parasite genotype interactions. In such systems, certain hosts are resistant to one subset of the parasite’s genotypes, while other hosts are resistant to a different subset.

**Methods:** To test for genotype by genotype interactions between malaria parasites and their anopheline vectors, different genetic backgrounds (families consisting of the F1 offspring of individual females) of the major African vector Anopheles gambiae were challenged with several isolates of the human malaria parasite Plasmodium falciparum (obtained from naturally infected children in Kenya).

**Results:** Averaged across all parasites, the proportion of infected mosquitoes and the number of oocysts found in their midguts were similar in all mosquito families. Both indices of resistance, however, differed considerably among isolates of the parasite. In particular, no mosquito family was most resistant to all parasites, and no parasite isolate was most infectious to all mosquitoes. These results suggest that the level of mosquito resistance depends on the interaction between its own and the parasite’s genotype.

**Interpretation:** This finding thus emphasizes the need to take into account the range of genetic diversity exhibited by mosquito and malaria field populations in ideas and studies concerning the control of malaria.

**O-178**

Plasmodium falciparum population structure in the four major African anopheline vectors: A study in a high endemicity equatorial forest malaria focus [MIM-ZA-81024]

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**Introduction:** African malaria foci are characterized by a great vectorial and parasite diversity. In endemic areas, the four major Anopheles vectors are often sympatric, infected with mixtures of Plasmodium species and, for Plasmodium falciparum, by mixtures of distinct genotypes. To elucidate the role of vector hosts on parasite populations structure, we have analysed the
distribution of oocyst neutral variability within the four major vectors sampled in an afican equatorial forest highly endemic malaria focus.

Methods: Entomological surveys were carried out in November 2002 (wet season) and March 2003 (dry season) in the perurban locality of Simbock, in the degraded equatorial forest of South Cameroon. Adult Anopholes mosquitoes belonging to the four major african vectorial species (An. gambiae, An. funestes, An. nili and An. moucheti) were captured at night inside and outside 10 houses. Specimens were identified using morphological keys, and midguts dissected and checked for the presence of oocysts. Infected midguts were stored in 80% ethanol, along with the carcasses. Oocysts were individually dissected on an inverted microscope and genotyped at seven microsatellite loci. Allelic frequencies were extracted from single oocysts and genotyped. We found a huge allelic diversity, with a mean number of alleles per locus of 13.7, ranging from 7 to 19. Mean unbiased expected heterozygosity in the mosquito, species, and seasonal structuring of each species is mostly a function of bio-climatic domains and habitat preferences. A total of 1.742 and 1.631 Anopheles mosquitoes belonging to the four major vector species were collected and checked for the presence of oocysts in November 2002 and March 2003, respectively. A total of 104 and 142 oocysts were dissected from 23 and 36 infected midguts of the four vector species. DNA was extracted from single oocysts and genotyped. We found significant heterozygote deficits in the oocyst populations, overall (Fis = 0.09; p < 0.0001) and for both wet (Fis = 0.16; p < 0.0001) and dry (Fis = 0.05; p < 0.05) seasons. Hierarchical analyses of genetic differentiation between P. falciparum oocysts from the four vector species, taking into account mosquito, species, and seasons structuring revealed no species effect on the distribution of oocyst genotypes (FS/A = 0.019; p = 0.283), but a strong mosquito (FM/S = 0.305; p < 0.001) and a seasonal (FA/T = 0.016; p < 0.05) effects.

Interpretation: Analysis of oocysts within vectors revealed uneven genotypic and allelic distributions in parasites sampled from the two seasons, as well as between and within mosquitoes. P. falciparum mating system and structure in a malaria focus will be discussed.

O-179 The distribution of the M and S forms of the malaria vector Anopheles gambiae s.s. in Nigeria [MIM-KI-88653]

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(1) Department of Zoology, University of Ibadan, Ibadan, Nigeria; (2) Public Health Division, Nigerian Institute of Medical Research, Lagos; (3) Department of Biological Science, State University of New York, At Buffalo, Buffalo, USA

Introduction: The genetic heterogeneity of Anopheles gambiae s.s., the most efficient and widespread malaria vector in sub-Saharan Africa is revealed both by chromosomal inversions and molecular makers. Sequence analysis of rDNA regions led to the characterization of two molecular forms of An. gambiae, named M and S-form. We earlier reported the sympatric presence of these forms in the holoendemic region of south western Nigeria; here we report their occurrence across seven ecological zones of the country.

Methods: Adult mosquitoes were collected using human baited landing catches and pyrethrum spray catches (WHO, 1975), for three consecutive days per month, between June and December 2003, in twelve localities of Nigeria. Mosquitoes identified morphologically as An. gambiae complex using the identifi cation key of Gilles and Coetzee (1987), were individually stored in silica gel-filled tubes and kept at −20 °C until further processing. DNA was extracted from wings and legs of each individual mosquito by a phenol-chloroform protocol (Yan et al., 1997) and species identification was made by PCR (Scott et al., 1993). Samples were further identified as belonging to M and S molecular forms according to PCR-RFLP method described by Fanello et al. (2002).

Results: Two species of the An. gambiae complex were found sympatric in Nigeria (N=1129), these are An. gambiae s.s. (72.3%) and An. arabiensis (27.7%). The distribution of each species is mostly a function of bio-climatic domains and habitat preferences. A total of 476 An. gambiae s.s. were characterized, out of which 43.9% were S form and 56.1% were the M form. The relative frequencies of M and S forms of An. gambiae s.s. were statistically different (p < 0.001) in humid forest and arid savanna zones collections. Each of S and M forms was prevalent over the other in at least one
locality in each of the seven zones. In three localities either one or the other molecular forms, but not both were exclusively observed. In the remaining nine localities both M and S forms were found sympatric, but one molecular form inconsistently predominated over the other. M forms prevail over S form in 6 out the 12 localities, 5 of which were in the northern arid savanna zones. Conversely, S form was predominantly identified in four localities, four of which were in the southern forest zones. No hybrid between the forms was found.

Interpretation: The complex distribution of the two molecular forms of An. gambiae s.s. from southern humid forest to northern arid savannas of Nigeria supports the hypothesis of ecological niche partitioning to reduce competition in areas where the two forms coexist.

O-180
Micro- and meso-geographic analysis of chromosomal inversion polymorphism of Anopheles funestus from Burkina Faso [MIM-WG-417438]

W. Guelbeogo, O. Groukho, E. Diboulo, N. Besansky, N. Sagnon, C. Costantini
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Introduction: Results from previous cytogenetic and molecular studies on Anopheles funestus are in agreement with the existence in Burkina Faso of two taxonomic units (Folonzo and Kiribina) with limited genetic flow and contrasting degrees of chromosomal polymorphism. To investigate the effect of distance and karyotype on the genetic structure and the level of population differentiation in An. funestus from Burkina Faso, we examined the pattern of chromosomal polymorphism at micro and meso-geographic scale.

Methods: For the micro-geographic survey (village-level), indoor-resting half-gravid females were collected during the course of one week from 39 compounds dispersed over a 4 km² area within Koubri, a village characterized by the abundance and diversity of An. funestus breeding sites, which is located 35 km southwards of the capital Ouagadougou. Similarly, for the meso-geographic (regional level) analysis, indoor-resting half-gravid An. funestus were sampled for two months yearly during two successive years from 11 villages aligned along a transect approx. centred in Ouagadougou, and ranging 300 km on a west–east axis spanning the same eco-climatic region.

Results: At the micro-geographic scale, significant genetic differentiation among compounds was observed (Fst = 0.07; P < 0.001). Higher inversion frequencies were found in compounds near the border of a large swamp, while they markedly decreased in compounds near an artificial lake mostly devoid of emergent aquatic vegetation only 1–2 km from the swamp. Both water reservoirs are good potential breeding sites for An. funestus. Significant genetic differentiation among villages was observed also at the meso-geographic scale, when all populations were considered across all inversions (Fst = 0.23; P < 0.001). Most villages presented contrasting levels of chromosomal polymorphism, with fewer showing intermediate inversion frequencies; however, villages with a higher or lower degree of polymorphism were spatially arranged in patches rather than in a pattern compatible with genetic intergradation. However, when the analysis was performed for each chromosomal form (Folonzo and Kiribina) separately, a significant decrease of Fst values was observed at the two geographical level of the survey.

Interpretation: Under the chromosomal forms scenario, our results suggest that the distribution of the two taxonomic units in Burkina Faso is mainly governed by micro-spatial environmental factors presumably associated to the presence of alternative larval habitats.

28. Roll Back Malaria

Thursday 17 November 14:30–16:30 — Mahogany Hall

Chairs: Fatoumata Nafo-Traore (Geneva) and Kamini Mendis (Geneva)

O-181
Are we rolling back malaria? Measuring progress toward targets in Africa [MIM-EK-0]

E. Korenromp, J. Miller, B. Nahlen
World Health Organization, Roll Back Malaria Department, Geneva, Switzerland
Abstracts / Acta Tropica 95S (2005) S1–S506

Introduction: Roll Back Malaria aims to halve the malaria burden by 2010 compared to 1998 levels. In 2000, African countries adopted the targets of 60% coverage of target groups, especially children under-5 and pregnant women, with insecticide-treated mosquito nets (ITNs) and prompt effective anti-malarial treatment, and of pregnant women with intermittent preventive treatment (IPT). This talk outlines the methods and data used for monitoring progress towards this goal and targets in sub-Saharan Africa.

Methods: Relevant data on intervention coverage and disease impact available to WHO/RBM come from: (i) reports from national health information systems (HIS) on cases and deaths attributed to malaria and other causes in clinics; (ii) household surveys such as the nationally representative Demographic and Health Surveys (DHS) and Multiple Indicator Cluster Surveys (MICS) on intervention coverage and all-cause under-5 mortality; and (iii) demographic surveillance sites (DSS) that monitor disease and death burden including malaria continuously over time in selected small-scale, mainly rural, populations. 16 DHS and 30 MICS planned in Africa in 2005–2006 will measure under-5 mortality and the coverage of ITNs and prompt effective treatment.

Results: Only a minority of malaria patients are seen and recorded in clinics. National HIS data do not, then, paint a reliable picture of overall burden, but they are important for monitoring malaria’s burden on health systems. Due to fluctuations in recording completeness and access to diagnosis and care, proportions of hospital admissions and deaths attributed to malaria are better HIS indicators than absolute numbers of cases or deaths. Population-level data are indispensable. Since malaria directly accounts for ±18% of under-5 deaths in Africa and contributes to many additional under-5 deaths in synergy with other infections, low birth weight and anemia, all-cause under-5 mortality is a relevant indicator. The detection of mortality impact is always delayed, however, because birth history data reflect the mortality level of on average 2.5 years before a survey. The prevalence of childhood anaemia and malaria parasitemia are potentially useful additional survey-based impact indicators. For correct interpretation, mortality trends must be evaluated alongside trends in intervention coverage. In countries that are approaching coverage targets, evaluation of trends in malaria-specific mortality, including in DSS, becomes relevant.

Interpretation: Given available data and recent considerable increases in coverage – especially of ITNs – in many African countries, an impact of improved malaria control under Roll Back Malaria is expected to become detectable between 2005 and 2010.

O-182
No abstract received.

O-183

R. Mandike, F. Molteni, R. Njau, E. Kahigwa, A. Simba, S. Msade, M. Marero, A. Mwita, C. Fanello

(1) National Malaria Control Programme, Dar es Salaam, Tanzania; (2) Italian Co-operation National Malaria Control Programme, Dar es Salaam, Tanzania; (3) WHO Country Office, Tanzania

Introduction: An effective system for monitoring the progress of the current efforts in combating malaria is critical for the success of Roll Back Malaria. Critical information such as mortality data, use of ITN, appropriate case management, and access to effective anti-malaria drugs, chemoprophylaxis and preventive treatment for pregnant women are needed for better planning and implementation of malaria control strategies.

Methods: Nine sentinel districts were selected to represent different geographical and malaria transmission areas in Tanzania. Adapted RBM WHO-AFRO monitoring and evaluation tools were used for community and health facility surveys. Four health facilities were randomly selected per each district and about 1400 households in eight communities were randomly selected and visited. A first survey has been carried out in 2001, a second in 2003 and a fourth in 2005.

Results: In the nine districts the impact indicators are showing little or no improvement over the five-year period. Morbidity and mortality attributed to malaria represented 44% and 41% of all deaths and 42% and 40% of all admissions recorded in health facilities in the first and last survey, respectively. However, some of...
Abstracts / Acta Tropica 95S (2005) S1–S506

the outcome indicators, such as ITN coverage and IPT, are showing a substantial progress over the five years (15–26% and 29–49%, respectively). First line antimalarial drugs availability raised from 29% of health facilities in 2001 to 97% in 2003. Health education materials and national treatment guidelines were found, respectively, in 43% and 47% of health facilities in 2001 and in 83% and 97% in 2003. At community level the children under five years of age appropriately treated for a febrile episode within 24 h from the onset were 11% in 2001 and 27% in 2003. The latest results from the ongoing 2005 survey will be presented.

Interpretation: The surveys conducted in 2001, 2003 and 2005 are valuable tools for monitoring the progress towards the Abuja RBM and the Tanzania Malaria Medium Term Strategic plan (2002–2007) targets.

O-184
No abstract received [368640]

O-185
Roll Back Malaria Programme in Zambia—Early evidence for dramatic success [MIM-PT-73454]

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Introduction: Following the inception of the Global RBM Initiative in 1998, Zambia adopted a multi-pronged approach aimed at introducing cost-effective evidence-based malaria control interventions. Through this, Indoor Residual Spraying, targeted ITN Programs and use of Artemisin Based Combination Therapies using Coartem® were begun. Resource mobilisation using HIPC and GFATM Funding was introduced in order to effect this. We report here early evidence of a dramatic change associated with this RBM strategy.

Methods: Data on paediatric admissions and specifically two impact indicators, the number of under-5 malaria cases and malaria deaths were obtained and verified from Macha Mission Hospital in southern Zambia. This is a 200-bed rural referral hospital in an endemic area of malaria transmission, which has also had an active malaria research programme. All patients with a discharge diagnosis or recorded death of malaria, have confirmation of that diagnosis by a blood smear. Hospital data were analysed, and compared to that obtained from referring rural health centres in the catchment area surrounding the hospital. Data obtained from the Zambian Health and Medical Information Services (HMIS) for the same time period were also analysed.

Results: Although malaria cases have always been quite seasonal in Zambia, and associated with the rains from November to May, hospital admissions for malaria in under-5’s showed a dramatic decrease in the 2003–2004 season. This has continued for the 2004–2005 season. For the period mid 1999 to mid 2003, the average annual number of under 5 malaria cases was 1508, while for the 2003–2004 period, the total was 444, a 71% decrease. Analysis by quarter shows that for the first quarter of the year, there was greater than a four-fold decrease in malaria admissions for 2004, when compared to the previous four years (2000–2003), and this has been repeated in 2005. Review of data from rural health centres in the Macha Hospital catchment area shows a similar trend. Deaths in children under-5 due to malaria have shown an equivalent dramatic decrease in 2003–2004 compared to previous years, and there appears to be a similar decrease in 2004–2005. Local and national data are being analysed and will be presented at the conference. Review of RBM initiatives in the catchment area shows that while drug availability and distribution using Coartem® has been effective, ITN distribution has not been widely implemented, nor has IRS been used.

Interpretation: The dramatic decrease in under five malaria morbidity and mortality at Macha Hospital since the RBM strategy was begun, may be unrelated to the introduction of ACT, but we believe that this preliminary data lends credence to a causal relationship.
Technical, behavioural and bureaucratic issues in ITN implementation: A review of some myths, assumptions and gaps in the evidence [MIM-JL-118795]

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

Introduction: Although the idea of putting pyrethroids on mosquito nets is now more than 20 years old, there are still big gaps in our technical knowledge. ITN project managers are often surprised at how much their programme routines are based on assumptions lacking evidence or contradicted by the evidence. The aim of this review is to identify the most practically important of these gaps and assumptions, and to summarise the evidence available.

Methods: Issues were selected for discussion because they are either (a) frequently encountered in local projects and local operations, or (b) relevant to national-level decisions concerning resource allocation, prioritisation and strategic planning. The evidence considered includes entomological, epidemiological, behavioural and survey data.

Results: Freshly treated nets do not need to be dried flat or in the shade. The best way to get an even insecticide deposit is probably to dry them quickly and with little folding. There is probably no minimum level of ITN coverage needed to achieve a “community effect”—rather, the strength of the effect probably just increases with increasing coverage. The survey question “Has your net ever been treated?” is used to measure progress towards international ITN coverage targets, but has not been adequately validated. In net-using households, the members of the family most likely to be using the net are the youngest children. Adult women are next. Adult men and the older children are the least likely to be under the net. We know surprisingly little about the real lifespan of nets in routine use. Some nets made by local tailors in Africa are better in quality, durability and appearance than nets which fit WHO specifications. Overall, such local nets have probably saved more lives than ITNs from projects. Global net-production capacity is said to be inadequate for future programme needs, but this reflects our unnecessarily restrictive fabric specifications, and our failure to seek out small local producers.

Interpretation: Some questions are important but difficult to answer, and are unlikely to be addressed in the M&E components of routine national programmes. In some cases, the evidence already exists, but planners are unaware of it, or do not know how to use it.

29. Bio-ethics symposium
Friday 18 November 11:00–13:00—Bubinga Hall
Chairs: Wen Kilama (Dar es Salaam) and Angela Wasunna (New York)

S-1 Protecting research participant in Africa
G.B. Tangwa
Department of Philosophy, University of Yaounde 1, Cameroon

Introduction: Africa is currently under the heavy burden of epidemics and other life-threatening diseases. This heavy disease burden, in combination with other negative factors, such as poverty, illiteracy, ignorance, unawareness, insecurity, wars, instability, etc., put the very existence of the continent into serious jeopardy. There is therefore an urgent need to change, reverse or ameliorate this catastrophic situation.

Methods: It is unquestionable that conducting medical research aimed at finding medicines, cures, and vaccines to lighten Africa’s disease burden, is one of Africa’s greatest needs of the present moment. But research in such a context needs to scale very high ethical hurdles on account of the multifarious vulnerabilities of Africa’s populations. What are these ethical hurdles and can they be scaled? Research on human subjects anywhere at any time runs ethical risks of harming, exploiting, cheating or otherwise unfairly treating humans. The guiding ethical principles for any such research are the following: respect for persons and their autonomy, beneficence, non-maleficence, and justice.

Results: These principles have often been recognized more in their breach than observance. Are they likely to be recognized and respected in Africa in its present context and situation? The catalogue of medical research harms, abuses, and malpractices, that occurred in Nazi
Germany and that first came to light during the Nuremberg Trials (1947) and those that occurred in the USA about the same time though coming to light only many years afterwards, when added to contemporary scandals like the TROVAN Pitzer meningitis trials on Children in Nigeria (2001), the Tenofovir affair with prostitutes in Cameroon, etc., are not such as to permit a firm affirmative answer to the above question. It is not easy anywhere at any time in any domain to refrain from taking advantage of the weak, needy, poor, ignorant, and vulnerable. This difficulty is abundantly exemplified in the history of colonization, slavery, capitalism, governance, etc. How much more difficult then would it be to overcome this difficulty in the domain of medical research where a lot of partly contradictory aims and motives are combined?

Interpretation: The task may be difficult but not impossible. There is demonstrable good will and increasing interest in biomedical ethics, an interest which should help to counteract the prima facie conflict of interest of researchers, since their first loyalty understandably is to science and not to ethics.

30. Innate immunity

Friday 18 November 11:00–13:00—Iroko Hall

Chairs: Jean Langhorne (London) and Elie Mavoungou (Lambarane)

O-187 Peripheral blood dendritic cells in Kenyan children with acute falciparum malaria [MIM-BU-29088]


(1) Centre for Tropical Medicine, Nufﬁeld Department of Clinical Medicine, University of Oxford, UK; (2) Kenyan Medical Research Institute/Wellcome Trust Research Laboratories, Kilﬁ, Kenya

Dendritic cells are important for the initiation and maintenance of adaptive immune responses. In humans, suffering from acute infectious diseases, only peripheral blood dendritic cells are accessible to investigation but not dendritic cells located in lymphoid tissue. However, together with in vitro studies, the frequency and function of peripheral blood dendritic cells can elucidate the role of these cells during acute disease.

We investigated the frequency and function of peripheral blood dendritic cells in Kenyan children suffering from acute Plasmodium falciparum malaria, during convalescence and in healthy children during the dry season.

I will give an overview and discuss results of our studies on the frequency and function of peripheral blood dendritic cells in Kenyan children suffering from acute P. falciparum malaria.

O-188 Differences in status of T lymphocytes but not of monocytes between cerebral malaria and severe malaria anaemia in African children [MIM-CB-436968]

P. Boeuf, S. Loizon, G. Awandare, M. Addae, J. Koffi, B. Goka, L. Hvid, O. Puijalon, B. Akamori, C. Behr

(1) Institut Pasteur, Paris, France; (2) Noguchi Memorial Institute for Medical Research, Legon, Ghana; (3) Rigshospitalet, Copenhagen, Denmark; (4) Korle-Bu Teaching Hospital, Accra, Ghana

Introduction: Both local cytoadhesion of infected red blood cells and systemic inﬂammation contribute to pathogenesis of severe malaria. The cascade of stimulation and the nature of the immunocompetent cells involved are unknown. Previous work of our network showed that cerebral malaria (CM) and severe anaemia (SA) in Ghanaian children are associated with a distinct inﬂammatory cytokine imbalance, suggesting that they are distinct immunopathological syndromes.

Methods: We have conducted ex vivo studies to explore the possible contribution of circulating monocytes and T lymphocytes to the cytokine imbalance in CM and SA. In order to better document the cytokine proﬁle, we ﬁrst developed a new real time RT-PCR technique allowing quantitative cytokine proﬁling adapted to small blood volumes. The phenotype of circulating monocytes was investigated by flow cytometry (level of HLA-DR and CD16 expression), and their intrinsic capacity of cytokine production in a short time stimulation assay (maximum levels of TNF and IL-10 produced) was evaluated.
Results: There was no significant difference in CM and SA patients after correction for age and parasitaemia. In contrast, CM and SA patients had different T cell status, evidenced using flow-cytometry and mRNA profiling. Interpretation: Taken together, these results indicate that differences in T lymphocytes rather than in monocytes may account for the different TNF/IL10 imbalance in CM and SA. The potential role of regulatory T cell will be discussed.

O-189
Red blood cell polymorphisms modulate the antibody response to *P. falciparum* in Senegal [MIM-JS-134373]

J. Sarr, S. Pelleau, C. Toly, J. Guitard, L. Konate, P. Deloron, A. Garcia, F. Migot-Nabias
(1) Institut de Recherche pour le Développement, Dakar, Senegal; (2) Université Cheikh Anta Diop, Dakar, Senegal; (3) Institut de Recherche pour le Développement, Paris, France

Introduction: The evidence of protection afforded by red blood cell polymorphisms against either clinical malaria or *Plasmodium falciparum* infection levels varies with the study sites and the conditions of malarial transmission. But no clear implication of an antibody-related effect has yet been established. We investigated the impact of sickle-cell trait, G6PD deficiency, alpha-thalassemia and ABO blood groups on the antibody response against *P. falciparum*.

Methods: Venous blood was drawn in June 2002 in 413 children aged from 2 to 10 years from a rural area of Senegal before the annual malaria transmission season. ABO blood groups were determined by serology, and sickle-cell trait, G6PD deficiency (G6PD A- variant) and alpha-thalassemia by molecular genotyping. Plasma IgG and IgG subclasses directed to recombinant proteins from the two serogroups of the Merozoite Surface Protein 2 (MSP2/3D7 and MSP2/FC27) and the ring-infected erythrocyte surface antigen (RESA) were determined by ELISA. Thereafter, a parasitological and clinical 18-month follow-up was carried out in these children.

Results: Children with sickle cell trait (AS) suffered from less malaria attacks than normal children (*p* = 0.01), although their parasite density was similar. The antibody response was dependent on age and to a lesser extent to the village of residence. IgG3 responders to all proteins, IgG responders to RESA and MSP2/3D7, as well as IgG2 responders to RESA and IgG1 responders to MSP2/3D7, presented enhanced mean parasite density (all *p* < 0.008). The levels of IgG and IgG3 to MSP2/3D7 were negatively associated with the risk of occurrence of a malaria attack during the following transmission season (*p* = 0.04). As compared to normal children, sickle cell trait carriers presented lower levels of IgG and IgG3 to MSP2/3D7 and of IgG1 to RESA (all *p* < 0.03). In a similar way, G6PD A- girls had lower levels of IgG and IgG3 to MSP2/FC27 than G6PD normal girls (both *p* = 0.004). Neither ABO blood groups nor alpha-thalassemia was associated with the antibody response.

Interpretation: IgG and IgG3 to MSP2/3D7 are not protective by themselves due to their association with parasite density. AS children are protected against mild malaria attacks through another mechanism than the cytophilic antibody response to MSP2/3D7.

O-190
Enhanced IgG antibody responses to *Plasmodium falciparum* variant surface antigens in Gabonese children with sickle cell trait [MIM-GC-311608]

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Introduction: Sickle cell trait protects against severe *Plasmodium falciparum* malaria in young African children, which explains the relatively high penetrance of this mutation in sub-Saharan African communities exposed to high rates of infection with *P. falciparum.* A
number of mechanisms have been proposed to mediate the protection afforded by the sickle cell trait, however, definitive proof of immunological correlates of the protective effects of HbAS has proven elusive.

Methods: We used flow cytometric methods to investigate whether antibodies directed to parasite-derived variant surface antigens (VSA) on the membrane of infected erythrocytes contribute to this protection. We measured anti-VSA IgG responses directed to two heterologous parasite isolates in 458 Gabonese children aged between 6 months and 10 years.

Results: Logistic regression analyses showed a highly significant association (P < 0.00001) between carriage of the sickle cell trait and the presence of anti-VSA IgG responses to both isolates. The pattern of IgG isotype-mediated anti-VSA responses was dependent on the origin of the isolates. In children with the sickle cell trait IgG1 and IgG4 predominated in the profile of responses to VSA of a parasite isolate from a child with severe malaria, but not in that to VSA of a parasite isolated from a child with mild malaria. The frequency of IgG2-mediated responses directed to VSA of both parasite isolates increased with age, while such an age-related increase in the frequency of IgG3-mediated responses was only evident for the parasite isolate from the severe case.

Interpretation: The results suggest that individuals with the sickle cell trait develop enhanced levels of cross-reactive anti-VSA antibody responses compared to their matched counterparts with normal hemoglobin.

O-191
Is resistance to Plasmodium falciparum infection in sickle cell trait (AS) individuals related to the presence of high frequency of haptoglobin 2-1? [MIM-558822]
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Introduction: Many studies showed evidence for partial protection of AS individuals from falciparum parasitaemia. One obvious puzzling question is why sickle cell disease (SS) does not show the same clinical resistance to falciparum malaria as (AS) individuals. Previously we found an association between the high frequency of Hp1-1 and susceptibility to falciparum infection while Hp2-1 and 2-2 confirmed resistance. In this study we attempt to clarify the role of Hp phenotypes in this protection.

Methods: Sixty sickle cell disease, 30 sickle cell trait individuals and 20 healthy control individuals (AA) were screened for their haptoglobin phenotypes using the 4.7% polyacrylamide gel electrophoresis separation of sera (mixed with erythrocyte haemolysate) followed by benzidine staining of the gel.

Results: Haptoglobin phenotypes distribution showed a highly significant difference between the three groups (P = 0.00001, χ²-test). The sickle cell disease individuals (SS) had high frequencies of Hp1-1, 80% had Hp1-1, 20% had Hp2-1 and none of them had Hp2-2, while in the sickle cell trait group 40% had Hp1-1, 20% had Hp2-1 and none had Hp2-2. In the healthy control individuals 45% had Hp1-1, 40% had Hp2-1 and 15% had Hp2-2.

Interpretation: We postulated that the protection of sickle cell trait (AS) against malaria infection may be related to the high frequency of haptoglobin phenotype 2-1 (60%).

O-192
Suppression of IL-12 in children with malaria is due to hemozoin-induced overproduction of IL-10 by circulating blood mononuclear cells [MIM-DP-143308]
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**Introduction:** Protective immunity against malaria is regulated by coordinated production of proinflammatory cytokines, such as interleukin (IL)-12 and tumor necrosis factor (TNF)-α, and anti-inflammatory cytokines, such as IL-10 that limit proinflammatory responses. Since parasitic products (e.g., hemozoin, malarial pigment) alter monocyte-derived cytokines, hemozoin-induced overproduction of IL-10 and TNF-α was investigated as a molecular mechanism to explain IL-12 suppression in malarial anemia (MA).

**Methods:** IL-12p70, IL-10, and TNF-α levels were determined by ELISA in plasma and cultured peripheral blood mononuclear cells (PBMCs) from children residing in a holoendemic area of *Plasmodium falciparum* transmission in Kenya. Cultured PBMCs and CD14+ monocytes from healthy U.S. donors were stimulated with lipopolysaccharide (LPS) and interferon (IFN)-γ, or LPS and IFN-γ with hemozoin or synthetically prepared hemozoin, β-hematin. Supernatant levels of IL-12p70, IL-10, and TNF-α were determined by ELISA, and IL-12p35, IL-12p40, IL-10, and TNF-α transcripts were quantified by real time RT-PCR. IL-10 neutralizing antibodies restored hemozoin-induced suppression of IL-12p70, while TNF-α neutralizing antibodies had no effect on IL-12p70 production. Addition of IL-10 neutralizing antibodies to cultured PBMCs from children with malaria also increased IL-12p70 production.

**Interpretation:** Suppression of circulating IL-12 in children with malaria is due, at least in part, to hemozoin-induced overproduction of IL-10 by monocytes, suggesting that hemozoin may be a primary cause of cytokine dysregulation in children with malaria.

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**O-193**

**Malaria transmission blocking immunity: Absence of complement-mediated inhibition of early *P. falciparum* development in *An. gambiae* [MIM-LG-9396]

L. Gouagna, M. Kolé, R. Sauerwein, J. Verhave, W. Eling, C. Boudin

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**Introduction:** Complement is one of the effector mechanisms of host immunity against malaria parasites. However, there is no evidence of antibody-dependent activity of host complement in *Plasmodium falciparum* transmission model. Here, we used endemic sera to test the relation between antibody response against Pfs230 and Pfs 48/45 and transmission blocking activity in the membrane feeds. We further test the hypothesis that complement is associated with transmission blockade process of *P. falciparum* within its vector.

**Methods:** Groups of *Anopheles gambiae* were given infectious bloodmeal prepared with non immune sera and pair-matched serum sample from malaria endemic area, both in the presence and absence of active host complement. Although the NF54 parasite strain was used in infection experiments, endemic sera came from naturally infected *P. falciparum* gametocyte carriers previously recruited from cross sectional surveys among residents of Mengang (100 km from Yaounde). Competition ELISA to antibodies against Pfs48/45 and Pfs230 was used to correlate seropositivity with transmission capacity.

**Results:** Twenty four endemic sera were tested, most of which showed high percentage of complement-mediated lysis in vitro whilst having little complement-dependent effect on inhibition of sporogonic development. Active complement in malaria negative control serum did not show any significant effect on parasite infectivity to mosquitoes, compared to the activity of complement depleted sera. Although the majority
(60%) of immune sera reduced oocyst production by 80% compared to the control, there was no association between complement and the reduction of either the preoocyst development or the overall oocyst intensity. Seropositivity by competition ELISA to antibodies against Pf48/45 (48%) and Pf230 (29%) did not correlate with transmission capacity, probably because antibody recognition to these sexual antigens was limited in the sera tested.

Interpretation: These results indicate that the effect of naturally acquired immunity in the transmission reduction process against *P. falciparum* is not enhanced by the presence of host complement as previously shown in animal Plasmodium models.

31. Vector control

**Friday 18 November 11:00–13:00—Mahogany Hall**

Chairs: Lucien Manga (Yaounde) and Martin Akogbeto (Cotonou)

**O-194**

**Insecticide treated materials: An evolutive concept**

[MIM_JH_487999]

*J. Hougard*

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Introduction: In the early 1980s, insecticide treated materials (ITMs) was mostly experimented for mosquito bednets, that are now considered as an effective tool to reduce malaria morbidity and overall infant mortality, particularly in Africa. Treated curtains were evaluated near after, particularly in Kenya and Burkina Faso. Although they were effective in both countries, they were not effective enough to be use alone but rather as an additional protection method to insecticide treated nets (ITNs).

Methods: The concept of ITMs was then progressively extended to other materials, especially thanks to the latest developments in the field of bio-active fibres and fabrics, that allowed insecticide industry and net manufacturers to develop long-lasting and wash resistance treated materials. At the present time, long-lasting ITMs have a range of potential applications, particularly with the development of new tools that can be used at a large scale, such as curtains, plastic sheeting’s, blankets, tents, hammocks, battlefield uniforms, etc. However, each of these intervention methods cannot be used in every situation, as it depends on ecologic and sociologic factors.

Results: For example, impregnated hammocks are suitable to protect people, as forest workers, from exophagic and exophilic vectors, as *Anopheles dirus*. Insecticide treated tents are recommended to protect sleepers from endophagic and exophilic vectors, as *An. gambiae* or *An. stephensi*, particularly in case of emergency situations. Insecticide Treated Battlefield uniforms protect soldiers operating under tropical field conditions, particularly non immune troops, but their impact on malaria transmission has not been yet clearly demonstrated. Recently, the efficacy of an experimental long lasting insecticide treated plastic sheeting (ITPS) was tested successfully in experimental huts against *An. gambiae*, as a potential alternative method to indoor residual spraying (IRS). New avenues of research are actually experimented to improve the sustainability of such vector control interventions. Studies carried out by our team in West Africa consist to use two different ITMs at the same time, either to better manage pyrethroid resistance (by treating one fabric with one insecticide and the second one with another insecticide), or to provide an extra personal protection (by adding or even synergizing the respective efficacy of each treated material).

Interpretation: These research projects clearly show that insecticide treated materials are an evolutive concept that will certainly more and more contribute to decrease malaria burden, particularly in Africa.
Does long term protection with insecticide treated curtains (ITCs) have an impact on the prevalence of drug resistant malaria parasites? [MIM-DD-105808]

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(1) Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso; (2) London School of Hygiene and Tropical Medicine, London, UK

Introduction: Vector control measures have been postulated to delay acquisition of immunity to malaria. If true, this may increase treatment-seeking, and impact on the prevalence of drug-resistant parasites.

Methods: We tested this hypothesis in nine villages in Burkina Faso, which had used ITCs for 6–8 years, and nine villages that had never used ITCs. Blood samples were collected from 1035 children aged 6–59 months seeking care at local health facilities and from a random sample of 588 children drawn from the 18 communities. The genetic markers of resistance to CQ and SP were detected by PCR.

Results: The proportions of children harbouring parasites carrying the pure wild type alleles at the pfcrt76 and the pfmdr1–86 gene loci, single mutations at the pfmdr1–86 or the pfcrt76 locus, and mutations at both gene loci were similar in protected and unprotected villages. In sick children, parasites carrying the pfcrt76T allele were observed in 42.5% of ITC users and 39.5% of non-users (OR = 1.09; 95%CI 0.79, 1.52; P = 0.27).

The proportions of children with parasites carrying the pfmdr1-86Y mutation were similar with 31.3% and 28.6% (OR = 1.195%;CI: 0.74, 1.64; P = 0.58). The community survey produced similar results. The distributions of parasites carrying mutant dhfr (N51I, C59R and S108N) or dhps (A437G and K540E) alleles were also similar in the two groups of villages. The triple dhfr mutation was observed in 11.6% versus 11.8% of children in protected and control villages, but no double dhps mutation was observed.

Interpretation: Children using ITCs were not more likely to be carrying parasites with mutant alleles associated with resistance to CQ and SP than children not using ITCs. In this setting, ITC use was not associated with increased anti-malarial drug pressure on the parasite population.

Operational field studies for community-based malaria vector control using plant-based mosquito repellent blend in Western Kenya [MIM-AS-187530]

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Introduction: This paper summarizes the major accomplishments of an operational field study on community-based malaria control using plant-based mosquito repellents, bednets and chemotherapy in integrated approach in East Gembe location of Suba District, Western Kenya. The primary objective of the project was to assess the impact of improved delivery of fumigant from a plant (Ocimum kilimandscharicum), used traditionally to repel mosquitoes, on malaria vectors and transmission at the village level.

Methods: In the pre-intervention phase of the project, entomological, parasitological and anthropological studies were carried out in the study villages (Kisamba and Kamasa). In the intervention phase, the study area was divided into four blocks, plant fumigation alone, bednets alone, plant fumigation and bednets, and no intervention control group. The impact of the interventions was evaluated by indoor sampling of mosquitoes and parasitological surveys at the pre-intervention and intervention periods.

Results: Entomological studies revealed that reductions of Anopheles gambiae s.l. due to plant applications were 56.18% and 62% for plant fumigation without and with bednets, respectively. PCR identification of the An. gambiae s.l. showed that, most of the samples collected during the intervention phase were An. gambiae s.s. (71.4%), and only 28.6% were An. arabiensis. However, during the pre-intervention period, most samples were identified as An. arabiensis (77.1), and 22.9% of the total samples were An.
Abstracts / Acta Tropica 95S (2005) S1–S506

**O-198**
Combination of a non-pyrethroid insecticide and a repellent: A promising approach for malaria vector control

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Introduction: With the spread of pyrethroid resistance in most mosquito species and the lack of alternative compounds for public health, the search for new strategies allowing a better control of “resistant populations” has become a priority. In that context, we investigated under laboratory conditions, the efficacy of non-pyrethroid mixture combining a repellent (diethyl toluamide or DEET) and a carbamate (propoxur) to control Kdr-resistant mosquitoes.

Methods: Irritancy, knock down effect (KD) and mortality were measured for each insecticide and repellent, alone and in mixture, using standard WHO test kits against susceptible and pyrethroid-resistant strains of Aedes aegypti. The mode of action of repellents was also investigated by measuring the effect of DEET on the dorsal unpaired median (DUM) neurons of the American cockroach Periplaneta americana using the patch-clamp and cell imaging techniques.

Results: DEET-propoxur combination showed the same features as deltamethrin at the LD100 (irritancy, knock down effect and mortality) against susceptible mosquitoes because of a strong synergism between these two compounds (mortality and KD effect). With the pyrethroid-resistant strain, the toxicity of deltamethrin was fairly low (4%) but the toxicity of the mixture remained high (94%). Electrophysiological studies demonstrated the neurotoxic activity of DEET in the central nervous system occurring through an increase of the intracellular Ca2+ concentration in the DUM neuron.

Interpretation: The neurotoxicity of DEET in insects may explain the synergistic interactions observed with the non-pyrethroid mixture. Further studies will now investigate the efficacy of repellent and carbamate/Ops “two in one” treated nets for malaria prevention.

**O-199**
Evaluation of new insecticides and long lasting treatments for nets and other materials used in malaria vector control and personal protection

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Introduction: World Health Organisation (WHO) recommends synthetic pyrethroids for impregnation of bednets. Practical obstacles to sustainable malaria vector control measure include emergence of pyrethroid...
resistant mosquitoes and inadequate re-treatment of bednets. This study aims to evaluate alternative insecticides and new tools for malaria vector control and personal protection, to detect insecticide resistance mechanisms and to establish new impregnation processes on long lasting materials.

Methods: This evaluation was carried out in experimental huts at Mabogini, Moshi, Northern Tanzania where the predominant malaria vector is Anopheles arabiensis between December 2004 and March 2005. Bednets and sheets treated with permethrin (500 mg/m²) manually and through industrial processes (long lasting treatments), were evaluated in each of the three experimental huts by a Latin square design. The construction and operation of the huts is as per earlier description by Smith (1963). Two sleepers per hut slept from 20:00 until 06:00h and in the morning dead and alive mosquitoes were collected in paper cups. After classification by abdominal conditions malaria vector species were held for 24 h to observe mortality.

Results: The deterrent effect of permethrin (500 mg/m²) on hut entry of An. arabiensis were as higher in permethrin net and sheet, 77% and 40% than olyset net and sheet, f(49% and 2%. The increased exophily of An. arabiensis due to repellence effect of permethrin (500 mg/m²) treated sheet and net were 11% and 3%, respectively, and slightly similar to Olyset sheet and net, 12% and 4%, respectively. Blood feeding rate reduction for An. arabiensis were low in huts with conventionally permethrin (500 mg/m²) treated sheet than in Olyset sheet, f(10% and 13%, respectively. Mortality rates of An. arabiensis were 22% and 30% in permethrin sheet and net, 32% and 42% in Olyset sheet and net, respectively. In contrast, C. quinquefasciatus showed low deterrent effect, repellent effect and blood feeding rates than An. arabiensis and had the same mortality rates due to both conventionally treated materials and olyset sheet and net.

Interpretation: Conventionally permethrin treated materials were more effective than long lasting treatments with permethrin in terms of deterrence and repellence against An. arabiensis. Ongoing data will be presented.

O-200 Retention and use of free insecticide-treated nets by pregnant women in the displaced communities in northern Uganda [MIM-GR-402428]

A. Kilian, A. Bell, S. Meek, G. Root, J. Muhiru, J. Komakech, A. Collins

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Introduction: Free insecticide-treated nets (ITN) were distributed to pregnant women attending ANC services in Northern Uganda, where a large proportion of the population is internally displaced by conflict. Six months later targeting, retention, and use of these nets were assessed.

Methods: Four internally displaced persons (IDP) camps out of 13 were selected as a convenient sample based on security and accessibility criteria as well as geographical representation. From the registers of distributed ITNs in the ANC clinics names of recipients were randomly selected. The number sampled from each camp was proportionate to the number of distributed ITNs, on average 19.0%. Women were visited in their homes as indicated in the records. Those women identified were interviewed after giving verbal consent using a pre-coded questionnaire which assessed socio-demographic data, availability, source and use of any mosquito nets in the households. During the interview the data collectors sought permission to physically inspect the ITN.

Results: Out of 274 nets followed up, the fate of 229 could be determined and 188 were found in the possession of the target group giving a targeting rate of 82.1% (95% confidence interval [CI] 76.5–86.8). Depending on whether health clinic registers or the information given by the women was considered true, 96.1–88.6% of nets were correctly distributed by health staff and 92.6–85.5% were retained by the ANC clients. However, only 89 nets had been used the previous night by one of the women resulting in a utilization rate of 38.9% (95% CI 32.5–45.5). One third of the nets (34.6%)
were still found in the original package, 32.5% were unpacked but not hung up and 33.0% were found hanging over the bed. The most important factor influencing whether the net was unpacked was having delivered the baby (odds ratio 2.91, 95% CI 1.48–5.75), but it was independent of explanation of correct use by health staff or educational status of the woman. Additional information on ITN use through radio, health staff or friends increased the likelihood of unpacking the net only after delivery (OR 2.72, 1.04–7.09) but not before (OR 1.40, 0.59–3.31).

Interpretation: We conclude that while distribution through health workers and retention by the women were adequate, actual use of nets was not and intensive communication efforts are required to improve this situation.
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1. Prevention of malaria

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

1A Knowledge and use of mosquito nets for prevention of malaria in Nigeria [MIM-RA-75952]

R. Aliyu
Faculty of Medical Sciences, University of Jos, Plateau State, Nigeria

Introduction: The study was conducted to ascertain the knowledge and level of use of mosquito nets and insecticide treated nets (ITN) and to determine the various factors that hamper their use in five selected malaria endemic communities in Jos, Plateau State, Nigeria. The communities are Angwan Rogo, Tudun wada, Gangare and Layin sarki.

Methods: Five malaria endemic communities were selected at random in Jos, Plateau State for the study, Angwan Rogo, Tudun wada, Gangare and Layin sarki. Family heads including both parents or their representatives were interviewed using a pre-tested interviewer administered questionnaire. The results were then compiled and analyzed.

Results: 80.3% of the respondents in general have a good knowledge of malaria and the use of mosquito nets to prevent malaria. Only 23.7% know about ITNs. 69.7% stated that malaria is a priority problem while 21.6 chose poverty. Only 16.7%, 13.2%, 9.1%, 11.5%, 16% of the respondents from the five communities have ever purchased any type of net. 79.8% in all communities indicated their willingness to buy ITN for the prevention of malaria. There were very high levels of prioritization of the disease. The use of ITNs was strongly advocated for by 89% of the respondents.

Interpretation: The interpretation of the results were done using spread sheets after the compilation of the data.

2B Clinical presentation of malaria during pregnancy and postpartum period among women from a rural area of Southern Mozambique [MIM-AB-43015]

A. Bardají, C. David, C. Romagosa, S. Amos, L. Bruni, S. Sánchez, P. Alonso, C. Menéndez
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Introduction: Pregnancy is associated with an increased susceptibility to malaria and there is some evidence that puerperal women compared with non pregnant women are also at a higher risk for malaria infection. Maternal anaemia, low birth weight and prematurity are the most frequent adverse effects of the infection during pregnancy, and the development of malaria and/or parasitaemia is higher in puerperal women. However, little is known on the clinical presentation of malaria episodes among these groups.

Methods: All pregnant or puerperal women attending the antenatal services at Manhiça Health Center with clinical complaints were enrolled through a Passive Case Detection system since August 2003. A standard questionnaire was administered and a blood smear, filter paper and haematocrit done if a predefined clinical criteria suggestive of clinical malaria were met. These were the following: axillary fever (T ≥ 37.5 °C), referred history of fever in the 24 h, pallor, arthromyalgias, headache and history of convulsions. Anaemia and severe anaemia were defined as Hb levels <11 g/dl and <7 g/dl, respectively.

Results: During this period 2864 pregnant and 149 puerperal women have been seen at the clinic, of them 2190 pregnant and 105 puerperal women had criteria for blood testing and malaria parasitaemia was present in 687 of pregnant women (24%) and in 38 of puerperal women (25%). The most common complaint in those with malaria parasitaemia was headache followed by arthromyalgias and history of fever, in both groups. Axillary fever was found in 21% of pregnant women and 24% of puerperal women (both groups with malaria parasitaemia). Anaemia and severe anaemia was found in 44% and 2.2% of pregnant women, respectively, and in 53.6% and 14% of puerperal women. Three hundred
and thirty-four (12%) of the women had severe malaria and required admission to hospital. Interpretation: Uncomplicated clinical malaria is frequent both in pregnancy and puerperium. Malaria and anaemia prevalence were higher in puerperal than in pregnant women, emphasising the need to extend malaria control measures over the puerperal period.

3C Prevention of malaria outbreaks in Burundi Highlands, Karuzi Province [MIM-DB-250250]

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Introduction: In the last decade, Burundi faced increase in malaria incidence in the highlands (>1600 m) ending in a major malaria outbreak in 2000. Drug resistance, environmental changes were the main driving forces of this epidemic. The objective of this four year program is a sustainable prevention of malaria epidemics in highlands through a reduction of the malaria vector population and malaria transmission, a screen effect for population living on the hills and a reduction in human reservoir.

Methods: The strategy is based on one round per year of indoor residual spraying (IRS) targeting both in time (before the months most suitable for transmission, August and September) and in space (houses near the valleys). Since 2002, each year, 23% of the houses (about 16,700) in the province have been sprayed with residual insecticide (deltamethrin) covering 95% of the target population in the intervention areas. Every year, two cross sectional studies (randomised clusters) are done in the intervention and control areas: one before and one after the IRS round to assess and document the impact of the intervention on vectors and human reservoir. Ethical clearance was acquired for this study.

Results: A drastic reduction in vector densities was observed after the first spraying campaign in the treated areas. The density drops from an average of 10 anophelines/house to less than 0.2 in the treated areas. The transmission has also slowed down. Less than 0.4% of the houses were found with infectious mosquitoes compared to 5.5% in the control areas (average of the five surveys) and no infectious mosquito was found during the last survey in the treated areas. The decrease of the proportion of human parasite carriers is slower than expected. After the second year of the campaign, we saw a significant reduction of 20% for the malaria prevalence between the lowland sprayed and lowland control.

Interpretation: Our program has a high impact on the vector population and on malaria transmission, however, the human reservoir in parasite is decreasing more gradually. With effective treatment and well targeted vector control we expect to avoid outbreaks.

4A ITN programmes evaluation in RDC [MIM-TB-79424]

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Introduction: In DRC, malaria is endemic and a significant source of morbidity and mortality. In 2001, DRC endorsed Abuja Declaration and the National Malaria Control Program (PNLP) initiated to protect children and pregnant women and to reduce poverty DRC objectives are 60% household with at least one ITN, 60% children sleeping under ITN and 60% pregnant women sleeping under ITN. With some partners the Ministry of Health are implementing ITN in some health zones for more than 1 year different distribution approaches used by partners. Than evaluation of these appears necessary.

Objectives: To evaluate coverage and equity of distribution. To identify factors influencing use of net,
and strengths and weakness of different programmatic approaches.

Methods: Methodology Surveys Conduct community-based surveys in nine Health Zones (Kinshasa, Mbuji Mayi, Tshikaji, Pawa, Kisangani, Kimpese, Lodja, Vanga and Lubumbashi). Interviews and documentary review Health zones responsible interviewed.

Results: ITN household possession: 14–49%. Proportion of pregnant women using ITN: 5–49%. Proportion of children sleeping under ITN: 5–36% malaria prevention is the principal factor influencing ITN use preceding nuisance. Cost is the principal barrier to ITN acquisition.

Interpretation: Different partners use different approaches. Distribution is not equitable in different groups. Coverage in progress in DRC But new is consensus needed between PNLP and partners.

5B

Intermittent malaria treatment and iron supplementation for control of malaria and anaemia in infants in forest belt of Ghana: A randomised trial

[ MIM-EB-63609 ]

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Introduction: Malaria-related anaemia is a major contributor to infant mortality. Hence more effective strategies to control malaria and anaemia in infancy are required. In perennial malaria transmission areas, an association between falciparum malaria and anaemia exists and malaria control improves survival and reduces incidence of severe anaemia. Consequently, a strategy to control these problems was implemented in rural Ghana through intermittent malaria treatment and daily iron supplementation.

Methods: A double-blind placebo-controlled randomised control trial was conducted in the Afigya-Sekyere district of Ghana. The study assessed effectiveness of intermittent malaria treatment given at 10 weeks, 14 weeks and 9 months through the EPI programme and daily iron supplementation from 10 weeks to 12 months in the control of malaria and severe anaemia in infancy. Haemoglobin was determined using haemocue and malaria parasitology was determined through blood films. Passive surveillance was done through health facilities using outpatient visits and health facility admissions. The sample size was 450 infants per arm, making a total of 1800. The field operations were conducted from May 2001 to August 2004.

Results: The final sample size achieved was 1791. Drug administration was as follows: 1791 dose 1, 1785 dose 2, 1734 dose 3. Forty deaths were reported. Baseline characteristics were similar in all four arms of the trial. Preliminary analysis for anaemia has been completed and results showed reduction in incidence of anaemia. Protective efficacy for anaemia at 12 months of age was as follows: SP+ Iron [51.5 (36.3, 63.1); p < 0.001], SP alone [25.0 (4.9, 40.9); p < 0.02] and Iron alone [28.5 (8.9, 43.8); p < 0.01]. P. falciparum parasitaemia in school children treated with SP at 28 days of follow up was 42% compared 2% for mefloquine. Analysis for clinical malaria, monthly follow-up, passive surveillance and side effects is ongoing.

Interpretation: Preliminary results from the field trial in Afigya-Sekyere district of Ghana showed a significant impact on severe anaemia in infants. Effects on clinical malaria will be available after data analysis. Recommendations will await final results.

6C

A double blind randomised placebo-controlled trial to measure the potential of intermittent treatment with artesunate plus sulfadoxine/pyrimethamine (SP) to prevent malaria in Senegalese children

[ MIM-BC-33099 ]

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Introduction: We have evaluated a new control method for these vulnerable children—intermittent preventive treatment, which involves administration of a full dose of anti-malarial treatment at defined times without prior testing for malaria infection.

Methods: We conducted a randomised, placebo-controlled, double blind trial of the impact of sea-
sonal, intermittent preventive treatment on morbidity from malaria in Niakhar, a rural area of Senegal. We enrolled 1136 children aged 6 weeks to 59 months who received one dose of artesunate plus sulphadoxine–pyrimethamine or placebo on three occasions during the malaria transmission season. The primary outcome was first or single episode of clinical malaria detected through active or passive case detection.

Results: The study arms were well matched on entry into the trial. Over 13 weeks of follow-up, the intervention led to an 86.3% [95% CI (81–90%)] reduction in clinical episodes of malaria.

Interpretation: Intermittent preventive treatment in children using artesunate and sulfadoxine–pyrimethamine is a promising control tool for preventing malaria in areas with seasonal transmission.

7A Malaria illness prevention and treatment in pregnancy: Implications for malaria control in south east Nigeria [MIM-NE-254514]

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Introduction: Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions throughout the world. Prevention, prompt and effective treatment is a critical element of malaria control. The pattern of malaria prevention and treatment during pregnancy needs to be known to enable appropriate intervention

Methods: A cross-sectional household questionnaire survey was conducted in three districts of Enugu East LGA in south east Nigeria with 730 women aged 15 and 49 years who were currently pregnant or were pregnant in the last one year. We sought Information on their socioeconomic and demographic status and use of malaria prevention and treatment services the last time they were pregnant.

Results: A total of 518 women (71%) reported that they had been ill during the present or a recent pregnancy. Out of these, 321 (62%) had malaria alone, 80 (24.9%) had a combination of malaria and other illnesses and 42 (13.1%) had illnesses other than malaria. Formal health facilities (hospital, clinic and health centre) were the most frequently patronized places of treatment. A high percentage of the women (38.6%) received treatment at home and a few of them (6.5% and 1.5%) patronized patent medicine dealers and the traditional healers, respectively. Daprim (pyrimethamine) was the most commonly used chemoprophylactic agent, by 350 (47.9%). Reported use of chloroquine was 12.5%, while only 5.5% and 0.96% used sulphadoxine–pyrimethamine (Fansidar) and proguanil (Paludrine), respectively. Only 5.1% of the pregnant women used insecticide treated bed nets.

Interpretation: Malaria is common in pregnancy but use of preventive measures is low. A majority of the pregnant women used formal antenatal care services, which offers an opportunity to encourage them to use intermittent preventive treatment with SP and ITNs.

8B Predictive value of P. falciparum asymptomatic carriage on malaria attacks in a cohort of Senegalese children [MIM-AG-20306]

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Introduction: The definition of criteria predictive of a mild malaria attack (MMA) could have important consequences on the medical care of children in endemic areas. The aim of this study was to evaluate the predictive value of an asymptomatic P, falciparum infected thick blood smear on the occurrence of a MMA during the 9 following days, in a cohort of Senegalese children.

Methods: The study was conducted in a seasonal transmission area, at the beginning (September) and at the
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end (November) of the transmission season 2002. Each child was classified according to its clinical and parasitological status. An asymptomatic carrier (AC) was defined as parasitaemic, without fever or any clinical sign. All ACs were followed up and their axillary temperature was measured every three days. A MMA was defined as fever (>37.5°C) associated with parasitaemia over 2500/μl. Survival analysis was performed and risk estimates for ACs to present MMAs were calculated by Cox proportional hazards model.

Results: At the beginning of the transmission season, 5.4% (8/147) of MMAs occurred among ACs versus 1% (4/382) among non-carriers (relative risk = 5.32; IC = [1.56–18.15], p = 0.008). Asymptomatic carriage was still associated with MMAs in univariate survival analysis when less restrictive criteria were used to define a MMA (fever and parasitaemia >0/μl) but was not any more taking into account a fever occurrence alone. We made no evidence of a pyrogenic threshold existence in our population. No clinical MMA was detected in the second period (end of transmission season), probably because children developed their immunity crossing the transmission season.

Interpretation: Our results indicate that *Plasmodium falciparum* asymptomatic carriers are more likely to develop malaria attacks than non-carriers and that these children should be treated in priority.

9C
*Plasmodium falciparum* gametocytæmia in children 0–15 years in Bolifamba: Influence of age, sex, haemoglobin AS and anaemia [MIM-FJ-386078]

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Introduction: Malaria has its greatest impact in children <5 years in endemic areas. Asymptomatic untreated children usually develop gametocytæmia. Studies elsewhere have reported *Plasmodium falciparum* gametocyte prevalence to be between 14 and 17%. Being the determining factor in malaria transmission because of an increase in infective mosquitoes, we sought to evaluate the prevalence of *P. falciparum* gametocytæmia in Bolifamba, so as to give a clue to future intervention strategies including drug studies.

Methods: From July to December 2004, blood was collected from 258 children and their parasitaemia, gametocytæmia, Hb genotypes (AA, AS) and PCV determined. The children were grouped as gametocytæmic (n = 35) or not gametocytæmic (n = 223). They were also grouped by age (years) as 0 ≤ 1 (n = 2), 1 ≤ 3 (n = 40), 3 ≤ 6 (n = 95), 6 ≤ 10 (n = 76), 10–15 (n = 45); sex as male (n = 138), female (n = 120) and Hb genotype as AA (n = 212), AS (n = 46). Anaemic cases (60) were subdivided by PCV (%) as severe (<15), moderate (15–20) and mild (21–29). Fever was recorded as axillary temperature >37.4°C. Speciation was done by nested PCR after extraction of parasite DNA from blood.

Results: The prevalences of gametocytæmia, Hb AS, fever and anaemia were 13.6%, 17.8%, 36.8%, and 23.3%, respectively. A significant difference was recorded in the prevalence of gametocytæmia between months (P<0.01), the highest being in September. Between age groups, it was not significantly different (P = 0.12), though children below 5 years were generally more gametocytæmic than older ones. Males were generally more gametocytæmic, though insignificantly (P = 0.12). Almost all HbAS (39) children were not gametocytæmic. Although children with mild anaemia were more gametocytæmic, there was no significant difference with anaemia severity. Hb AS children had lower parasitaemia than HbAA children (P < 0.01).

Interpretation: The peak transmission months of malaria in Bolifamba, correspond to the highest gametocyte prevalence in children. The association of gametocytæmia with age and sex though weak, needs to be considered in epidemiological studies.

10A
Elucidating the role of glycosylphosphatidylinositol (GPI) of *Plasmodium falciparum* origin in malaria pathology using synthetic GPI [MIM-FK-504252]

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Introduction: The glycosylphosphatidylinositol (GPI) of *Plasmodium falciparum* origin is the major toxin in malaria disease. We have previously shown that syn-
thetic GPI from *P. falciparum* can serve as an effective anti-toxin vaccine in a rodent model (1). Local high concentration of *P. falciparum* GPI causes exaggerated proinflammatory immune response leading to organ dysfunction and life threatening pathological conditions.

**Methods:** A series of chemically synthesized, fully lipidated GPIs varying the number of carbohydrate units were employed to elucidate the molecular mechanism responsible for GPI toxicity. Using the well established murine macrophage cell-line RAW264.7 we investigated TNF-alpha secretion, NO production and NF-κB translocation to determine the minimal GPI sequence capable of eliciting an immune response. In addition, we used macrophage cell extracts and an affinity column carrying synthetic *P. falciparum* GPI to identify cellular interactors involved in the genesis of GPI-based toxicity.

**Results:** Synthetic GPI could induce secretion of TNF-alpha from macrophages in significant amount and this induction could be abolished by preincubating the cells with anti-Toll-like receptor 2 (TLR2) antibodies. **Interpretation:** This indicates that the Toll-like receptor 2 is probably the receptor involved in GPI signalling/toxicity.

**11B**

Improving child survival using insecticide treated nets (ITNs) in Kilombero district Tanzania [MIM-ML-163002]

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**Introduction:** Malaria is a leading cause of health service utilisation and is top in both in and outpatient statistics in Tanzania killing about 100 children every day. Insecticide treated nets (ITNs) are proven tools for malaria control. It reduces malaria episodes and increasing child survival. Plan, IHRDC and health promotion unit jointly implemented a community based behaviour change communication for ITNs use in Kilombero district.

**Methods:** Public information campaign involving trade/interpersonal communication and promotion campaigns were conducted which included halo-halo publicity, direct marketing, and net treatment demonstrations by the IHRDC staff, local drama and video shows. Mothers were trained in malaria prevention and control, symptoms, home care and treatment and the benefits and risks of net treatment when attending MCH clinics. Questions were asked after sessions to capture the understanding of individuals. Retail sales agents were trained to teach their customers about the benefits and risks.

**Results:** A total population of 27,930 including adults and children were covered during the trade/interpersonal behaviour change communication and promotion campaigns held during the period under the review. Twenty-one video shows on malaria prevention with emphasis on net treatment were made to community at different villages. Eleven MCH clinics were covered during the special campaign and a total of 3466 mothers voluntarily attended in the special information, education and promotion campaign.

Increased knowledge on malaria and its prevention among the community was noted from questions asked from selected participants in the audience. Increased demand of ITNs resulted to increased net ownership in household levels. This in turn resulted in increased child survival due to reduction of malaria illness at household level.

**Interpretation:** Malaria still remains number one health problem in children under five years old in the district. Further efforts to expand coverage and sustain the demand for insecticide net treatment is required.

**12C**

Efficacité comparée de deux schémas de prévention contre le paludisme au cours de la grossesse à Faladi (Kati)-Mali [MIM-DM-23598]

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Introduction: La chimio prophylaxie par la chloroquine (CQ) telle qu’elle est pratiquée actuellement se heurte à de nombreux problèmes en zone endémique: - Faible observance liée aux contraintes du traitement; 15.4% d’observance sur le plan national; - Progression de la chimiorésistance à la CQ. Face à ces problèmes il est nécessaire de trouver un traitement plus efficace moins contraignant à coût bas. Les espoirs sont de ce fait portés sur la sulfadoxine-pyriméthamine (SP).

Methods: De juin 2003 à mai 2004 nous avons mené une étude comparative de deux schémas de traitement prophylactique anti-paludique au cours de la grossesse qui consistait à administrer deux doses curatives de chloroquine (CQ) ou de sulfadoxine-pyriméthamine (SP) au deuxième et au troisième trimestres de la grossesse en évitant le 9ème mois pour la SP. L’objectif était de comparer l’efficacité de ces deux schémas chez les femmes enceintes et les nouveau-nés. Nous avons travaillé dans le village de Faladié (cercle de Kati) situé à 80 km de Bamako.

Results: L’étude a porté sur 301 femmes parmi lesquelles 150 ont reçu la chloroquine et 151 la SP. A l’inclusion les deux groupes étaient comparables sur l’ensemble des paramètres de jugement socio-démographiques et paludométriques utilisés. A l’accouchement nous avons constaté une réduction de l’infection palustre de 31.6% dans le groupe CQ et de 74.3% dans le groupe SP; le taux d’anémie a baissé de 52.9% chez les femmes soumises à la CQ et de 77.9% chez celles qui ont reçu la SP; l’infection placentaire était de 20.6% dans le groupe CQ contre 8.3% dans le groupe SP (p = 0.004); 16.73% des nouveau-nés avaient un faible poids à la naissance dont 70.45% étaient issus du groupe CQ.

Interpretation: Nous concluons que la SP est plus efficace que la CQ dans la prévention du paludisme chez la femme enceinte et que le TPI par la SP doit être adopté pour mieux protéger la mère et l’enfant contre le paludisme gestationnel et ces conséquences.

13A
Culture des culicinés et essai de test d’efficacité des moustiquaires imprégnées de deltaméthrine dans la ville de Douala [MIM-VM-427455]
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Introduction: Les moustiquaires imprégnées sont actuellement adoptées comme outils privilégiés dans le contrôle du paludisme. Cependant, leur utilisation gagnerait à être optimisée; d’où la nécessité de mettre en place des systèmes de culture de moustiques au laboratoire pour les tests d’efficacité de celles-ci.

Methods: Un système de culture des culicinés (Anophèle et culex) a été mis en place dans notre laboratoire. Les femelles gorgées étaient nourries avec une solution de saccharose à 10%. Les larves étaient cultivées dans les milieux contenant du riz à l’ordre de 60 larves pour 10 g de riz par litre d’eau. La température du milieu était maintenue entre 27–29°C.

Results: Le taux d’éclosion était de 73%. La durée du cycle gonotrophique était de 4 à 5 j. Le passage du stade œuf au stade adulte durait de 7 à 8 j. Le sex-ratio était de 1. Pour le test d’efficacité, la mortalité dans les moustiquaires imprégnées était 9 fois supérieure à celle obtenue dans les moustiquaires non imprégnées. L’imprégnation protégeait l’animal des piqûres 14 fois plus que les tulles non imprégnées. Cependant cette efficacité diminuait lorsque les tulles imprégnées étaient lavées. La mortalité diminuait de 13% et 25% lorsque les tulles étaient respectivement à eau non savonneuse et à eau savonneuse. La protection était aussi diminuée de 3% et 13% respectivement avec les tulles lavées à eau et à eau savonneuse.

Interpretation: La moustiquaire imprégnée protège contre les vecteurs. L’eau savonneuse, enlève l’insecticide fixé sur la moustiquaire. Cette étude suggère que la distribution des ces moustiquaires doit être accompagnée des mesures adéquates pour leur utilisation.
The burden of malaria in pregnancy in Ifakara, Tanzania—An area of high ITN coverage \[MIM-AM-198838\]


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Introduction: Malaria in pregnancy has been associated with adverse outcomes for both mother and newborn. Infection rates have been consistently demonstrated to be highest in women in their first and second pregnancies. To assess the magnitude of the burden of malaria in pregnancy in an area of high insecticide-treated nets (ITN) coverage, we examined parasitemia outcomes among women not receiving intermittent preventive treatment (IPT) in a community where ITN coverage was high beginning in 2003.

Methods: Over one year, 413 pregnant women were recruited into this study upon presenting for delivery at the designated district hospital in Ifakara, Tanzania. We restricted enrolment to women in their first and second pregnancies who had not been given IPT at any time in the previous 12 months. Malaria blood films were prepared from cord blood and the placenta and women answered questions about fever episodes, antimalarial drug consumption and ITN use. We compared the outcomes of reported fever or malaria, and umbilical cord and placental malaria parasitemia against the use of ITN or antimalarial drugs and the place of domicile. Results were compared with findings from previous studies of malaria in pregnancy completed in the same area.

Results: Primigravid women accounted for a majority of those enrolled in the study (62%); as did women younger than 18 years (59%). 178 (43%) women reported experiencing fever or malaria during their pregnancies, and nearly all (91%) took some form of medication. Overall, 103 (25%) women reported using an antimalarial drug and nearly all of these instances (n = 91, 88%) occurred in women who reported fever (p = 0.0001). Overall net coverage was very high with 366 (87%) women reporting that they had slept under a bed net during their pregnancy. However, only 49 (13%) women could accurately report that their net had been treated within the previous year. Cord blood tested positive for malaria parasites in only 1 case, and placental malaria was documented in only 33 cases (7.9%). This is substantially lower than the prevalence observed in the same area before intensive promotion of socially marketed ITNs began in 1996. Given the low rate of placental malaria, it was not possible to measure a statistically significant protective effect of net use (p = 0.20).

Interpretation: Despite not receiving recommended IPT during pregnancy, women in this study experienced very low prevalence of placental malaria at delivery. High utilization of ITNs and antimalarial drug consumption may contribute to this observation.
Goutte épaisse et hématocrite ont été mesurées mensuellement et l’apposition placentaire a été réalisée à l’accouchement. L’étude 3 a été menée dans la banlieue de Dakar auprès des prestataires de CPN et des femmes enceintes. Les entretiens portaient sur la connaissance du paludisme et sa prévention, la pratique du TPI, les effets secondaires de la SP et le vécu du TPI.

Results: Etude 1: Aucun cas d’effet secondaire grave lié à la prise de SP n’a été noté. Les effets secondaires mineurs: prurit (1,6%), céphalées (5%) ont été rarement observés. Les fonctions rénales et hépatiques n’ont pas été perturbées. Toutefois il semble exister une tendance à la diminution du taux de plaquette en l’inclusion et l’accouchement ($p = 0.07$). Nous n’avons pas noté une augmentation de l’incidence des avortements, des malformations comparativement aux années précédentes. Aucun cas d’ictère néonatal n’a été noté. Les études 2 et 3 sont en cours et permettront de déterminer: (1) l’incidence des accès palustres et de l’anémie chez les femmes enceintes sous TPI, (2) la prévalence de l’infection palustre placlentaire, (3) la prévalence des petits pois de naissance, (4) le vécu de la stratégie TPI par les prestataires et les femmes enceintes, (5) le pourcentage de femmes ayant pris les 2 cures de SP.

Interpretation: Le TPI-SP est bien toléré mais des études sur une plus grande cohorte et une surveillance plus rapprochée sont nécessaires. Ces études fourniront des éléments objectifs de base pour l’évaluation régulière stratégie TPI-SP.

16A Bio-prospecting for botanical mosquito repellents from East African flora [MIM-IN-228312]


Introduction: Due to the absence of an operational malaria vaccine, parasite resistance to anti-malarial drugs, and resistance of malaria transiting mosquitoes to insecticides, alternative methods for disease control have to be investigated. Reduction of host-vector contact is one such method that is less susceptible to resistance development. In the last 6 years, we have been bio-prospecting for botanical mosquito repellents from East African flora.

Methods: Essential oils from over 150 plants were investigated against laboratory reared Anopheles gambiae s.s. Repellency of selected plant essential oils from different ecological zones was assessed. The efficacy of three traditional deployment modes (burning, thermal expulsion, intact potted plants) for selected botanical repellents was investigated. The chemical components of repellent essential oils were identified by GC–MS and coinjection. Single components and artificial mixtures of major and/or bioactive constituents were assayed. Selected bioactive components were formulated (creams, coils, candles) singly and assayed.

Results: Twenty plant essential oils exhibited good repellency with over 200 chemical components identified, and about 30 repellent compounds noted. There was more than one repellent compound in each essential oil, and no single component could reproduce the repellency of the whole mixture. In some cases, the repellency of the plant essential oil could be reproduced from reconstituting a few major or bioactive constituents. Repellency of similar essential oils varied with ecological zones. Quantitative variation of bioactive components was noted. Thermal expulsion was better than direct burning of botanical repellents. One essential oil mixture was formulated into a topical cream with 89% protection after 8 h in durational repellency assays. Similarly, eight of the bioactive components were formulated into topical creams, and three (99% protective efficacy) selected for development into commercial repellents. One was developed into a topical repellent cream with 99.9% protection after 8 h at 10% (w/w), and is better than DEET (<15%, w/w). The repellent coil (0.8%, w/w) performed as well as pyrethrin-based commercial ones but slightly worse than α-allethrin. Repellent candles performed poorly, giving 26% protection at 10% (w/w).

Interpretation: Repellency of the plant essential oils is due to blend effects, and varies with the ecological zone due to quantitative differences in the bioactive compounds. Thermal expulsion is effective for the deployment of botanical repellents.
17B
Indoxacarb and chlorfenapyr: Alternative insecticides with potential for use on nets against pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* mosquitoes [MIM-RN-268086]

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**Introduction:** The pyrethroids were the last major group of insecticides to become available for malaria vector control. The evolution and spread of pyrethroid resistance threatens to undermine present gains. There is an urgent need to investigate insecticide alternatives to supplement the pyrethroids for use on insecticide treated nets. The Gates Malaria Partnership has been working with WHO and industry to identify agricultural insecticides that might be adapted for vector control. We report here on an evaluation of the insecticides indoxacarb (an oxadiazine) and chlorfenapyr (a pyrole).

**Methods:** The performance of these insecticides was studied using conventional laboratory bioassays and semi-field systems (tunnel tests) that allows fuller expression of the vector’s behavioural responses to insecticide on netting. Tests were carried out against susceptible and insecticide resistant mosquitoes carrying kdr (pyrethroid resistance) and Ace-1 insensitive acetylcholinesterase (organophosphate resistance) mechanisms.

**Results:** Bioassay tests against resistant adults and larvae showed no cross resistance of indoxacarb or chlorfenapyr to strains of *An. gambiae* bearing the kdr or Ace-1 mechanisms. A matrix of dosages ranging from 50 to 1000 mg/m² and exposure times from 3 to 24 min were tested on netting against *An. gambiae*. Indoxacarb did not cause irritability even at the highest concentration, whereas chlorfenapyr was irritant resulting in lower mortality at higher dosages. The toxic activity of both insecticides was rather slow and delayed mortality was observed between 24 and 72 h. Activity on netting was similar against pyrethroid susceptible and resistant strains. Insecticidal efficacy and protection from mosquito biting, as measured in tunnel cages against *An. gambiae* with guinea pigs as bait, showed high mortality with chlorfenapyr 100 mg/m² and indoxacarb 250 mg/m² but little or no inhibition of bloodfeeding; however, many of those that fed died within 48 h. Experimental hut trials of these insecticides are now underway.

**Interpretation:** New generation insecticides will not necessarily have all the useful characteristics such as rapid knockdown or repellency associated with pyrethroids. However, the toxicity and residual activity of pyroles and oxadiazines indicate they might prove useful as supplements to the pyrethroids.

18C
Intermittent preventive treatment in schools: Malaria parasitaemia, anaemia and school performance [MIM-KN-409248]


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**Introduction:** Although malaria risk is greatest in early childhood, significant numbers of school-aged children remain at risk from malaria morbidity. Asymptomatic infection also contributes to anaemia reducing concentration and learning in class. An effective intervention in infants and pregnant women, IPT could be a valu-
able addition to school health programmes. A trial in W Kenya examines whether IPT in schools can reduce parasitaemia and anaemia amongst school children and improve school performance.

**Methods:** A placebo-controlled trial in schools in Bondo District, W Kenya: an area of intense perennial transmission. Thirty schools were randomly selected to take part in the trial and allocated to intervention or control arms. IPT is administered three times per year, given once per term, in intervention schools. Mass treatment with anthelminthics is given in all schools. Children with individual informed parental consent are eligible for treatment (age range 6–16 years). Pre- and post-intervention surveys will establish haemoglobin level, prevalence and intensity of *Plasmodium falciparum* and intestinal helminth infections, and nutritional status. Class-based attention tests are conducted amongst children in standards 5 and 6 on the day prior to clinical assessment.

**Results:** The results of the recent baseline surveys undertaken in February–March 2005 will be presented. The prevalence and intensity of *P. falciparum* infection in primary schoolchildren living in an area of intense, perennial transmission will be described for 30 schools, as well as levels of anaemia and co-infection with intestinal helminths. Cross-sectional data on concentration and performance in class will be discussed in relation to haemoglobin level, malaria parasitaemia and infection density.

**Interpretation:** The findings will be discussed in relation to malaria control in schools.

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**19A Mosquito net coverage in some locations in the copperbelt province of Zambia [MIM-EN-152262]**

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**Introduction:** Malaria infects not less than 200 million people each year world wide. In endemic countries young children (below 5 years) and pregnant women are especially vulnerable to severe infections. The enormous total number of lives and days of labour lost, the costs of treatment of patients and the negative impact of the disease on development, make malaria a major social and economic burden. Use of treated mosquito nets is one of the recommended strategies for malaria prevention in Zambia.

**Methods:** We interviewed pregnant women and children under the age of five years in some locations that were targeted for indoor residual spraying (IRS) in the year 2003 in the copperbelt province.

**Results:** Out of 459 pregnant women who were interviewed, only 153 (33.3%) said they sleep under a mosquito net. In children, under the age of five years, out of 1252 only 439 (35.1%) were reported to be sleeping under mosquito nets.

**Interpretation:** Mosquito net coverage is very low in these locations. We recommend that studies to establish factors that contribute to low net coverage be conducted.

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**20B IPTi through the EPI system: Community response to a new preventive measure and evaluation of the interactions with the EPI system in Mozambique [MIM-RP-310320]**

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**Introduction:** This anthropological study, linked to a trial of IPTi in Mozambique, was intended to investigate the acceptability of IPTi and possible interactions with EPI (e.g. does IPTi affect community attitudes to EPI, and how does knowledge of EPI affect people’s perceptions of IPTi?).

**Methods:** In-depth interviews, focus group discussions and participant observation with mothers of infants participating in a trial of IPTi, community members, clinic staff.

**Results:** People were familiar with the concept of immunisation due to various traditional practices, though they were unaware of precisely which diseases were prevented by EPI. EPI was initially accepted because it was thought to be obligatory and then became routinised. There were initially serious obstacles to the IPTi trial, but when these were overcome, IPTi rapidly became accepted and routinised as part of EPI. Prior familiarity with SP, and the fact that many
women were able to make health care decisions independently of husbands and relatives was an important factor in this. Interpretation: Initial problems with acceptance of IPTi do not appear to have had any long-term negative effect on people's attitudes towards the health facilities or their acceptance of EPI. People are well aware of the difference between EPI and IPTi.

21C Predicting the impact of intermittent preventive treatment for infants on the spread of drug resistant malaria [MIM-WP-321425]

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Introduction: Intermittent preventive treatment in infants (IPTi) is designed to give a full treatment regimen of an antimalarial drug to infants at the time of routine vaccinations during the first year of life. In two published trials, IPTi has been shown to be effective in reducing the number of clinical episodes of malaria relative to control groups. It is an open question whether or not administering drug to uninfected, non-immune individuals could accelerate the spread of drug resistant parasites.

Methods: We developed a mathematical model which predicts the spread of fully- and partially-resistant malaria parasites in response to changes in individual drug use. In particular, we modeled the interaction between host immune status, drug pharmacokinetics, drug use patterns and transmission of resistant parasites. This model allows variable and seasonal transmission. It represents development of immunity as proportional to transmission intensity. By integrating a model of drug use, including both treatment and prevention, with a model of parasite fitness, we were able to translate individual drug use into predictions about community-wide transmission of resistant parasites.

Results: In at least one trial of IPTi, there was a significant increase in the frequency of drug-resistant infections in the treated group relative to the control group. It is not known if this reservoir of resistant infections would increase the community-wide transmission of resistance. This is a difficult issue to address experimentally due to the size and duration of IPTi trials as well as the intractability of resolving spread of resistance due to treatment of malaria episodes versus IPTi when the same drug is used in both contexts. With the model we developed, we were able to distinguish between spread of resistance due to treatment of symptomatic infections and that due to IPTi. We showed that IPTi could accelerate the spread of resistant parasites, but this effect was only likely to be significant in areas of low or unstable transmission. We saw significantly different rates of spread of resistant parasites in low versus high transmission regions, which is consistent with epidemiological observations. IPTi had a greater impact on the spread of partially resistant than fully resistant parasites. The model also predicted that drugs with shorter clearance times are less likely to contribute to spread of resistance due to IPTi.

Interpretation: Resistance should be monitored during trials of IPTi. The risk of accelerating spread of drug resistant malaria parasites will depend on the intensity of transmission. Results from one epidemiological setting should not be extrapolated to another.

22A In vitro susceptibility of Plasmodium falciparum to the drugs used to treat severe malaria, and to prevent malaria in Comoros Union and Madagascar [MIM-MR-43900]

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Introduction: We used the in vitro isotopic method to monitor the sensitivity of Plasmodium falciparum to quinine, mefloquine and cycloguanil.

Methods: We tested fresh isolates of P. falciparum, collected from patients living in urban, suburban and rural areas and suffering from uncomplicated malaria in 2001, against at least one of the antimalarials cited above. In both countries all of the successfully tested isolates were sensitive to quinine (N=243) and to cycloguanil (N=67).

Results: The mean IC50 ranged from 85.7 to 133.7 nM for quinine. For cycloguanil, the mean IC50 ranged from 1.4 to 20.2 nM and the highest IC50 value (102.5 nM) was recorded in Comoros. Only 0.9%
of the informative isolates from Madagascar were mefloquine-resistant (0/18 in Comoros). The mefloquine mean IC50s were 8.2, 14.1 and 11.6 nM, respectively, in the rural, suburban and urban areas of Madagascar, and 5.9 nM in Comoros. A positive correlation was found between quinine and mefloquine IC50s ($N=127$, $r=0.48$, $p<10^{-6}$), but in vitro mefloquine was 6–16 times more potent than quinine. No correlation was noticed between the activities of quinine and cycloguanil or between the activities of mefloquine and cycloguanil. These results are reassuring, and also demonstrate that the use of mefloquine- and cycloguanil-based antimalarials is justified to prevent malaria in both countries, mainly in the case of travellers.

**Interpretation:** These results are reassuring. Nevertheless, above all, there is a need to assess the therapeutic effectiveness of mefloquine.

### 23B

**The effect of bacillus Calmette Guerin vaccine on malaria morbidity—A randomised trial [MIM-AR-23900]**

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**Introduction:** Observational studies from West Africa have reported that the BCG vaccine improves the overall child survival, including to non-related diseases. BCG is a strong immune stimulator and if it reduces the overall child mortality, this effect would be also probable against malaria, a disease considered to be one of the major causes of deaths in Guinea-Bissau.

**Methods:** Children at 19 months of age were randomised to receive BCG re-vaccination or not. The primary end-point was malaria episodes at the hospital outpatient clinic and at health centres between June and December 2003. Secondary end-points included anaemia, hospital admissions and Pl. falciparum parasitaemia at the beginning and the end of the malaria transmission season.

**Results:** We randomised 1433 children. Anaemia was very common with 78% of children having Hb $<11$ g/dl. BCG revaccination at 19 months of age did not reduce the rate of first consultation due to malaria nor malaria with a parasite density $5000/µl$.

**Interpretation:** The findings did not support the hypothesis that BCG revaccination at 19 months of age reduces malaria morbidity.

### 24C

**KO Tab 1-2-3: A ‘dip-it-yourself’ long-lasting insecticide formulation for converting conventional nets into wash-resistant insecticidal nets [MIM-MR-147539]**

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**Introduction:** Insecticide treated nets (ITN) are an effective method of controlling malaria. To remain effective, ITN need to be re-treated with pyrethroid about once a year. Bayer environmental science (BES) has developed a long-lasting formulation (KO-Tab 1-2-3) that renders the insecticide treatment wash resistant and can be applied in the home or community.

**Methods:** BES commissioned LSHTM to evaluate the performance of polyester nets treated with their long-lasting formulations alongside a conventionally treated net (treated with standard KO-Tab) and PermaNet. After subjecting the treated nets to up to 30 washes using WHO-recommended procedures, performance was measured using three types of assay: ‘3 min exposure’ and ‘median time to knockdown’ bioassays and chemical assay using high pressure liquid chromatography.

**Results:** The conventional ITN was stripped of deltamethrin within 5–10 washes and insecticidal efficacy in bioassay declined to suboptimal levels. With PermaNet and KO Tab 1–2–3 the loss of deltamethrin was much slower – insecticide content halving within 20 washes – and there was no loss of efficacy even
after 30 washes. The claim for KO-Tab 1–2–3 being a long-lasting insecticide treatment was confirmed.

**Interpretation:** This offers the prospect of conventional nets being made into long-lasting insecticidal nets through simple dipping in the home. This single development should transform the malaria control landscape in Africa and have a major impact on malaria.

**25A**

**Racing against malaria: The role of the SADC Military Health Services (SADC MHS) [MIM-AT-134130]**

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**Introduction:** In Southern Africa malaria remains a significant military threat, a threat to cross-border public health and a leading cause of morbidity and mortality, however, access to information on the dangers of malaria remains low especially to communities in inaccessible locations. To raise awareness against malaria among the Southern Africa communities and the SADC military, SADC countries; Angola, Botswana, Malawi, Mozambique, RSA, Swaziland, Tanzania, Zambia and Zimbabwe organized a vehicle convoy, the “Race Against Malaria (RAM)” that drove to Da-es-alaam (Tanzania) to commemorate the Africa Malaria Day on 25 April 2003. Goal: To create massive awareness for scaling up action against malaria in Southern Africa. General objective: To raise the local, national, international profile of malaria, developing public and community awareness, mobilizing resources for strengthening malaria control capacity, strengthen civil–military, public–private-partnerships for malaria control. Military Health Services (MHS) from Lesotho, Malawi, Mozambique, RSA, Tanzania and Zimbabwe participated in the RAM. Aim: To support the SADC campaign and pool together civil–military resources against malaria. The strategic focus for MHS participation was to strengthen civil–military alliance in the fight against malaria and make available idle military human and logistical resources to increase coverage rates for achieving Abuja targets by 2010. MHS participation would prepare the SADC military for joint support and rapid response to epidemics and emergencies in Southern Africa.

**Methods:** RAM Coordination: A RAM co-ordinators committee which included MHS focal points was established to organize and direct all RAM activities. RAM coordination was achieved through e-mail, telephone, teleconferences, fax and meetings. The task of the military was: to mobilize MHS participation, planning the route map, route safety, distances between stops, distances covered/day, work/rest schedules, speed limits; distances between cars, meal/refuelling/sleep over stations, vehicle recovery; medical cover; immigration and customs clearances; convoy security including overall convoy command and control.

**Results:** Achievements: Large communities were mobilized and Malaria control materials (ITN’S, drugs, insecticides, T-shirts, posters, billboards) distributed in all RAM districts along the RAM routes; community awareness heightened and ground gained in the fight against malaria but it requires consolidation. Lessons learned: Civil–military collaboration can be maximized during epidemic/emergencies and major public health campaigns; military human and logistical resources can be used for public health campaigns and poverty alleviation; however, there is need for rules of engagement for MHS participation in malaria control activities.

**Interpretation:** Conclusions: Civil–military partnerships and pooling together of resources in malaria control is possible; RAM planning and execution was a huge success but without military participation it would have been impossible. Post-RAM gains: Inter-country and cross-border initiatives in malaria control gaining strength; massive demand for ITN’S, IRHS and protection against malaria by communities and the military; increased access to health care and anti-malarial drugs; discussions on harmonization/standardization of interventions; establishment of rapid response teams for epidemics/emergencies by the military and increased availability of funds and malaria control resources from governments and partners.
The effect of intermittent preventive treatment with amodiaquine on variant-specific antibody responses to *Plasmodium falciparum* in infants [MIM-LV-2364]

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Introduction: Intermittent preventive treatment in infants (IPTi) has proven highly effective in reducing malarial fevers and anaemia. Meanwhile, IPTi may compromise development of malarial immunity, normally acquired as a result of repeated infections. We examined this question by comparing variant-specific antibodies to *Plasmodium falciparum* in infants receiving amodiaquine (AQ) or placebo during a randomized placebo-controlled trial of IPTi in a highly endemic area in north-eastern Tanzania.

Methods: Infants aged 12–16 weeks attending Mother and Child Health clinics were randomised to receive three doses of either AQ or placebo every 60 days during a 6-month period. Antibodies were measured in plasma samples collected during the course of the study, a complete set of samples and clinical information was available from 35 infants in the AQ-group and 37 infants in the placebo-group. Levels of IgG with specificity for variant surface antigens (VSA) were measured with flow cytometry, using three in vitro cultivated *P. falciparum* isolates originating from Tanzanian children, and by ELISA using a recombinant VAR4-CIDR1 domain of *P. falciparum* Erythrocyte Membrane Protein 1 (PfEMP1).

Results: Preliminary analyses indicate no statistically significant differences between the two treatment groups. With regard to the ELISA data, the proportion of infants recognizing VAR4-CIDR1 was 58.3% in the AQ-group and 47.1% in the placebo-group before the intervention. Half way through the study, 60.9% in the AQ-group and 60.0% in the placebo-group showed reactivity, and at the end of the intervention period the protein was recognised by 62.8% and 90.9% (*P* = 0.36) in the AQ and placebo-group, respectively. At the end of the study, median levels of IgG to the VAR4-CIDR1 was 26.3 ELISA Units (EU) in the AQ-group and 27.9 EU in the placebo-group (*P* = 0.91). The proportion of infants who showed anti-VSA reactivity in the flow cytometry assays was lower compared to those positive to anti-PfEMP1 ELISA tests, but again no statistical significant difference was observed between the infants receiving AQ or placebo.

Interpretation: Infants receiving AQ IPTi had slightly lower levels of variant-specific antibodies than those receiving placebo, but the difference was statistically insignificant. Further studies are needed to know if IPTi delays acquisition of malarial immunity.

2. Parasite biology

27C

*Plasmodium falciparum* gametocyte persistence and within-host dynamics in an area of unstable malaria in Gedaref, eastern Sudan [MIM-AA-11988]

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Introduction: The persistence and within-host dynamics of gametocyte production in *Plasmodium falciparum* clones was studied in inhabitants of Asar village, Gedaref State, which is an area of highly seasonal malaria transmission in eastern Sudan. Five cross-sectional surveys were conducted over a 12-month period, from the transmission season of October 1998 through the dry season until the next transmission season of October 1999.

Methods: Samples collected during these surveys were examined by microscopy and RT-PCR for the presence of gametocytes. RT-PCR was used to detect the expres-
sion of two genes that encode gametocyte-specific proteins, PfS25 (zygote-ookinete surface protein) and PfG377 (gamete surface protein), in parasites sampled from individuals throughout the one year period. PfS25 was initially used to detect the presence of gametocytes in an infection. PfG377, being highly polymorphic, was subsequently used to examine the diversity of alleles among the detected gametocytes. The studied parasite population was genotyped by PCR-amplification of the polymorphic regions of the merozoite surface proteins 1 and 2 (MSP1, MSP2) and the glutamine rich protein (GLURP) genes.

Results: All the isolates with microscopically detectable gametocytes produced RT-PCR products for PfS25. Patent gametocytaemia was very low during the dry season; however, a high proportion of people who contracted the infection during the transmission season maintained sub-patent gametocytaemia detectable by RT-PCR through the following dry season in the apparent absence of mosquito transmission. Consistent gametocytaemia was retained as a chronic infection for more than 9 months. Genotyping of parasites in multiclonal infections showed considerable fluctuation of gametocyte production by individual clones within a single infection. Nevertheless, not all multiple clones produced gametocytes simultaneously. The majority of the non-gametocyte carriers harboured single clone infections (57.6%) and only 5.9% harboured three clones. On the other hand, in most of the gametocyte carriers two clones were detected which represented 47.6% while 8.7% harboured three clones. The mean minimum number of clones (MNC) was significantly higher \( (P<0.05) \) in gametocyte carriers (1.65) compared to non-gametocyte carriers (1.48).

Interpretation: Multiclonal infections are more likely to produce gametocytes. These active gametocytes persisting until the rains infect mosquitoes and allow recombination between clones providing the definite source of the genetically complex malaria outbreaks.

28A
Alternate pathways for erythrocyte invasion are commonly used by Plasmodium falciparum isolates from Senegal [MIM-AA-1989]

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Introduction: Invasion of human erythrocytes by Plasmodium falciparum involves numerous molecular interactions. Laboratory lines vary in the extent of their utilization of different ligand-receptors, which have been defined as alternative invasion pathways. The extent of use of alternative invasion pathways within a single parasite population in vivo may identify dominant invasion pathways that would be useful for vaccine development.

Methods: We first determined the prevalence of polyclonal infections among the patients, using nested PCR of the highly polymorphic MSP1 locus using standard typing methods. We analysed the K1, MAD20 and RO33 allelic families of MSP1. We conducted first round invasion assays \( (n=30) \), as well as invasion assays on culture-adapted parasites \( (n=11) \) obtained from the same Senegalese population.

Results: From the 97 samples isolated from Pikine \( (EIR=1) \) 68% had single infection and 35% double infection. We show that \( P. falciparum \) parasites, both fresh isolates and lab-adapted lines from Senegal routinely use alternative invasion pathways, as evidenced by the ability of these parasites to invade erythrocytes modified by treatment with neuraminidase or chymotrypsin. Senegalese parasites appear to be highly dependent on a trypsin-sensitive receptor for invasion.

Interpretation: Alternative invasion pathways are commonly used in vivo with a large dependence on a trypsin-sensitive receptor. The clonal population in this setting will permit powerful analysis of the mechanisms underlying the use of alternative invasion pathways.
29B Modifications occur in erythrocyte membrane surface properties following malaria parasite invasion [MIM-NA-156060]

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Introduction: To show that modifications in ligand-erythrocyte surface receptor interactions occur after malaria parasite invasion, *Plasmodium falciparum* infected erythrocytes were labeled with cell surface ligands, WGA and EMA. Based on the pattern of ligand binding, which was indicative of modifications in WGA-erythrocyte surface interactions following malaria parasite invasion, the effect of limited ankyrin digestion on band 3-WGA interactions was examined using calpain/Ca2+-treated ghosts as an in vitro model.

Methods: *P. falciparum*-infected erythrocytes were labeled with FITC-wheat germ agglutinin (WGA) and eosin-5-maleimide (EMA). Cell surface fluorescence associated with FITC-WGA and EMA binding was assessed by flow cytometry. To propose a possible mechanism for WGA-erythrocyte band 3 interactions in *P. falciparum*-infected erythrocytes, the effect of limited ankyrin digestion on band 3-WGA interactions was examined using calpain/Ca2+-treated ghosts.

Results: Although *P. falciparum* ring stage-infected erythrocytes had some ability to bind WGA, binding was completely abolished in mature stage-infected erythrocytes. However, all *P. falciparum*-infected erythrocytes were EMA labeled, irrespective of parasite stage of development. From our results, we infer that limited digestion of erythrocyte ankyrin by some *P. falciparum*-derived proteases may generate conformational changes on surface domains of band 3 and as a consequence, preclude WGA interaction with erythrocyte band 3.

Interpretation: Removal of ankyrin domains, as is the case during proteolysis of ankyrin by a stage-specific protease during the mature stage of parasite growth, plays a key role in modulating extra-erythrocytic ligand binding to erythrocyte band 3.

30C Functional analysis of a polyclonal B cell activator in *Plasmodium falciparum* [MIM-AC-271350]

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Introduction: Polyclonal B cell activation and hypergammaglobulinemia are prominent features of human malaria. We have recently identified the cysteine-rich interdomain region 1alpha (CIDR1alpha) of the plasmodium falciparum Erythrocyte Membrane protein 1 (PfEMP1) as a T cell independent polyclonal B cell activator and immunoglobulin binding protein. The mechanisms behind immunoglobulin binding are not fully understood and their elucidation using CIDR1alpha is the major aim of this work.

Methods: CIDR1alpha soluble fusion proteins and CIDR1alpha stable transfected cell lines have been obtained by cloning from a recoded DNA sequence of PfEMP1 from the parasite clone FCR3S1.2. The binding pattern to immunoglobulins is analysed by ELISA and immuno fluorescence. In order to determine binding sites, domain swap immunoglobulins (compromised between human and murine) are used combining Biacore to the previous methods.

Results: CIDR1alpha binds to Fab and Fc fragments of human immunoglobulins and to immunoglobulins purified from sera from different animal species. This binding pattern is similar to that of the polyclonal B cell activator Staphylococcus aureus protein A. IgM binding domains of CIDR1alpha harbors potential alpha-helical structures (amino acids 550-700) and similar contact residues as those present in protein A. Competition ELISA assays demonstrate that the binding affinity of CIDR1alpha to human IgM and IgG is relatively low though enough to lead to B cell receptor signalling.

Interpretation: These results suggest that CIDR1alpha binds to B cells via the Fab fragment of immunoglobulins expressed at the B cell surface. The signature imposed by CIDR1alpha stimuli during B cell activation has to be further investigated.
31A

Variant expression and sequence polymorphism in Plasmodium falciparum adhesive ligands within a population of Senegalese parasites [MIM-CJ-853922]

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Introduction: Invasion of human erythrocytes involves multiple ligand-receptor interactions. The erythrocyte binding antigen (EBA), and the Reticulocyte binding protein homologue (PfRh) families have been implicated in this process. Members of these families show variant expression in different Plasmodium falciparum laboratory isolates that has been linked to the use of alternative invasion pathways. Alternative invasion pathway use by different in vivo isolates may also be attributable to variant expression.

Methods: We have conducted first round invasion assays (n = 30) and show that P. falciparum isolates from Senegal routinely use alternative invasion pathways in the field, defined by the ability of these parasites to invade erythrocytes with receptors limited by enzyme treatment with either neuraminidase, trypsin, or chymotrypsin. We have also analyzed expression and sequence polymorphisms of PfEBA and PfRh proteins to determine whether these are associated with the use of specific invasion pathways.

Results: P. falciparum isolates from Senegal routinely use alternative invasion pathways. We observe that PfEBA and PfRh ligands are variantly expressed between different isolates at both the mRNA and protein levels. The expression of the different parasite ligands does not appear to be coordinately regulated. We have also observed significant size polymorphisms in the PfRh ligands corresponding to specific sequence polymorphisms in regions previously postulated to be of functional significance.

Interpretation: The use of alternative invasion pathways is indeed a parasite-derived strategy within a clonal parasite population. Such complex variant expression could result either as a mechanism for immune evasion or in response to host erythrocyte polymorphisms.

32B

Great genetic diversity of Plasmodium falciparum in Cameroon with Limited variation of the 5′ cis-control region of Pfs25 [MIM-PM-302033]


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Introduction: Genetic recombination during sexual reproduction within Plasmodium sp. contributes to parasite diversity and generates drug resistance through variant off-springs and altered gene expression of certain surface markers. The pfs25 gene involved in the setup of gametocytogenesis is a candidate antigen in transmission blocking vaccine. This study was investigated for polymorphisms within the 5′-cis-control region in field isolates from different ecotypes in Cameroon.

Methods: Symptomatic patients (n = 552) and asymptomatic healthy school children (n = 107) with a positive smear and from savanna (Dschang and Nkambe), forest-savanna mosaic (Yaounde), upland forests (Fontem and Kumba), lowland forest/littoral (Limbe) and different altitudes, 400 m (Mutengene), 700 m (Molyko) and 1000 m (Soppo) above sea level were included. Parasite DNA was extracted by the phenol/chloroform method or by the chelex method. Polymorphisms within pfs25, cg2, msp-1, msp-2, glurp, dhfr/dhps and Pfcrt genes were investigated by the PCR or PCR-RFLP. Putative control elements of the 5′-cis control regions of Pfs25, were identified by PCGENE software and enzymes were selected whose sequences produced or abolished restriction sites by mutations.

Results: Women and men of all age groups were represented in the study. Parasites densities ranged from 40 to 200,000 parasites/μl. Malaria infection was mainly caused by P. falciparum with sporadic occurrence of P. malariae and mixed infection with P. falciparum + P.
ovale (5.26%) or *P. falciparum* + *P. malariae* (2.64%) in Molyko at 700 m above sea level. There were seven alleles of msp-1, 15 of msp-2 and 15 of glurp with no significantly allele in Mutengene, Molyko or Soppo. For cg2-w polymorphism, zonal differences in the band size exist with a 560 bp alleles highly represented in Limbe and Nkambe but with different msp-1 allelic profiles. Analysis of the Pf25 5′ cis-control region, identified only one polymorphism (0.002%) that abolished an RsaI restriction site as part of the initiation sequence TTTCTGTAC, located 40 bp downstream of the ATG. 

**Interpretation:** Analysis of the 5′ cis-control sequence of Pf25 revealed minimal variation of the promoter region amid great zonal differences in parasite population. Altitudinal differences in parasite populations were not easily discernable.

### 33C
**Impact of genetic complexity on longevity and gametocytogenesis of *Plasmodium falciparum* during the dry and transmission-free season of eastern Sudan** [MIM-EN-8928]

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**Introduction:** Malaria in eastern Sudan is characterized by limited seasonal transmission, with the majority of the year remaining transmission-free. Some inhabitants who contract malaria during the transmission season retain long-lasting sub-patent infections throughout the dry season which are the probable source of gametocytes that persist in the dry season to initiate transmission the following year.

**Methods:** We have monitored *Plasmodium falciparum* infection prevalence and gametocyte production during the dry season, and examined the impact of parasite genetic multiplicity on infection longevity. A cohort of 38 individuals who were infected with *P. falciparum* in November 2001 was monitored monthly by microscopy and PCR until December 2002. Reverse transcriptase polymerase chain reaction of the pf377 gene was used to detect sub-patent gametocytes. In addition, all isolates were examined for msp-2 alleles and pf377 gene, and the mean number of parasite clones per infection was estimated accordingly.

**Results:** We found that the relationship between parasite multiplicity and survival of asexual infection was highly significant (31.37, *P* < 0.01). Overall, the parasite clearance rate in the single clonal infection group was nine times faster than in the multiple-clone group. Whereas 50% of single-clone infection had cleared within 5 months, 65% of multiple infections remained until the end of the monitoring period (13 months). Patients with mixed gametocyte genotypes sustained their gametocytes for a significantly longer period of time than those with a single genotype (7.66, *P* < 0.01).

*P. falciparum* gametocyte infections that were genetically homogeneous were cleared three times faster than those consisting of multiple clones. Large proportion (40%) of the cohort retained gametocytes throughout the dry season. The majority of patients retained *P. falciparum* asexual infection for 9 months and asexual infection for at least 7 months.

**Interpretation:** Genetic multiplicity of *P. falciparum* significantly influenced longevity of asexual infection and its gametocyte production. Gametocytes from mixed genotype *P. falciparum* infections persisted three times longer than those from single genotype, suggesting that genetic diversity promotes persistence.

### 34A
**Investigating molecular mechanisms of erythrocyte invasion by *Plasmodium falciparum* in Kenya** [MIM-SN-45262]

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**Introduction:** Erythrocyte invasion by *Plasmodium falciparum* involves the use of a number of specific receptor-ligand interactions between the erythrocyte surface and the blood-stage merozoite. On the parasite side, proteins that bind to erythrocytes include erythrocyte binding antigen-175 (EBA-175), EBA-140 and EBA-181, that contain a Duffy binding-like (DBL)
Another family of ligands comprises members of the reticulocyte binding-like homologs (PfRh) family: PfRh1, PfRh2a, PfRh2b and PfRh4.

Methods: We analysed 33 Kenyan isolates collected from 13 severe and 20 uncomplicated malaria cases that were cultured until the mature schizont stage. RNA, DNA and protein were extracted from RBC pellets enriched with schizonts at 1% parasitemia. We developed real-time RT-PCR assays to quantitate the relative expression of the different Rh transcripts (1, 2a, 2b, 3 and 4) among isolates, using TaqMan technology. Furthermore, we have raised murine sera against different Rhs that allow us to look at protein levels using immunofluorescence assays (and Western Blots when possible). We are also analyzing parasite genomic DNA for sequence polymorphisms in the EBA and Rh ligands molecules, to test for associations with severity of disease.

Results: We were able to extract RNA from 100 μl PCV at 1% parasitemia that was subsequently used to determine the relative amount of expression of different members of the Rh family of genes. Due to polymorphisms and/or homology between Rhs most of the primers do not span exon-exon boundaries making it necessary to DNase treat the RNA samples. We found this to be a critical step since it may influence RNA quality. We found high variability in the amount of RNA extracted from similar volumes of RBC at 1% parasitemia. For relative quantification we used 10–50 ng of total RNA/PCR reaction using seryl-t-RNA synthase as endogenous control. Dilutions of Dd2 gDNA were used to generate standard curves. We observed a variation of profile of Rh expression across samples and are investigating a possible association with the severity of disease. We are determining the minimum amount of RBCs needed to detect Rh proteins by Western Blot with the murine sera generated against Rh2a, Rh2b and Rh4. These Abs will also be used in IFAs on slides from the field cultures. We have tested these sera in IFAs with 3D7 cultures to confirm merozoite localization. gDNA will be used to identify allelic variations: SNPs in ebas; and SSRs and indels in Rhs.

Interpretation: This study characterises the molecular basis of invasion in mild and severe field isolates. We focused on the Rh family of ligands at RNA and protein level whereas at DNA level we also looked at allelic variations in the EBA family.

Relative fitness of wild type and mutant pfcrt parasites: An inference from direct in vitro growth competition experiments [MIM-SN-32913]

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Introduction: Recent work from Malawi, where chloroquine (CQ) was replaced with SP as a first line antimalarial in 1993, has shown a dramatic decline in the prevalence of CQ-resistant parasites in the field. This suggests that CQ resistance-conferring mutations in the pfcrt gene come at a cost to parasite fitness. We hypothesized that the fitness cost of harbouring mutant pfcrt might be manifested by reduced asexual stage replication rate of mutant pfcrt parasites relative to the wild type parasites.

Methods: We performed in vitro growth competition experiments between parasites carrying wild type and mutant pfcrt genotypes and assessed survival of each genotype by detecting its presence in competition cultures with a polymerase chain reaction-based procedure.

Results: We found that, in the absence of CQ pressure, asexual erythrocytic parasites carrying the mutant pfcrt allele multiply more efficiently than those with wild type pfcrt. These findings suggest that CQ-resistant parasites have a survival advantage over sensitive parasites during the asexual erythrocytic stage.

Interpretation: The loss of pfcrt mutants in Malawi must implicate a pfcrt dependent loss of fitness at some other point in the parasite’s life cycle.

Investigating the role of PfRab5 in endocytosis in Plasmodium falciparum [MIM-LO-174325]

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Introduction: Plasmodium falciparum endocytically takes up erythrocyte haemoglobin and transports it to its food vacuole via vesicles. Endocytosis is regulated by GTP-binding Rab proteins that are localised to specific
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membrane bound compartments. Rab5 is well characterised in the endocytic pathway of eukaryotic cells. It is a key component of the protein complex responsible for trafficking vesicles from the plasma membrane to the early endosome and the homotypic fusion of early endosomes.

Methods: Rab5 homologues have been identified in *P. falciparum*. They are PfRab5a, 5b and 5c these homologues are being investigated during the parasite’s erythrocytic cycle. We have cloned PfRab5a, 5b and 5c cDNA, which are 708, 624 and 645 bp in size, respectively. PfRab5 genes, mRNA and proteins have been studied using Southern, Northern and Western Blot analysis, respectively. Their localisation in the cell is being investigated using antibodies raised against recombinant PfRab5 proteins and by introducing Green Fluorescent Protein (GFP)-tagged PfRab5 proteins into 3D7 strain parasites using a plasmid vector designed for *P. falciparum* transfections.

Results: PfRab5a, 5b and 5c are present as single copy genes and show a differential expression pattern over 48 h. However, for each PfRab5 the bulk of expression was during the trophozoite stage of parasite development. The PfRab5 proteins are present in the parasite. Initial confocal microscopy imaging of infected erythrocytes stained with an antibody raised against PfRab5a showed cytosolic localisation in trophozoite stage parasites.

Interpretation: Rabs control distinct steps of vesicular transport and due to their specificity they are used as subcellular markers. The characterisation of PfRab5 will be a valuable tool in identifying subcellular compartments in the parasite cell.

37A
A new tool for the development of vaccines and drugs targeting severe *Plasmodium falciparum* malaria [MIM-PP-184212]


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Introduction: Although the pathogenesis of *Plasmodium falciparum* malaria is still not completely resolved, it is agreed that the sequestration of malaria infected erythrocytes (iRBC) is a contributing factor. We have developed a new animal model for the immediate sequestration of iRBC in immunocompetent rats. The model have been successfully used to monitor how the sequestration can be inhibited by immunization against an adhesive ligand on the iRBC surface and by injection of heparin devoid of anticoagulant activity.

Methods: Human iRBC, cultured in vitro and purified in a single step over a magnet, were labelled with 99mTc and injected into the tail vein of Sprague–Dowley rats. The distribution of the iRBC was monitored dynamically using whole body imaging or separate imaging of the lung subsequent to organ extraction. The anti-sequestration capacity of inactivated heparin was tested either by co-injection with the iRBC or by injection into the tail vein 3 min after the injection of iRBC. In separate experiment rats were immunized with recombinant Semliki forest virus particles expressing the DBL1a domain of the adhesive ligand PIEMP1 and challenged with different strains and clones of iRBC.

Results: iRBC of different strains and clones sequestered avidly in the rat in vivo while uninfected human erythrocytes did not. The highly rosetting and multi-adhesive clone FCR3S1.2 sequestered in high numbers while its non-adhesive sister-clone FCR3S1.6 did not. The sequestration of FCR3S1.2 iRBC was shown to be significantly reduced by removal of the adhesive ligand PIEMP1 prior to injection or by co-injection with soluble receptors. A CD36 binding 3D7 clone (3D7AH1S2), selected by micromanipulation of iRBC binding to CD36 expressing cells, sequestered in very high numbers in vivo while its non-CD36 binding mother-clone showed poor sequestration in the rat. Co-injection of periodate inactivated heparin at 10 mg/ml reduced the sequestration of FCR3S1.2 iRBC by 80% while post injection 3 min after the iRBC resulted in a more modest reduction of 45%. Inactivated heparin was also used in conjunction with different wild malaria isolates demonstrating a reduction in sequestration ranging from 10 to 40%. Immunization reduced the level of sequestration by 50–75% as compared to GST-immunized animals when homologously challenged with FCR3S1.2 iRBC.
Interpretation: Sequestration in the rat can be used to monitor the effect of drugs and vaccines targeting adhesive events. Hence, this syngeneic animal model provides a crucial tool for the development of vaccines and adjunct therapies for severe malaria.

38B Binding sites on Inter cellular adhesion molecule-1 (ICAM-1) for Plasmodium falciparum clinical isolates [MIM-HP-127554]
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Introduction: Of the endothelial adhesion molecules that are receptors for Plasmodium falciparum infected erythrocytes (IEs), ICAM-1 is of interest because its expression is up-regulated in severe malaria. ICAM-1 is an 80–110 kDa variably glycosylated membrane glycoprotein consisting of five immunoglobulin (Ig)-like domains that is expressed on the surface of a wide range of cell types. Previous studies have characterised the binding site for IEs, which overlaps, but is distinct from, the binding sites for LFA-1 and human rhinovirus, and has a high level of overlap with the binding site for fibrinogen. A natural genetic polymorphism of ICAM-1 at position 29 of the BC loop termed ICAM-1Kilifi, produced by the replacement of a lysine with a methionine residue, is found at high frequency in Africa where it affects disease severity. A recent study using a panel of ICAM-1 mutant proteins, consisting of single amino acid substitutions covering much of the P. falciparum binding region of ICAM-1, and three ICAM-1-binding laboratory parasite lines with different binding avidities under flow and static conditions revealed subtle differences in the contact residues used by the laboratory isolates (Tse et al., 2004). What we don’t know, however, is how clinical isolates bind these ICAM-1 mutant proteins. The aim of this study, therefore, was to test two ICAM-1 mutant proteins for their ability to bind paediatric clinical isolates under static conditions and compare the results with previous data collected under the same conditions using laboratory isolates. We used the S22/A mutant, ICAM-1Kilifi (K29/M) and reference ICAM-1 (ICAM-1ref) as a control.

Methods: Briefly, static protein-binding assays were performed as described previously (Berendt et al., 1992) using two ICAM-1-Fc spotting concentrations of 25 and 50 mg/ml, which had previously been shown to be within the dynamic range for detecting differences in adhesion. Proteins were spotted in triplicate and in duplicate dishes for each experiment. The IE receptor CD36 was also included as a control and to investigate any relationship between ICAM-1 and CD36 adhesion

Results: Data analysis is in the process to come up with meaningful interpretation and conclusions.

Interpretation: Data analysis is in the process to come up with meaningful interpretation and conclusions.

39C The impact of Plasmodium berghei berghei on male reproductive indices of albino mice—Preliminary report [MIM-YR-77740]
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Introduction: Malaria is a devastating parasitic disease, which affects many people worldwide. It remains one of the greatest causes of illness and death in the world and particularly in Africa. Surprisingly, malarial chemotherapy has been associated with adverse effects on reproductive functions. However, the impact of malaria parasite on reproductive functions has not been fully elucidated, hence the present study.

Methods: Forty male Swiss albino mice (mean weight, 18.85 g), divided into eight equal groups were infected with Plasmodium berghei berghei through inoculation with 107 parasitized red blood cells for 1, 2, 3, 4, 5, 6 or 7 days, respectively. Group Do served as the control and was not infected with the Plasmodium. Animals were sacrificed from day 1 up to day 7 after infection. Cau-
dal epididymal sperm motility, counts and morphology were determined. The body weight and wet weight of spleen, liver, testis, epididymis and kidneys were determined. Haematological indices were also determined on blood samples from all the mice.

**Results:** The results showed a progressive duration-dependent decrease in sperm motility, sperm count and the percentage live/dead spermatozoa ratio ($P < 0.01$) in parasitized mice. Significant decrease in serum testosterone and increase in cortisol levels were recorded ($P < 0.05$) in parasitized mice when compared with the controls. There was also a progressive decrease ($P < 0.05$) in the red blood cell count and packed cell volume. However, there was a progressive increase ($P < 0.01$) in the white blood cell count and weight of the spleen and liver. There was no significant change in weight of the testis and epididymis.

**Interpretation:** The results suggest that *P. berghei* could depress fertility indices in mice.

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**41B**

Transfection of *Plasmodium knowlesi* in the baboon provides a new system for analysis of parasite expressed transgenes and host–parasite interface [MIM-HS-534603]

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**Introduction:** *Plasmodium knowlesi* infection in baboons and rhesus monkeys was comparable and some clinical symptoms of the baboons and *P. falciparum*-infected humans were similar. Pyrimethamine was successfully used to select for transfected parasites. In addition, in vivo characteristics of transfected parasites in *P. anubis* and Macaca mulatta were similar. The above information was used to successfully transfect *P. knowlesi* in *P. anubis* to express host interferon gamma, an important cytokine during malaria infection. Host–parasite interaction of transfected parasites was characterised.

**Interpretation:** These studies provide *P. anubis/P. knowlesi* as a suitable system for transfection and analysis of host–parasite interaction.

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**42C**

Allele frequency based tests of neutrality on eba-175 and ebi-1 in *Plasmodium falciparum* populations from Kenya and Thailand [MIM-FV-66780]

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**Introduction:** Erythrocyte binding proteins encoded by the eba gene family in *Plasmodium falciparum* play a role in erythrocyte invasion, and may be targets of acquired immunity. The existence of divergent paralogous eba and preliminary evidence of different selective pressure on these genes provide a compelling opportunity for a comparative analysis. Molecular population genetic methods provide a powerful tool for
detecting signatures of selection and demographic processes acting on natural populations.

Methods: We sequenced Region II, consisting of two tandemly duplicated Duffy Binding Like (DBL) domains, F1 and F2, of eba-175 and ebl-1 from Kenyan and Thai isolates. Allele frequency distributions of nucleotide polymorphisms for each gene within the Kenyan and Thai populations were examined using Tajima’s and Fu and Li’s indices. Departures from neutral expectations result in a positive value for each intra-population statistic index: Tajima test calculates $D$, which equals the difference between the observed average pairwise diversity and the expected nucleotide diversity under neutrality derived from the number of segregating sites, $S$. Fu and Li’s test reveals an excess or lack of singletons.

Results: Sequence polymorphisms of *Plasmodium falciparum* region II for eba-175 and ebl-1 were examined in Kenyan and Thai isolates. The average nucleotide diversity index was 0.0003 for ebl-1 in the Kenyan population and 0.0004 in the Thai population and 0.003 for eba-175 in both populations. The nucleotide frequency distribution was tested for statistical departure from neutral expectations. Tajima’s $D$ value was significantly different from zero for ebl-1 with $D = -2.35$ ($P < 0.01$) in the Kenyan population and $D = -1.85$ ($P < 0.05$) in the Thai population. A consistent statistically significant negative value was detected for ebl-1 in both populations when using the other indices. A Tajima’s $D$ positive value was observed for eba-175 with $D = 1.11$ in the Kenyan population and $D = 1.59$ in the Thai population although none of these values reached statistical significance. A consistently positive trend was observed for the other indices as well.

Haplotype frequencies for F2 domain of eba-175 were compared in the two populations in order to design recombinant F2EBA-175 allelic variants based on predictions from the pattern of sequence polymorphisms which will be used to investigate allele specific reactivity in two Kenyan cohorts of children.

Interpretation: Different signatures of selection were detected at the two loci, eba-175 and ebl-1, examined in both Kenyan and Thai populations, suggesting they are undergoing different selective pressures. Immuno-epidemiological studies on eba-175 will follow.

3. Immuno-epidemiology

**Posters 43–75**

Presenting authors of A-posters should be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

43A

No abstract received [MIM-PM-18438]

44B

Hyperreactive malarial splenomegaly (HMS) in malaria endemic area in eastern Sudan [MIM-MA-173190]

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Introduction: Hyperreactive malaria splenomegaly is a condition affecting adults in certain malarious areas. The present study was carried out in Kassala state, eastern Sudan where HMS is considered to be in highly prevalent. The objective of the study was to describe the epidemiology and magnitude of disease in the area. The study was designed to help local clinicians to differentiate HMS cases from other diseases causing splenomegaly and to apply modern investigative methods particularly Species-specific PCR for detection of malaria parasites DNA in HMS cases.

Methods: A cross sectional study was carried out in four health centers in Kassala state between January and March 2004. Eighty-seven of total 1010 medical cases were identified HMS cases using the major diagnosis criteria. IgM concentration was measured in all subjects using nephelometric method and all samples were screened for malaria parasites using both blood films and polymerase chain reaction (PCR).

Results: Fifty-five (63%) were male and 32 (37%) female with mean age 28 years. All cases suffer from abdominal pain in upper left quadrant and all with palpable spleens (10-26 cm) from the costal margin. Serum concentration of IgM in all subjects was above the threshold of the mean value plus 2SD for 30 asymptomatic normal individuals from Kassala. Seventy-two patients (83%) were anaemic. Microscopical examination of blood film only revealed *Plasmodium falci*
parum gametocytes in one case (1.14%) but when use nested PCR for identification of the four Plasmodium species, PCR detected *P. falciparum* DNA in other 12 cases (15%).

**Interpretation:** This study indicates that the prevalence of HMS in Kassala state was 8.61% of all cases reported in four Kassala health centers. Along with the clinical manifestations and estimated of IgM was found to be helpful in the diagnose of HMS. Further more the results shown there a considerable percentage of HMS were harboring low asymptomatic malaria parasite.

**45C**

**Role of antibody affinity in conferring protective immunity to *P. falciparum* malaria [MIM-ra-195168]**

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**Introduction:** Most studies on humoral immunity of exposure to malaria have concentrated on the quantity of antibody produced to particular antigens. Relatively few have measured the quality of this response in relation to exposure and age. In some cases the quality of the antibody response (subclass/specificity) can be significant factors conferring protection. Here we examine the evolution of the affinity of responses to various antigens with age and exposure.

**Methods:** Antibody avidity (as a measure of affinity) was measured by an ELISA comparing antibody binding in the presence and absence of Ab-Ag dissociating agents. Assays specific for malaria antigens (MSP-119, MSP-2 and AMA-1) were first optimized using a set of well characterized sera from The Gambia. Subsequent analyses have concentrated on samples collected in 24 villages, arranged in six altitude transects in the highlands of north-eastern Tanzania. Avidity data will be correlated with antibody prevalence and isotype as well as standard malariometric and demographic variables.

**Results:** Assay optimisation allowed the definition of an avidity index, expressed as the proportional reduction in antibody bound following treatment with guanidine. In preliminary data measuring the evolution of avidity in Gambian sera, where malaria transmission intensity is moderate, no correlation between age and avidity index was observed (*n* = 233). This analysis is now being extended to samples from a large cross-sectional survey in north-eastern Tanzania. Here, large altitude differences allow similar populations exposed to widely different malaria transmission to be studied to determine how age and exposure influence the development of a mature antibody response. Previous work has established that both age and exposure are independently significant for the development of subclass preferences, while preliminary data suggests that exposure predominates in generating the breadth of antibody repertoire. Few studies have attempted to measure the quality of this response and to track the way in which this evolves with exposure and age, factors which are anyway difficult to disentangle in endemic settings. These data will be presented.

**Interpretation:** We have previously demonstrated that both subclass and specificity are important for protection but antibody affinity remains low in endemic settings. These data will be presented.

**46A**

An epidemic and immunity in a population living in an island where malaria was eliminated for 10 years [MIM-MA-104720]


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**Introduction:** In malaria control, resurgence after intervention is a major concern. It is generally thought the herd immunity would soon fade if parasites are eliminated from the area. Vanuatu consists of 80 islands with unstable malaria. A previous study on Anetium island demonstrated that mass chemotherapy and vector control was able to eliminate malaria in 1991. Now a few cases of malaria have been reported.
**Methods:** To investigate an apparent epidemic and whether immunity is maintained/lost in different population groups living in an area where malaria was temporarily eliminated for 10 years, we have continued to follow up malaria situation and immunity of the local population living on the three islands of Vanuatu: Maewo, Malakula (no intervention), Aneityum (intervention) and Futuna (no malaria). Surveillance is necessitated for a new outbreak on an emergency basis.

**Results:** Before the reported outbreak on Aneityum island, where transmission was interrupted, seroepidemiological studies suggested that without antigenic stimulation immunologic memory will still remain for many years especially in adults previously repeatedly exposed to malaria. The local health team conducted a mass blood survey in March 2002 and found about 20 P. vivax cases, which were confined to children under 10 years old. Some of these positive cases showed febrile attack during the survey.

**Interpretation:** The results suggest that immunologic memory lasts for a long time without re-exposure in individuals who have been exposed to malaria for many years in isolated areas.

**47B**

The epidemiology and consequences of malaria infection in primary school children in the Muea area and its environs [MIM-NA-363565]

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**Introduction:** In endemic areas, immunity to malaria is rarely sterile. Children above 5 years of age are often found asymptomatically infected. Malaria is a common cause of school absenteeism (WHO, 1996). Children lose time from school and suffer throughout life from effects on cognitive development and education levels attained. The prevalence of asymptomatic malaria and its consequences in school children in Muea and its environs was assessed by carrying out a cross-sectional study from April to June 2002.

**Methods:** A total of 246 school children from four primary schools aged 3–16 years old and of both sexes were enrolled into the study after parental consent. Finger prick blood samples were collected from them for the assessment of the presence and species of Plasmodium parasites and haematocrit (packed cell volume) values. After blood collection, 144 children were followed up for a period of one month to find out the consequences of a malaria infection in them in terms of school days lost.

**Results:** Out of a total of 246 blood samples examined, 241 of them were positive for the malaria parasites giving a prevalence rate of 97.96%. The highest prevalence rate of the disease (100%) and geometric mean parasite density (1520 parasites/μl of blood) occurred in the 5 years age group and this decreased slightly with an increase in age. The variation in prevalence rates and the geometric mean parasite densities in the different age groups were not significantly different ($\chi^2 = 0.45$, $P = 0.798$ and $F = 2.63$, $P = 0.074$, respectively). The infection rate was higher in the females (99.19%) than in the males (96.72%), but the difference was not significant ($\chi^2 = 1.88$, $P = 0.169$). Plasmodium falciparum was the predominant Plasmodium species (93.08%), followed by $P$. malariae (52.03%), $P$. ovale (42.68%) and $P$. vivax-like parasites (33.33%), and occurred in mixed infections of two, three or four species combinations. Generally, mean PCV values increased with an increase in age and decreased with the level of parasitaemia. The prevalence of anaemia (PCV 20–29%) was generally low (10.8%) and there were no cases of severe anaemia (PCV < 20%). From follow up data, each sick child was found to lose an average of 1.53 school days per month.

**Interpretation:** Malaria was found to be a major cause of school absenteeism and poor performance following anecdotal reports from teachers.

**48C**

Antibodies to Plasmodium falciparum antigens vary by age and antigen in children in a malaria holoendemic area of Kenya [MIM-KC-31452]


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Introduction: Antibodies are important in protection against infection and disease due to Plasmodium falciparum, but the frequencies of antibodies to multiple P. falciparum antigens in children are not well characterized.

Methods: Immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to the vaccine candidate antigens circumsporozoite protein (CSP), thrombospondin-related adhesive protein (TRAP), liver-stage antigen-1 (LSA-1), apical membrane antigen-1 (AMA-1), erythrocyte binding antigen-175 (EBA-175) and merozoite surface protein-1 (MSP-1) were measured by ELISA in 110 children 0–50 months of age in a malaria holoendemic area of Kenya.

Results: A similar pattern was seen for IgG antibodies to CSP, TRAP, AMA-1 and EBA-175: high frequencies (70–90%) in children 0–4 months of age, a decrease in children 5–20 months of age (35–71%), and progressive increases in children 21–36 and 37–50 months of age (53–80% and 60–100%, respectively). In contrast, IgG antibodies to LSA-1 were infrequent in children 0–4 months of age (5%) and increased with age to 64%, and IgG antibody frequencies to MSP-1 were similar across age groups (26–52%). IgG antibodies to all antigens were predominantly of the IgG1 and IgG3 subclasses. Frequencies of IgM antibodies to all antigens were low in children 0–4 months of age (0–15%) and increased with age (24–56% in the oldest children).

Interpretation: In children in a malaria holoendemic area, IgM antibody to all P. falciparum antigens increases with age and increased exposure. The pattern of age-related IgG frequencies to P. falciparum antigens varies significantly according to antigen.

Estimating malaria transmission using serological markers of malaria exposure [MIM-PC-17952]

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Introduction: Besides playing an important role in protection, anti-malarial antibodies constitute a record of infection history. The age-specific prevalences of malaria-specific antibodies within a community therefore reflect cumulative exposure to malaria over increasing periods of time, stretching further into the past with each successive age group. It should therefore be possible to reconstruct information about transmission intensity from cross-sectional serological surveys.

Methods: We have compared the prevalence of IgG antibodies to three recombinant Plasmodium falciparum asexual stage antigens (AMA-1, MSP-2 and MSP-119), using standard ELISA methods, with parasitological measures of transmission intensity. The populations studied included individuals of all ages living at varying altitudes and encompassing a range of transmission intensities from hyper- to hypo-endemic in North-Eastern Tanzanian. Large variations in transmission over short distances have allowed us to study the effects of transmission and age within populations of uniform ethnic background living under similar conditions.

Results: The prevalence of antibodies to MSP-119 was significantly more closely correlated with altitude than either point prevalence malaria parasitaemia or single measures of haemoglobin concentration. The analysis of age-specific seroprevalence rates enabled us to differentiate recent (seasonal) changes in transmission intensity from longer term transmission trends and, using a mathematical model of the annual rate of seroconversion, we were also able to estimate the
longevity of the antibody response. These figures could be matched to an existing model of the dependence of EIR on altitude, showing how a serological survey might supplement or replace conventional EIR measurements, especially in locations with low transmission where EIR determination is problematical. We have also investigated how such serological data might be more easily collected.

**Interpretation:** Serological tools allow us to detect temporal variations in malaria transmission and will be valuable for monitoring changes in endemicity and the effectiveness of control programmes, particularly in areas of unstable and sporadic malaria.

**50B**

**IL4 polymorphisms and IgE levels on malaria-endemic islands in Vanuatu** [MIM-MD-272385]


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**Introduction:** Recent findings suggest that the susceptibility to malaria is associated with genetic variants in the IL4 promoter region, resulting in the upregulation of serum IgE. In this study, we investigated the mutant allele frequencies at positions $-590$ and $+33$ of the IL4 gene and total and *Plasmodium falciparum* -specific IgE levels on three islands with variable malaria endemicities in Vanuatu: Malakula (meso-endemic), Aneityum (meso-endemic with intervention) and Futuna (non-endemic).

**Methods:** As part of malariometric surveys conducted on these islands between 1997 and 1998, we collected blood on chromatographic filter paper, extracted DNA from a randomly selected sub-sample and performed PCR on these samples. Thereafter, we used the method of pyro-sequencing to type polymorphisms at positions $-590$ and $+33$ of the IL4 gene. Levels of total and *P. falciparum*-specific IgE were determined in the study subjects from Malakula and Aneityum using ELISA.

**Results:** A total of 878 and 750 samples were typed for $-590$ and $+33$ positions. In Malakula, Aneityum and Futuna, T allele frequencies were 0.27, 0.39 and 0.28 for IL4 $-590$ and 0.39, 0.48 and 0.39 for IL4 $+33$, respectively. Genotype distributions of both positions were in agreement with the Hardy-Weinberg equilibrium ($p < 0.05$) and there was a strong linkage disequilibrium between the two alleles ($p < 0.001$). For both mutant alleles, higher frequencies were detected in Aneityum than in Futuna ($p < 0.05$). In Aneityum, there was a significant association between the carriage of C $+33$T allele and increased levels of *P. falciparum*-specific IgE ($p < 0.05$). However, these relations were not observed in Malakula. The observed mutant allele frequencies lay between higher values in Asian populations and rather lower values in Caucasians.

**Interpretation:** Our findings suggest that IL4 promoter polymorphism may be one of the genetic factors that explain relations between malaria disease and IgE.

**51C**

**Effect of intervention with insecticide impregnated thobs on the humoral immunity to *Plasmodium falciparum* in El Rahad area, Western Sudan** [MIM-SE-4460]

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**Introduction:** Malaria infection gives rise to host responses regulated by innate and adaptive immune system. In residents of endemic areas, the infection induces immune responses, involving IgM, IgG and other immunoglobulin isotypes. Immune response is rarely sterile and related to low parasitemia, episodes of the clinical disease and the complex life cycle of the parasites. The impact of intervention with impregnated Sudanese thobs on the development of protective (semi) immunity is controversial so this study investigated the effect on the humoral immunity to *Plasmodium falciparum* in El Rahad area.

**Methods:** The study population was selected from two villages in the Rahad area of Western Sudan. Elrigaila village (study village) was given impregnated Thobs
where a total of 391 individuals were randomly selected as study group and another 110 controls from Elrahad village (control village). Individuals were grouped into four age groups (0–4, 5–9, 10–14 and ≥15 years). Five parasitological surveys and samples collection for humeral immunity were conducted throughout the study period. One survey, as a baseline survey before intervention, and four follow-up surveys, every six months for two years, after impregnated Thobs were distributed and in use at the study village. At the same time these surveys were applied in the control village.

Results: The impregnated thobs was found to be highly acceptable by the community as observed by the continuous use of the thobs. The effect of the use of impregnated Thobs was found to significantly reduce the prevalence of malaria in the study group from 36% at baseline survey to 6% at the end of study. The reduction in malaria prevalence in the study group was also significant (P = 0.000) as compared to the control group especially at the transmission season. There was a highly significant difference (P = 0.000) in the IgG levels between cases and control during the last four follow ups except the first one (0 month). IgG levels to P. falciparum MSP-1 antigen was higher in Impregnated Thobs users than non users (P = 0.000). IgG levels to P. falciparum NANP was lower in impregnated Thobs users than non users (P = 0.000), as for this antibody to be produced, it needs continuous and frequent exposure to the parasite. There was no significant difference between parasitologically positive and negative individuals and the IgG levels to both antigens. Age: There was a significant difference between age groups in the IgG levels to NANP but it was not significant to MSP-1.

Interpretation: Low exposure to malaria infection increases the level of IgG to MSP-1, but IgG levels to NANP needs frequent exposure to be produced. The promotion of the impregnated Thobs must be viewed with concern in high endemic areas with stable malaria transmission and on large-scale population trials.

52A
Immunogenicity of a C-terminal fragment of the Plasmodium falciparum antigen Pf332 [MIM-BH-1382300]

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Introduction: The Pf332 antigen is a 750 kDa protein present on the surface of parasitized red blood cells (pRBC) during the schizont stage. Its expression appears to be crucial for parasite survival and has been shown to harbor target epitopes for parasite growth inhibitory antibodies. We have cloned a C-terminal fragment, termed C231, which contains 231 amino acids and a putative T-cell epitope. C231 expressed as a recombinant protein was used to investigate the immunogenicity of this region of Pf332.

Methods: The DNA used for cloning originated from the 3D7 laboratory strain of Plasmodium falciparum. Primers used for amplification were designed from Pf332 sequence (TIGR database). The C231 fragment was expressed in Escherichia coli BL21(DE3) using a plasmid vector PAff10c, and the His-tagged recombinant protein was purified by affinity chromatography on a Ni-NTA column. For analytical purposes, antibodies against C231 were produced in rabbits. Antibody binding to C231 was analysed by ELISA and immunoblotting and their parasite reactivity was studied by immunofluorescence and in vitro parasite growth inhibition assays. The occurrence of antibodies against C231 during natural P. falciparum infections was detected by ELISA using sera from Liberian and Senegalese donors.

Results: As revealed by SDS-PAGE, C231 was expressed as 43 kDa polypeptide (predicted molecular weight of 29.4 kDa). The recombinant protein was highly immunogenic in rabbits, giving antisera of high titers (average 3.7 × 106) by ELISA and strong reactivity in immunoblotting. The rabbits’ antibodies also showed good reactivity with the native protein, as revealed by their reactivity with pRBC in immunofluorescence (average end titre of 63 × 103), giving a dot-like staining previously seen with other Pf332 reactive antibodies. Furthermore, the IgG of the C231 antisera was efficient in inhibiting P. falciparum growth in
vitro (50% inhibition, at 180 μg/ml). The recombinant C231 antigen was well recognized by African sera in immunoblotting and showed high reactivity with sera from Liberian and Senegalese donors in ELISA (average logtitre of 2.59 and 3.51, respectively). While the antibody reactivity of the Senegalese sera to crude *P. falciparum* antigen was predominantly of the cytophilic IgG1 and IgG3 subclasses, the C231 reactive antibodies were to a substantial proportion, also of IgG2 subclass. While the levels of C231 reactive antibodies increased by age, the levels of antibodies reactive with crude antigen were similar in all age groups.

**Interpretation:** Our study shows that recombinant C231 protein is immunogenic and there is induction of C231-reactive antibodies during natural *P. falciparum* infection. This study confirms and extends our data on Pf332 as a target for parasite neutralizing immune responses.

**53B**

**Association between protection against clinical malaria attacks and antibodies to vaccine candidates antigens in endemic area of Burkina Faso [MIM-NI-26328]**


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**Introduction:** Antibodies have been shown to play an important role in the development of premunition and many studies have suggested that human antibodies of cytophilic subclasses are particularly critical in this respect. Studies conducted in different geographical areas have shown that high GLURP and MSP3 cytophilic antibodies are predictors of protection against malaria attacks. To confirm these findings, a study was conducted in children under 15 years, in an area of seasonal malaria transmission.

**Methods:** The target population for this study was children aged 0.5–15 years living in the village of Balonguen. Prior to malaria transmission season (June), 5 ml venous blood were taken from each target child and plasma used for antibody measurements.

An active case detection of clinical malaria case was performed from early July to the end of October 2003. The levels of antibodies (IgG, IgM and IgG subclasses) to the selected antigens (MSP3, GLURP and MSP19) were determined by an indirect ELISA in children with haemoglobin AA type.

**Results:** Children were considered to have a clinical malaria episode only if they had a measured temperature $\geq 37.5$°C and asexual parasitemia of $\geq 5000$ parasites/μl. Four hundred and twenty-two were haemoglobin AA but 297 were eligible for this study, 175 (58.9%) had no episode of clinical attack and were considered “protected” and 122 (41.1%) had at least one malaria attack and were considered “susceptible”. Except for IgG4 to MSP119 and GLURP, IgG, IgM and IgG subclasses increased with age. In general level of antibodies to the three antigens were inversely proportional to the number of malaria attacks. IgG and IgM levels to MSP3 and GLURP were significantly higher in protected than in susceptible children, but similar in the two groups for MSP3.19. Levels of IgG subclasses to MSP3 and to GLURP were higher in protected than in susceptible children, leading to a significantly higher ratio of cytophilic to non-cytophilic subclasses ([IgG1 + IgG3]/[IgG2 + IgG4]) in the protected individuals compared with the susceptible (MSP3: 2.5 versus 1.8, $P < 0.001$; GLURP: 2.1 versus 1.8, $P = 0.002$). IgG2 and IgG3 to MSP119 levels were significantly high in protected group but the ratio of cytophilic to noncytophilic subclasses was similar in both groups (2.4 versus 2.4, $P = 0.96$).

**Interpretation:** Our data confirmed that cytophilic antibodies to GLURP and MSP3 are strong surrogates of protection against clinical malaria in area where malaria is hyperendemic.

**54C**

**Co-infection parasitaire par *P. falciparum* et *S. haematobium* en zone rurale au Sénégal [MIM-AJ-334647]**

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**Introduction:** Des études antérieures ont suggéré que les sujets porteurs d’helminthes intestinaux ou urinaires étaient plus fréquemment atteints d’infections
ou d’accès palustres. Notre étude avait pour objectif de vérifier cette hypothèse chez les enfants de moins de 15 ans en cas de co-infection par *P. falciparum* et *S. haematobium* dans le centre du Sénégal, zone rurale où le paludisme est de transmission faible et de type saisonnier instable, la bilharziose urinaire est endémique.


**Results:** L’oviurie générale de *S. haematobium* a été de 50%. La prévalence d’autres helminthes intestinaux tel que *A. lumbricoides* a été inférieure à 10%. La prévalence d’infection par *P. falciparum* a varié entre 47 et 76%. 85% (154/184) des GE de sujets reçus à la consultation pour suspicion d’accès palustre étaient positives à *P. falciparum*. L’analyse croisée des résultats suggère l’existence d’une association entre infection par *S. haematobium* et infection par *P. falciparum*. Cette relation semble charge-dépendante car plus importante chez les sujets présentant de fortes ovi-uries. A l’inverse, les porteurs de charges moyennes semblaient présenter moins d’accès palustres que les sujets non infectés.

**Interpretation:** Nos données suggèrent une association entre infection palustre et infection par *S. haematobium*. Ces données nécessitent d’être confirmées dans d’autres sites avec le développement conjoint d’études immunologiques pour déterminer les mécanismes en cause.

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**55A**

**Differential serum expression of the CC-chemokines MCP-1, MIP-3a, and RANTES in Ugandan infants with severe or uncomplicated malaria [MIM-TE-25345]**

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**Introduction:** Manifestations of malaria infections in children range from the mild or asymptomatic (uncomplicated) to fatal severe and complicated malaria. The major goal of the present study was to investigate the possible association of specific serum chemokines with severe/complicated and uncomplicated malaria, respectively, in Ugandan children under 5 years old. Our hypothesis is that distinct chemokines might be associated with the various forms of malaria.

**Methods:** A case-control study of severe malaria in children under 5 years old was carried out at Apac Hospital in Northern Uganda. A commercial antibody array containing membrane-bound antibodies against 78 cytokines was used to probe the expression of host factors in serum pools of children with severe malaria anemia or asymptomatic malaria. Quantitative ELISA kits were employed to measure individual infant serum levels of monocyte chemotactic protein-1 (MCP-1) macrophage inhibitory protein-3a (MIP-3a) and regulated upon activation, normal T cells expressed and secreted chemokine (RANTES) in order to confirm the results of the antibody arrays.

**Results:** A significantly higher proportion of cytokines were detected in the serum pools of infants with severe malaria anemia compared with that in the serum pool of infants with asymptomatic malaria. There was a predominance of MIP-3a, MCP-1, interleukin-6 (IL-6), and interleukin-10 (IL-10) in the serum pools of infants with severe malaria anemia, as revealed by the antibody array. Mean MCP-1 and MIP-3a serum levels in infants with severe malaria were significantly greater than those in sera of children with asymptomatic malaria (3.8- and 2.5-fold, respectively). By contrast, mean RANTES serum levels in children with asymptomatic malaria was significantly greater (2.1-
fold) than that in sera of children with severe malaria. MCP-1 levels were significantly positively correlated with parasite density; MIP-3 alpha levels were significantly negatively correlated with temperature and positively correlated with titer of IgG antibodies against the serine rich antigen 5 (SERA5) in asymptomatic children. Serum levels of RANTES were significantly positively correlated with titer of IgG antibodies against SERA5 and EBA175.

**Interpretation:** MCP-1 and MIP-3a might be important correlates of severe malaria while RANTES might be a marker of convalescence or immunity. Functional studies of these chemokines in infant malaria will provide insights about their roles in disease or immunity.

**56B**

The effects of insecticide treated bed nets on the immune responses to *P. falciparum* antigens in Children in Western Kenya [MIM-JK-133518]

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**Introduction:** The use of bed-nets, especially in high malaria transmission areas, could lead to alterations in the acquisition and maintenance of malaria-specific immunity in young children, consequently shifting the burden of disease to older children. The effect of bed nets on humoral and cellular immune responses to well characterized malaria vaccine candidates antigens were investigated in young children.

**Methods:** Antibody responses to *Plasmodium falciparum* pre erythrocytic antigens, Circumsporozoite Protein (CSP) and liver stage antigen (LSA-1) and blood stage antigen (MSP-1) were tested by standard enzyme linked immunosorbent assay (ELISA) in a total of 2779 children under three years of age and enrolled in three morbidity cross surveys conducted before and after the bed nets trial. In addition, blood samples from a subset of children enrolled in two cross-sectional surveys were tested for the cellular proliferative response to LSA-1 and MSP-1 antigens by thymidine incorporation assay.

**Results:** 14 and 22 months after the introduction of bed-nets, frequencies and levels of total IgG and IgG subclasses 1–3 to LSA-1 and total IgG to CSP were significantly lower in children from bed net villages than in children from control villages ($P<0.001$ for all). In contrast, the frequencies of total IgG and IgG1 to MSP-1 antigen were significantly higher in children from bed-net villages than in children from control villages at 14 months ($P=0.0069$ and 0.029, respectively) but not at 22 months after the bed net intervention.

**Interpretation:** The results obtained from this study suggest that, the use of ITNs could improve the acquisition of protective clinical immunity in young children and the transplacental transfer of anti-CSP antibodies in areas of high malaria transmission.

**57C**

A detailed study of the kinetics of antibody responses to *Plasmodium falciparum* merozoite antigens [MIM-SK-10044]

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**Introduction:** Understanding the natural history of antibody responses to malaria antigens is important in elucidating the mechanism underlying immunity to malaria. However, limited temporal monitoring of malaria responses in previous studies did not facilitate detailed analysis of kinetics of the responses. As such, little data is available on this subject. Here we describe a detailed study on the kinetics of antibody responses to malaria merozoite antigens among Kenyan children.

**Methods:** Plasma samples taken from 40 children (age: 4–107 months; median = 28.5 months) during admission to Kilifi District Hospital with uncomplicated malaria and 1, 2, 3, 6, 9 and 12 weeks after treatment were simultaneously assayed by ELISA for IgG1 and IgG2 antibodies against recombinant *P. falciparum* MSP1-19, the two allelic variants of MSP2, EBA175 region II and AMA-1.
Results: There were wide variations in the kinetic of responses among the children and between responses to different antigens. IgG1 dominated the responses among these children. A child’s repertoire and isotypic complexity of responses increased with age. Each child displayed unique kinetics of responses although there were some similarities. While some children already had antibodies to some of the test antibodies at admission \( (n=11) \) others made sharp responses one week after admission \( (n=19) \). In both cases the responses declined sharply after the peak. Application of an exponential decay model indicates that the responses had a half-life of less than 8 days regardless of isotype. The rest of the children \( (n=10) \) did not make any IgG1 or IgG3 response to any of the test antigens. Re-parasitisation in 14 children was associated with boosting of some responses albeit not to the initial peak levels but did not affect the kinetics of responses in another 13 children.

Interpretation: (1) Multiple factors, including age, determine an individual’s response to malaria antigens. (2) Antibody responses to malaria antigens are very brief regardless of isotype. (3) Acute malaria may cause unresponsiveness to malaria antigens.

58A
The role of T cell and cytokine responses in malaria anaemia: Implication for the acquisition of immunity [MIM-SK-490197]


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Introduction: Malaria anaemia is very life threatening and may be affected by T cell, cytokine and antibody responses to the disease. Circulating Th1/Th2 cytokines have been implicated in the pathology of malaria and in the induction of protective antibodies that can prevent parasites from inducing Th1 cytokines (IFN-g and TNF-a) associated with fever. Low production of Th2 cytokines (IL-5) may contribute to the destruction of erythrocytes by malaria parasites.

Methods: In a 4-year longitudinal field-based study, we determined the T cell levels by immunohistochemistry, cytokines (IL-5, IFN-g and TNF-a) and IgG antimalarial antibodies to total parasite protein by ELISA and also looked at possible relationship with disease and protection in patients in Simbok, a village population chronically exposed to malaria, in southern forested Cameroon.

Results: Of the 457 subjects studied, 24% were feverish more than once. CD4+/CD8+ ratio was low in anemic \( (2.5 \pm 2.5) \) compared to non-anemic \( (3.1 \pm 2.4) \) patients: \( P=0.03 \). It was also low \( (2.7 \pm 2.2) \) in patients with fever \( (T>37.5 \degree C) \) compared to those without \( (3.7 \pm 2.8) \): \( P=0.008 \). In addition, the level of CD4+/CD8+ ratio increased with the episode of fever \( (r=0.53) \). The presence of malaria parasites coincided with low IL-5 levels, regardless of the clinical status \( (P<0.05) \). In addition, the mean IL-5 was lower in anemic \( (6.4 \pm 8.4 \text{ pg/ml}) \) than in non-anemic patients \( (19.0 \pm 53.5 \text{ pg/ml}) \): \( P=0.001 \), while the level of TNF-a in slide positives was higher than in slide negatives \( (p=0.004) \). It was also high in anemic \( (77.2 \pm 90.6 \text{ pg/ml}) \) compared to non-anemic patients \( (58.7 \pm 52.7 \text{ pg/ml}) \): \( P=0.049 \). High plasma levels of TNF-a and IFN-g coincided with the severity of malaria. Antimalarial IgG levels were lower in slide positives than in slide negatives \( (P<0.05) \). It was also low in anemic \( (OD=0.77 \pm 0.26) \) compared to non-anemic patients \( (OD=0.84 \pm 0.26) \): \( P=0.02 \).

Interpretation: Our findings showed that high plasma levels of TNF-a and IFN-g coincided with the severity of malaria, while high IL-5 and antibody titres coincided with protection.
Cellular and humoral immune responses to P. vivax Merozoite surface protein 9 (PvMSP9) in naturally exposed individuals from Rondônia State-Brazil


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Introduction: Merozoite surface protein-9 of Plasmodium vivax is present in several malaria species, and it is related to a P. falciparum protein also known as ABRA. We have demonstrated that a P. vivax monoclonal antibody and polyclonal antiserum against the native P. cynomolgi MSP-9 inhibit erythrocyte invasion of P. vivax merozoites in vitro and that PvMSP-9 is immunogenic in mice. In this study we evaluate the antibody and T cell reactivity to PvMSP-9 in individuals naturally exposed to malaria infections.

Methods: In a cross-sectional study carried out in Porto Velho, Rondônia state, Brazil, cells and sera from individuals living in Ribeirinha (N=188), a riverine native community along the Madeira river a tributary of the Amazon river and a transmigrant community living in a rural area close to Porto Velho (N=122), were assessed for IFN-γ and IL-4 cellular response by ELISPOT using PvMSP-9 synthetic peptides and for IgG antibodies response to PvMSP-9 recombinant proteins by ELISA.

Results: Our preliminary data shows that individuals naturally exposed to P. vivax malaria infections presented a cellular immune response to 6 of the 11 PvMSP-9 derived peptides. However, the cytokine profile were different between the two communities. In Ribeirinha, the native population, the frequency of positives individuals was similar for IFN-γ and IL-4 and most immunogenic peptides were MSP9A (19%) and MSP9H (22%) for IFN-γ and MSP9L (18%) and MSP9K (18%) for IL-4. In colina, the transmigrant population, the frequency of positive individuals was mainly for IFN-γ and the most immunogenic peptides were MSP9E (25.5%) and MSP9H (21%).

Interpretation: Studies are in progress to evaluate the antibody response. The association between cellular and humoral responses may provide information on the characteristics of acquired immunity to this P. vivax antigen and its potential as a vaccine candidate.

Plasma IgG level to VAR4, a conserved Plasmodium falciparum erythrocyte membrane protein 1, predict protection against anaemia and febrile malaria


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Introduction: Antibodies to Plasmodium falciparum surface antigens such as erythrocyte membrane protein 1 (PIEMP1) and merozoite surface proteins (MSP) mediate malaria immunity. In the large family of PIEMP1s, some can be divided into subfamilies. We recently identified a semi-conserved VAR4 that is associated with severe infection in young children. We monitored malaria morbidity in villages with high and medium transmission and compared IgG levels to PIEMP1s, some can be divided into subfamilies. We recently identified a semi-conserved VAR4 that is associated with severe infection in young children. We monitored malaria morbidity in villages with high and medium transmission and compared IgG levels to PIEMP1 and MSP1 in susceptible and protected children.

Methods: We enrolled 640 individuals below 60 years from two villages of different transmission intensity and obtained their baseline malariometric indices and blood samples. We followed them longitudinally for 7 months to detect malaria fevers and to monitor haemoglobin levels. At the initiation of the study, the plasma IgG levels to two PIEMP1 constructs (VAR4 and VAR9) and two MSP1 constructs (MSP1-19 and MSP1 block 2) was measured by ELISA. We analysed separately the association between antibody levels and febrile malaria or anaemia using logistic regression.
model controlling age and other covariates by transmission intensity.

Results: The point prevalence and density of *P. falciparum* was higher in Mkokola (for 2-year-old children 86.7% and 2801 parasites/μl, respectively) than in Kwamasimba (16% and 1288 parasites/μl). Similarly, prevalence and levels of antibodies to surface antigens were higher in Mkokola than Kwamasimba and positively correlated with age (*P* < 0.001). After controlling for age and other covariates the risk of developing malaria fever during the study was reduced among the children with a measurable VAR4 response in the high transmission village [adjusted odds ratio (AOR): 0.51 (0.29–0.89), *P* = 0.018]. The risk of having anaemia on admission was also reduced in VAR4 positive individuals [AOR: 0.49 (0.29–0.88), *P* = 0.016] for the high transmission village and [AOR: 0.33 (0.16–0.68), *P* = 0.003] for the medium transmission village. The plasma levels of IgG against VAR9 and MSP1 were not associated with protection.

Interpretation: To our knowledge this is the first demonstration of an association between anti-malaria antibody levels and anaemia. Thus, if the associations between anti VAR4 and protection are causal, the malaria vaccine candidacy of VAR4 is strengthened.

61A Characterisation of the naturally occurring antibody response to *Plasmodium falciparum* erythrocyte membrane protein 1 (PIEMP1) [MIM-cm-190904]

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Introduction: The surface of *Plasmodium falciparum*-infected RBC is modified by the insertion of parasite-encoded proteins; the most extensively studied family is PIEMP1. PIEMP1 undergoes antigenic variation and mediates cytoadherence of pRBC, underpinning the virulence associated with *P. falciparum*. Protection following exposure to a parasite displaying a specific variant of PIEMP1 is associated with antibodies against that variant. However, the nature of this protective response remains poorly understood.

Methods: Domain and interdomain regions of PIEMP1 from a laboratory parasite, A4, were expressed as recombinant proteins and used to screen, by ELISA, individuals from two areas within Kilifi district, differing in transmission intensity. Antibodies were purified against each domain and their reactivity against pRBC surface assessed. The presence of asymptomatic parasitaemia within each cohort was then related to the ability of children to recognise the surface of erythrocytes infected with parasite isolates displaying specific PIEMP1 through flow cytometry. The interaction between having detectable parasites and the presence of anti-pRBC surface antibodies led to the identification of a subgroup of children susceptible to clinical malaria.

Results: Antibody recognition of recombinant proteins corresponding to the domains of A4-PIEMP1 has given us insight into the acquisition of PIEMP1-domain specific antibodies with age and transmission. It has demonstrated differences in antibody acquisition between domains, individuals and areas and shown a boosting of antibody responses in the presence of asymptomatic parasitaemia. It has also highlighted the age-associated acquisition of responses and identified differences between the domains in terms of rate of acquisition. The affinity-purified domain-specific antibodies have demonstrated recognition of the intact A4-infected pRBC surface. By associating the presence of detectable parasites at the end of a low transmission season within each cohort with the ability to recognise the intact pRBC surface we have identified a susceptible subgroup of children and by correlating individual responses to different isolates we show that these responses may be targeting a conserved antigen.

Interpretation: Differences exist between individuals in the acquisition of PIEMP1 domain-specific antibodies. Interaction between asymptomatic parasitaemia and anti-pRBC antibodies identifies a population at risk and these antibodies may target a conserved antigen.
**62B**
Etude de la prévalence du paludisme chez les écoliers de la ville de Kinshasa [MIM-CN-57105]

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**Introduction:** Les modifications de l’écosystème de grandes villes africaines ont entraîné une migration d’anophèles de centres vers leurs périphéries majorant le risque au centre pour tous les groupes même ceux jugés non à risque. Ainsi pour évaluer l’émergence de ce phénomène à Kinshasa, ville à transmission stable, nous avons voulu déterminer la prévalence du paludisme chez les écoliers de cette ville.

**Methods:** De novembre 2003 à février 2004, période de haute transmission à Kinshasa, une étude transversale a été menée dans cette ville subdivisée en 4 strates. Chaque strate a été représentée par une école tirée au sort. Grâce à un échantillonnage systématique avec progression arithmétique de 4 et après consentement des parents, 400 enfants ont été inclus sur un total de 1608, tous habitant les alentours des écoles. Un palpé de la rate a été réalisé selon Hackett, une goutte de sang capillaire prélevée pour la confection d’une goutte épaisse et d’un frottis mince. Deux groupes ont été récés à savoir ceux âgés de 6 à 10 ans et d’autres de 11 à 15 ans. Une comparaison des moyennes et fréquences a été faite ainsi qu’une recherche de corrélations.

**Results:** L’indice plasmodique était de 32% avec un odd ratio de 1,78 pour les enfants de 6 à 10 ans. *Plasmodium falciparum* a été retrouvée dans 99% de cas et aucun gamétocyte n’a été relevé. Plus de 50% d’écoliers ont une densité parasitaire de plus de 1000/mm³ et ce en faveur du groupe d’âge de 6 à 10 ans (X² = 7,33, p = 0,026). En plus, 20% d’enfants ont une splénomégalie présente avec une rate hypertrophiée moyenne (RHM) de 1,95 pour le groupe de 6 à 10 ans et de 1,56 pour les enfants de 11 à 15 ans (test F de Snedecor = 9,87, p = 0,002). En présence d’une splénomégalie, la goutte épaisse était fréquemment positive (X² = 15,37, P = 0,000). En fonction de la localisation de ces écoles, la densité parasitaire ainsi que l’indice splénique relevés au centre de la ville n’ont pas été différents de ceux retrouvés en périphérie de la ville (X² = 8,37, p = 0,007).

**Interpretation:** La ville de Kinshasa demeure dans une zone meso-endémique avec un faciès stable. La décroissance avec l’âge des indices plasmodique et splénique et de la densité parasitaire suggère que l’immunité s’acquiert probablement au-delà de 10 ans.

**63C**
Consequences of *P. falciparum* and HIV co-infection in Zimbabwean patients [MIM-TM-52712]

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**Introduction:** Les pathologies de nombreuses infections, auto-immunes et maladies malignes sont influencées par les profils de production de cytokine dans des réponses pro-inflammatoire (Th1) et anti-inflammatoire (Th2). L’existence de co-infection de malaria et HIV est une autre menace portant sur l’efficacité du traitement de la malaria et les possibilités de progression à l’AIDS. L’objectif principal était de comprendre et de démontrer l’immunologie de la co-infection malaria et HIV.

**Methods:** Adult patients between the ages of 15 and 50 were recruited for examination on presentation at Nyamhunga Clinic, Kariba. The patients were diagnosed for *Plasmodium falciparum* using thick blood smears and then the levels of parasitaemia were determined. HIV infection was also determined. Re-examination was carried out the following day then on day 3 and 7 days after treatment. Full blood counts were also carried out on the patients. Cytokine profiles were detected using ELISAs after stimulations of whole blood with a PHA mitogen.

**Results:** Of 177 adults examined, 83.05% (147/177) were positive for *P. falciparum* parasitaemia of which 25.42% (45/177) were HIV positive. The body temperature was statistically similar regardless of parasitaemia density. Treatment with chloroquine lowered the fever regardless of being infected or not. About 20% (35/177) had fever persistent from day
one up to three days post treatment of which 71.43% (25/35) of these were HIV-P. falciparum co-infected. The individuals with P. falciparum-HIV co-infection had less than 10,000 parasites per μl compared to HIV uninfected (U = 408.0, Z = −1.956, p < 0.05), and with persistent fever. Similar levels of IL-10 were detected in both groups of patients that had malaria and HIV co-infected. However, levels of inflammatory cytokines were elevated in HIV and malaria or co-infected (U = 1154.0, Z = −4.121, p < 0.0001; and U = 1021.5, Z = −4.674, p < 0.0001) for TNF-α and IFN-γ, respectively. Similarly, the co-infected had elevated inflammatory cytokines (U = 126.5, Z = −4.939, p < 0.0001 and U = 20.0, Z = −6.185, p < 0.0001) for TNF-alpha and IFN-gamma, respectively. The individuals who were not HIV infected had levels of IL-10 comparable to TNF-alpha and IFN-gamma. Interpretation: Inflammatory cytokines dominate during infection and co-infection of HIV and malaria. Lower parasitaemia is common in HIV and malaria co-infected, whilst HIV negative individuals would require heavy parasite load to be clinically sick.

64A
Fetal immune response to malarial antigens in Yaounde, Cameroon [MIM-68328]
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Introduction: In malaria endemic areas, Plasmodium falciparum infection in pregnant women often results in the sequestration of parasites in the placenta, creating a situation where malarial antigens could enter fetal circulation. The exposure of the fetus to malarial antigens may lead to activation or tolerance of T and B cells. The current study evaluated the frequency of lymphocyte priming to malarial antigen in newborns in Yaounde, Cameroon.

Methods: 120 cord blood samples were collected between June and November 2002. Cord blood mononuclear cells (CBMC) were purified and cultured in vitro (1) in the presence of PHA, schizont extract and a pool of class II CSP and MSP-1 peptides for lymphocyte proliferation and cytokine production, and (2) in the absence of antigens for malaria-specific antibody production. Parasitaemia was determined by thick and thin cord blood smears and polymerase chain reaction (PCR). Maternal cell contamination of cord blood was assessed by PCR using DNA microsatellite. Antibody and cytokine production were determined by suspension array technology. Malaria specific antibodies were determined using recombinant AMA-1 and MSP-142, CSP peptide and schizont extract.

Results: Overall, among the CBMC samples 93% proliferated in response to PHA, 54% to schizont extract, 9% to MSP-1, and 0% CSP. The MP extract induced production of IFN-γ, IL-12 and IL-2 in 20%, 13%, and 33% of CBMC samples, respectively, and IL-4, IL-5, IL-10, and IL-13 in 4%, 18%, 76%, and 63%, respectively. In response to MSP-1, 31% of the samples produced IL-13, 4% IL-10, 4% IFN-γ, 4% IL-12, 7% IL-2, and none produced IL-4 or IL-5. Overall, 47.5% of the CBMC supernatants contained IgG antibody to MP, 3% to RESA, 41% to MSP-142, 52% to AMA-1 and 0% to CSP. AMA-1-specific antibodies were predominantly IgG1, whereas MSP-142-specific antibodies were IgG1, IgG2 and IgG3. Data showed that the prevalence of IgG antibodies specific for malaria antigens was significantly higher in cord CBMC culture supernatants from babies born to primi- and secundigravida (89%) than that of babies born to multigravida (69%) (P < 0.001). Also, 61% of P. falciparum PCR positive cord samples were from babies born to primi- and secundigravida women.

Interpretation: These results suggest that in malaria endemic area, upon encounter with malarial antigens, fetal B cells can be activated and isotype-switch before birth and T cells can be activated and differentiate into memory cells.
Seroepidemiological estimation of Plasmodium falciparum transmission intensity in north-eastern Tanzania [MIM-DM-18408]

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Introduction: Malaria is the leading cause of morbidity and mortality among young children and pregnant women in Tanzania. Plasmodium falciparum merozoite surface protein 1 (MSP1), a protein expressed by merozoites in the erythrocytic stage is the major surface antigen that invades erythrocytes. MSP1 is a potential malarial vaccine candidate. This study aimed at determining acquisition of anti-MSP1 antibodies, as a proxy to P. falciparum transmission intensity.

Methods: As part of an ongoing longitudinal study for monitoring malaria morbidity in north-eastern Tanzania, a cross-sectional survey was conducted in two villages (Kwamasimba and Mkokola) in Korogwe district involving individuals below 60 years of age. Venous blood was collected in EDTA tubes in March 2004; plasma was separated and stored at −20°C before analyses. Vital malariometric indices were determined, and anti-MSP1 IgG analyzed by ELISA to detect reactivity to recombinant protein MSP119 batch 43. Control plates were coated with glutathione S-transferase and mean ELISA units plus 2 standard deviations from sera of 19 Danes naïve to malaria were used as negative controls. Cut-off positive reactivity was determined based on this value.

Results: A total of 640 individuals were involved in this study from both villages. Prevalence of P. falciparum was significantly different in the two villages. Kwamasimba [16.1%; (95% CI: 12.5–20.6) and Mkokola 63.5%; (95% CI: 58.0–68.6), P < 0.001]. Anti-MSP1 IgG were positively associated with age [r = 0.2, P = 0.0003 in the moderate and r = 0.3, P = 0.001 in the intense transmission village]. The cut-off value for the negative control was 1.19 arbitrary ELISA units.

Interpretation: Acquisition of anti-MSP1 antibodies depends on age and transmission intensity. The protective effect of antibodies against the test construct and against other parts of MSP-1 will be evaluated.

Characterization of peripheral blood lymphocyte subsets in acute Plasmodium falciparum and P. vivax malaria infections at Wonji Sugar Estate, Ethiopia [MIM-Dm-355140]

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Introduction: Immunity against malaria and the immunopathogenesis mechanism is very complicated and not fully understood. Assessing the profile of immune cells during malaria infections could help therefore to understand further immunopathogenesis mechanism of malaria. Thus, we investigated the absolute counts and proportions of lymphocyte subsets (CD4+, CD8+, B and NK cells) in acute Plasmodium falciparum (pf) and P. vivax (pv) malaria patients. The findings were also correlated with parasite densities.

Methods: Three-colour flow cytometry was used for enumerating the immune cells. After slide smears stained with 3% Giemsa, parasite species were detected using light microscopy. Data was analysed using STATA and SPSS softwares.

Results: A total of 204 adults of both sexes (age >15 years) were included in the study. One hundred and fifty-eight subjects were acute malaria patients, of whom 79 (50%) were infected with pf, 76 (48.1%) infected with pv and 3 (1.9%) infected with both pf and pv. The remaining 46 subjects were healthy controls without detectable parasitemia. WBC count in pf patients was lower than in controls (P = 0.015). Absolute counts of CD4+, CD8+, B, CD3+ and total lym-
Phocytes were also decreased in both pf (P < 0.0001) and pv (P < 0.0001) malaria patients. Exceptionally however, NK cell count was not affected by either pf or pv infections. No difference was found in CD4%, CD8% and CD3% in pf or pv, whereas B and total lymphocyte percentages were lower in both pv (P = 0.002, P < 0.001) and pf (P = 0.008, P < 0.0001) malaria patients. Except with B cells, there was a negative correlation of CD4+ (r = −0.34, P = 0.003), NK (r = −0.43; P = 0.026), CD3+ (r = −0.30; P = 0.011) and total lymphocyte (r = −0.32; P = 0.05) counts with the asexual stage parasite densities of pf but not of pv. Interpretation: Our results showed acute malaria infection causes a depletion of lymphocyte populations in the peripheral blood. Thus, special consideration should be given when dealing with malaria patients.

67A Antibody acquisition against apical membrane antigen 1 in a cohort of children and the relationship to malaria exposure and protective immunity [MIM-CM-40691]

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Introduction: The Plasmodium falciparum merozoite protein apical membrane antigen 1 (AMA1) is essential for red blood cell invasion. AMA1 specific antibodies (Ab) can inhibit parasite growth in vitro and may play an important role in protective immunity against malaria disease. In order to more clearly understand the relationship between antibodies to AMA1, malaria exposure, and protective immunity, we have investigated the acquisition of Ab to AMA1 in a cohort of Kenyan children followed over 3 years.

Methods: Sera was obtained from a longitudinal cohort of 300 children aged 0-10 years old, in a malaria-endemic coastal district of Kenya. Venous blood samples were collected at 6 monthly intervals from May 2002 to October 2004. The children were monitored weekly for symptomatic illness and malaria. Antibodies to recombinant AMA1 (3D7) expressed in Escherichia coli were measured in serum samples by ELISA. Sera from 12 non-exposed donors were used as reference controls.

Results: Preliminary data analysis shows that the percentage of samples positive for 3D7 AMA1 antibodies at each time point was relatively constant with a mean of 42%. An OD value greater than the mean + 3SD of non-exposed controls was defined as positive for antibodies. Considerable fluctuation was observed in antibody levels among individuals over the study period. For those samples present at all six test points, there appear to be four main antibody profiles: (a) initially high antibody levels which fall and are then followed by a several-fold increase, (b) initially low antibody levels with a subsequent increase over time, (c) persistently low levels throughout the study period, and (d) persistently high antibody levels during the study period. Fourteen percent of these samples were positive for antibodies for the first time during May 2003. The acquisition of antibodies and changes in their levels will be related to episodes of malaria, age, parasitemia at time of sampling and other factors. We will examine antibodies to multiple AMA1 allelic forms and test sera for the ability to inhibit P. falciparum growth in vitro.

Interpretation: AMA1 antibody levels varied over time and related to age and malaria exposure. Measurement of antibodies at a single time point may not reflect the capacity to mount a specific response, nor adequately account for prior exposure to malaria.

68B Estimating the sequestered parasite load in severe malaria patients using both host and parasite markers [MIM-LO-23716]

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Introduction: The virulence of the malaria parasite Plasmodium falciparum is due in part to its ability to cytoadhere to vascular endothelium. Our inability to
Methods: We initially evaluated potential biochemical markers of sequestered load by comparing them with estimates of the sequestered load from a statistical model fitted to longitudinal patterns of peripheral parasite densities in a series of 22 patients from coastal Kenya with severe *P. falciparum* malaria. The markers comprised the host factors: haematocrit, circulating host DNA, sTNF-R75 and parasite derived products HRP2, pLDH, pigments and circulating parasite DNA. Results: The suitability of these markers for determining sequestered loads in patients on quinine treatment was assessed by comparing with predictions from a statistical model fitted to parasite dynamics. The observed peripheral parasitaemia, plasma levels of sTNF-R75 and circulating parasite DNA were most strongly correlated with estimates of sequestered loads on admission. However, the dynamics of both sTNF-R75 and circulating pDNA during follow-up were very different from those of the estimated sequestered mass. Interpretation: On the basis of these analyses we are not able to endorse the use of any of these markers for estimating the sequestered load in partially immune patients on admission.

69C Characteristics of anti-MSP1 antibodies passively transferred from pregnant mothers to their infants [MIM-CO-51824]

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Introduction: Infants in malaria endemic regions are first exposed to malaria from the placenta. It is known that children may be protected in the first few months of life through passive transfer on immunity from their mothers, but the factors that determine the length of protection and the course of infection in the infant remain unknown. We studied the passive transfer of anti-MSP1 specific antibodies from mothers to their infant and the immunity in infants through the first year of life. Methods: We enrolled 189 pregnant women in their second and third trimesters in a longitudinal study and followed up their infants (n = 62) from birth through the first year of life. The pregnant women were followed-up monthly until delivery. At delivery, maternal peripheral blood, cord blood, placental blood and placental tissue were obtained. The children were also followed monthly, from six weeks after birth till >1 year of life. For all samples, both at delivery and monthly, parasitaemia was determined by giemsa-stained thick blood smears. The levels of anti-MSP-119, and processing inhibitory antibodies were measured by ELISA and western blots. Results: Anti-MSP1 titres in children were lower than in their mothers with a mean log antibody titre of 2.1 Parasitaemia ranged between 0 and 80 parasites/µl blood in the children. Parasitaemia in the infants within the first 3-months of life was asymptomatic with the first clinical case occurring between the fifth and sixth months of life in most cases. We have observed a trend of two major malaria peaks in the first year of life. The lower peak at 5 months which corresponds with a major anti-MSP-1 antibody peak that might be indicative of the primary immune response of the infant to the malaria parasite. The second peak of parasitaemia however correlated with a lower MSP-1 antibody titre, showing the importance of secondary immune response of these infants to malaria. For the first time we have observed that passive transfer of MSP-119 specific processing-inhibitory antibody from a pregnant mother to her offspring was possible. Interpretation: Our findings highlighted the trend of malaria infection in infants in the first year of life and the central role and importance of the primary and secondary immune responses in infants to malaria infection.
70A  
The IgG subclass distribution of naturally acquired antibodies to Plasmodium falciparum merozoite surface protein 119 in children [MIM-YO-81600]

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Introduction: Plasmodium falciparum merozoite surface protein-119 (MSP-119) is a prime candidate for a blood-stage malaria vaccine. Antibodies against MSP-119 have been shown to prevent erythrocyte invasion by P. falciparum. IgG subtypes have been shown to have an effect on malaria outcome.

Methods: Blood samples were collected from children 10 days to 15 years in the months of January to March (n = 369) and October to November (n = 351), 1999, corresponding to the dry and rainy seasons, respectively. Parasite densities were determined by microscopy. Enzyme linked immunosorbent assay (ELISA), was used to determine the total IgG and IgG subtypes. SPSS and Excel were used to analyse the data.

Results: Anti-MSP-119 IgG subclass IgG1–4 incidence were, 84.4%, 66.2%, 84.4%, 55.8% in the dry season and 87%, 73.9%, 97.8%, 45.7% in the rainy season, respectively. IgG1 and IgG3 had the highest mean antibody titres in the dry season and at the end of the rainy season. There was an increase in mean antibody titres for IgG1, IgG2 and IgG3 at the end of the rainy season. This increase was only significant for anti MSP-119 IgG3 (p < 0.05). In the dry season there was a positive correlation between IgG1, IgG2, and IgG3 with age, while IgG4 showed negative correlation. However, in the rainy season there was a positive correlation between IgG2 and IgG4 (non-cytophilic antibodies) with age; while IgG1 and IgG3 (cytophilic antibodies) showed a negative correlation. The mean IgG1 and IgG3 titres for malaria negative individuals were higher than those of malaria positive individuals in the dry season while in the rainy season, the mean IgG1 and IgG4 titres for malaria negative individuals were higher than those of malaria positive individuals.

Interpretation: The study shows that anti-MSP-119 IgG1 and IgG3 may play a protective role in the anti-malarial immunity of children. The results also show that seasonal variation affects the immune response to MSP-119.

71B  
Allele-specific antibody reactivities to MSP-3 and protection from malaria [MIM-FO-44148]

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Introduction: Malaria is a major health challenge facing sub-Saharan Africa. A vaccine is the ideal long-term solution for resource-poor countries, but there is as yet no effective one. Field studies conducted in malaria endemic areas help to understand naturally acquired immunity to malaria and to identify potential candidate molecules for inclusion in a vaccine. This study investigated whether antibody responses to Merozoite surface protein 3 (MSP-3) are associated with protection from malaria.

Methods: Study participants (n = 536) were enrolled at the start of a malaria transmission season. A blood sample was taken for peripheral parasitaemia and serum was stored. Participants were followed up weekly for the ensuing 26 weeks with both active and passive case detection of clinical episodes of malaria. Allele-specific antibody reactivity to MSP-3 (K1-like and 3D7-like alleles) was determined by direct and competition ELISA. The isotypic pattern of this response was investigated by ELISA. The prevalence and amount of antibody was then compared in those developing disease and those remaining disease-free (protected) during follow-up. Allele frequencies of MSP-3 in the parasite population at the time of the study were also determined.

Results: Overall, 76% of individuals tested had reactivity to one or the other (or both) alleles of MSP-3, with a larger proportion responding to the 3D7-like antigen, compared to the K1-like antigen (68% versus 56%, chi squared test, p < 0.001). In agreement with this, the prevailing parasite genotype at the MSP-3 locus was the 3D7-like allele (75% versus 25% for K1-like). Both the
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prevalence and titre of anti-MSP-3 antibodies increased significantly with age. Individuals that had reactivity to both alleles responded predominantly to allele-specific rather than conserved epitopes. IgG3 was the principal isotype produced, followed by IgG1. The age-adjusted results indicate that seropositivity to the K1-allele of MSP-3 is significantly associated with a lower risk of malaria but only in individuals that are parasitaemic (malaria slide positive) at the time of serum sampling, OR 0.23, \( p = 0.001 \). Notably, in aparasitaemic individuals (malaria slide negative), seropositivity to either allele was associated with a higher risk of malaria, OR 2.64 and 2.56 for K1 and 3D7, respectively, \( p = 0.02 \) for both.

Interpretation: Allele-specific antibody reactivity to MSP-3 is protective in parasitaemic individuals but increases the risk of malaria in slide negative persons. Parasitization status should be considered when evaluating vaccine candidates and in vaccine trials.

72C

*P. falciparum* gametocyte carriage and sexual stage immunity in a malaria endemic area of Burkina Faso [MIM-AO-432848]


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Introduction: Plasmodium gametocytes are responsible for malaria transmission from man to mosquito, whilst asexual stages are responsible for clinical manifestations. To develop appropriate malaria transmission blocking strategies, it is relevant to understand the profile of gametocyte carriage and the dynamics of sexual stage immunity in populations living in endemic area.

Methods: We have carried out five cross sectional surveys in six villages from 2002 to 2003. To assess gametoctye carriage and sexual stage immune response in the area, malaria diagnosis was done on 4616 blood films for each developmental stage of the parasite and 709 plasma samples from children and adults were analyzed for antibodies against CS (sporozoites), GLURP (sexual stage) and Pf48/45 (sexual stage). These data have been collected in different periods of malaria transmission to assess the seasonal variation in parasitic carriage and humoral response to malaria specific antigens. A comparison between the microscopy and the QT-Nasba technique (a new molecular quantitative technique for malaria parasites count) was performed.

Results: The entomological inoculation rates (EIR) was 0 infective bite per person-month in the dry season versus 22 in the wet season (range 1–55). Prevalence of gametocyte carriage proportionally increased with asexual stage parasitaemia. Microscopically, gametocyte carriage (prevalence and density) in children under 5 years was significantly higher compared to elders above 15 years (\( p = 0.00 \)). In contrast to the asexual stages, the prevalence of gametocyte did not show seasonal variation in any age group. CS and GLURP antibodies increased with age whilst Pf48/45 antibodies decreased. Neither CS nor Glurp antibodies nor Pf48/45 antibodies did show a seasonal variation. The QT-Nasba showed significantly higher gametocyte prevalences independently of age compared to microscopy.

Interpretation: The prevalences of both gametocytes and Pf48/45 antibodies were higher in children. Anti-Pf48/45 antibodies associate with transmission blocking activity, therefore, both children and adults contribute to the human reservoir of malaria infection.

73A

IgG antibodies to merozoite surface antigens are associated with recovery from chloroquine (CQ)-resistant *P. falciparum* in Gambian children [MIM-CS-62139]

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Introduction: To define molecules and epitopes suitable for malaria vaccine candidates, a frequently used strategy is to examine their association with protection in endemic residents. However, definition of such
Protective immunity is difficult. We have examined the hypothesis that recovery of patients with CQ-resistant parasites treated for mild P. falciparum malaria is associated with humoral response to putative candidate vaccine antigens, and could serve as a measure of effective clinical immunity.

Methods: Sera from children screened for malaria and treated with chloroquine (CQ) in 28 day trials conducted in The Gambia were assayed by ELISA for IgG to the merozoite surface antigens apical membrane antigen (AMA) 1, merozoite surface protein (MSP) 119 and double and triple mutants of MSP-119. Responses to treatment were defined following WHO recommendations. Briefly, clinical failures were defined as the absence of severe malaria and absence of parasitaemia with fever after day 3 and parasitological failures were presence of parasitaemia on days 14 or 28. CQ-resistant parasites were defined by the presence of the PfcrtThr allele at enrolment.

Results: Plasma, parasite genotyping and complete response to treatment data were available from 78 patients; 44 sampled on day 0, twelve of whom were sampled again on day 7, and an additional 32 patients sampled only on day 7. In the majority of patients, clinical failures were defined as the absence of severe malaria and absence of parasitaemia with fever after day 3 and parasitological failures were presence of parasitaemia on days 14 or 28. CQ-resistant parasites were defined by the presence of the PfcrtThr allele at enrolment.

Antigen specific IgG was more frequent at screening in children who recovered particularly for the MSP1-19 triple mutant (age-adjusted odds ratio 0.18 (95% CI 0.03,0.97) P = 0.013) and less so for AMA-1 (age-adjusted odds ratio 0.32 (95% CI 0.05,1.87) P = 0.168) with the other MSP1-19 antigens giving values between these two. In plasma samples taken 7 days after treatment from children harbouring CQ-resistant parasites, IgG titres to MSP-119 wild type and triple mutant were significantly higher in children who recovered and remained parasite free. Moreover, in children who were parasitaemic on days 14 or 28, there was a significant linear trend between parasite density and IgG to both MSP-119 wild type and triple mutant, independently of age.

Interpretation: These results support the case for MSP-119 as a malaria vaccine candidate. We have also shown that the ability of treated children to clear resistant parasites is a useful functional test of immunity.

Impact of malaria intermittent preventive treatment in infants on their development of naturally acquired immunity [MIM-DQ-17974]

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Introduction: Intermittent preventive treatment in infants (IPTi) consisting of three doses of sulfadoxine-pyrimethamine (SP) given through the EPI scheme has been shown to reduce clinical malaria and anaemia. However, the mechanisms whereby IPTi may influence protective immunity against malaria in infants remain unclear. In the context of an ongoing IPTi trial in Mozambique we aim to investigate whether this malaria control strategy may have an effect in the development of naturally acquired immunity in children.

Methods: Details on the design of the main efficacy IPTi trial are provided in an accompanying abstract (Macete et al). For the immunology ancillary study, a subgroup of 200 children was selected to evaluate the immune responses to Plasmodium falciparum during the first 2 years of life. Capillary blood samples were drawn at four cross-sectionals (5, 9, 12 and 24 months) from children who received IPTi or placebo and immune responses were compared at these time-points. Antibody responses specific for P. falciparum antigens, including levels of IgG, IgG1, IgG2, IgG3, IgG4 and IgM, were measured in plasma samples by ELISA. Cellular responses were assessed by levels of selected Th1 and Th2 cytokines (e.g. IFN-γ, IL-4, IL-10), using flow cytometry-based methods.

Results: Up to date, the three first cross-sectional have been completed and we have obtained and processed blood samples at 5.9, and 12 months for immunological assessment from 325 study participants. Children are continued to be followed up to 24 months of age. Since all children entered the immunology study at the same age (5 months) and the randomisation into placebo and SP treatment groups was a block randomisation, a similar age distribution and a similar number of children is expected in each group. Data on the antibody and
cytokine responses to *P. falciparum* detected in both cohorts up to one year of age will be presented at the conference.

**Interpretation:** Data on immune responses will be compared between the intervention groups, and in relation to clinical outcomes. Understanding the mechanisms whereby IPTi prevents malaria and influences immunity is key if it is to be used as a malaria control tool.

75C Acquisition of protection to malaria correlates with patterns of antibody responses to potential vaccine antigens in children from Dielmo, Senegal [MIM-AT-74682]

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**Introduction:** In Dielmo, Senegal, naturally acquired immunity to *Plasmodium falciparum* was studied through a ten years longitudinal follow up including clinical, parasitological and entomological surveys. Through years, children progressively showed a decreased rate of malaria attacks indicating acquisition of a relative increasing protection. The aim of this study was to analyse the concomitant acquisition of antibody responses to *P. f.* vaccine candidate antigen in relation to the acquisition of protection.

**Methods:** Study site and subjects: In Dielmo, Senegal, a long-term prospective study of naturally acquired immunity to *P. f.* has been conducted for 10 years with the informed consent of the villagers, providing longitudinal clinical, parasitological and entomological data. In addition, blood samples were obtained each year for biological studies. Material 342 sera from 50 children (age 1–14 years), with one sample/year for each subject Antigens: Total extract of *P. f.* 0703 (a local *P. f.* isolate), MSP3TLP, LSA3TLP, GLURP/R0, R23 Method: Ab responses were tested by ELISA assays for IgM, IgG, IgG1, IgG2, IgG4 Statistical analysis Relationship between antibody responses and the risk of malaria attack was tested using a Poisson regression model.

**Results:** Epidemiological data showed that age is significantly associated with a decrease in incidence of malaria attacks among children in the different age groups from 1 to 7 years. Analysis of Ab responses showed different dynamics of IgG and IgM responses depending upon the Ag tested. Logistic regression model taking into account the effect of age, transmission and Ab responses indicated that clinical protection was predominantly associated with IgG1 and IgG3 responses to different Ag. In addition, some non-cytophilic Ab responses were found associated with an increased risk of malaria attack. Analysis of individual responses showed that protection corresponds to different patterns of responses to the Ag tested. In some subjects a decrease in malaria attacks numbers occurred in parallel to the acquisition of Ab responses to a particular given Ag whereas a positive immune response to several Ag seemed to be needed in others subjects to reach the same status. A high heterogeneity in responses was observed from one individual to the other with – Individuals who develop early responses versus those who acquire late responses – Individuals who have substantial responses to several Ag versus those who have limited or no responses to various Ag.

**Interpretation:** Our results suggest that protection occurs through different patterns of immune responses. As different Ag can be the targets of protective immune responses, this raises the issue of the potential efficacy of a vaccine derived from a single *P. f.* Ag.

4+6: Bio-ecology, behaviour and transmission

**Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday**

76A Microclimatic changes due to deforestation affect the duration of the gonotrophic cycle of *Anopheles gambiae* in the western Kenya highlands [MIM-YA-32813]

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Introduction: Deforestation is known to increase local ambient temperatures and this can cause an increase in the rate of blood meal digestion, reduce the length of the gonotrophic cycle and increase malaria transmission. The effects of microclimate change due to land use change, in the Western Kenya highlands on the duration of the gonotrophic cycle of Anopheles gambiae was investigated.

Methods: Cages containing 100 four-day-old mated female A. gambiae were blood fed and hanged in houses in deforested and forested highland areas of Iguhu, Kakamega district, western Kenya, with three cages in each land use type. After 24 h period, each mosquito was aspirated into a paper cup containing an egg-laying pad. Each morning the cups were inspected to find out if eggs had been laid. The number of mosquitoes that laid eggs per day was recorded. Indoor and outdoor temperature and humidity were measured using a Hobo data logger fixed unto the side of the cage. The study was carried out during the hot dry season from February to March and repeated in cold rainy season (June–July) 2004.

Results: Average ambient temperature in the deforested area in western Kenya highlands was 0.5 °C higher than the forested area over a 10-month period. During the dry season, deforestation increased the mean indoor temperatures by 1.8 °C, and shortened the duration of the first and second gonotrophic cycles by 1.7 days (59%) and 0.9 days (43%). During the rainy season, the average indoor temperature in the houses located in the deforested area was 1.2 °C higher than that in the forested area. The duration of the first and second gonotrophic cycles was shortened by 1.5 days (17%) and 1.4 days (27%). The relative humidity in the deforested area fluctuated substantially while the humidity in the forested area was more stable.

Interpretation: This study showed that deforestation increases the indoor temperatures and shortens the length of the gonotrophic cycle, which will lead to increased biting frequency and an increased risk of malaria transmission.

Distribution, anthropophilic and infection rates of malaria vectors in southern and south-eastern Mauritania’s regions, 2003 [MIM-HB-25536]

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Methods: This study was undertaken in October and November 2003, in order to know the presence of malaria vectors, their distribution, anthropophilic and infection rates and their implication in malaria transmission. Five regions including 21 localities were prospected. Mosquitoes were sampled by pyrethrum spray catch method. The source of blood meals was determined after BEIER et al. (1988) procedure and the head-thoraces of mosquitoes were tested by ELISA for Plasmodium falciparum, P. malaria and P. ovale CS antigen (WIRTZ et al., 1987). The species from the An. gambiae complex and molecular forms of An. gambiae s.s. were identified by PCR according to SCOTT et al. (1993) and FAVIA et al. (2001), respectively.

Results: 647 anopheline specimens belonging to three species were collected. An. gambiae s.l. was the most common species (92%) followed by An. pharoensis (5%) and An. funestus (3%). An. gambiae s.l was collected in all regions visited. An. funestus was observed only in Trarza region. The analysis of blood meals from blood fed females has shown that no significant difference was observed between the anthropophilic rates of An. gambiae s.l. within the five regions (4.57, d.f. = 4, p = 0.34). All An. funestus and An. pharoensis were fed, respectively, on Human and Ovine hosts. The molecular identification of the species of the An. gambiae complex has shown the predominance of An. arabiensis only one An. arabiensis from 394 females of An. gambiae s.l tested was found to be positive with P. falciparum antigen in (Tezekra) Assaba region.

Interpretation: This study shows the involvement of An. arabiensis in malaria transmission in Mauritania. However, it must be stressed that the low infection rate observed contrasts with the suspected malaria burden in Mauritania. Despite the low sample size, it can be a result of a zoophilic tendency of the anophe-
line species as a high proportion of blood meals was taken on two different hosts mainly others than Human. The hypothesis of malaria transmission by anopheine species other than those already described cannot be excluded with regards of the current knowledge from the epidemiological situation. In many centres, due to the lack of parasitological analysis, only clinical diagnosis is made for malaria. Hence, with the important symptomatic cases attributed to malaria, a thorough attention has to be made to other circulating disease like hemorrhagic fevers in the light of their frequent emergence.

78C Malaria vectors and transmission dynamics in the coastal area of Cameroon [MIM-JB-16572]
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Introduction: Very little is known about the dynamics of malaria transmission in the coastal area of Cameroon. An. melas is purported to be an important malaria vector along the coast of many West African countries. Its importance and contribution to malaria transmission in coastal Cameroon, is yet to be clearly defined, due to insufficient information on its abundance and infectivity. For the first time, we have described the malaria vectors and the dynamics of malaria transmission in coastal SW Cameroon.

Methods: During 432 human nights in 12 months, mosquitoes captured indoors on human volunteers were identified morphologically to groups and sibs of the Anopheles gambiae complex identified using the polymerase chain reaction (PCR). Vector parity rates were determined and infectivity detected using the double monoclonal antibody Enzyme-linked Immunosorbent Assay and the presence of sporozoites confirmed by PCR. Malarialometric indices (plasmodic index, gametocytic index and parasite species prevalence) were determined in three age groups (<5 years, >5 <15 years, >15 years) followed once every three months.

Results: A total of 2773 malaria vectors comprising An. gambiae (78.2%), An. funestus (17.4%) and An. nili (7.4%) were captured. No An. melas was caught biting humans. Generally, vector abundance, biting rate and infectivity correlated rainfall patterns. Monthly sporozoite rates were higher in An. gambiae than An. funestus and An. nili. Entomological inoculation rates (EIR) varied by locality and season, increasing with rainfall. There were 287, 160, and 149 infective bites/person annually in Tiko, Limbe and Idenau, respectively. An. gambiae was responsible for 72.7%, An. funestus for 23% and An. nili for 4.3% of the transmission. Malarialometric indices depicted a perennial mode of transmission and more intensely during the wet season. P. falciparum was the predominant parasite species accounting for over 98% of all infections. The plasmodic index (PI) decreased with increasing age. Mean parasite loads ranged from 2,120 to 103,000/μl of blood. The periodicity of heightened EIR coincided with periods of increased PI. All gametocytes found were P. falciparum and only children less than 5 years constituted parasite reservoirs.

Interpretation: Our data show that malaria transmission is perennial and that An. melas is not an important malaria vector in coastal Southwest Cameroon. It provides a baseline for the planning and implementation of malaria vector control activities in this area.

79A Influence of cortisol and prolactin in the susceptibility to Plasmodium falciparum malaria during pregnancy [MIM-MB-0]
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Introduction: Increased susceptibility of pregnant women to malaria is due to parasites sequestration in placenta or to depression of the immune system com-
ponents associated with increased production of hormones or proteins. Cortisol depresses cellular immune response during pregnancy. Prolactin might play a role in T helper dysequilibrium observed in malaria. We assessed prolactin and cortisol concentrations throughout pregnancy related to parity and Plasmodium falciparum infection.

Methods: Thirty-six primiparous and 23 multiparous attending their first antenatal visit at the Albert Schweitzer Hospital in Lambaréné between March 2003 and October 2003 were enrolled. They were seen between 15 and 19 weeks of gestation (T1), between 20 and 27 weeks (T2), between 28 and 37 weeks (T3) and at the delivery (T4). As a control group, 23 healthy nonpregnant women living in the same area and from the same age group were recruited. Maternal venous blood samples (2 ml) were collected on heparin in sterile tubes at T1–T4. Malarial infection was assessed. The plasma concentrations of cortisol and prolactin were estimated by use of the Minividas V. B. 02.96 system.

Results: The prevalence of P. falciparum infection was highest between the 28th and 37th weeks of pregnancy. Primigravidae were significantly more likely to be infected than multigravidae. Although the cortisol concentration increased from 20 weeks of gestation until the delivery in all women, it remained higher in primigravidae than in multigravidae. No difference in cortisol concentrations was found between non pregnant women and multigravidae at inclusion. Cortisol concentrations were significantly higher in primigravidae women than in multigravidae women between 20 and 25 weeks’ gestational age (166 ng/ml versus 132 ng/ml, respectively), between 28 and 37 weeks (226 ng/ml versus 188 ng/ml). Conversely, plasma prolactin levels were highest in multigravidae women. Cortisol and prolactin concentrations both increased with the stage of pregnancy (P = 0.01 and P < 0.01, respectively). There was a significant association between cortisol concentration and P. falciparum infection on the one hand and strong correlations with parasite load in P. falciparum-infected primigravidae women on the other hand (rho between 0.35 and 0.45 with P < 0.01).

Interpretation: There is a causal relationship between high cortisol levels and increased susceptibility to malaria in primigravidae women.

80B
High incidence of bacteria in Anopheles gambiae s.s. mosquitoes [MIM-CB-168636]
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Introduction: A symbiont that has the potential of being useful to express anti-Plasmodium proteins in mosquitoes will require one present in both immature and adult stages. The aim of the present study therefore was to isolate, identify and characterize bacterial symbionts in Anopheles s.s. mosquitoes, and ultimately to select one which is universal in all the life forms.

Methods: Mosquito larvae and pupae samples were collected from six locations in Ghana and some reared to adults. Wild adult An. gambiae mosquitoes from different geographical sites were also studied. Each specimen was surface sterilized before used. Scott et al. (1993) PCR method was used for sibling species identification. Bacteria detection was first carried out using universal eubacteria 16S and 23S rDNA primers then WOLB16SF1/WOLB16SR1 and ftsZ1/ftsZ2 primers were used on positive reactions to detect Wolbachia sp. In silico analyses of DNA sequences of Escherichia coli and Pantoae agglomerans were performed and the results compared with those obtained by restriction analysis of the amplified 16S and 23S rDNAs with six enzymes.

Results: Of the 395 PCR positive specimens, 373 (94.4%) were identified as An. gambiae s.s. These consisted of 274 (73.5%) adults, 28 (7.5%) pupae and 71 (19.0%) larvae. DNA fragments of the predicted sizes were successfully amplified in 81.8% (305/373) and 75.9% (283/373) of the specimens using the 16S rDNA and 23S rDNA primers, respectively. Bacterial DNA sequences were amplified from all life form stages, from both wild and laboratory reared adults irrespective of the geographical origin. Out of 281 specimens, PCR positives for both 16S and 23S rDNA primers, respectively. Bacterial DNA sequences were amplified from all life form stages, from both wild and laboratory reared adults irrespective of the geographical origin. Out of 281 specimens, PCR positives for both 16S and 23S rDNA primers, respectively. Bacterial DNA sequences were amplified from all life form stages, from both wild and laboratory reared adults irrespective of the geographical origin. Out of 281 specimens, PCR positives for both 16S and 23S rDNA primers, respectively.
revealed that none of the products could be that of either *Escherichia coli* or *Pantoea agglomerans*.

**Interpretation:** Since a high number of bacteria occur naturally in wild mosquitoes, it may be possible to modify anopheline vector competence by introduced or indigenous bacteria.

81C

**Malaria transmission dynamics and urbanization in the equatorial forest region of south Cameroon**

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**Introduction:** During the 20th century, ecological upheavals resulting from urban development in Africa, led to extensive modification of malaria epidemiology. We report here results of a follow-up study of malaria transmission conducted in the town of Mbalmayo 45 years after Adam’s studies of 1955. Sampling was also conducted in the village of Olama, 15 km away in a rural environment. This study design allowed us to assess the impact of urbanization on malaria transmission dynamics.

**Methods:** Adult mosquitoes were collected from February 2000 to June 2001 by human landing catches from 19:00 to 06:00 and by pyrethrum spray catches. The head and thorax of female anophelines were tested for the presence of circumsporozoite protein of *Plasmodium falciparum*, *P. malariae*, and *P. ovale* by ELISA. The entomological inoculation rate was calculated by multiplying the human biting rate from the landing catches by the CSP rate for each sampling period. Females belonging to the *An. gambiae* complex were identified to species using the PCR technique described by Scott et al. (1993). Specimens identified as *An. gambiae* were then tested for the M and S molecular forms following the diagnostic PCR-based assay of Favia et al. (2001).

**Results:** A total of 5743 females anopheline belonging to seven species were collected during this study. Malaria vectors were *Anopheles gambiae* Giles (M and S forms) and *An. moucheti* Evans in both areas, together with *An. funestus* Giles in Mbalmayo. One *An. marshallii* (Theobald) specimen was also found infected by *Plasmodium falciparum* Welch in Olama. *Anopheles moucheti* was the most abundant anopheline species caught in Olama while *An. gambiae* was most abundant in Mbalmayo. All these vectors were highly anthropophilic as indicated by the fact that only 5 of 201 blood meals analysed had been taken from non-human hosts. *Plasmodium falciparum* was the only malaria parasite species found in Mbalmayo, while *P. malariae* Laveran was also found in Olama. Malaria transmission was perennial throughout the study period in both Mbalmayo and Olama. The annual entomological inoculation rate was estimated at 129 infective bites/person/year in Mbalmayo, and 322 in Olama.

**Interpretation:** Comparison with data published in 1955 before expansion of the town, showed the impact of urbanization on malaria transmission dynamics in this equatorial area. Such changes have to be considered when implementing sustainable control measures.

82A

**The epidemiological importance of secondary malaria vectors in Cameroon**

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**Introduction:** Malaria transmission in Africa is a complex system. Despite decades of research, transmission dynamics is only superficially understood and further knowledge is required to improve the control of the disease. In the present report, we highlight the role of so-called “secondary” malaria vectors in participating to the overall parasite transmission intensity in sites in Cameroon, through a retrospective analysis of surveys conducted in the OCEAC laboratory between 1998 and 2003.
Abstracts / Acta Tropica 95S (2005) S1–S506

Methods: Longitudinal and cross-sectional studies were undertaken in 16 localities belonging to different bioclimatic domains throughout Cameroon, ranging from Sahelian savannas in the North to the equatorial forest in the South. Mosquitoes were collected by human landing catches, indoor pyrethrum spraying and aspiration in outdoors resting sites. They were identified to species using morphological characteristics and molecular diagnostic tools when available (for major vector species complexes). Infectious status of female specimens was assessed either by directly looking for Plasmodium sporozoites in the salivary glands, or by the ELISA technique for detection of the circumsporozoite protein in the head and thorax.

Results: Over 41,000 female anophelines belonging to 17 morphological species were collected between October 1998 and March 2003. Major vectors including Anopheles gambiae, An. arabiensis, An. funestus, An. nili and An. moucheti represented about 91% of the total anophelines fauna referenced in the OCEAC database. Beside these major malaria vectors, malaria parasites or the CS protein was found in nine species: An. ovengensis, An. carnevalei, An. coustani, An. hancocki, An. marshallii, An. paludis, An. pharoensis, An. wellcomei and An. ziemanni. Mean infection rate of secondary vectors (1.25%) was significantly lower than that of major vectors (3.06%) (chi-square = 36.58; d.d.l=1; p < 0.001). An. wellcomei and An. ziemanni were widely distributed in both forest and savanna environments, while the other species were localised in specific area. An. pharoensis and An. ovengensis were repeatedly found infected by Plasmodium falciparum, and contributed substantially to the total malaria transmission intensity in some areas where they were abundant. Both species have strong exophilic and/or exophagic habits that might save them from available means of vector control directed against endophilic and endophagic malaria vectors.

Interpretation: Although recent efforts on malaria research and control have focused on major vector systems, genetically competent anophelines species are numerous in Africa and contribute to the overall complexity of malaria transmission dynamics and epidemiology.

83B Spatial distribution of newly established Anopheles funestus (Diptera: Culicidae) populations and first evidence as a malaria vector in the Senegal River basin [MIM-ID-148572]

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Introduction: Anopheles funestus is one of the most important vectors of malaria in Africa and in some places is the main vector. In Senegal, this species has been found in all bioclimatic areas and its role as a malaria vector demonstrated in Sudanian and Sudano-Guinean zones but not in the northern drier part. Following its re-invasion in this latter area after 30 years of absence, the spatial distribution, bionomics and role in malaria transmission of the newly established populations were studied.

Methods: Entomological cross-sectional investigations were conducted in August and September 2003, in the Senegal River basin, the Ferlo and Lake Guiers areas. Mosquitoes were collected by pyrethrum spray catch. The trophic preferences, anthropophilic rates as well as Plasmodium infection rates were studied by ELISA.

Results: The study showed that An. funestus had a focalised distribution in the lower Delta of the Senegal River where it coexisted with An. gambiae s.l. In the Lake Guiers area and the low valley of Ferlo, An. funestus was almost the only malaria vector present with several available breeding sites whereas only An. gambiae s.l. In the Lake Guiers area and the low valley of Ferlo, An. funestus was almost the only malaria vector present with several available breeding sites whereas only An. gambiae s.l. was present in the inner valley of Ferlo. The mean anthropophilic and circumsporozoite infection rates by Plasmodium falciparum were, respectively 86% (CI 95% = 79–93) and 0.11% (CI 95% = 0–2.11).

Interpretation: Our data demonstrate that An. funestus is well established in the Senegal River Basin. Such re-colonization is probably due to environmental changes following the implementation of an anti-salt dam. Studies on its involvement in transmission and population genetics are in progress.
Malaria transmission dynamics and the diversity of anophelines vector populations in Tibati, Cameroon

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Introduction: The huge variability of malaria epidemiology observed throughout Africa is mirrored by the genetic and bionomical diversity of the vector system involved in parasite transmission. In many places, several vector species and populations transmit malaria simultaneously or replace each other seasonally. We provide an example of such diversity, where classical morphology, genetics and molecular methods were combined to provide refined assessment of the epidemiology of malaria transmission.

Methods: Longitudinal entomological survey of malaria vectors was conducted in Tibati, a village within the forest-savanna transition area in Cameroon, from June 2002 to June 2003. Mosquitoes were collected monthly by landing catches and indoor pyrethrum spraying. Female anophelines specimens were identified morphologically. Species and molecular forms within the Anopheles gambiae complex and species within the An. funestus group were identified using available PCR-based molecular diagnostic tools. Ovaries of half gravid An. gambiae and An. funestus females were dissected for karyotyping. The head and thorax of all female anophelines specimens were dissected and screened for the presence of Plasmodium falciparum CSP using an ELISA technique.

Results: A total of 7532 anophelines belonging to 10 morphological species were collected in Tibati. Based on results from the CSP-ELISA technique, seven species contributed to malaria transmission estimated at 194 infective bites/person/year. Malaria transmission dynamics was highly variable across the village, with a cluster of intense and year round transmission on the immediate border of the permanent lake that surrounds the village where the bulk of anophelines species diversity and abundance was observed. An. funestus was present throughout the year, with a peak in abundance during the dry season. It was the major malaria vector, accounting for 63% of the total transmission, followed by mosquitoes from the An. gambiae complex (24%). The S-molecular form of An. gambiae was predominant (89%), and occurred together with the M-form and An. arabiensis. The highly exophilic An. pharoensis was very abundant during the dry season and accounted for an overall 8.5% of malaria transmission. Cyto genetic analysis revealed a mixture of chromosomal forms in both An. gambiae s.s. (Mopiti, Savana, Forest) and An. funestus (Folonzo, Kirhina), with strong deficits in heterokaryotypes suggesting the presence of differentially adapted subpopulations.

Interpretation: Our survey of the vector system involved in malaria transmission in Tibati highlights the need for in-depth studies on the bionomics and genetics of African malaria vectors to provide the knowledge required for improving malaria control over Africa.

A screening of malaria mosquito preference for bacteria

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Introduction: An approach to battle malaria is by genetic modification of bacteria living inside the malaria mosquitoes. The bacteria would be modified with a gene encoding an anti-parasitic factor that kills the parasite. In our laboratory we are trying to identify suitable bacteria, and the midgut flora of Aedes aegypti and Anopheles mosquitoes have been screened. To investigate if any of the bacteria isolated can attract or repel the mosquitoes, we have developed a screening method based on sugar feeding.

Methods: Aedes aegypti, Anopheles gambiae and Anopheles arabiensis mosquitoes are used in the experiments, which are based on a dual-choice test of solutions coloured red and green with food-colours. Three bacteri a isolates from Ae. aegypti, characterised as Klebsiella pneumoniae, Pantoea stewartii and Bacillus luis sp., were tested separately against 10% sugar.

85A
Results: None of the mosquito species showed significant preference for one colour over the other. As expected, the mosquitoes preferred to feed from 10% sugar solution compared to both water and 1% sugar solution. The experiments show that this method, which is based on odour and/or taste responses, can be used in screening for mosquito preference of smell and taste. This method is easy to perform and cost efficient. The preliminary bacteria test on *Ae. aegypti* did not give a clear indication of preference for any of the bacteria species used so far. Further experiments need to be carried out. Also, *An. gambiae* and *An. arabiensis* preference for bacteria isolated from these mosquito species (see Abstract by Jenny Lindh) will be tested.

Interpretation: We have developed a screening method with the purpose of studying mosquito preferences. Three bacteria species isolated from the midgut of *Ae. aegypti* have been tested with this method, none are clearly attractive or repellent to the mosquitoes.

86B

Variations saisonnières de la productivité des larves de *Anopheles funestus* dans un gîte permanent à Nkolbisson (Yaoundé, Cameroun) [MIM-TG-4895]

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Introduction: Le paludisme constitue avec le VIH/SIDA, les maladies les plus importantes sur les plans clinique et économique. Le paludisme est responsable d’environ 300 à 500 millions de nouveaux cas cliniques chaque année en Afrique subsaharienne. Les travaux que nous avons effectués, visent à évaluer la productivité de *An. funestus*, vecteur important du paludisme.


Results: Nous trouvons que la différence des densités mensuelles des larves n’est pas significative si nous tenons compte des 18 mois. Cette différence devient cependant significative entre les mois de forte pullulation des larves (mars, décembre) et les mois de faible pullulation (octobre, mai). Par contre, les variations saisonnières des densités des populations larvaires sont significatives d’une saison à une autre. Ainsi nous notons que les densités des larves sont élevées pendant les 2 saisons sèches; la petite (juillet-mi-aout) avec 347 larves par récolte, et la grande (mi-novembre-mi-mars) avec 370 larves par récolte. Ces densités sont faibles pendant les 2 saisons des pluies; la petite (mi-mars-mi-juin) avec 211 larves par récolte, et la grande (mi-aout-mi-novembre) avec 119 larves par récolte.

Interpretation: Les densités larvaires sont sous l’influence de la pluviométrie ($r = -0.57; p = 0.003$). Les fortes pluies ainsi que leur fréquence élevée sont à l’origine de la baisse des populations des larves de *A. funestus*. Les pluies espacées les relèvent.

87C

Implications of mosquito biting patterns for malaria control in Dar es Salaam, Tanzania [MIM-YG-0]

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Introduction: Little is known about mosquito feeding behavior in cities even though half of the African population will live in cities or towns by 2015. We hypothesize that malaria-vector mosquitoes have started changing their biting behavior in response to increasing coverage of bed nets and house screening in urban Dar es Salaam, Tanzania.

Methods: In order to see if some Anopheles species are changing their biting behavior in urban areas, we will conduct hourly human landing catch during the whole night indoors and outdoors over a period of 9 weeks after the long rainy season. We will also use
household survey data describing human behaviour and access to prevention measures to estimate the overlap of mosquito biting activity with the exposure of human residents.

**Results:** We aim to evaluate where and when residents of Dar es Salaam are most at risk of contracting malaria. This will be accomplished by overlapping the nightly activity profiles of humans and vector mosquitoes, as well as the interventions that aim to separate them.

**Interpretation:** These results will enable evaluation of personal protection provided by bed nets and house screening against *Anopheles* mosquitoes and malaria transmission in Dar es Salaam.

**88A**

**Malaria transmission dynamics in two different agro-ecosystems of central Côte d’Ivoire: Entomological and parasitological investigations [MIM-KG-3112]**


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**Introduction:** In a recent study, we have recently noticed that location of human habitation to irrigation rice fields, and the practice of double rice cultivation influence transmission dynamics of malaria in rural part of central Côte d’Ivoire. Malaria prevalence rates and the number of fever cases attributable to malaria have also been measured among children aged 0–15 years, and related to the overall entomological inoculation rate of the both villages between February 2002 and August 2003.

**Methods:** The study was carried out in the village of Tiémélékro (geographic coordinates: −4.167 W, 6.500 N) and Zatta (−5.395 W, 6.884 N), located in central Côte d’Ivoire. As a first step, mosquitoes were collected by human night bait catches and pyrethrum knock-down spray sheet collections at four sentinel sites in each of the two study villages. As a second step, we carried out clinical and parasitological surveys among children aged 2–14 years. Data collection included basic demographic indicators, access to clean water and improved sanitation and common protective measures against malaria. In addition, geographical coordinates of potential malaria mosquito breeding sites were mapped, using a hand-held global positioning system (GPS, Magelan, 302).

**Results:** Anopheles gambiae s.l. was the predominant mosquito and the key malaria vector throughout, followed by *An. funestus*. In the village where a large rice paddy is located in close proximity, we observed a marked decrease in the entomological inoculation rate (EIR) of *An. gambiae* s.l. from 789 in the year 2002 to 2038 in the following year. In the other village where subsistence farming and intensified vegetable farming are the predominant agricultural activities, more stable transmission dynamics were observed. We found that *Plasmodium falciparum* is the most important malaria parasite in these epidemiological settings. The difference between the numbers of presumptive clinical malaria cases in the two villages over the entire study period was not significant. We found a high proportion of negative blood slides among clinically diagnosed malaria cases at the dispensary in one of the two villages (63.3%), underscoring the problem of the non-specificity of clinically relevant malaria signs. At a parasitaemia threshold of 5000 parasites/µl, the proportions of clinical malaria cases in this village were significantly lower (27.5%). The corresponding percentage in the other village was 25.0%.

**Interpretation:** Our entomological and parasitological findings are probably explained by the interruption of rice irrigation in the village of Zatta in 2003, and showed the importance for improved clinical algorithms for malaria diagnosis.

**89B**

**Variability of malaria transmission intensity in a rural area of Burkina Faso [MIM-EI-270816]**

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**Introduction:** The entomological inoculation rate (EIR) is one of the parameters measuring malaria transmission intensity, which is also used to evaluate the impact of malaria control measures. The clinical profile of
Malaria was shown to vary with different levels of EIR. The EIR is generally assessed at village or regional level, however it is known to vary in time and space. We aimed to quantify EIR’s spatial and temporal variability in a rural village of Burkina Faso where malaria transmission is seasonal.

Methods: In Tensobentenga, 30 km east of Ougadougou, a large artificial lake provides the main breeding site for mosquitoes. The village was subdivided in 3 strata according to clusters of households and distance from the lake. Zone 2 was the furthest from the lake, zone 3 the nearest. Zone 1 had more temporal breeding sites than zone 2. A longitudinal entomological survey, using indoor CDC light-traps was performed in 13 positions in the whole village. The sporozoite rate (s) for *P. falciparum* of *An. gambiae* s.l. and *An. funestus* was determined using a sandwich ELISA. Specific biting rates (ma) were calculated as the geometric mean of trap yield. Accordingly, the EIR was determined for each of the three strata as the product mas.

Results: During the high transmission season, the mean entomological inoculation rate due to both *An. gambiae* s.l. and *An. funestus* was 74, 33, and 125 infective bites per person per month in zones 1, 2, and 3, respectively. Sporozoite rates of *An. gambiae* s.l. were 8.8, 9.5 and 9.2% in the 3 strata. They were 7.4, 10.3 and 9.1% for *An. funestus*. Although these values are similar, especially in the case of *An. gambiae* s.l., it is noteworthy that stratum 2’s figures are consistently the highest for both species. Mean biting rates due to both species were 876, 361, and 1464 bites per person per month, respectively, i.e. two to four times higher in zones 1 and 3 than zone 2. These values were close to 0 during the low transmission (dry) season. Even within the same stratum, transmission intensity varied from one collection position to another.

Interpretation: Biting rates and sporozoite rates were correlated in opposite ways to the abundance of breeding sites. However, the larger variance of biting rates between strata made the risk of malaria transmission four-fold higher near the lake than zone 2.

**90C**

**Distribution pattern in the dry season of members of the *An. gambiae* complex in an area of The Gambia by PCR identification [MIM-MJ-318080]**

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Introduction: In The Gambia prevalence of asymptomatic parasitaemia remains around 30% throughout the year (Greenwood 1987) but malaria morbidity is very low in the dry season. Sexual parasite point prevalence rises from 1.3% in June to 9% in November, which correlates well with the increase in the mosquito population. This study was conducted around the Farafenni area of The Gambia, to look at the distribution pattern of members of the *An. gambiae* complex during the dry season.

Methods: Mosquitoes were collected forth nightly (March–June) using CDC light traps, spray sheet collections and room searches from dwelling huts, animal shelters, grain stores, and disused wells in four villages within the Farafenni DSS area. In addition, artificial breeding ponds were created within the village to attract ovipositing mosquitoes. The Anophe-line mosquitoes collected were then identified using the polymerase chain reaction (PCR) method using primers for the three main species in the area, *An. melas*, *An. arabiensis* and *An. gambiae* s.s.

Results: A total of 2500 Anophelines collected from the huts and disused wells were identified by PCR. Preliminary results indicate 90% of those identified as *An. melas*, 5% as *An. gambiae* s.s., 1% as *An. arabiensis* and the rest did not show any bands. Only in two villages (Dai Mandinka and Jajari) did Anopheline mosquitoes lay eggs in the artificial breeding ponds created in the village. Overall, 116 samples were identified by PCR (42 from Dai and 74 from Jajari) and 12% of these were identified as *An. melas*, 52% as *An. gambiae* s.s. and 21% as *An. arabiensis*. *An. melas* seem to be the predominant species in the dry season in this area, but *An. gambiae* and *An. arabiensis* take advantage to breed where there is a potential breeding site available. This may also contribute to their rapid regeneration when there are lots of potential breeding sites at the starts of the rainy season.
Interpretation: An. melas predominates in the dry season and appears to be an opportunistic breeder. Low levels of transmission in the dry season could be associated with its prevalence since it is a poor vector.

91A
Dry season ecology of members of the An. gambiae complex in an area of The Gambia [MIM-MJ-68152]

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Introduction: In The Gambia malaria transmission occurs mainly in the wet season (June–November), and is very low or absent in the dry season. A study was conducted to look at the ecology of the different members of the An. gambiae complex during the dry season and to investigate whether the availability of breeding sites restrict the occurrence of An. gambiae s.s.

Methods: Mosquitoes were collected fortnightly (March–June) using CDC light traps, spray sheet collections and room searches from dwelling huts, animal shelters, grain stores, and disused wells in four villages within the Farafenni area. In addition, small artificial freshwater breeding ponds were created within the village to attract ovipositing mosquitoes. The Anopheline mosquitoes collected were then identified with a polymerase chain reaction (PCR) method using primers for the three main species in the area, An. melas (salt water breeder), An. arabiensis and An. gambiae s.s.

Results: A sample of >2000 Anophelines collected were identified by PCR. Approximately 90% were An. melas, 5% as An. gambiae s.s. and approximately 1% as An. arabiensis. Temperatures this period, on average ranges from 30 to 42 °C and no rainfall was recorded throughout. Only in two out of the four villages (Dai Mandinka and Jajari) did Anopheline mosquitoes lay eggs in the artificial breeding ponds. Larvae were collected and reared in the insectary to adulthood, and 116 of those emergent Anophelines were identified by PCR (42 from Dai and 74 from Jajari). 52% of these were identified as An. gambiae s.s., 21% as An arabiensis and 12% as An. melas.

Interpretation: An. melas is the predominant species in the dry season in this area, but An. gambiae and An arabiensis take advantage of potential breeding sites. This is likely to contribute to their rapid regeneration at the start of the rainy season.

92B
Malaria transmission in two villages of the mandara mountains (extreme north province, Cameroon) [MIM-AJ-36076]

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Introduction: Cross-sectional entomological surveys of malaria vectors and transmission intensity was carried out in two villages of the Mandara mountains in the Extreme North province of Cameroon. Both villages have different eco-geographic characteristics: Godola is at an altitude of 450 m and Mokolo at 930 m. Standard entomological indexes were assessed in the rainy season (August 2003, September 2004) and once in the dry season (November 2003).

Methods: Mosquitoes were collected by night landing catches on volunteers and indoor pyrethrum spray catches. The anophelines species were identified morphologically and using the rDNA-PCR and PCR-RFLP assays to characterize members of the An. gambiae complex and molecular forms, respectively. The CSP infection rate and the feeding diets of vector species were determined by immunoenzymatic techniques (ELISA).

Results: An. gambiae s.l. and An. funestus were the two malaria vectors identified in both villages. Species
identification within the An. gambiae complex showed that it was composed of 86% of An. arabiensis and 14% of An. gambiae s.s. Both M and S molecular forms of An. gambiae were present in both sites, with a mean relative ratio of 10S:2M. The biting rate of An. gambiae was comparable in both villages (26 bites per man per month), whereas that of An. funestus is twice greater in Godola (8 b/m/m) than in Mokolo (4 b/m/m). The human biting rate of both vectors varied significantly across the seasons. The entomological inoculation rate was 4.5 times greater in Mokolo (3.51 infected bites per man per month) than in Godola (0.78 ib/m/m). Malaria transmission occurred in the dry season as well as in the rainy season in both villages, but with a greater intensity during the rainy season.

Interpretation: The existence of dams around the village of Mokolo might explain differences we observed between the two villages belonging to the same epidemiological strata by providing year-round breeding sites for the locally mosquito population.

93C
Malaria transmission in Nairobi, Kenya? [MIM-SK-391617]
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Introduction: The increase of population mobility arising from the migration from the rural to urban centers has not only contributed to the development of slum areas but also the introduction of malaria into areas considered malaria-free. Two clinical studies conducted in Nairobi, Kenya, located at over 1500 m above sea level, suggests that previously unrecorded malaria transmission could be occurring in Kibera, a sprawling slum estate reported to be the largest in sub-Saharan Africa. 
Methods: An intensive mosquito surveillance and testing program was undertaken in Kibera, a sprawling slum estate within the city boundaries of Nairobi, Kenya, in east Africa. Kibera is reported to be the largest slum complex in sub-Saharan Africa. From January 2001 to December 2003, day resting indoor (DRI) collections were made from 120 houses chosen randomly within the southern central section of Kibera. Houses were global position satellite (GPS) mapped and collections made on weekdays for 3 years. Larval habitats were identified and sampled 5 days a week using WHO standard dippers.
Results: In total, 176,994 mosquitoes were collected in the following numbers: 176,910 Culex fatigans, 2 Anopheles gambiae s.s., and 82 An. arabiensis. Mosquito population peaks were noticed during the long rains in April to May and the short rains in November and December. Out of the total number of Anopheles spp specimens collected, 99% were identified by PCR as Anophelles arabiensis and 1% as An. gambiae s.s. Blood-fed Anopheles mosquitoes were tested for their meal sources by blood-meal ELISA: 91% fed on human blood; 6% on both dog and human; and 3% fed on sheep. However, none was found positive for Plasmodium falciparum. Concurrent with the adult mosquito DRI collections, extensive larval sampling revealed Anophelines breeding in polluted water along the only stream passing through the study area. 95% of Anopheline larvae reared in the insectary to adulthood were identified by PCR as Anophelines arabiensis.
Interpretation: Malaria vectors known to feed mainly on cattle elsewhere in Kenya are breeding in the polluted water in Nairobi and are predominantly feeding on humans, thus indicating a shift in the ecology and behavior of this species.

94A
Entomological indicators for malaria transmission in urban Ghana, West Africa [MIM-E-142475]
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Introduction: Urban malaria is receiving increased attention due to the rapid, often uncontrolled, urbanization that is changing malaria epidemiology. We have been conducting studies in urban Ghana since October 2002 to determine the impact of urban agriculture (UA) on malaria transmission risk and to identify the most vulnerable sections of the human population. As part of the study we estimated transmission risk using entomological indicators.
Methods: From September to December 2003 a combination of human bait (landing) catches (HBC) and pyrethrum spray catches (PSC) were undertaken in communities in Accra, close to and without urban agriculture (UA). In addition, several rounds of PSC were performed in the city of Kumasi. The HBC and PSC data are analysed to investigate the association between UA, entomological inoculation rate (EIR) and human biting rate (HBR). In the 2nd phase of the study impregnated bednets were introduced in a community in Accra in 2004. Light traps were set fortnightly for 1 year in this intervention community and a contiguous control community. The light trap data are analyzed to investigate the associations between the intervention and anopheline densities.

Results: Preliminary results of HBC indicate an average nightly HBR of 101.1 for Culex and of 9.7 for Anopheles spp. For both species the HBR was three times higher in UA then U areas (156.9 versus 45.1 \((p < 0.001)\) and 14.7 versus 4.6 \((p = 0.02)\) in UA and U for Culex and Anopheles spp., respectively). Nearly all (99.5\%) of the anopheles caught were An. gambiae s.l. To date all An. gambiae s.l. have been identified as An. gambiae s.s. by PCR. Analysis of sporozoite infection is in progress and will be used to calculate and investigate EIRs. The PSC resulted in very low number of mosquitoes, an average number of anopheles per room of less then 1. Although low in both cities the number in UA was double the number in the U communities (0.3 and 0.1 per room in Accra and 1.2 and 0.6 in Kumasi for UA and U, respectively) with large variation between houses. Preliminary analysis of the light trap data indicate nearly all anopheles to be An. gambiae s.l. with a small number of An. funestus. There were great heterogeneities in the distribution of anopheles with marked variation both between areas as well as between houses. A multivariate analysis is being done to determine which factors most influence the distribution of anopheles in these urban settings.

Interpretation: The results of the different surveys help us to predict transmission risk in this urban environment. In addition, comparison between different methods will be used to discuss the suitability of the different methods for use in the urban setting.

95B Malaria transmission in Sahel zone: Which markers are reliable? [MIM-RL-196417]
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Introduction: Estimation of malaria transmission may be performed by entomological survey and malaria prevalence in human population. The malaria prevalence alone is usually not considered as a relevant marker of the transmission because of its fair correlation with entomological data. However, that fact lies on data from continuous and high transmission tropical humid African zones. Indeed, the respective values of these markers need to be regarded according to the different transmission zones.

Methods: To better document this issue, a cross-sectional study to estimate the transmission was performed in Niger during two annual rainy seasons and dry seasons, across a transect according to three malaria transmission levels: sudanian savanna, sahelian and near-Sahara zones. In four villages in each area aggressive and resting vectors were sampled and parasite detected among asymptomatic carriers by thick and thin blood films in age-classed population. Vector species were morphologically assessed or with help of PCR in case of sibling species. Infected vectors were quantified by CSP-ELISA. Variability of the markers was assessed with special attention to spatial relation.

Results: Globally and as expected, both parasite prevalence and entomological parameters differ according to the three bioclimatic zones and among the seasons with malaria transmission decreasing when the dryness is getting higher. However, at a finer spatial level, some discrepancies observed between prevalence and entomology compel us to consider specific local situations, specially in the Sahel. Notable asymptomatic carriage was found in considered as unstable malaria zones and focus of high transmission lie in the Sahelian zone. Environmental factors appear to be of prime importance in entomological marker difference when parasite carriage seems to smooth the variability at the human level.

Interpretation: The respective values of these markers need to be regarded according to the characteristics of the organisms: a short vector life-time linked with
environment and a long duration of parasite carriage as a sum of inoculations at the human level.

96C

Adult anopheline ecology and malaria transmission in irrigated areas of south Punjab, Pakistan [MIM-CM-430641]

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Introduction: Malaria in the Punjab is classically unstable and epidemics occur at the intervals of 8–10 years. Heavy rainfall in monsoon season has been implicated as a possible explanation for the variation in malaria transmission. The objective of the present study was to understand the relationship of population dynamics of adult anophelines and malaria transmission in different irrigation related environmental conditions.

Methods: Three villages were selected along an irrigational distributory in South Punjab reflecting a range of different environments, from severely waterlogged to desert conditions. On fortnightly basis mosquitoes were collected from 20 pre-selected bedrooms per village from April 1999 to March 2000 using pyrethroid spraycatch method. To include other potential anopheline resting sites, animal sheds and vegetation within a 1000 m radius of the village were also sampled by using Backpack Aspirator. Other sites encountered by the field team were also sampled on an ad hoc basis. To estimate the prevalence of malaria in study area, house-to-house fever surveys were also conducted on monthly basis.

Results: Overall, Anopheles subpictus was predominant (49.6%), followed by An. stephensi (40.4%), An. culicifacies (6.8%), An. pulcherrimus (2.0%) and An. peditaeniatus (1.1%). Majority (98.8%) was collected from indoor resting sites, confirming endophilic behavior of anophelines in Pakistan. Only An. pulcherrimus and An. peditaeniatus were collected from outdoor resting sites. The females of three main species, remained gonotrophically active all year-round and unfed, blood-fed, semi-gravid and gravid females were collected in each month. Interesting contrasts in the anopheline fauna collected from the different villages were also observed. The village situated at the head of irrigation system (waterlogging) profusely yielded An. stephensi (84.2%) and An. culicifacies (83.8%) while from the dry village situated at tail of system 66.4% collection comprise of An. subpictus, An. stephensi, An. culicifacies and An. subpictus populations peaked in August, September and October, respectively. Monthly fever case surveys reported low malaria prevalence and all cases reported from the village with waterlogged conditions (2.9). An. stephensi and An. culicifacies were also most abundant in the same village.

Interpretation: The overall low prevalence could be attributed to the low densities of Pakistan’s primary malaria vector, An. culicifacies. However, the role of An. stephensi in this situation needs to be further clarified.

97A

The role of vector species in the changing malaria pattern of the southern Punjab, Pakistan [MIM-MM-113725]

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Introduction: Despite the absence of large-scale vector control activities in Punjab, malaria situation now days is much improved. This raises the question whether the irrigation related environmental changes that have taken place during past decades influenced the vector species abundance or composition and low level of malaria transmission in the Punjab.

Methods: The routinely government-collected entomological data for the period 1970–1999 from district Bahawalnagar, Punjab was analysed. Collections were made from living rooms, storerooms and animal sheds.
In more than 95% of the cases, the collection method was the pyrethroid knock down spray catch. Percentage of positive rooms and density per positive room were used in the analysis. The outcome of interest was the change in abundance of *An. stephensi* relative to *An. culicifacies* over the time. Data of groundwater depth and salinity level was also collected from respective department to analyse relationship between species composition and malaria transmission.

**Results:** A significant shift in vector species composition was noted. The abundance of Anopheles stephensi increased gradually relative to *An. culicifacies* over the past 30 years. All five sub localities within the Bahawalnagar district showed same pattern of increasing relative abundance of *An. stephensi*. The density of females per positive room showed a similar pattern, a change in dominance from *An. culicifacies* to *An. stephensi* over the period 1970–1999. An increase in saline content of water and soil since early 1980’s was confirmed.

**Interpretation:** This shift could have been due to the large-scale ecological changes took place in Punjab over the time, where irrigation-induced waterlogging with related salinization had created favourable environment for the more salt-tolerant *An. stephensi*.

98B

Anopheles merus Dönitz (Diptera: Culicidae): preliminary implication as malaria vector in Zimbabwe [MIM-HM-56028]

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**Introduction:** Malaria vector studies showed the predominance of Anopheles merus Dönitz among the *An. gambiae* complex at Masakadza in Gokwe South District, Zimbabwe. The occurrence of *An. merus* was associated with the presence of brackish water habitats (32.76% seawater). Elsewhere in the country, *An. merus* has a known patchy and insignificant occurrence. The purpose of the study was to determine the role played by *An. merus* in malaria transmission.

**Methods:** Mosquitoes were collected in 2001–2002 from Masakadza (17°04’ S × 28°36’ E), a malaria endemic area in Gokwe South District, Zimbabwe. Adult anopheline mosquitoes were collected from human landing catches, indoor resting, exit window traps and pit shelters. Preserved specimens were identified to species level using the polymerase chain reaction method. Head and thorax segments were processed for circumsporozoite antigen using the enzyme linked immunosorbent assay (ELISA) technique.

**Results:** Human landing collections showed *An. merus* was the dominant species of the *An. gambiae* complex accounting for 89.6 and 72.3% of collections indoors and outdoors, respectively. Corresponding proportions for *An. arabiensis* were 10.4% indoors and 25.5% outdoors. ELISA tests showed *An. merus* was sporozoite positive for *Plasmodium falciparum* as follows: 0.0333 in December 2001 (n = 30), 0.0072 in April 2002 (n = 139) and 0.0292 in May 2002 (n = 137). At this locality, the infection rate for *An. arabiensis*, the principal malaria vector, was 0.0128 in April 2002 (n = 78) but was undetected in February 2001 (n = 13), December 2001 (n = 20), and May 2002 (n = 67).

**Interpretation:** *An. merus* played a greater vectorial role than *An. arabiensis*. This was the first instance *An. merus* has been implicated in malaria transmission in southern Africa. Further studies are necessary to determine the impact of insecticide treated nets and house spraying on *An. merus* at Masakadza.

99C

Breeding of malaria vectors in irrigated agriculture: A study in southern Punjab, Pakistan [MIM-MM-5798]

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**Introduction:** The decreasing effectiveness of conventional malaria vector control methods has led to the re-
emergence of environmental management as a possible alternate strategy for the reduction of vector breeding and malaria transmission. The objective of the study was to assess the importance of irrigation system in the generation of malaria vectors, as a first step towards identifying and designing environmental management interventions.

Methods: Three villages were selected along an irrigation distributory in South Punjab to investigate the potential linkage between irrigation system and malaria transmission. The selected villages reflected a continuum of habitats from severely waterlogged to desert conditions. All surface water bodies in and around the selected villages were surveyed on fortnightly basis from April 1999 to March 2000. The samples were characterised according to exposure to sunlight, substratum, presence of vegetation, fauna and physical water condition (clear/turbid/foul). Water temperature, dissolved oxygen (DO), electro-conductivity (EC) and pH were also noted in situ.

Results: A total of 37,982 Anopheles larvae of six species were collected from 2992 samples taken from irrigation/agricultural and village/domestic aquatic habitats. Anopheles subpictus Grassi sensu lato was by far the most abundant (74.3%), followed by An. culicifacies Giles s.l. (4.1%), An. stephensi Liston s.l. (2.6%), An. pulcherrimus Theobald (1.8%), An. peditaeniatus Leicester (0.3%) and An. nigerrimus Giles (0.1%). The species composition also differed among the villages. In the waterlogged village An. culicifacies followed by An. stephensi were predominant species while An. subpictus was predominant in the desert village. Malaria vectors, Anopheles culicifacies and An. stephensi were mainly recorded in irrigated and waterlogged fields and communal village drinking-water tanks. Habitat characteristics correlating with occurrence of anophelines were physical water condition and presence of fauna, particularly predators. Occurrences of Anopheles immatures were not significantly correlated with water temperature, DO, EC or pH.

Interpretation: In south Punjab, where the rainfall is low, it should be possible to reduce the anophelines breeding through water management in canal-irrigation system.

100A

Estimating population size, dispersal and survival for Anopheles gambiae and Anopheles funestus on the Kenyan coast by mark release recapture methods [MIM-JM-1400]

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Introduction: Information on the characteristics of mosquito populations is of paramount importance in understanding the epidemiology and ecology of malaria and for planning vector control. We conducted mark release recapture experiments with emergent Anopheles reared from larval stages. The objective was to determine adult dispersal capabilities, estimates of the population size and survival probability of An. gambiae and An. funestus in an area of perennial malaria transmission on the Kenyan coast.

Methods: Mark release recapture experiments were conducted with An. gambiae and An. funestus mosquitoes at two sites, Jaribuni and Mtepeni in Kilifi, Kenya. Larvae from natural habitats were reared in a field insectary and emergent adults marked with fluorescent dye and released at the original habitat. Recap- ture by manual aspiration of indoor resting females and human landing catches started a day after release. Recaptured mosquitoes were screened for colour, classified by gonotrophic state and identified by PCR. The Lincoln index method and the exponential model were used to estimate the population size, and the daily survival probability, respectively. The mean dispersal distance from the point of release was also estimated.

Results: Only An. gambiae s.s and An. funestus were recaptured during indoor collections by manual aspiration, and by human landing catches, both indoors
and outdoors. A recapture rate of 24.6% was recorded at Jaribuni and 4.33% at Mtepeni. The mean population size of *An. funestus* was estimated as 12,485 (74%) and *An. gambiae* s.s as 4386 (26%) in Jaribuni, while in Mtepeni, the population size of *An. gambiae* s.s and 9.6% (95% CI = 0.89–1.00) for *An. funestus*. At Mtepeni, estimates of daily survival probability were 0.95 (CI = 0.87–1.00) for *An. gambiae* and 0.83 (CI = 0.79–0.87) for *An. funestus*. The mean dispersal distance was 393.3 m for *An. gambiae* and 384.3 m for *An. funestus* in Jaribuni, and 274 m for *An. gambiae* and 9.7 m for *An. funestus* in Mtepeni. On the overall, maximum dispersal distance recorded was 661 m. Interpretation: The results provide insights into how physical and ecological differences contribute to malaria epidemiology and suggest female age and proximity of the human dwellings to larval habitats as important factors affecting mosquito dispersal.

101B Monitoring seasonal variations in malaria transmission in a low endemicity area in Tanzania [MIM-OM-248768]

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Introduction: Detailed knowledge of malaria transmission will allow better targeting of malaria interventions. In particular, in areas of low seasonal transmission longitudinal monitoring of factors related to increases in transmission will identify optimal times in which to intervene. We have collected data on meteorological, demographic, entomological and parasitological data over the course on 1 year in one village to describe its malaria epidemiology and identify those at risk.

Methods: Each house in the village of Msitu wa Tembo in Lower Moshi was mapped and censused. The village dispensary was used to verify and treat cases of malaria in this and other nearby villages. Meteorological data were collected from the nearby TPC sugar plantation. An entomological inoculation rate (EIR) was calculated from weekly light trap catches in the village. Malaria cases were mapped by residence using GIS software and the incidence of cases by season and distance to breeding site calculated.

Results: Rainfall was below the 10 year seasonal average though peaked in April and May. Entomological monitoring showed that 99% of malaria vectors caught were *An. arabiensis*. Predictably peak vector densities followed peaks in recent rainfall. There was an overall sporozoite rate of 0.0005 (3/5634) generating an EIR of 3.4 infectious bites per person per year. The distribution of malaria cases in children under 15 showed a clear pattern with the majority of cases (53/102) occurring in the 3 months after the rainy season. Throughout the year the risk of malaria was significantly higher in those children living in houses 1700–1400 m from a breeding site (OR 2.1, 95% CI 0.94–4.4) and <1400 m (OR 3.5, 95% CI 1.6–7.1). Further analysis on risk for infection and incidence will be presented.

Interpretation: Our data show that local malaria transmission is highly restricted especially in the low transmission season. This will allow better targeting of interventions including transmission blocking vaccines which may be particularly effective in this area.

102C Sporozoite rates in *Anopheles* spp., and *P. falciparum* inbreeding coefficient in the Lower Shire Valley, southern Malawi [MIM-TM-10205]

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Introduction: There is a lack of data on malaria transmission in Malawi. Recent entomological studies in southern Malawi have shown that *An. gambiae* s.s, *An. arabiensis* and *An. funestus* were common but their roles in malaria transmission are not known. It is also hypothesised that frequent cross-mating occurs within *P. falciparum* populations in highly malaria endemic areas.

Methods: To determine entomological indices of malaria transmission, mosquitoes were collected using pyrethrum knockdown catches (PKDs) in the lower
Shire Valley, southern Malawi over a period of 52 weeks between January 2002 and January 2003. Mosquito abdomens were dissected and examined under a dissecting microscope for the presence of oocysts. PCR methods were used to identify members of the An. gambiae sensu lato and to detect sporozoites in their salivary glands. A direct ELISA was used to determine the source of the mosquito blood-meals. Single P. falciparum oocysts were genotyped at three microsatellite loci: ta109, ta1 and ara2.

Results: To determine entomological indices of malaria transmission, 7713 Anopheles were collected and of these, 100 (infection rate of 1.3%) carried oocysts. Of 3792 examined An. gambiae s.s, An. arabiensis and An. funestus had sporozoite rates of 11.68, 3.31 and 4.61% and human blood indices (n=883) of: 99.2, 85 and 96.3%, respectively. Using the frequency of alleles at different microsatellite loci, an inbreeding co-efficient of 0.44 (0.32-0.56; 95% CI) (n=192) was estimated from individual P. falciparum oocysts. Interpretation: An. gambiae s.s. is the main malaria vector in the area. And P. falciparum populations are 60–70% outbred indicating high rates of outcrossing.

Interpretation: An. gambiae s.s is the main malaria vector in the area. And P. falciparum populations are 60–70% outbred indicating high rates of outcrossing.

103A
Monte-Carlo simulation model of mosquito choices up odour plumes to alternative hosts [MIM-LN-157025]

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Introduction: We present an individual-based simulation model of zoophilic mosquitoes presented with a choice of two odour plumes at varying distance from each other. Our simulation is based on the following assumptions: (i) every mosquito appears on a plane ‘downwind’ and flies up odour plumes originating from two points ‘upwind’, C1 and C2, which correspond to odour sources from N1 human subjects and N2 mammalian hosts, respectively; (ii) a mosquito may locate an odour source if it comes under the influence of either plume; the probability of its flight up one or the other is described by fractional-linear functions dependent on a set of values of model parameters that reflect the number of individuals from each source and relative attractiveness of human and mammalian hosts; an insect that is outside the zone of behavioural influence of the plumes, has zero probability of locating the source; (iii) the insect’s movement below the influence of a plume is a stochastic process with exponential distribution, each step being dependent on the perception of the odour signal; the flight of an insect that loses the signal is considered equivalent to movement parallel to and away from the zone of influence [A and B] on x-axes.

Methods: Within this set of assumptions, we modelled the relationship between the number of mosquitoes that arrive at odour sources C1 and C2 with varying number of hosts (human or animal) and the relative distance between these sources. Computer experiments, each involving large number of insects comprising 100,000–1,000,000 individuals, were carried out.

Results: The most interesting result was the finding that the number of mosquitoes that arrive at the source of human odour is dependent on its distance from animal odour source; that is, it has a non-linear and non-monotonous pattern. This confirms our expectation that, at a given mosquito population density, significant diversion of insects between alternative hosts occurs at certain spatial relationships between competing plumes. A smaller or greater gap than a certain optimum does not facilitate net diversion of incoming insects from one to the other.

Interpretation: The present study represents the first report of a theoretical treatment of the spatial relationship between different proportions of competing hosts of zoophilic mosquito and the distribution of the insects between these hosts. Previous studies had looked at the effect of the number of animals (and blood meals) on mosquito survival and, therefore, on the disease levels.
104B
Anopheles funestus (Giles, 1900), la riziculture et le paludisme dans la région forestière ouest de la Côte d’Ivoire [MIM-BN-46368]

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Methods: Trois agrosystèmes ont été considérés: les bas-fonds non-aménagés (R0), les bas-fonds aménagés avec une culture de riz par an (R1) et les bas-fonds aménagés avec deux cultures de riz par an (R2).

Results: Sur un total de 38 632 moustiques récoltés au cours de 936 hommes-nuits de captures sur sujets humains, 88.2% ont été des anophèles. Anopheles funestus a représenté 34% des anophèles capturés. Cette espèce a constitué respectivement 25, 6, 40 et 32% des anophèles récoltés dans les villages des agrosystèmes R0, R1 et R2. Les taux de piqûres ont été de l’ordre de 2 p/h/an dans les villages de l’agrosystème R1 et 21 p/h/an dans les villages de l’agrosystème R2.

Anopheles funestus a été présenté toute l’année dans les trois agrosystèmes, les taux d’agressivité les plus élevés ont été enregistrés pendant la saison des pluies, entre mai et octobre. Les taux de parturition ont été déterminés pour l’ensemble des agrosystèmes R0, R1 et R2.

Interpretation: Ces observations démontrent qu’Anopheles funestus est présent et peut être responsable d’une importante transmission palustre en zone rizicole, ce qui n’avait pas été observé en zone de savane du sud du Burkina Faso et du nord de la Côte d’Ivoire.

105C
A survey of the Anopheles funestus (Diptera Culicidae) group and their role in malaria transmission in Nigeria [MIM-AO-80180]

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Introduction: The main vectors of malaria in Africa belong to the Anopheles gambiae complex and the Anopheles funestus group. Both are groups of morphologically similar species containing both vectors and non-vectors. While information on species distribution of the An. gambiae complex is well documented, the species composition of the An. funestus group is still being determined for much of Africa. In this study, we determined members of the An. funestus group at 16 localities across Nigeria.

Methods: Mosquitoes sampling were carried out at 16 localities across five ecological zones: mangrove, forest, forest-transitional, guinea and Sudan savanna. Indoor resting anophelines were collected from human dwellings, supplemented with outdoor collections made from artificial pit shelters. Mosquitoes identified morphologically as members of the An. funestus group were analysed using a published multiplex PCR assay that can identify the five main species within the group. Specimens were tested for their blood meal source and for Plasmodium falciparum infection using ELISA.

Results: Of the 2240 specimens examined morphologically as belonging to the Anopheles funestus group, 1864 were positively identified by PCR. The analysis showed the presence of at least three species: Anopheles funestus Giles, Anopheles leesoni Evans and Anopheles rivulorum Leeson. An. funestus s.s. was the most predominant (>62%) occurring in more than 60% of the study localities with no apparent relationship to a particular ecological zone. An. funestus and An. leesonii were focal in their distribution predominantly in the
transitional ecotype. *An. funestus* was the only member of the group found positive for *Plasmodium falciparum* with an overall infection rate of 2.1% in the mangrove, 6.8% in the forest, 2.6% in the transitional, 1.8% in the guinea and 1.2% in the Sudan savanna ecotype.

**Interpretation:** *An. rivulorum* is the second most abundant species of the group and can be mistaken at its adult stage for the main vector: *An. funestus*. This emphasizes the need for correct species identification within a malaria vector control programme.

106A

**Behaviour and population dynamics of Anopheles gambiae s.l. in a malaria endemic area in southwestern Nigeria** [MIM-OO-35460]

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**Introduction:** *Anopheles gambiae* Giles and *Anopheles arabiensis* Patton are good vector of malaria parasites and known to coexist in most part of Africa. Here we report the population dynamics and their behaviour in a malaria endemic area where little information exist.

**Methods:** Mosquitoes were sampled in four villages in a forest area in southwestern Nigeria. Sampling was done monthly indoor and outdoor using standard mosquitos’ collection techniques from January to December 2004. Specimens were preserved on desiccated silica for both morphological and PCR identification.

**Results:** Of the 920 *Anopheles gambiae* group collected 490 (53.3%) were *An. gambiae* Giles while 430 (46.7%) were *An. arabiensis* Patton. The outdoor collections showed 60.2% of the overall collection to be *An. arabiensis* while daytime indoor resting collections were predominantly (>55%) *An. gambiae* s.s. The overall man-biting rate of *An. gambiae* s.s. was 17.5 bites/man/night as against 6.7 bites/man/night for *An. arabiensis*. *An. arabiensis* also accounted for >67% of the wet season collections while *An. gambiae* s.s. was predominant in the dry season (P < 0.01).

**Interpretation:** The differences in the density and behaviour of these species highlight the need for an integrated approach to malaria vector control where both species coexist.

107B

**Patterns and seasonality of malaria transmission in a rural endemic area in middle Ghana (Kintampo district)** [MIM-DD-12896]

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**Introduction:** Malaria is the most threatening public health disease in sub-Saharan Africa. Approximately 80–95% malaria cases and related deaths in the world are recorded in Africa. Most areas in sub-Saharan Africa receive over 300–800 infective bites/person/year. The pattern and seasonality of malaria transmission is an important indicator for control measures and drug or vaccine evaluations. The middle belt of Ghana has a forest-savannah ecological setting and a seasonal rainfall pattern.

**Methods:** The communities were divided into sixteen clusters. The CDC light trap catch (LTC) was used to collect mosquitoes in rooms of randomly selected compounds from the Kintampo demographic surveillance system (KDSS) data base. Traps were set weekly and occupants of any room with a light trap were given an untreated bed net to sleep in for that night. Anopheline vectors were morphologically identified into species. Heads and thoraces of the two major vectors of malaria, *Anopheles gambiae* and funestus, were checked for the presence of circumsporozoite (CS) antigens of *P. falciparum* using the sandwiched ELISA method. About 200 corresponding legs of *An. gambiae* species were also checked by PCR to identify the sibling species within the complex.

**Results:** A total of 664 LTCs captured 19835 mosquitoes. Anopheline vectors comprised 35.2% *funestus*, 10.6% *gambiae*, 1.8% *rufipes* and 0.1% *phaoranis*. Non-anophelines captured comprised 51.3% Culex and 1.0% Aedes species which were subsequently discarded. *An. funestus* (AF) and *An. gambiae* (Ag) had 1.5 and 4.7% proportions, respectively.
A total of 8,418 samples were assayed by CS-ELISA. An entomological inoculation rate (EIR) of 269ib/p/y was calculated in this area for the 1 year period of the study (November 2003–2004). EIR peaked during the wet months (April–November) with an average of 214ib/p/y. During the dry months (December–March) an average EIR of 54ib/p/y was sustained. Inoculation rates by specie by season reveals the following: Ag (81ib/p/y), Af (65ib/p/y) during the minor wet season (July–November) and Ag (45ib/p/y), Af (9ib/p/y) during the major wet season (March–June). The dry periods sustained an EIR of 13ib/p/y and 41ib/p/y for Af and Ag, respectively. An overall EIR by specie clearly shows An. gambiae (166ib/p/y) to have a high inoculation rate than that of An. funestus (102ib/p/y). A proportion of legs of An. gambiae are being taken through PCR to discriminate the dominant species within the complex.

**Interpretation:** An all year round malaria transmission was observed. The two major malaria vectors contribute at different times of the year to transmission. However, An. gambiae remains the main vector contributing immensely to transmission despite its low numbers.

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**Biting behaviour of malaria vectors in relation to community behavior in sleeping and usage of insecticides treated bed nets in Sudan [MIM-05-50127]**

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**Introduction:** Large-scale implementation and sustainable use of Insecticide Treated Nets (ITNs) needs to overcome several obstacles linked to human behaviour and vector biting behaviour. ITNs are more likely to affect late biters than early biters. People may be bitten before going under the bednet as many mosquitoes are active after sunset when people gather together to relax or watch television. The peak biting time of the local vector might be expected to be an important determinant of ITN effectiveness. Therefore, a study was carried out to investigate the association between the biting time of the malaria vector(s) and the sleeping hours of the community in Sudan.

**Methods:** A cross-sectional study was carried out in Sudan during September 2003 to March 2004 to study the association between the biting times of the malaria vector(s) and the sleeping times of communities of five different epidemiological sites. The biting cycle of Anopheles gambiae s.s. was studied using human landing collection during the period 19:00–07:00h (from sunrise to sunset); and the physiological age of the hourly-collected biters using parity was determined. The knowledge, attitudes and practices (KAP) of household members regarding sleeping habits; use of insecticide treated bednets (ITNs) and other protective measures were also evaluated.

**Results:** The peak biting cycle of An. gambiae s.l. was observed between 20:00–21:00 and 24:00–01:00. Although no significant hourly variation existed, hourly-dissected females showed a predominance of nulliparous females in age composition throughout the night (ranged from 1.5 to 4.0-folds). Excluding data from the Nyala region (4.9%), the parity rates in all the study regions ranged between 22.0 and 34.3%. Children under 5 years of age sleep before 22:00, in contrast to other age groups that sleep after 22:00. Cumulative distribution of parous biters (i.e. older which are probable to be infective) during exposure and bed times periods considering the sleeping habit of children under 5 years indicates that parous biters has decreased slightly from 60 to 40%. Usage and perception of bednets also differed significantly between the study areas, with a range of 20–89.3% of interviewed households using bednets regularly. Of the interviewed mothers, 52–88.9% agreed that bednets are protective measures against malaria. However, 44.3% of respondents said they were using nothing to protect themselves against malaria and only 14.5% said they were using ITNs.

**Interpretation:** Children under 5 years are probable to be protected by their habit of early sleeping in relation to other age groups in case they used ITNs. Other protective measures are necessary to protect late-sleeping individuals.
5. Drug discovery and development

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

109A Cajachalcone: The antiplasmodial compound identified in Cajanus cajan leaf extract [MIM-EA-18792]

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Introduction: Malaria, one of the diseases caused by protozoa is responsible for the high rate of morbidity and mortality in the developing world, especially in the tropical countries. It is estimated that malaria is the cause of the death of between 1.5 and 2.7 million people each year with renewed increase in parasite resistance. The search for new antimalarial drug is essential and requires identification of new biochemical targets for drug development and new chemical entities.

Methods: In the course of the identification and evaluation of potential antimalarial components from the Nigerian ethnobotany, Cassia siamea was selected for evaluation. The bioassay-guided fractionation of the crude methanol extract of C. cajan leaves was done in vitro using the multi resistant strain of Plasmodium falciparum (K1) in the parasite lactate dehydrogenase assay. Isolation of compound was achieved by a combination of chromatographic techniques while the structure of Cajachalcone was elucidated by spectroscopy.

Results: The ethyl acetate fraction was most active with an IC50 of 13.3 μg/ml, from which Cajachalcone: 2′,6′-dihydroxy-4′-methoxy chalcone with IC50 of 2.0 μg/ml was isolated and characterised.

Interpretation: The results have confirmed the ethnomedical use of C. cajan as an antimalarial plant. C. cajan could be a possible source for the discovery of antimalarial agents.

110B Antimalarial prenylated chalcones from the twigs of Dorstenia barteri var. subtriangularis [MIM-FB-41120]

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Introduction: In the search of new biologically active agents against the deadly malaria parasite, Plasmodium falciparum, a Cameroonian medicinal plant, Dorstenia barteri var. subtriangularis (Moraceae) was investigated.

Methods: Two new diprenylated chalcones bartericin A 1 and B 2 [1], and four known natural products, stipulin 3, 4-hydroxylonchocarpin 4, isobavachalcone 5 and kanzonol B 6 were isolated from the twigs of Dorstenia barteri var. subtriangularis (Moraceae) using chromatographic techniques. The structures of the purified compounds were elucidated by spectroscopic methods, mainly 1D and 2D-NMR spectroscopy. Drugs were evaluated in culture against strain W2 of P. falciparum.

Results: Compounds 1, 3 and 4 were found to have significant antiplasmodial activities in vitro, with relatively low IC50s (2.155, 5.137 and 3.360 μM, respectively).

Interpretation: The results achieved were found to be promising. Further investigations will be carry out to evaluate the toxicity and other pharmacological properties of the evaluated compounds.
In vitro screening of antimalarial activity of *Trigonella foenum-graecum* [MIM-PG-303240]

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**Introduction:** Malaria, one of the devastating parasitic infectious diseases remains uncontrolled to date due to lack of effective parasite, vector control strategies, spread of multidrug resistant strains of *Plasmodium* and adverse side effects of the existing antimalarial drugs. The need for alternative efficient antimalarial drugs has initiated intensive efforts for developing new drugs from indigenous plants. Here, an in vitro analysis of antimalarial activity of *Trigonella foenum-graecum* is reported.

**Methods:** Crude extracts of *Trigonella foenum-graecum* leaves were prepared with 50 and 80% ethanol followed by fractionation using hexane, ethanol, butanol, chloroform and ethyl acetate. All the fractions were subjected to screening tests to detect the presence of flavonoids, alkaloids, terpenoids, saponins, tannins, steroids and cardio amino glycosides. In vitro antimalarial assay of the crude extract and fractions was carried out using laboratory adapted chloroquine sensitive and resistant *P. falciparum* isolates. The assay followed was schizont maturation inhibition assay. From dose response curve, IC50 values were calculated from plot of the probability of chloroquine activity and logarithm of drug concentration by linear regression analysis.

**Results:** Screening tests revealed the presence of alkaloids and tannins in ethanol extracts, steroids and terpenoids in hexane extracts and saponins in water extracts, respectively. The ethanol fractions seemed to possess profound antimalarial activity with an IC50 value of 8.75 ± 0.35 g/ml and 10.25 ± 0.35 g/ml against chloroquine sensitive and resistant *P. falciparum* isolates. The assay followed was schizont maturation inhibition assay. From dose response curve, IC50 values were calculated from plot of the probability of chloroquine activity and logarithm of drug concentration by linear regression analysis. Results: Screening tests revealed the presence of alkaloids and tannins in ethanol extracts, steroids and terpenoids in hexane extracts and saponins in water extracts, respectively. The ethanol fractions seemed to possess profound antimalarial activity with an IC50 value of 8.75 ± 0.35 µg/ml and 10.25 ± 0.35 µg/ml against chloroquine sensitive and resistant *P. falciparum* isolates. The parasitaemia was found to decrease with increasing concentration of the extract reflecting an inhibitory activity on parasite replication. This may be indicative of a significant potential for isolating purer compound. Crude plant extracts that showed lower activity upon fractionation have yielded purer compounds with potent antimalarial activity. Being the first report on in vitro antimalarial effect of *Trigonella foenum-graecum* further studies can be carried out for the isolation of active principle and elucidation of chemical structure for exploring the possibility of using the component as oral/parenteral drug for treating malarial infection.

**Interpretation:** The results of the study are encouraging. Further studies on isolation of active principle, elucidation of chemical structure of active principle from ethanolic extracts can be carried out for possible drug discovery and use as antimalarial agent.
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gametocytes on days 0, 3, 7 and 14. Treatment emergent adverse events were recorded from clinical assessments and evaluation of blood parameters. Adults gave blood samples via an indwelling cannula for pharmacokinetic analysis.

Results: Between June 2003 and January 2005, 116 adults and 107 children with acute uncomplicated P. falciparum malaria were recruited from two sites; Blantyre, Malawi and Farafenni, The Gambia. Results will be available in May 2005, and data will be presented during this meeting. Due to the more frequent sampling strategy achievable in adults, the primary analysis will be performed on the adult dataset, with the child data analysed independently. Comparisons will be made between the four treatment groups by PC90 and parasite viability at 12 h. Additional summaries by treatment group of treatment failures, gametocyte prevalence, reduction in temperature and adverse event data will also be generated. The choice of artesunate dose to combine with the established dose of CPG-DDS will be determined primarily by the efficacy parameters PC90 and parasite viability, but will also take into consideration the safety signals and gametocyte data. Interpretation: The dose of artesunate chosen from this study to combine with CPG-DDS will be carried forward into the phase III programme for CDA, due to commence in the second half of 2005.

113B

In vitro activity of proteasome inhibitors against Plasmodium falciparum field isolates from Lambaréné, Gabon [MIM-AK-253950]

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Introduction: P. falciparum becomes resistant against an increasing number of antimalarials. Therefore, it is of great importance to develop new treatment strategies. Several inhibitors have been synthesized that block proteasome function efficiently and specifically. As the first inhibitors come into clinical use, we tested a panel of proteasome inhibitors in P. falciparum. Pro-teasomes are particularly important for the survival of Plasmodia due to structural features of plasmodial proteins. Here we show that epoxid-based compounds are highly active against growth of P. falciparum.

Methods: Blood samples (81) were collected from malaria patients in the Albert Schweitzer Hospital, Lambaréné, Gabon. Inclusion criteria were: parasitaemia of P. falciparum between 1000 and 120,000/μl, no intake of antimalarials the preceding month, age between 1 and 15 years and oral informed consent. Blood samples were used immediately to test drug sensitivity following standard protocols. Briefly, 96-well test plates were predosed with epoxomicin, MG132, lactacystin and bortezomib as well as with artesunate and chloroquine. Parasitaemia of culture was adjusted to 0.05% and hematocrit was set at 1.5%. Blood-medium mixture (200 μl) was added to each well of predosed test plates and incubated 37 C. After 72 h, plates were freeze-thawed twice and 100 μl of each well was transferred onto ELISA plates to determine parasite growth by measuring histidin-rich protein 2 (HRP2) levels.

Results: Eighty-one P. falciparum isolates were tested for their in vitro susceptibility to the proteasome inhibitors epoxomicin (n = 22), MG132 (n = 21), lactacystin (n = 11) and bortezomib (n = 12) as well as to the control drugs chloroquine (n = 43) and artesunate (n = 43). The epoxid-based inhibitor epoxomicin and the aldehyd-based inhibitor MG132 were the most active proteasome inhibitors against P. falciparum growth. Geometric mean IC50 (95% CI) of epoxomicin and MG132 was 8.1 nM (6.8–9.6 nM) and 33.5 nM (26.0–43.2 nM), respectively. In contrast, activity of lactacystin and bortezomib was very low. Mean IC50 of lactacystin was 689.9 nM (350.9–1356.4 nM). Borte-zomib, a proteasome inhibitor that is currently tested for treatment of neoplasia, had a mean IC50 of 442.1 nM (112.6–1735.7 nM). Mean IC50 of chloroquine and artesunate was 109.3 nM (89.6–133.3 nM) and 1.0 nM (0.7–1.4 nM), respectively. More than 90% of P. falciparum isolates were resistant to chloroquine. Cross-resistance of each proteasome inhibitor to artesunate and chloroquine was measured by pairwise correlation of log-transformed values. Neither epoxomicin, MG132, lactacystin or bortezomib versus artesunate nor versus chloroquine were significantly correlated.
Interpretation: Epoxid- and aldehyd-based proteasome inhibitors are highly active against growth of *P. falciparum*. Epoxid-based molecules are promising candidate compounds for the development of *P. falciparum* specific inhibitors.

**114C**

Caffeine enhances the solubility, partition coefficient, and total parasite clearance of Halofantrine

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**Introduction:** The aqueous solubility of halofantrine, a phenanthrene methanol anti-malarial agent widely used for the treatment of drug resistant *Plasmodium falciparum* malaria, is practically very low, and of concern as it exhibits an inconsistent and dose limiting oral bioavailability.

**Methods:** In this study, the effect of caffeine on the solubility of halofantrine has been investigated at physiologic temperature and various pH using the HPLC, unlike previous studies carried out at room temperature. The effect of caffeine on the partition coefficient was also investigated using the Leo Hansch method. The anti-malarial activity of halofantrine against five clinical isolates of *Plasmodium falciparium*, from children in the Ibadan community, was investigated using the WHO invitro micro-test method.

**Results:** Caffeine increased the solubility of halofantrine at all the pH values (1.2, 5.9, 7.4), with the highest increase recorded at pH=5.9, at which the solubility increased more than a thousand times (i.e from 0.1133 ± 0.025 mg/ml to 124.7 ± 6.9 mg/ml), just within 1 h. This increase is about two times higher than the increase observed at room temperature (483 times increase), within 72 h, at the same pH in a previous study. Caffeine also increased the partition coefficient of halofantrine at this pH (about twice), further amplifying the effect. Caffeine however, did not increase the mean potency of halofantrine (IC50 increased by 81 ± 7.2%) on the isolates tested, but seems to increase the total parasite clearance of halofantrine (MIC decreased by 48.1%).

**Interpretation:** Caffeine could be investigated and optimized, to see if the enhancement character on the solubility, partition coefficient, and parasite clearance could find useful application during the formulation of halofantrine products.

**115A**

Antiplasmodial activity of tiliroside isolated from *Croton lobatus* L. [MIM-LI-9744]

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**Introduction:** In a search for new plant-derived biologically active compounds against malaria parasites, tiliroside, a compound isolated from methanol extract of aerial part of *Croton lobatus* L. (Euphorbiaceae), a plant commonly used in Benin by traditional healers for the treatment of malaria, was tested for its antimalarial activity.

**Methods:** Quantitative assessment of in vitro antimalarial activity against the K1 resistant strain was determined by means of the microculture radioisotope technique based upon the method previously described by Desjardins et al. (1979) and modified by Ridley et al. (1996). The assay uses the uptake of [3H]hypoxanthine by parasites as an indicator of viability. Continuous in vitro cultures of asexual erythrocytic stages of *Plasmodium falciparum* were maintained following the methods of Trager and Jensen (1976).

**Results:** Analysis of methanol extract of aerial part by liquid chromatography, gel filtration and nuclear magnetic resonance identified tiliroside (kaempferol-3-O-(6″′-p-coumaroyl)-b-d-glucoside) as the active compound which had an IC50 value of 4.24 µg/ml.

**Interpretation:** This result shows that tiliroside may be a good lead against *P. falciparum* as antimalarial agent and also, support the traditional use of the plant for the treatment of malaria.
Assessing antimalarial efficacy in Africa: Longitudinal trials as a public health tool [MIM-ML-394772]

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Introduction: Disease and death caused by malaria in Africa are increasing. Drug resistance is one of the main factors in this disturbing trend. Efforts to devise effective strategies to deter the spread of drug-resistant malaria have not succeeded yet. Replacing ineffective regimens with effective ones requires knowing both the efficacy and longer-term health benefits of treatment regimens. Little is known about the medium and long-term efficacy and benefits of newer combination therapies.

Methods: The problems of defining and monitoring resistance and antimalarial drug treatment outcomes will be discussed in hopes of clarifying the issues and identifying ways to move forward. Strategies to improve measurement of resistance and treatment outcomes, collection and use of information on resistance, and potential approaches to deter and reduce the impact of resistance, will all be considered.

Results: The epidemiological setting and the goals of monitoring determine how antimalarial treatment responses should be measured. Efficacy studies using short-term clinical and parasitological response to a single treatment do not provide the information needed for policy decisions in Africa. Molecular surveys serve a limited role. Longitudinal studies, with incidence of uncomplicated malaria episodes as the primary endpoint and anemia and severe malaria as secondary endpoints, provide the best information on which to base treatment policy changes. Standard in vivo efficacy studies and molecular surveys are better suited for ongoing efficacy monitoring.

Interpretation: Different antimalarial treatment alternatives are appropriate in different settings and longitudinal trials will be important tools in the era of combination therapy.

Promising in vitro antimalarial activity of Annickia kummeriae, a rare Tanzanian medicinal plant [MIM-HM-83601]

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Introduction: In order to contribute to new drugs discovery for malaria, we investigated Annickia kummeriae, a traditional medicinal plant used in Tanzania for the treatment of malaria and convulsions in children.

Methods: Crude extracts and some fractions of A. kummeriae leaves, stem and root-barks were tested for their antimalarial activity against the multi-drug resistant P. falciparum K1 strain in vitro using the radioactive method of Dejardins et al; whereas the In vitro cytotoxicity level against mammalian cell lines were determined using the Alamar blue assay.

Results: The crude methanolic extracts exhibited the highest antimalarial activity with IC50 values from 0.14 to 0.36 μg/ml. Crude dichloromethane extracts shown high activity with IC50 values 0.30–0.36 μg/ml, whereas, the petroleum spirit crude extracts shown moderate activity with IC50 values ranging from 1.85 to 4.65 μg/ml. All of the crude extracts exhibited very low cytotoxicities against mammalian cell lines in vitro with IC50 values ranging from 10.5 to 72.0 μg/ml with favourable selectivity indexes (SI) which ranged from 10.5 to 187.7 μg/ml. Vacuum liquid chromatographic fractionation of the methanolic extract of the stem bark resulted to nine antimalarial fractions with IC50 values ranging from 0.09 to 3.8 μg/ml against the multi-drug resistant P. falciparum strain K1 in vitro with more safe cytotoxicity IC50 values ranging in between 8.9 and 90 μg/ml (SI, 24.0 to 775.5 μg/ml).

Interpretation: This is the first report of in vitro antimalarial activity of this plant. The observed high anti-
malarial activity of the crude extracts and their fractions as well as the very low cytotoxicity level exhibited, render these extracts and fractions to be candidates for the isolation of compounds which could develop into new lead structures for drug development programs against drug resistant malaria.

118A
Antiplasmodial agents from the leaves of *Glossocalyx brevipes* [MIM-JM-260015]
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Introduction: *G. brevipes* Benth (Monimiaceae) is a shrub with hairy stems and leaves, growing in the humid rain forests of West and Central Africa. In Cameroon, macerated leaves are added to antifever preparations. In our continuous search for new antimalarial agents from plant sources, CH$_2$Cl$_2$/MeOH (1:1) extract of the leaves of *G. brevipes* that revealed moderate antiplasmodial activity in vitro was investigated.

Methods: The air-dried and powdered leaves of *G. brevipes* were macerated in a mixture of CH$_2$Cl$_2$/MeOH. The extract was partitioned for alkaloids. Vacuum liquid chromatography of the neutral portion (150 g) on SiO$_2$ using a gradient of EtOAc in hexane gave 60 fractions (500 mL each) which were combined on the basis of TLC profiles. Chlorophyll was removed from each group by gel permeation through Sephadex LH-20 (CH$_2$Cl$_2$/MeOH [8:2]). Purification was done on SiO$_2$ or Sephadex LH-20, leading to five compounds which were characterized by 1D and 2D NMR and other analytical techniques. The in vitro antimalarial assays were performed (at Walter Reed Army Institute) using the semi-automated microlodilution technique described by Desjardins et al.

Results: Judicious analysis of NMR and other analytical data enabled us to characterize three new homogentisic acid derivatives and identify two known alkaloids. Below are names, physical constants and antiplasmodial activities of the compounds isolated. D-6 is the chloroquine susceptible strain while W-2 is the chloroquine resistant strain in brackets. Methyl 2-((1′b-geranyl-5′b-hydroxy-2′-oxocyclohex-3′-enyl) acetate (1): colourless oil; MF C$_{19}$H$_{28}$O$_4$ (320); Antiplasmodial activity: 2.20 M [for D-6] (6.64 M [for W-2]). 2-(1′b-Geranyl-5′b-hydroxy-2′-oxocyclohex-3′-enyl) acetic acid (2): colourless oil; MF C$_{18}$H$_{26}$O$_4$ (306); Antiplasmodial activity: 4.78 M [for D-6] (8.34 M). Methyl 2-((1′b-geranyl-5′b-hydroxy-4′b-methoxy-2′-oxocyclohexyl) acetate (3): colourless oil; MF C$_{20}$H$_{32}$O$_5$ (352); antiplasmodial activity: (not tested). Liriodenine (4): yellow crystals, mp 275–276°C; MF C$_{17}$H$_9$O$_3$N (275); antiplasmodial activity: 4.82 M (8.63 M). Aristololactam BII (5): yellow crystals, mp 256–257°C; MF C$_{17}$H$_{13}$O$_3$N (279); inactive. Standards: Chloroquine 0.02 M [for D-6] (0.54 M) and Mefloquine 0.05 M (0.02 M).

Interpretation: Conclusion: Compound 1 showed strong antimalarial activity while 2 and 4 revealed moderate activity. These active compounds confirm the use of *G. brevipes* in antifever preparations.

119B
Antiplasmodial activity and toxicity of extracts and products from selected medicinal plants used in Cameroon [MIM-MN-187588]
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Introduction: The emergence and spread of drug resistant malaria has stimulated the search for new drug leads for this devastating disease. To identify new leads, thirty-nine extracts from twenty-one medicinal plants and four pure compounds isolated from *Turreanthus*...
**Introduction:** In the search for anti-infective agents from natural sources, we have collected and identified plant materials used in West and Central African ethnomedicine in the treatment of infectious diseases including fevers and resistant malaria. The present study investigates the antimalarial activity of 1200 plant extracts belonging to 80 plant families and 253 species, aiming at identifying the most effective plant extracts and new natural product compounds as potential antimalarial agents.

**Methods:** Plant materials used in Nigerian and Cameroonian ethnomedicine in the treatment of malaria were identified and collected. Extracts of these plants were tested for in vitro antimalarial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The technique measures the ability of the extracts to inhibit the incorporation of [G-3H] hypoxanthine into the malaria parasites.

**Results:** Our results indicate that several medicinal plants used in traditional medicine against fever and/or malaria revealed strong antiplasmodial activity in vitro: Of the 1200 plant extract samples from 80 plant families tested for antiplasmodial activity, 53% showed remarkable activity. Of the active species 25% were tested against *P. falciparum* strains for the first time. The assays indicate that extracts from members of the families Annonaceae, Apocynaceae, Fabaceae, Simaroubaceae, Zingiberaceae, Monimiaceae and Euphorbiaceae inhibited the growth of CQ-sensitive (D6) and CQ–resistant (W2) clones of *Plasmodium falciparum*. The most active extracts on both strains were from 17 plant species with IC50 < 30 μg/mL. Phytochemical analysis of these plants revealed the presence of different classes of secondary metabolites. The diverse antimalarial compounds isolated from two Afromomum spp., *Araliopsis tabouensis*, *Enantia chlorantha*, *Glossocalyx brevipes*, *Hyptis suaveolens*, *Milletia griffoniana*, *Morinda lucida*, *Odyendyea gabonensis*, *Penanthes longifolius*, *Picralima nitida*, *Renelminia cincinnata*, *Spaethodea campanulata* and *Xymalos monospora* will be discussed in relation to their uses in African traditional medicine.

**Interpretation:** These results provide evidence that some of the plants might indeed be potential sources of new antimalarial agents, effective against chloroquine-resistant *Plasmodium falciparum*. 

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**120C**

**In vitro antimalarial activity of plants used in traditional medicine in west and central Africa [MIM-CO-512765]**


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**Introduction:** In the search for anti-infective agents from natural sources, we have collected and identified plant materials used in West and Central African ethnomedicine in the treatment of infectious diseases including fevers and resistant malaria. The present study investigates the antimalarial activity of 1200 plant extracts belonging to 80 plant families and 253 species, aiming at identifying the most effective plant extracts and new natural product compounds as potential antimalarial agents.

**Methods:** Plant materials used in Nigerian and Cameroonian ethnomedicine in the treatment of malaria were identified and collected. Extracts of these plants were tested for in vitro antimalarial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. The technique measures the ability of the extracts to inhibit the incorporation of [G-3H] hypoxanthine into the malaria parasites.

**Results:** Our results indicate that several medicinal plants used in traditional medicine against fever and/or malaria revealed strong antiplasmodial activity in vitro: Of the 1200 plant extract samples from 80 plant families tested for antiplasmodial activity, 53% showed remarkable activity. Of the active species 25% were tested against *P. falciparum* strains for the first time. The assays indicate that extracts from members of the families Annonaceae, Apocynaceae, Fabaceae, Simaroubaceae, Zingiberaceae, Monimiaceae and Euphorbiaceae inhibited the growth of CQ-sensitive (D6) and CQ–resistant (W2) clones of *Plasmodium falciparum*. The most active extracts on both strains were from 17 plant species with IC50 < 30 μg/mL. Phytochemical analysis of these plants revealed the presence of different classes of secondary metabolites. The diverse antimalarial compounds isolated from two Afromomum spp., *Araliopsis tabouensis*, *Enantia chlorantha*, *Glossocalyx brevipes*, *Hyptis suaveolens*, *Milletia griffoniana*, *Morinda lucida*, *Odyendyea gabonensis*, *Penanthes longifolius*, *Picralima nitida*, *Renelminia cincinnata*, *Spaethodea campanulata* and *Xymalos monospora* will be discussed in relation to their uses in African traditional medicine.

**Interpretation:** These results provide evidence that some of the plants might indeed be potential sources of new antimalarial agents, effective against chloroquine-resistant *Plasmodium falciparum*. 

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121A
In vivo antimalarial activity of methanol extract of Adansonia digitata bark in a mice model [MIM-OO-4536]

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Introduction: Malaria is one of the highest cause of morbidity and mortality in the developing world. Due to an increase in parasite resistance, there is a need to develop newer agents for the treatment of malaria or novel chemical structures with different mechanism of action from existing antimalarial drugs. The plant Adansonia digitata commonly known as baobab tree, its ethnobotanical use as antimalarial drug is well known throughout the West Africa region.

Methods: Chloroquine sensitive Plasmodium berghei berghei parasite NK65 clones was used to assess the in vivo antimalarial activity of the methanol extract of A. digitata in male albino mice, in a 4 day suppressive test. Animals were inoculated intraperitoneally with infected blood suspension (0.2 ml) containing 106 parasitized erythrocytes on day zero (D0). Different doses (100 mg–700 mg/kg per day) of extract were administered orally to the mice. Drug and normal saline 0.9% were used as control. The administration of drugs took place from day 0 to day 3. On day 4, blood smears were made from the tail of the mouse and percentage parasitemia assessed to find the percentage inhibition of the extract.

Results: The methanol extract of A. digitata at 100 and 700 mg/kg gave 34.8 and 49.2% suppression of parasitaemia, respectively. The administration of drugs took place from day 0 to day 3. On day 4, blood smears were made from the tail of the mice and percentage parasitemia assessed to find the percentage inhibition of the extract. The methanol extract of A. digitata at 100 and 700 mg/kg gave 34.8 and 49.2% suppression of parasitaemia, respectively. The administration of drugs took place from day 0 to day 3. On day 4, blood smears were made from the tail of the mice and percentage parasitemia assessed to find the percentage inhibition of the extract.

Interpretation: The results of the antimalarial test indicated that A. digitata bark extract had little but significant inhibitory activity on P. berghei berghei in vivo.

122B
Lead antiplasmodial substances from some Cameroonian medicinal plants [MIM-PT-34914]

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Introduction: Natural products drug discovery has proven productive in the identification of lead antimalarial substances. The inventory and collection of plants used traditionally to treat malaria led to selection of 122 species, and after preliminary biotests, extracts from 15 of them displayed significant activity. Araliopsis tabuensis, Odyendyea gabunensis and Penianthus longifolius were the most active, some of the isolates displayed activities close to those of the reference compounds.

Methods: Plant materials were identified by botanists and collections were carried out in a sustainable manner. Dried powdered plant material was subjected to different extraction techniques. The extracts were evaluated in the antimalarial screen applying continuous in vitro cultures of asexual erythrocyte stages of two Plasmodium falciparum strains (D-6, chloroquine-sensitive, from Ghana and W-2, chloroquine-resistant, from Indochina). Hypoxantine was used as index of inhibition of parasite growth. Chloroquine, mefloquine and artemisinin were used as reference molecules. A variety of chromatographic methods were applied for antimalodial-guided separation of compounds and the structures were elucidated using modern spectroscopic techniques.

Results: Extracts from 15 plants that were screened positive against D6 and W2 strains with IC50 ranging between 101.54 and 4808.19 ng/mL. Antiplasmodial-guided fractionation of the most active extracts gave the following results: The stem bark of A. tabuensis (IC50 895.65 and 1042.1 ng/mL for the D6 and W-2 strains, respectively) yielded 13 alkaloids of which araliopdimerine-A was the most active with IC50 values of 34.1 ng/mL and 17.4 ng/mL for the D6 and W-2, respectively. From the stem bark of O. gabunensis (IC50 111.9917 (D6) and 101.5394(W2) ng/mL), three indole alkaloids with modest activity and one quasi-
Aralipdimerine could be a candidate for antimalarial drug development.

Interpretation: These results justify the use of the plants in traditional medicine to cure malaria. Alanthinone could be a candidate for antimalarial drug development. Aralipdimerine A. palmatine and jatrorrizine can also be seen as antiplasmodial leas.

123C Evaluation of some Benin medicinal plants for their in vitro antiparasitic activity against 3D7, W2 and K1 Plasmodium falciparum strains [MIM-AT-911544]

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Introduction: The crisis of drug resistance in malaria increases the need of new drugs discovery. In this investigation, twelve extracts from fourteen native plant species were evaluated for their antimalarial activity in vitro, by assessing their ability to inhibit the growth of Plasmodium falciparum’s chloroquine resistant strains W2 and K1 and chloroquine sensitive strain 3D7.

Methods: Air dried plant material of each species (A. mexicana, C. rotundifolia, P. amarus, T. geniculata) was ground and 5 g of the powder was used to obtain the different extracts. This extracts are tested for their ability to inhibit the growth of P. falciparum from ring stage to schizont stage in continuous cultures. Initial concentration of each plant extract was 128 μg/ml diluted with two-fold dilutions to make 8 concentrations, the lowest was 1 μg/ml. After 24–36 h of incubation of the parasites with each dilution at 37°C in a candle jar, thin blood films were made and stained with Diff-Quick Stain. The number of schizonts was counted in each well and IC50s are determined graphically.

Results: Strain 3D7: The highest antiparasitic activity against 3D7 strain was found for the ethanolic extract of An. mexicana (IC50 = 3.15 μg/ml). Seven other extracts showed good antiparasitic activity against the chloroquine sensitive strain 3D7 (IC50 < 10 μg/ml). The methylene chloride extract of P. amarus and the methanolic extract of T. geniculata showed mild antiparasitic activity: respectively, 14.53 and 19.16 μg/ml (10 μg/ml < IC50 < 20 μg/ml). Strain K1: the methylene chloride extract of A. mexicana had a IC50 of 3.12 μg/ml only two other extracts (methanolic extract of A. mexicana and ethanolic of C. rotundifolia) showed good antiparasitic activities against the chloroquine resistant strain K1 (IC50 < 10 μg/ml). The ethanolic, methanolic and methylene chloride extracts of C. rotundifolia and the methanol extract of P. amarus, showed mild antiparasitic activity against the chloroquine resistant strain K1 with, respectively, 18.69, 11.11, 15.94, 16.45 and 19.16 μg/ml as IC50s (10 μg/ml < IC50 < 20 μg/ml). Strain W2: The ethanolic extract of An. mexicana showed the highest activity against W2 strain. All the other extracts showed low or no activity against this strain. (IC50 > 20 μg/ml).

Interpretation: The four species investigated in this study, showed high in vitro activities with one or more extracts against at least one of the three strains (IC50 < 5 μg/ml). More biochemical investigations on these plants may probably conduct to new drugs.


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Introduction: Depuis vingt ans les études de chimiosensibilité en vitro de Plasmodium falciparum, sont
réalisées par radioactivité. Ce test a permis une standardisation et le screening de nombreuses molécules. Nous proposons ici une nouvelle stratégie de mesure du développement parasitaire, orientée vers cette phase de re-invasion. Le but de cette étude est d’évaluer la faisabilité de cette méthode lors de l’utilisation de souches sauvages, présentant des parasitomés et des taux de leucocytes variables.

Methods: Cette méthode propose d’utiliser un marquage des acides nucléiques pour quantifier les parasites et identifier le stade. L’utilisation du thiazole orange et de l’hydroethidine, permet de séparer les différents stades du parasite. La nécessité de passer rapidement un grand nombre d’échantillons (et de dilutions) et d’analyser les données manuellement, rendait fastidieux l’utilisation du cytometre. Les derniers cytomètres, comme ceux du Facscalibur BD, permettent de concevoir des procédures automatiques, notament grâce au passeur de plaques 96 puits. Nous avons fait un test isotopique parallèle. Les parasites ont été cultivés en présence de concentrations croissantes de Chloroquine et les concentrations inhibitrices 50 ont été comparées.

Results: L’incorporation d’Hypoxanthine tritiée, se fait pendant le stade trophozoïte du parasite, qui est également la cible de nombreux médicaments actuellement utilisés. Cependant il génère des déchets radioactifs, mal pris en compte dans les pays du Sud et explore plus difficilement d’autres mécanismes d’action des molécules, comme l’inhibition de re-invasion. Le double marquage thiazole orange (marqueurs des cellules vivantes ou mortes)—hydroéthidine (intercalant des acides nucléiques) a permis grâce au passeur de plaques du cytomètre Facscalibur BD de différencier et de quantifier les stades. Le logiciel de pilotage assure une adaptation automatique des fenêtres d’analyse et la génération de statistiques. Une automatisation est donc possible assurant en même temps une meilleure reproductibilité des données. Nous avons utilisé cette procédure parallèlement au test isotopique sur 48 h, dont le protocole comprend un lavage des hématies et le retrait du buffy coat. La souche Palo Alto (HB3) a été utilisée comme référence. Pour étudier l’impact des leucocytes et des réticulocytes sur l’IC50 calculée, les parasites ont été mélangés à des échantillons de sang contenant des quantités croissantes de leucocytes.

Interpretation: Cette étude montre la très bonne corrélation des IC50 calculés pour la chloroquine. C’est une méthode de recherche particulièrement adaptée à l’étude des nouvelles molécules agissant sur des stades variés du parasite.

125B
Antiplasmodial agents from Xylopia parviflora seeds

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Introduction: ‘X. parviflora’ (Annonaceae) is a shrub growing in the savanna region of West Cameroon. Its seeds are abundantly used as a condiment and in antifever preparations. Six diterpenes isolated from the seeds showed antiplasmodial activity.

Methods: The air-dried, finely ground seeds were macerated in methanol. Chromatographic separation and purification led to six diterpenes, whose structures were elucidated with the help of 1D and 2D NMR and other spectroscopic and physical methods. Antiplasmodial bioassays were carried out with the six pure compounds.

Results: Spectroscopic analysis showed that all these diterpenes are known and of the kaurane series, and identified as ent-kaur-16-en-19-oic acid (1), ent-kaur-16-ol (2), ent-15β-acetoxy-kaur-16-en-19-oic acid (3), ent-14-hydroxy-kauran-19-oic acid (4), ent-15-hydroxy-kauran-19-oic acid (5) and ent-15-hydroxy-kauran-19-oic acid (6). IC50 indicated in vitro activity varying from 12.0 to 23.0 μg/mL on chloroquine-sensitive (HB3) clone, and between 7.0 and 19.0 μg/mL on chloroquine-resistant (Dd2) clone. Cytotoxic tests revealed very low cytotoxicity (CC50 of 21.8 to >50 μg/mL or CC10< of 18.0–40.8 μg/mL).

Interpretation: In vivo tests have to be performed to confirm the use of this plant in antifever preparations.
Parasitoses intestinales et morbidité palustre en milieu côtier lagunaire au Bénin, Afrique de l’Ouest

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Introduction: Au Bénin, l’affection représente la première cause de consultation dans les formations sanitaires. Le groupe cible est représenté par les femmes enceintes et les enfants de moins de 5 ans. Chez ces derniers, les parasitoses intestinales constituent également une menace permanente. Nous avons voulu vérifier dans cette étude si les parasitoses intestinales n’influencent pas la morbidité palustre chez les enfants.


Results: La prévalence des parasitoses intestinales dues aux helminthes est de 13.5%. L’infestation est plus fréquente chez les enfants de sexe masculin (18.1%) que ceux de sexe féminin (10.0%). Chez les enfants por-

teurs de parasitisme intestinal, l’indice plasmodique est de 56.5%. Cet indice représente 39% chez les enfants non-porteurs de parasitisme.

Interpretation: Les parasitoses intestinales semblent favoriser le paludisme infestation en milieu côtier lagunaire. Il ne s’agit cependant que d’une tendance à confirmer sur l’analyse des résultats de dépistage actif d’accès palustre.
heterodimerization partner and homolog), and BID (apoptotic death agonist) in infected murine brain. Western blot and qRT-PCR analyses showed significantly up-regulated expression of Caspase-3 protein and mRNA in infected murine brain, and postmortem human CM brain. In particular, the maximal Caspase-3 expression was in the cerebellum. The proportion of TUNEL-positive cells in infected mice was significantly higher (>20% of apoptotic cells), than in controls (0–3%). Apoptotic cells were observed mainly in the granule neuron regions of the cerebellum, but not in the Purkinje cell regions. There was significantly less apoptosis in brainstem and cerebrum at peak parasitemia. The brain sections of uninfected mice displayed only few apoptotic cells (0–3%), consistent with normal cell-cycle events. P. berghei-infected erythrocyte (IE)-conditioned medium and infected mouse plasma induced significantly more apoptosis in HBVEC (>10% at a minimum threshold of 0.005 μL conditioned medium/ml with a LD50 at approximately 5.0 μL/ml), than controls exposed to unconditioned medium or uninfected mouse plasma.

Interpretation: Our findings suggest that parasite-derived apoptotic factors may mediate apoptosis in the cellular components of BBB in CM. Further proteomic and functional studies to identify these factors and associated signal transduction pathway are in progress.

128B Decreased production of macrophage migration inhibitory factor (MIF) is associated with susceptibility to severe malaria in African children [MIM-472625]


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Introduction: One of the most lethal complications of Plasmodium falciparum malaria is severe anemia, yet the mechanisms and molecular mediators involved in the pathogenesis are largely undefined. Studies in murine models of malaria have suggested that increased levels of macrophage migration inhibitory factor (MIF) contribute to the development of malarial anemia (MA) by suppressing erythropoiesis. The aim of this study was to investigate the role of MIF in the pathogenesis of P. falciparum MA in children.

Methods: Plasma MIF levels were examined by ELISA in children (age 3 years, n = 125) presenting at the Siaya District Hospital with acute P. falciparum malaria with varying degrees of anemia. Children with cerebral malaria were not included in the study. Parasitemia was determined using Giemsa-stained blood smears and complete hematological measures were determined by a Beckman Coulter Counter. Children with malaria were classified into uncomplicated malaria (UM; hemoglobin, Hb < 11.0 g/dL), mild MA (MlMA; 8.0 < Hb < 11.0 g/dL), moderate MA (MdMA; 6.0 < Hb < 8.0 g/dL) and severe MA (SMA; Hb < 6.0 g/dL) based on Hb only. Healthy, parasite-free, age-matched controls (HC; Hb < 11.0 g/dL) were recruited from the childhood vaccine program at the outpatient clinic.

Results: There were no statistically significant differences in age, gender, and parasitemia among the groups. Plasma levels of MIF were non-significantly decreased in the MlMA and MdMA groups, and significantly decreased in the SMA group (p < 0.05) relative to the HC and UM groups. Circulating MIF levels were non-significantly decreased in the SMA group versus the MlMA and MdMA groups. In contrast to findings in murine models of malaria, MIF was positively associated with Hb concentrations (r = 0.2, p = 0.04), but non-significantly correlated with red blood cell counts (r = 0.1, p = 0.12). Categorization of the malaria-infected children according to parasite density revealed that plasma MIF levels were non-significantly lower in children with low density parasitemia (LDP; < 10,000 parasites/μL) and significantly lower in children with high density parasitemia (HDP; > 100,000) compared to the HC group (p < 0.05). There were no significant differences in MIF levels between the HDP and LDP groups. Furthermore, MIF had no significant correlation with parasitemia (r = −0.1, p = 0.27). Taken together, our data suggest a protective role for MIF against severe anemia.

Interpretation: Decreased MIF production is associated with increased malaria disease severity, suggest-
ing that elevated MIF production may be required for effective control of parasitemia and protection against development of SMA.

129C
Regulatory T cells limit the cellular response to CS and TRAP encoding candidate malaria vaccines in humans [MIM-SD-8756]
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Introduction: Regulatory T cells (Treg) have been shown to be essential for controlling immune responses and preventing self reactivity. Treg also play a role in limiting the immune response to infectious diseases. Although there is some evidence for Treg involvement in malaria immunity their full role remains to be elucidated. Human vaccination trials provide an opportunity to study both effector and regulatory immune responses to malarial antigens before and after the intervention.

Methods: We investigated the contribution of Treg to immune responses in malaria-naive volunteers receiving a series of prime-boost immunisation regimens with either the circumsporozoite (CS) protein or thrombospondin-related adhesion protein (TRAP). Gene expression of a panel of key cytokines and immune markers was measured by real time RT-PCR in antigen-stimulated PBMCs from several timepoints. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is constitutively expressed on Treg and is likely to play a role in Treg-mediated suppression. The effect of CTLA-4 (CD152) blocking antibodies on immunogenicity was measured by ex vivo interferon-gamma ELISPOT and real time RT-PCR.

Results: The expression of FoxP3, a transcription factor specifically expressed by Treg cells, was found to be upregulated in some subjects following vaccination. Addition of antibodies to CTLA-4 (CD152) to the cells at the time of antigen stimulation enhanced immunogenicity and resulted in altered gene expression.

Interpretation: Our findings provide evidence that immune responses to vaccination against malaria may be attenuated by Treg. Further elucidation of this suppressive response may promote strategies to improve suboptimal anti-malarial immunity.

130A
P. falciparum malaria in Senegal: Numbers of msp2 genotypes are related to the antibody response to MSP2 [MIM-FM-144963]
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Introduction: Host genetic factors and specific antibody response may modulate the presence of distinct parasite populations. It has been suggested that asymptomatic and especially polyclonal P. falciparum infections protect against disease caused by a new infection. We determined the multiplicity of P. falciparum infections in asymptomatic Senegalese children and related it to clinical, parasitological, host genetic and immunological data.

Methods: A cross-sectional study was carried out in a rural area of Senegal among 413 children aged from 2 to 10 years, who were asymptomatic for malaria. Venous blood was drawn in June 2002 before the annual malaria transmission season. ABO blood groups were determined by serology, and sickle-cell trait, G6PD deficiency (G6PD A-variant) and alpha-thalassemia were determined by molecular genotyping. Isolates from P. falciparum carriers were genotyped by nested-PCR of the msp2 block 3 using primers specific for the 3D7 and FC27 allelic families. Plasma IgG and IgG subclasses directed to recombinant proteins from the two serogroups of the Merozoite Surface Protein 2 (MSP2/3D7 and MSP2/FC27) were determined by ELISA.

Results: Forty-four per cent of children harboured P. falciparum parasites. Of those, 36% harboured parasites from the 3D7 msp2 allelic family only, 25% parasites from the FC27 family only and 39% clones from both allelic families. The multiplicity of infection (MOI) was 2.5, 38% of samples containing a single genotype. Neither age nor red blood cell polymorphisms were related to MOI. But MOI was positively associated with parasite density ($P < 0.0001$) and tended to be higher among children who did not suf-
fer from a malaria attack during the following malaria transmission season ($P = 0.12$). Children harbouring clones from one allelic family (either 3D7 or FC27) presented higher levels of IgG, IgG1 and IgG3 against recombinant protein from the same allelic family than the carriers of clones from the other allelic family (all $P < 0.01$).

**Interpretation:** Red cell polymorphisms had no effect on MOI. Cytophilic antibodies against MSP2 proteins resulted from the asymptomatic persistence of polyclonal infections during dry season. Such antibodies had a limiting but not a neutralizing effect on parasites.

**131B**

**Effects of Plasmodium species clearance on the cd4 count and viral load of hiv infected individuals in benin city, edo state, Nigeria [MIM-TM-212415]**

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**Introduction:** The association between HIV and malaria has important implications. Malaria and HIV are two of the commonest infections in sub-Saharan Africa and, to a lesser extent, in other developing countries. It is estimated that 29.4 million Africans are infected with HIV, whereas at least 500 million suffer from malaria each year. Therefore, any interaction between these two infections will be of major public health significance.

**Methods:** Twenty-one confirmed HIV positive infected individuals being treated for malaria with Dihydroartemisinin (Cotecxin) between the months of December 2004 and February 2005 were enrolled for the study, the subjects had not started the any antiretroviral therapy. Five millilitres of venous blood was collected from the subjects, thick and thin blood films were made and stained with Giemsa stained for parasites identification, count and speciation. The CD4 count and viral load of the subjects were assessed using Dynabeads T4-T8 Quantification Protocol (Dynal Biotech) and Amplicor HIV-1 Monitor Test, respectively (Roche) following the manufacturer’s instructions.

**Results:** Of the 21 patients studied, 17 (80.9%) were infected with *Plasmodium falciparum*, 4 (19.1%) suffered from *Plasmodium malariae* infection. No cases of *Plasmodium vivax* or *Plasmodium ovale* infection were recorded among the subjects. Before drug administration, patients with a CD4 count lesser than 200 cells/ml had a mean CD4 count of 180 cells/ml, a mean viral Load of 3.05 HIV-1 RNA log copies/ml and a mean parasitemia level of 17,500 parasites/ml of blood. Two days after completion of the drug dosage, the mean CD4 count indicated 110 cells/ml, while that of viral load was 3.85 HIV-1 RNA log copies/ml and the mean parasitemia level was 1500 parasites/ml of blood. On the other hand, patients with CD4 count above 200 cells/ml had a mean CD4 count of 290 cells/ml, a mean viral load of 2.65 HIV-1 RNA log copies/ml and a mean parasitemia level of 8500 parasites/ml of blood before drug administration. When reassessed 2 days after the complete drug dosage, the mean CD4 was 300 cells/ml, the mean viral load was 2.85 HIV-1 RNA log copies/ml and the mean parasitemia level was 500 parasites/ml of blood.

**Interpretation:** This study revealed that HIV patients with lower CD4 count recorded a higher parasitaemia compared to those with higher CD4 count. This could contribute to the high viral replication observed in such patients.

**132C**

**CD1 gene polymorphism is associated with resistance to Plasmodium falciparum malaria in Gabonese schoolchildren [MIM-LM-224910]**

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Introduction: Malarial toxins synthesized by the asexual intraerythrocytic stages of *Plasmodium falciparum* are defined as glycolipids that the predominant class is the glycosylphosphatidylinositols (GPIs). The possible ability for CD1 molecules to bind and present GPI antigens from *Plasmodium* parasites, lead to investigate whether CD1 genes polymorphism could be related to both asymptomatic and mild *P. falciparum* infection.

Methods: In order to analyse associations between CD1A and E gene polymorphisms and asymptomatic *P. falciparum* infection, a randomly group of 85 schoolchildren of village of Dienga (South Eastern Gabon) was followed clinically during 4 months (February–May 1995). In order to study CD1A and E polymorphisms with mild *P. falciparum* infection, 85 schoolchildren were also recruited in the same village and followed from February 1995 to March 1996.

Results: Statistical analysis failed to reveal any association between the CD1A and CD1E polymorphisms and the prevalence of asymptomatic infection, even if the number of patients with asymptomatic infection is higher in the group homozygous for the CD1E 02 allele than in the non-homozygous one. We found that CD1E 02 homozygosity is associated with low frequency of malarial attacks ($P = 0.015$, Chi-square test). In addition, the distribution of CD1 genotypes in children with 0, 1 and more than 1 malarial attacks, shows strong association between the CD1E 02 allele at homozygous state and resistance to multiple malarial attacks ($P < 0.005$, Chi-square test). The CD1A 01 allele also showed, albeit weak, an association with small mean number of malarial attacks.

Interpretation: The latter association may be due to its strong linkage disequilibrium with the CD1E 02 allele. Our results suggest that CD1E molecules are involved in antimalarial response eventually through a cell surface expression.

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I33A

Parasitological clearance following sulfadoxine–pyrimethamine (SP) treatment of infections with SP-resistant *P. falciparum* [MIM-SM-21248]

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Introduction: Resistance to the first-line malaria drug, sulfadoxine–pyrimethamine (SP), is widespread in Tanzania, and has been linked to mutations in the dihydrofolate reductase (dhfr) and dihydropteroate synthetase (dhps) genes of the parasite. However, in a low transmission area in northeast Tanzania, we observed a high proportion of successful SP-treatment cases, despite the parasites with mutations in these genes. We investigated the involvement of immunity in clearance of these SP resistant infections.

Methods: The study was carried out in two clinics in lower Moshi, Tanzania in 2003 and 2004. Patients of all ages presenting with malaria symptoms and blood films with >1000 parasites/ml of blood and treated with SP were enrolled and assessed after 7, 14 and 28 days. Parasites from blood samples on presentation were genotyped at loci associated with resistance to SP in the dhfr and dhps genes. Serum antibody levels to MSP-119, MSP-2, GPI and AMA-1 were evaluated in ELISA. Data analysis was conducted for serological predictors of parasitological cure. Treatment failure was defined as a single parasite in any follow-up blood film. Resistant infections were defined as those with both triple mutations in dhfr and double mutations in dhps genes.

Results: Of 227 individuals treated with SP who met inclusion criteria, 47 (20.7%) had parasites on any of the follow-up days – the majority of these were on day 14 (8.8%). Treatment failure was higher in the lower age groups ($\chi^2$ for trend 3.4, $P = 0.06$). In samples tested to date antibody responses to MSP-119 (o.d. >0.5) were correlated with successfully resolving...
infection ($\chi^2=5.6$, $P=0.02$). Of the samples typed to date 55.46% (66 of 119) had parasites with both triple mutations dhfr and double mutations in dhps genes. Of these 77.27% (51 of 66) successfully resolved infection after treatment with SP. Antibody response to MSP-119 was higher in those with the five mutations who cleared parasites than those who failed treatment though not significantly so (48% versus 23%; $p=0.1$). Data for antibody responses to GPI, MSP-2 and AMA-1 showed no correlation with treatment success. Laboratory analysis is currently underway to complete ELISA for antibody isotypes to MSP-1 and to constructs of variant surface antigens.

Interpretation: These results suggest that mutations in dhfr and dhps are not always associated with treatment failure and that the involvement of anti-MSP-119 antibodies is important in clearance of SP-resistant parasites.

134B
Host immunological factors involved in clearance of drug resistant falciparum malaria [MIM-DO-179865]

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Introduction: The prevalence of anti-malarial drug resistance measured by several in vitro methods and molecular methods exceeds the prevalence of clinical treatment failure in malaria endemic areas. Recent studies show that host factors may be involved in the clearance of chloroquine resistant parasite. The purpose of this study was to test the hypothesis that serum levels of specific cytokines are associated with in vivo clearance of drug resistant Plasmodium falciparum.

Methods: We used the standard WHO 14-day protocol to measure the efficacy of chloroquine treatment of uncomplicated falciparum malaria at two sites during the 2002 and 2003 rainy seasons. Serum was collected at enrollment and day 14. Pfcr K76T polymorphism prior to chloroquine treatment was determined by PCR/RFLP. Patients capable of clearing infections with pfcr 76T parasites were compared to those unable to clear these infections. Serum levels of pro-inflammatory and Th1–Th2 cytokines were measured at day 0 and day 14 using cyometric beads array ($n=238$, 6 months and older). We chose the median value of each cytokine level as the cut off according to enrollment and day 14 sera. For a given cytokine, the proportions of patients with a serum cytokine level above the cut off value were compared between the two groups.

Results: Our results show that at day 0, IFN-$\gamma$ levels were more frequently below the median titer in patients capable of clearing mutant parasites (140/301 (46.10%) versus 81/141 (57.45%); $p=0.032$). And at day 14, the level of IL-10 was negatively associated with the ability to clear resistant infections (91/242 (37.60%) versus 86/121 (71.07%); $p=0.000$). No statistically significant differences were found with any of the other cytokines tested.

Interpretation: While higher IFN-$\gamma$ and lower IL-10 appear to be associated with clearing resistant parasites, other humoral or cellular immune mechanisms may play a more important role in clearance.

135C
Analysis of helminthic infections and modulation of malaria morbidity and anaemia at population level [MIM-AR-80070]

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Introduction: In the tropics, people are co-infected with intestinal helminths and falciparum malaria. Helminths have been shown to modify the immune response to both parasitic and non-parasitic antigens non-specifically. They induce predominantly Th2 response and the balance between Th1 and Th2 cytokine is a
determinant factor for protection/pathology. We conducted a cross-sectional study to establish if co-infection could affect the course of evolution of uncomplicated falciparum malaria.

Methods: Individuals below 20 years were recruited into the study to compare the magnitude of indicators of malaria morbidity and anaemia between individuals co-infected and non-infected with intestinal helminths. Patients were examined for malaria parasites by thin and thick blood films gimsa staining. Those found positive were subsequently screened for the presence of helminths by direct examination of stool and urine. Haemoglobin was estimated by packed cell volume (PCV). We then assessed the magnitude of severity of malaria morbidity and anaemia indicators commonly associated with malaria between the two groups.

Results: From 1732-screened individuals, 344 (20%) were infected with malaria parasites. Among the parasitaemic, 182 (52.9%) were also found infected with intestinal helminths, 18 (5.2%) having mixed infections. The distribution of helminths was Ascaris lumbricoides (30.8%), hookworm (12.2%), Trichuris trichiura (9.0%) and Schistosoma haematobium (5.5%). Univariate analysis showed mean PCV of co-infected individuals was 1.69 lower compared to those with malaria alone ($p=0.024$). Log mean malaria parasite density was 1.08, ($p=0.04$) times higher in patients co-infected with schistomiasis compared to malaria alone. Nausea and diarrhoea were significantly prevalent in malaria group compared to co-infected group ($p=0.024$, $p=0.015$, respectively). Headache was significantly associated with co-infection compared to malaria alone group. These significant values observed in univariate analysis disappeared in logistic regression model analysis. Other symptoms and signs commonly associated with malaria morbidity had no significant difference between the two groups.

Interpretation: Our data can be interpreted to mean that the occurrence of helminth infections in malaria parasitaemic subjects had no effect on the cause of evolution of uncomplicated falciparum malaria.

136A
A study on the role of interleukin-10 and tumour necrosis factor-alpha promoter gene polymorphism in Plasmodium falciparum malaria severity [MIM-TR-20648]

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Introduction: The pathologies of many infectious, autoimmune and malignant diseases are influenced by the profiles of cytokine production in pro-inflammatory (Th1) and anti-inflammatory (Th2) responses. TNF-alpha and IL-10 were chosen as cytokines that play important roles in host immune responses to a variety of organisms. It was postulated that there could be a correlation between a genetic polymorphism and disease severity.

Methods: Patients were recruited at a local clinic in an endemic area of Kariba. They were diagnosed for Plasmodium falciparum using a thick smear and then levels of parasitemia were determined. Full blood counts were also carried out on the patients. Genotyping was carried out using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR).

Results: For IL-10 polymorphism, 98% (117) of the patients were homozygous genotype (AA at position -1082), which predicted high producers and only 2% were heterozygous (AG at position 1082), predicting medium producers. TNF-alpha promoter, however, showed more polymorphism with 7.4% being homozygous genotype (AA at position -308), predicting high producers, 25% heterozygous (AG at position 308) and 68% wild type genotype (GG at position 308), predicting low producers.

Interpretation: Results did not show a correlation between malaria severity and TNF-alpha/IL-10 genotype. Various associations of these gene polymorphisms with malaria severity in different populations underscore the complexity of host response to malaria.
Abstracts / Acta Tropica 95S (2005) S1–S506

137B Association of a promoter polymorphism in the gene encoding interleukin-12 p40 (IL12B) with cerebral malaria in a population living in Bamako [MIM-IS-157760]

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Introduction: Interleukin-12 (IL-12) is important in the generation of a Th1-biased immune response. IL-12 has been inversely associated with disease severity in human and murine malaria, and a polymorphism within the promoter region of IL-12 p40 subunit gene (IL12B) has been associated with fatal severe malaria outcome and reduced nitric oxide (NO) production. To better define the relationships between IL-12 and severe malaria, we investigated whether IL12B polymorphisms may be associated with cerebral malaria (CM) and severe malaria anemia (SMA), using family-based association approach.

Methods: Three polymorphisms were studied (an A TT repeat in intron 2, an A-C substitution in the 3′ untranslated region (UTR), and a 4 bp insertion-deletion within the promoter region of IL12B gene). The polymorphisms were genotyped in DNA samples from 583 Malian individuals from 190 nuclear families with at least one individual with CM (n = 118) or SMA (n = 72). Genotyping was analyzed with transmission disequilibrium test.

Results: The results indicate that the allele 2 (4 bp insertion) of IL12B promoter polymorphism was significantly overtransmitted from parents to affected children with cerebral malaria (p < 0.0007), and no preferential transmission of either allele of this polymorphism was observed in affected children with SMA. No association was found between 3′-UTR and ATT repeat polymorphisms and CM or SMA. This study indicates that the promoter IL12B polymorphism, or another polymorphism in linkage disequilibrium with it, could confer increased susceptibility to cerebral malaria in this population of Bamako.

Interpretation: This result suggests that Interleukin 12 plays a key part in the pathogenesis of severe malaria.

138C Genotyping of nitric oxide synthase2 promoter polymorphisms among selected ethnic groups in Eastern Sudan [MIM-IS-131796]

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Introduction: Different studies in Africa showed an association of inducible nitric oxide synthase (iNOS) gene promoter polymorphisms with protection from severe malaria. In eastern Sudan a two different ethnic groups, Hawsa and Masalit, are showing a great variation in the prevalence of malaria despite the same epidemiological setup. The purpose of this study is to investigate the prevalence of NOS2 promoter polymorphisms within Hawsa and Masalit population.

Methods: Subjects, from whom a written informed consent was obtained, were sampled using the toothbrush method. DNA was extracted from 50 healthy trios (parents and one sib) in Koka and Umshala villages, which are inhabited by two different ethnic groups, Hawsa and Masalit. Two different single nucleotide polymorphisms were screened NOS2–1173C/T and –954 G/C using amplification refractory mutation detection system (ARMS-PCR) and restriction fragment length polymorphism (RLFP), respectively.

Results: Twenty-two trios (A total of 66 individuals) from Koka village have so far been genotyped for NOS2–1173C/T polymorphisms by ARMS-PCR. (48.4%) were homozygous wild type (C/C), (48.4%) heterozygous C/T and (3.9%) homozygous mutant T/T. Genotyping is going on for the remaining samples and is underway for the Masalit. The correlation between NOS2 polymorphisms and malaria will be discussed.

Interpretation: Compared to other published data, these early results show higher prevalence of the NOS2–1173C/T polymorphism in the Hawsa population. This might explain the high prevalence of asymptomatic falciparum malaria in Hawsa population compared to others.
139A
The role of chemokines in malaria [MIM-JS-120456]

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Introduction: Malaria causes over 1 million deaths annually in Africa. The immunopathology of malaria is mediated partly by cellular and immunomodulator interactions involving cytokines and adhesion molecules. However, the role of chemokines and their receptors in malaria pathogenesis remains unclear. Studies utilizing murine models have implicated chemokines in microvascular recruitment of leukocytes, degradation of cell–cell junctions, and blood–brain barrier dysfunction relevant to malaria pathogenesis.

Methods: We analyzed mRNA and protein expression of RANTES and its receptors CCR1, CCR3 and CCR5 in post-mortem brain samples from CM, severe malarial anemia (SMA) and non-malaria (NM) deaths, using qRT-PCR and Western blot, respectively. Additionally, we analyzed alteration in immunomodulator gene expression in brain of P. yoelii 17XL-infected mice using cDNA microarray screening, followed by temporal comparison of mRNA and protein expression of RANTES and its corresponding receptors by qRT-PCR and Western blot, respectively. RANTES concentrations were measured by ELISA in plasma samples from malaria and non-malaria patients; and infected and uninfected mice. Mock and anti-RANTES antibody blocking functional experiments were performed.

Results: CCR1 mRNA expression was similar in all the human brain samples. Expression of RANTES, CCR3 and CCR5 mRNA was significantly higher in the cerebellar and cerebral samples from CM, than SMA and NM controls. The expression of RANTES mRNA (but not that of CCR3 and CCR5 mRNA) was also slightly higher in the samples of brain stem and hippocampus from CM, than controls. Western blots showed the expression of RANTES and CCR5 proteins in both cerebellum and cerebrum was significantly higher in the CM samples, than controls. Curiously, although the transcriptional analysis indicated up-regulation of CCR3 mRNA in CM cases, no CCR3 protein could be detected, by western blotting, in the same samples. RANTES, CCR1, CCR3, and CCR5 mRNA were up-regulated at peak parasitemia, and remained at steady state thereafter in the experimental mouse model. RANTES protein in the brain of infected mice was up-regulated compared with controls. RANTES was significantly upregulated in plasma of infected mice and malaria positive human subjects, compared with controls. The mock antibody treated mice had significantly higher parasitemia and significantly lower survival than the mice in which RANTES was blocked with anti-RANTES antibody.

Interpretation: Our findings suggest that immunopathology of CM is partly mediated by interactions between RANTES and its receptors. The role of other chemokines needs to be further examined in the search for key molecules mediating malaria pathogenesis.

140B
Parasite-mediated NFkB activation protects Plasmodium berghei-infected hepatocytes from Fas (CD95/Apo-1)-induced apoptosis [MIM-RT-137592]

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Introduction: To survive in the liver, Plasmodium sporozoites must protect the host cells from apoptosis. The liver stage of malaria is the target of a cellular immune response in which T cells play a major role. Fas (CD95/Apo-1) ligand-mediated apoptosis is a mechanism of cell-mediated cytotoxicity induced by T cells. We set out to test the hypothesis that sporozoites modulate the signal induced by Fas receptor triggering during the early stage of Plasmodium infection.

Methods: HepG2 cells or mouse primary hepatocytes were infected with P. berghei sporozoites for 18 h, followed by induction of apoptosis by the addition of oligomerized FasL-Fc. In HepG2 cells FasL-Fc was added in presence of cycloheximide (CHX). Apoptosis was assessed 8h later by flow cytometry of propidium iodide stained nuclei or Annexin-V...
stained cells. To determine which possible anti-apoptotic molecules are induced by the parasite, Western blot, luciferase reporter assays and RT-PCR were performed. To address the significance of parasite-mediated inhibition of apoptosis in host cells, infection studies were performed. Infected hepatocytes were either left untreated or apoptosis was induced. Cells were fixed and the number of parasites was assessed.

Results: Our results clearly demonstrate that sporozoites downregulate Fas-induced host cell apoptosis. The anti-apoptotic effect of sporozoite infection appears to be NFkB mediated. As early as 15 min post-infection, the NFkB inhibitor IkBa is phosphorylated and thus inactivated. NFkB luciferase reporter assays revealed a two to three fold induction of NFkB activity in infected HepG2 cells. Importantly, the anti-apoptotic effect mediated by sporozoites can be blocked by a chemical inhibitor of IkBa phosphorylation. We could assess several anti-apoptotic molecules controlled by NFkB which were upregulated in infected liver and hepatocytes. Pretreatment of hepatocytes with the IkBa phosphorylation inhibitor blocked sporozoite-mediated induction of NFkB-regulated anti-apoptotic proteins. As expected, the infection rate does not differ between control cells and anti-Fas treated cells. In contrast, blocking NFkB activation by the IkB phosphorylation inhibitor reduces the infection rate in anti-Fas treated hepatocytes.

Interpretation: Our data show that Plasmodium parasites inhibit Fas-induced apoptosis in hepatocytes. Furthermore, we suggest that sporozoite-induced NFkB activity plays a central role in apoptosis inhibition by upregulating different anti-apoptotic molecules.

141C Plasma levels of migration inhibitory factor (MIF) and transforming growth factor beta 1 (TGF-beta1) in uncomplicated and severe malaria patients [MIM-NY-19124]


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Introduction: Malaria remains the leading cause of morbidity and mortality in tropical countries. Much of the pathology of malaria is mediated by cytokines secreted in response to malarial toxins released upon schizont rupture. Cytokine balance may be the key determinant in malaria presentation and outcome. In this case-control study, we investigated the role of MIF and TGF-beta1 in the pathogenesis of malaria in children from the South West Province of Cameroon.

Methods: Febrile children 1–14 years of age attending some health care facilities for medical attention and healthy children of the same age group attending some community schools (controls) were recruited into the study and subsequently characterized as cerebral malaria (CM), severe malarial anaemia (SMA), uncomplicated malaria (UM) or healthy controls (HC) according to WHO standards. Plasma cytokine levels were measured by ELISA and compared between the different categories of study participants.

Results: The mean concentration of TGF-beta1 was significantly (P<0.01) different between the study groups with the highest levels (in pg/ml) occurring in HC (9652.8) followed by UM (35894.8), CM (35894.4) and SMA (18094.4). TGF-beta1 levels also correlated positively with parasite density. In contrast, no significant difference (P>0.05) was observed in the mean MIF concentration between the groups. A gradual increase in mean MIF concentration was observed from HC (312.4), through UM (633.6) and then CM (682.7) as expected but interestingly the mean MIF concentration in SMA (358.9) was lower than that in UM. There was no significant difference (P>0.05) in mean MIF to TGF-beta1 ratios between groups. There was also no significant difference (P>0.05) in parasite density between the study groups.

Interpretation: The markedly raised level of TGF-beta1 in HC and UM children when compared to severe disease children and the irregularity observed in MIF levels is suggestive of a role for the magnitude and timing of cytokine production in malaria pathogenesis.
8. Malaria epidemiology

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

142A

Le risque de paludisme transfusionnel en Côte d’Ivoire en 2004 [MIM-EB-206606]

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Introduction: La transfusion sanguine, geste médical dont le but est de sauver des vies humaines n’est malheureusement pas toujours sans risque pour le receveur. Si le risque infectieux reste une préoccupation permanente et justifiée pour les infections virales et bactériennes qui sont recherchées systématiquement par des tests biologiques spécifiques, ce n’est pas le cas des infections parasitaires notamment du paludisme qui est le plus souvent découvert de manière fortuite.

Methods: Cette étude transversale qui s’est déroulée de juin à août 2004 au Centre National de Transfusion Sanguine (CNTS) d’Abidjan a eu pour but d’estimer le risque de transmission du paludisme en Côte d’Ivoire lié aux poches de sang “Plasmodium falciparum” positives. Les donneurs de sang bénévoles, âgés de 18 à 60 ans et répondant aux critères (cliniques et biologiques) de bonne santé ont été sélectionnés. Chaque échantillon de sang a servi à confectionner une goutte épaisse et un frottis mince. Tous les échantillons sanguins ont été soumis aux tests sériologiques classiques de dépistage du CNTS.

Results: Les examens sérologiques effectués chez les 437 donneurs bénévoles ont permis d’éliminer 9 donneurs infectés. Ainsi, 428 donneurs ont pu être recrutés. 61% des sujets avaient un âge compris entre 20 et 29 ans. Il s’agissait principalement de donneurs célibataires et de sexe masculin 37 donneurs (soit un taux de 8.64%) étaient infestés par les hétéroatiques à l’examen de la goutte épaisse. Plasmodium falciparum a été la seule espece plasmodiale identifiée et tous les donneurs étaient asymptomatiques. La parasitémie variail 40 à 9120 trophozoïtes par microlitre de sang. 78.4% des donneurs avaient une parasitémie inférieure à 1000 trophozoïtes par microlitre de sang. La majorité des donneurs (57%) a déclaré ne pas avoir recours à la prevention contre le paludisme. Sur les 184 donneurs utilisant des mesures préventives, 79.35% ont préféré la pulvérisation intra domiciliaire d’insecticides.

Interpretation: Le risque de développer un paludisme post-transfusionnel demeure. Tous les malades transfusés devraient pouvoir bénéficier d’un traitement présomptif antipalustre, et les donneurs sensibilisés à l’utilisation de la moustiquaire imprégnée.

143B

Transmission of Plasmodium falciparum after treatment with first line antimalarial drugs [MIM-TB-466180]

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Introduction: Artemisinin based combination therapy (ACT) can reduce post-treatment gametocytaemia in Plasmodium falciparum infections and may therefore reduce malaria transmission. Several studies showed a substantial reduction in transmission after treatment with ACT. However, these studies relied on microscopic detection of gametocyte carriers. If submicroscopic levels of gametocytaemia are common and result in mosquito infection, previous studies may have led to invalid and overoptimistic conclusions.

Methods: This study determined treatment efficacy and post-treatment malaria transmission in a randomized clinical trial where Kenyan children (6 months-10 years) were treated with sulphadoxine-pyrimethamine (SP, n = 152), SP + amodiaquine (SP + AQ, n = 127), SP + artemunate (SP + AS, n = 174) or artemether-lumefantrine (AL, n = 75). The infectiousness of treated children was determined on day 14 post-treatment using a direct membrane-feeding (DMFA) set-up with locally reared mosquitoes.

Results: Adequate clinical response was observed in 55% (SP); 83% (SP + AQ); 85% (SP + AS) and 97% (AL) of the patients. When only gametocytaemic chil-
dren were included in the DMFA experiments, the estimated proportion of infectious children was 46% (SP), 35% (SP + AQ), 15% (SP + AS) and 14% (AL). However, we found that microscopy seriously underestimated the proportion of gametocyte carriers. We therefore also included a random sample of children (n = 100) in the DMFA experiments regardless of microscopical results. In these experiments, the proportion of infectious children turned out to be much higher than expected: 64% (SP); 35% (SP + AQ); 44% (SP + AS) and 60% (AL). The difference between the two estimates is attributable to children who were infectious to mosquitoes while harbouring submicroscopic gametocyte densities.

**Interpretation:** We show that post-treatment malaria transmission has been seriously underestimated and we have to redefine our knowledge on the infectious reservoir. The presented findings are sobering for future interventions aiming at reducing malaria transmission by the use of antimalarial drugs.

**144C**
Detection of malaria parasites by nested PCR in an area of seasonal malaria transmission in eastern, Sudan [MIM-LE-684046]

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**Introduction:** Malaria in Sudan is considered to be the major health problem in the country, yet our knowledge of the epidemiology of malaria in terms of morbidity and mortality in most part of the country is still unclear.

**Methods:** In the course of epidemiological and immunological baseline studies, a parasitological cross-sectional survey was after the transmission season, in two villages (Koka and Umsalal villages) situated on the bank of the Rahad river, Eastern Sudan. The area is characterized by seasonal but stable malaria transmission. Using both blood stained Giemsa slides and PCR techniques parasitological were used to determine the prevalence of malaria parasites in the area.

**Results:** Blood film parasite point prevalence of the two surveys were (26%), and (10%), after the rainy season and (1.2 and 1.1%) before the rainy season, in Koka and Umsalal villages, respectively. Eight percent of the reported malaria cases during these surveys were due to *P. falciparum*, the rest were due to *P. malariae*. No other malaria species were detected during the survey. The prevalence of both *P. falciparum* and *P. malariae* is markedly increased when nested Polymerase Chain Reaction method where employed for detection of DNA of malaria parasites in samples collected from the study subjects. The results showed that the PCR point prevalence were 43% and 13% of *P. falciparum* and *P. malariae*, respectively in Koka village, and PCR point prevalence of 10 and 4.4% for *P. falciparum* and *P. malariae* in Umsalal village, respectively.

**Interpretation:** This data constitutes the first report on the presence of *P. malariae* along the Rahad River in eastern Sudan, this area is neighbouring Dinder National Park, which might suggests a zoonotic cycle of transmission of *P. malariae* in the area. Furthermore, this data indicated the sensitivity of nested PCR methods in detection of other *plasmodium* species which are characterized by low asymptomatic infections.

**145A**
Spatial effect of bednet use on malaria mortality in the Kilombero Valley, Tanzania [MIM-LG-162435]

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**Introduction:** Insecticide treated nets (ITNs) were shown to have a high efficacy in reducing mortality and morbidity due to malaria in endemic regions. We applied spatial statistical approaches to look at the effect of treated nets on child and adult survival using a demographic surveillance system (DSS) carried out in 25 villages from Kilombero Valley on a total population of 80,000.

**Methods:** Using a Global Positioning System (GPS) the position of each house was determined and the distance between houses was calculated. The net coverage and the distance to the nearest health facility were included in the model as explanatory variables. We fitted Bayesian hierarchical models to estimate the association between risk factors and mortality in the presence of spatial correlation. We used a negative
binomial model with household-specific random effects to assess the effect of different risk factors on mortality. The spatial correlation was modelled by assuming that the random effects are distributed according to a multivariate normal distribution with an exponential covariance matrix depending on the distance between households.

**Results:** The mean bednet coverage in Kilombero Valley was 271.5 nets per 1000 inhabitants. We categorized the bednet coverage as low (0 nets/1000 persons), moderate (1–300 nets/1000 persons) and high (more than 300 nets/1000 persons). The distribution of households within the study area was: 4077 (40.5%) households in low, 3647 (31.5%) households in moderate and 3237 (28%) households in high bednet coverage areas, where the coverage was defined as nets within 50 m. The child mortality rates in the three bednet coverage areas were: 32.8/1000 persons in low bednet coverage, 30.1/1000 persons in moderate bednet coverage and 27.4/1000 persons in high bednet coverage. 69.939 individuals had information available on geo-location and socio-economic covariates. The factors related with the risk of death were sex, age, occupation of the head of the household, the number of bicycles, radio, bednets, treated nets, sleeping rooms and acres land, ownership of cows, goats, sheep, chickens, tin roof and toilet.

**Interpretation:** Our findings show that child mortality rates in areas with high bednet coverage were significantly decreased when the bednet coverage within 50 m was considered.

146B

**Challenges of malaria control: A critical study among pavement dwellers in urban India [MEM-SG-282394]**

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**Introduction:** Chennai in southern India contributes 85% of malaria cases to the state of Tamil Nadu. It particularly affects the poor pavement dwellers (PD) who live under the most difficult and impoverished conditions. Their exposure to malaria is several times greater than that of the other local people. This study was done to assess the ecological situation of urban pavement dwellers in relation to mosquitogenicity and to describe the protective measures practiced by them in a high malaria incident zone.

**Methods:** This descriptive study was conducted in the high malaria incident zone (Annual Parasite Incidence >21) in Chennai during the period October and December 2002. All head of the families who gave consent to the study were interviewed using a structured questionnaire. Details regarding their occupation, History of malaria among the family members in the past 6 months, habit of regularly using personal protection measures and their insight towards using treated bed nets were elicited. 74 families were interviewed. An ecological survey was made and photographs were taken on potential breeding sources for mosquitoes in the area.

**Results:** Each PD family had six to seven members. Almost 85% of them were employed as daily laborers, predominantly engaged as domestic workers (43%). About 92% of them reported every day mosquito nuisance and history of malaria among their family members was seen in 89% of the families to which 100% of them responded by using some repellants measures. Among repellants used, coils were very popular and rest used other methods. Usage of mosquito nets was not so popular and they felt it was costly and problematic in daily fixing in their dwellings; and another reason was inadequate living space and hence they cannot accommodate all the family members; Skin cream was inconvenient and mats was not feasible as their dwellings had no electricity. However, 81% of them committed that if bed nets are provided free of cost they will use it and they needed more than one net per family. Ecologically a large number of ornamental overhead tanks were identified, an immediate challenge before public health workers. They were unapproachable for anti-larval treatment and also there is no mechanism to correct the problems through regulating acts. Stagnant pools of waterways were seen in plenty in the area.

**Interpretation:** Pavement dwellers serve as a dangerous source of malaria transmission. Provision of cost-free bed net and education regarding their proper usage in their dwellings and stringent public health laws is recommended for effective malaria control.
Malaria and urinary schistosomiasis in Niger—links with anemia? [MIM-HH-223704]

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Introduction: Malaria and urinary schistosomiasis are commonly found pathogens in Niger. The two diseases are frequently and independently pointed out as causes of anemia but concomitant studies are scarce. As the controls of schistosomiasis and malaria are getting increased by national respective programs, the elucidation of respective and/or cumulative roles of these pathogens in anemia occurrence seems of prime importance to deal with a predicted impact on children health.

Methods: Data were collected from two sahelian villages presenting with large difference in Schistosoma haematobium prevalence but supposed identical malaria transmission. Sampling was performed during the dry season, when malaria incidence is low and the urinary parasite contamination past for several months. Parasite carriages were quantified among four class-age with urinary and feces egg counts, thick and thin blood films. Anemia was assessed with Hemocue® photometer. The malaria parasites genetic diversity was studied by MSP-1 and MSP-2 gene polymorphism.

Results: According to the WHO thresholds, anemia was very frequent: 56% in Minao and 38% in Alibou. In the 5–12-year-old children, urinary schistosomiasis was hyper-endemic (i.e. higher than 50%) in Minao and only 5% in Alibou. As expected in such dry zones, no Schistosoma mansoni egg was found and geo-helminths were very rare. The same malaria prevalence about 20% was found in the two villages. The relationship between malaria and urinary schistosomiasis co-infection and anemia are being analyzed both for prevalences and parasite loads. Genetic polymorphism data are currently in analysis process. At the individual level, neither the malaria nor the schistosomiasis parasite carriage could explain the observed anemia cases.

Interpretation: Although malaria transmission is endemic in all the coastal Provinces of Papua New Guinea, there has not been any study conducted to assess the malaria prevalence in different ecological settings in endemic areas. The objective of our study was to assess malaria prevalence existing in different ecological settings.

Ecological variability in malaria prevalence in two districts of East Sepik Province, Papua New Guinea [MIM-III-8616]

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Introduction: Although malaria transmission is endemic in all the coastal Provinces of Papua New Guinea, there has not been any study conducted to assess the malaria prevalence in different ecological settings in endemic areas. The objective of our study was to assess malaria prevalence existing in different ecological settings.

Methods: We conducted a cross-sectional malarialometric survey involving Community-Schools and the surrounding Communities in two districts of East Sepik Province. The epidemiological characteristics of malaria were studied covering four different ecological settings (inland plains, mountain fringes, coastal lowland, and Islands) over a 3-year period (2001–2004) during wet and dry seasons.

Results: A total of 11,276 blood smears for malaria microscopy was done with positivity rating of 24% (n=2707/11276). Plasmodium falciparum species accounted for 63.5%, followed by P. vivax (25.8%) and P. malariae (5.5%). Highest prevalence of asexual forms of P. falciparum parasitaemia occurred in the 1–9 years age group. Plasmodium vivax and P. falciparum gametocytaemia were observed more frequently in the 1–4 years age group while P. malariae parasitaemia was observed more frequently in the 4–10 years age group. Prevalence of parasitaemia for all species decreased with age from 9 years. The highest burden of Plasmodium falciparum parasitaemia was observed in the inland lowland communities, moderate in the mountains fringes with lower parasitaemia.
in the coastal and Island regions, while opposite trend was observed for \( P. \) vivax parasitaemia. Seasonal variability was marked in the mountain fringes than other areas.

**Interpretation:** Perennial transmission of malaria in inland lowland areas, moderate in coastal/island areas, while almost epidemic levels during wet season in mountain/fringes areas. Specific malaria control strategies may be designed for different ecological areas.

149B

*Plasmodium falciparum* strain characterization in highland and lowland areas of Muheza, northeastern Tanzania [MIM-DI-23359]

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**Introduction:** Different parasite strains have variable roles in the development of clinical malaria. Similarly, environmental factors such as altitude and climate have significant contributions to the development of clinical disease. This study was conducted as part of ongoing project on the epidemiology of malaria in Muheza district, northeastern Tanzania. It aims at providing information on *P. falciparum* genetic diversity, multiplicity of infection and their influence on febrile condition.

**Methods:** Random blood samples were collected from 1956 individuals aged 6 months to 45 years in Muheza district during short rains (November to December 2003). Out of those, 615 were parasitologically positive, of which 264 samples met the inclusion criteria for molecular characterization. The selected samples were from individuals who were febrile or afebrile, positive for *P. falciparum* with parasitaemia 400 parasites per \( \mu l \) of blood and had no danger signs such as chronic diseases and complicated malaria. Genotyping of the selected samples was done for Merozoite surface protein 2 (msp2) marker using polymerase chain reaction (PCR) and restriction digestion with *Hinfl*. STATA 7.0 was used for data analysis.

**Results:** Genotyping was successful on 192 samples with 46 (23.96%) highlands and 146 (76.04%) from lowland areas. Out of those, 27.08% (52/192) had parasites belonging to 3D7 family while 15.10% (29/192) had FC27, and 57.81% (111/192) were of both allelic families. Individual parasite family distribution in both strata (highland and lowland areas) was not significantly different (highlands: 3D7 = 59.3%, FC27 = 40.8% and lowlands: 3D7 = 66.7%, FC27 = 33.3%; \( p = 0.512 \)). The level of multiple infection (with both parasite families) was higher in lowland than highland areas (lowlands: 63.0% and highlands: 41.3%; \( p = 0.026 \)) but the trend of an individual being infected with both families was shown to increase in lowlands and vice versa in the highlands. Overall prevalence of fever was not significantly different in the two altitude strata (highlands = 34.8%; lowlands = 40.4%; \( p = 0.495 \)). Logistic regression model, adjusted for altitude strata and age showed that 3D7 was fairly associated with fever as compared to FC27 allelic family (OR = 2.56, 95% CI 0.89–7.37; \( p = 0.082 \)) while the combination of the two families was significantly associated with fever episodes as compared to FC27 family alone (OR = 2.93, 95% CI 1.10–7.86; \( p = 0.037 \)).

**Interpretation:** There was homogenous distribution of *P. falciparum* allelic families in low and high altitude areas, with 3D7 being more associated with fever episode. This information has to be considered when designing interventions such as malaria vaccine trials.

150C

Baseline Malaria Parasite Rates In Five Communities On The Copperbelt Province Of Zambia [MIM-NK-176030]

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**Introduction:** The Ndola and Kitwe District Health Management Teams (DHMTs) were scheduled to conduct Indoor Residual (IRS) in January 2004. However, since the overall impact of IRS was intended for the reduction of malaria cases in the community, it was necessary that a baseline study of the malaria parasite
rates be conducted. The study was carried out from 2nd to 24th December 2003.

Methods: Main objective to establish baseline prevalence of malaria parasites in Ndola and Kitwe. Specific objective to establish parasite rates in children under the age of 5 years and pregnant women in Ndola and Kitwe. Design The study design was cross-sectional. Study setting and population The study was conducted in five communities in Ndola and Kitwe. The categories of study subjects used were the pregnant women and children under the age of five. These subjects were accessed through the Health Centres that fall in the two districts of Ndola and Kitwe.

Results: Peripheral blood samples were collected from 334 to 121 pregnant women and 939 and 296 under five children in Ndola and Kitwe, respectively. Microscopy was used to detect infections. 19.46 and 23.97% pregnant women had malaria in Ndola and Kitwe, respectively. 21.9 and 31.08% under five children had malaria in Ndola and Kitwe, respectively. The prevalence of the malaria parasites was compared in the two towns. 99.6% of all infections in Ndola were Plasmodium falciparum and 0.37% was plasmodium ovale. In Kitwe all (100%) infections were Plasmodium falciparum.

Interpretation: Conclusion The baseline parasite rates were established but a post-intervention assessment will establish whether or not Indoor Residual Spraying (IRS) is an appropriate malaria preventive measure.

151A

Molecular detection of DHFR and DHPS genes from sporozoite infected mosquitoes [MIM-HM-25296]

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Introduction: Detecting and identifying malaria parasites in mosquitoes are crucial in estimating the efficacy of malaria control strategies. Among several methods which have been used include monoclonal antibodies, DNA probes and polymerase chain reaction (PCR). We are using monoclonal antibody ELISA to screen sporozoite positive mosquitoes and nested PCR to amplify dhfr and dhps genes from these positive samples and from some sporozoite negative samples.

Methods: We collected 1090 anopheline mosquitoes from Lupilo village in Ulanga district by indoor resting method. The mosquitoes were transferred to insectary and kept there for 10 days to ensure completion of sporogonic cycle. We examined 646 mosquitoes without their abdomen to minimize the interferences from blood and oocyst stages of Plasmodium. Individual mosquito was homogenized in Tris EDTA buffer and aliquoted into two halves one for ELISA and another for DNA extraction. Sandwich ELISA was used to detect circumsporozoite protein by using monoclonal antibody. DNA from positive and negative sporozoite samples were extracted by chelex-based method and subjected to nested PCR to amplify dhfr and dhps genes with 594 bp and 711 bp, respectively.

Results: Of 646 samples processed, 19 samples (2.94%) were positive for ELISA. Of these 19 samples, 3 were positive for PCR (dhfr). 36 samples (5.57%) which were negative for ELISA were found to be positive for PCR (dhfr). Of 39 samples where dhfr gene was detected, dhps gene was only detected from two samples. Most of these samples produce characteristically weak bands which may suggest low parasitaemia in most of these samples or poor DNA quality. This problem together with the failure to detect dhps in some samples where dhfr was detected, limited us to undertake further analysis such as screening all possible mutations in these markers of SP resistance. PCR inhibitors that are contained in anopheline mosquitoes may contribute to inhibitory effect to PCR. Nothing is known to whether chelex method is efficient enough to remove these inhibitors. However, the optimisation of extraction methods and PCR conditions are still underway to overcome these problems.

Interpretation: Our preliminary results support the fact that PCR is highly sensitive in detecting parasite DNA. The failure to detect these genes from many positive sporozoite samples may be due to melanin, inhibitors that are present in exoskeleton of mosquitoes.
152B
Age-specific malaria infections in communities exposed to different levels of malaria transmission intensity [MIM-WK-7572]
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Introduction: There is considerable debate about the relationship between the intensity of malaria transmission and the development of immunity against infection and the implications of this for malaria control.

Methods: We examined this relationship in 6 villages experiencing markedly different levels of transmission at different altitudes in the Usambara Mountains, Tanzania. Routine entomological collections were made over 12 months, and a cross-sectional parasitological survey carried out in each community at the end of the main rainy season. These villages represented a spectrum of transmission levels from holo-endemic malaria in the lowlands to hypo-endemic at the mountain peak.

Results: Prevalence of parasitaemia in children declined with increasing altitude; from 81.5% in the lowlands to 11.8% in the highest village. A similar, but more pronounced, relationship was also seen with spleen rates (86.2 versus 2.4%). At low to high exposure (2–91 infective bites/year) both infection and parasite densities were greatest in 2–3 years old, whereas where transmission was extremely low (<0.1 infective bite/year) all ages were at risk of infection. Importantly, high-density parasitaemia (>5000 parasites per ml) were confined largely to young children, irrespective of the general level of exposure in each village, suggesting that age-dependent immunity is important for controlling high parasite densities.

Interpretation: Although in holo- or hyper-endemic areas transmission control needs to reduce exposure substantially to achieve an impact on malaria prevalence and intensity, control efforts should have a negligible impact on the development of immunity to infection, and is unlikely to increase malaria risk in older age groups.

153C
Effect of indoor residual spraying on malarial parasitemia prevalence in children on Bioko Island, Equatorial Guinea [MIM-IK-219912]
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Introduction: A comprehensive package of malaria control measures has been initiated on Bioko Island, Equatorial Guinea (EG), since February 2004, funded by Marathon Oil Company in partnership with the government of EG. The measures consist of indoor residual spraying (IRS) and case management including the introduction of improved diagnosis and effective treatment based on combination therapy.

Methods: Between February and July 2004, a program of spraying domiciliary structures with pyrethroid insecticide was carried out. Case management was rolled out at health facilities from February 2005. A baseline and first annual follow-up household survey were conducted in March 2004 and 2005, respectively at 16 sentinel sites. Houses were selected from random coordinates using satellite images in urban sites and using systematic sampling from hand-drawn maps in rural sites. Household data and blood samples were collected. Each child <15 years was assessed for malarial parasitemia and haemoglobin level using rapid tests. More than 2500 children were tested in each survey. At baseline a questionnaire was used to ascertain under-5 all-cause mortality.

Results: At baseline site specific prevalence of infection with Plasmodium falciparum varied from 28 to 63% with a mean of 40% [95% CI 35–46%] for urban sites and 51% [95% CI 46–57%] for rural sites (p = 0.012). Under-5 mortality at baseline was estimated to be 15.3% [95% CI 13.5%–17.1%]. Initial results of the follow-up survey indicate that prevalence of parasitemia reduced by approximately one third compared to baseline (p < 0.05), and that more than 80% of houses were sprayed during the first spray round. Full results will be available at the time of the conference. Preva-
lence of infection in pregnant women was estimated to be 18% [95% CI 10%–24%].

**Interpretation:** Significant reductions in prevalence of malaria infection can be achieved through a well managed programme of IRS. Such a strategy should be complemented with effective case management using combination therapy to achieve sustainable malaria control.

154A

**A model for the epidemiological effects of pre-erythrocytic malaria vaccines [MIM-NM-32132]**

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**Introduction:** Field trials of malaria vaccines do not assess the community effects of vaccination, nor do they consider the overall effectiveness when only a proportion of the population are vaccinated. Model-based estimates need to consider both the dynamics of transmission and the effects of reducing exposure on the acquisition of immunity. We now propose a model of the effects of introduction of a pre-erythrocytic vaccine against *Plasmodium falciparum* into a malaria endemic population in Africa.

**Methods:** Our stochastic simulation model includes components of transmission, parasitology, and clinical epidemiology of malaria. We model the action of a pre-erythrocytic vaccine as a reduction in the force of infection. The various components have been fitted to field data, and evaluated against the results of field trials of pre-erythrocytic vaccines. We consider the consequences of a vaccination program lasting 20 years that introduces a pre-erythrocytic vaccine via the Expanded Programme of Immunisation (EPI) into a simulated population of 100,000 people.

**Results:** The results suggest that pre-erythrocytic vaccines with moderate efficacy will have a substantial impact on malaria morbidity and mortality during the first decade following their introduction, but have negligible effects on malaria transmission at levels of endemicity typical for sub-Saharan Africa. The main benefits result from prevention of morbidity and mortality in the first years of life, with the consequence that a vaccine with efficacy half-life of 2 years has almost the same benefit as one with half-life 10 years. The predicted effectiveness of vaccination is higher at lower transmission intensities.

**Interpretation:** Pre-erythrocytic vaccines with moderate efficacy, and a half-life of 2 years or more, can substantially reduce the malaria burden.

155B

**Urban malaria in the Sahel: Prevalence and seasonality of presumptive malaria and parasitaemia at primary care level in Chad [MIM-ON-210144]**

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**Introduction:** While it is reported that over 70% of malaria cases do not present initially to health facilities, malaria is still the most important reason for consulting a health service in many countries of Sub-Saharan Africa. This especially holds for private and public primary care facilities such as dispensaries, health centres and out-patient departments of hospitals where an important share of patients are diagnosed as malaria cases, based on a clinical examination only.

**Methods:** All patients consulting two private and two governmental providers on a randomly selected week day during a 9 month study period were included in the study. For those patients with presumptive malaria, a blood slide examination was done.

**Results:** Among the 1658 patients included in the survey, 47% were clinically diagnosed and treated as malaria cases. Malaria was more frequently diagnosed at the level of private providers. No clear seasonal patterns in presumptive malaria were observed. Among clinically diagnosed cases, 30% patients were positive for *Plasmodium falciparum* by thick film examination. Thus, false positive cases constituted more than 70% of the clinically diagnosed malaria cases. The highest positive prevalence rates were found at the end and shortly after the rainy season (44–47%) and the lowest ones during the dry season (2%).

**Interpretation:** Clinical diagnosis of malaria has a very low positive predicted value in this low endemicity, urban setting.
Malaria in the highlands of Uganda: Incidence, diagnosis and treatment [MIM-RN-69498]

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Introduction: Less is known about the incidence, diagnosis and treatment practices amongst populations exposed to low and unstable transmission. With epidemics occurring with increasing frequency in the East African Highlands, the epidemiological background and treatment-seeking behaviour in highland communities is of increasing interest. The incidence and distribution of malaria risk was examined in a rural area of the Ugandan highlands that has experienced recurrent malaria epidemics.

Methods: Active case detection was undertaken over 18 months in three communities in an epidemic-prone area of SW Uganda. All households were visited weekly, and malaria blood slide prepared from any family member with raised temperature or history of fever in the last 24 h. Clinical episode was defined as temp $\geq 37.5^\circ C$ and positive blood film. Demographic and geographic risk factors were examined. Information on treatment and diagnosis was also recorded at local peripheral health facility serving the study area. History of symptoms and treatment prior to attendance at health unit was obtained from patients with a presumptive diagnosis of malaria using a semi-structured questionnaire. Diagnosis was confirmed by a malaria blood film.

Results: The mean annual incidence in the three communities was 19.2, 35.6 and 36.5 cases per 100 person-years, with seasonal peaks occurring during the wet seasons. No malaria epidemics were recorded in the district during the period of the study. Clinical attacks were seen in all agegroups, although incidence gradually decreased with age from 40.2 cases per 100 person-years amongst children 0–5 years to 29.6 cases per 100 person-years amongst adults $>30$ years ($P < 0.001$). Asymptomatic parasite carriage was also observed. Most patients sought treatment within 1–2 days of onset of symptoms (78%). Of 1627 patients with a presumptive diagnosis of malaria, only 24% were slide-positive. All were given antimalarials, however a third of patients only received chloroquine (CQ), contrary to national drug policy introduced 24 months previously, which recommends combination of CQ plus sulphadoxine-pyrimethamine (SP). A quarter of patients had taken antimalarials prior to attending the health unit, obtained either from drug shops (53%), general shops (34%), private clinics (11%), traditional healers (1%) or friends (1%). Only 3% of treatments given outside the formal sector complied with national guidelines of CQ + SP.

Interpretation: Interpretation Failure to comply with national policy in both private and public sectors is of great concern in an era of rapidly-evolving drug policy. Patients, drug vendors, health staff—all need education on change.

Malaria amongst children below 12 years in tiko, Cameroon [MIM-an-69000]

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Introduction: Malaria being an acute febrile disease still remains the number one cause of morbidity and mortality amongst children in Sub Saharan Africa. Prevalence studies are important to better manage and control the disease. This study aims at studying the prevalence of malaria and its related effects in children below the age of 12 years attending the Cameroon development co-operation (CDC) Central Clinic, Tiko, Cameroon.

Methods: This study was carried out at the CDC central clinic, Tiko, to determine the prevalence of Malaria in children (0–12 years) attending the clinic in the months of February and March 2004. Children were clinically assessed for the presence of splenomegaly, anaemia, fever, diarrhoea, cough, headache and vomiting. History of treatment, area of residence, age and sex were obtained from parents and older children through interview and questionnaire. Capillary blood samples were collected from 101 children and analysed for haemoglobin concentration. Thin and thick blood...
Smears were prepared to detect and quantify malaria parasites.

Results: *Plasmodium falciparum* (30.69%) was the only species of malaria diagnosed microscopically. Children below the age of five were more infected with clinical malaria (38.6%) and malaria parasitaemia (40.4%) than children above this age. There was a positive correlation between these two parameters \((r > 0)\). Males were more infected (36.96%) than females (14.45%), though there was no significant difference by sex \((P > 0.05)\). There was no significant difference in mean parasite density and haemoglobin in the different age groups \((P > 0.05)\). There was a negative correlation between these two parameters \((r < 0)\). Anaemia was more prevalent in malaria positive (54.8%) than in malaria negative children (0.1%) while those with parasitaemia had severe anaemia. Fever was the most prevalent clinical feature amongst the children (52.5%) and especially amongst those positive for malaria \((P > 0.05)\).

Interpretation: *Plasmodium falciparum* and its attendant effects were more in children below 5 years of age. Since most children are treated at home prior to being brought to hospital, the community should be educated on the appropriate use of antimalarials.

**158B**

Low autochthonous urban malaria in Antananarivo (Madagascar) [MIM-LR-91221]

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Introduction: Late 1980s, the Madagascar highlands underwent an epidemic of *falciparum* malaria killing about 40,000 persons. Today, despite a very low malaria transmission in these areas, febrile episodes are over diagnosed as malaria attacks on the basis of clinical signs only even in urban zones. But malaria frequency among febrile episodes remains unclear. Thus, we carried out a study to detect malaria cases among outpatients in Antananarivo.

Methods: We conducted two cross-surveys in 43 urban health centres in Antananarivo in February 2003 (rain season) and in June 2003 (dry season). Consenting patients with clinically suspected malaria were included. Microscopy and malaria rapid diagnostic tests (RDT) were performed to diagnose malaria. Basic information was collected from patients in hope to identify the origin of the infection (introduced or autochthonous).

Results: In February, among 771 patients, 15 (1.9%) positive slides were recorded. All the four malaria parasites occurred: *P. falciparum* \((n = 12)\), *P. vivax* \((n = 2)\) and *P. ovale* \((n = 1)\). Only two cases, with *P. falciparum* were likely autochthonous (of local origin, in patients that did not travel out of Antananarivo during the 4 previous weeks). In June, of 739 blood smears examined, 11 (1.5%) were positive: *P. falciparum* \((n = 9)\) and *P. vivax* \((n = 2)\). Three cases of *P. falciparum* malaria could be considered as of local origin. During the June survey, the malaria rapid diagnostic test using dipsticks detecting pLDH allowed to diagnose the two case of *P. vivax* malaria. Irrespective of the season, malaria cases among febrile episodes are low in Antananarivo (<2%). Our results also demonstrate that malaria cases in Antananarivo are almost associated with a history of recent travel in coastal areas of high malaria transmission levels. Nevertheless, the presence of autochthonous malaria transmission was suspected from some localities with ricefields that are favourable for *An. arabiensis* breeding.

Interpretation: There is need to improve the access to malaria diagnosis tool. Since all the four species of malaria parasites co-exist in Madagascar, RDT detecting pLDH may be useful. Malaria case management will be also discussed in our presentation.
Clinical and parasitological characteristics of puerperal malaria [MIM-SA-23088]

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Introduction: Semi-immune women in endemic regions become vulnerable to malaria during pregnancy. Recent findings indicate that this increased risk might persist beyond delivery but the underlying mechanisms are poorly understood.

Methods: In a cohort study in Lambaréné, Gabon, 150 women were actively followed-up weekly for 10 weeks after delivery along with age and location matched non-pregnant women. Placenta biopsies and peripheral blood spots were genotyped using the merozoite surface antigen-2 and the subtelomeric variable open reading frame.

Results: Eleven cases of clinical malaria were observed in the postpartum group versus 1 in the control group (rate ratio: 9.8; p = 0.006). Eighteen women had Plasmodium falciparum parasitemia, compared to 6 in the control group (rate ratio: 2.7; p = 0.03). Five out of 16 women with parasitemia on follow-up (31%) had identical parasites in placenta and periphery and 11 (69%) were due to reinfection. Women remained equally susceptible throughout the first 10 weeks of observation. Bednet usage, chloroquine prophylaxis during pregnancy, malaria episodes during pregnancy, gravidity and age were not associated with acquiring parasitemia during follow-up.

Interpretation: Women in the puerperium have a considerably increased risk for malaria and parasitemia. This is due to both the re-appearance of P. falciparum infections acquired during pregnancy as well as an increased susceptibility to new infections.

Duration of Plasmodium falciparum infections [MIM-WS-355768]

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Introduction: The duration of Plasmodium falciparum infections within a host is a crucial determinant of the effects of preventative interventions. Surprisingly little is known about this because it is difficult to estimate it in areas where people are continuously re-infected. We consider the general problem of estimating this quantity when: (i) infection and clearance processes may depend on acquired immunity AND (ii) the means of detection has an unknown sensitivity which may also depend on acquired immunity.

Methods: (a) We use repeated cross-sectional prevalence data summarized by age-groups from three historical malaria projects in different endemic areas where transmission was interrupted by indoor residual spraying. We review the use of methods based on fitting exponential decay curves to the observed data, assuming that transmission is completely interrupted. (b) We use an immigration-death model, allowing for imperfect detection, to analyse molecular typing data from people whose infections are not treated, and who are exposed to new inoculations. Immigration refers to the acquisition while death refers to the clearance of a parasitic clone. This approach is tested on a panel dataset from a study of the dynamics of Plasmodium falciparum in Ghana.

Results: The analyses on the historical datasets where data were obtained by the use of optical microscopy suggest that Plasmodium falciparum can persist within a host for a period of 2–3 years. These estimates are much greater than the mostly widely quoted figures (of about 200 days). However, the same model for the underlying process can give very different results if it is fitted to data summarized cross-sectionally or longitudinally. The analyses of the molecular typing data obtained by PCR suggest that on average any individual residing in this holo-endemic area will acquire 16 new genotypes per year and that infection with any of
these genotypes lasts an average of 152 days. We also estimate that an average of 47% of the parasite clone present in a host can be detected in a finger-prick blood sample by PCR. When assessed by age, this quantity varied from about 60% in younger individuals to 10% in adults suggesting a greater influence of acquired immunity. We did not find any strong evidence suggesting that acquired immunity has much influence on the infection rate or the rate at which infections are cleared.

Interpretation: We find that the duration of Plasmodium falciparum infections is not highly influenced by acquired immunity. It can depend on the parasitological diagnostic tool and the survey methodology used and it can also vary depending on the endemic setting.

161B
Epidémiologie de la transmission du paludisme en saison sèche dans le cercle de Ménaka au nord-est du Mali [MIM-LS-7482]
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Methods: Nous avons conduit une étude traversale en mai 2004 dans le cercle de Ménaka au nord-est du Mali. Au total, 1328 sujets ont été inclus dans quatre communtes du cercle et cela sur neuf sites (9 dont 39.4% de l’échantillon est leucodermes. Nous avons procédé à une stratification de la population à étudier en 12 groupes de 108 unités statistiques à travers les neufs sites. La goutte épaissie et le test rapide de l’Optimal® DiaMed ont servi au diagnostic et à la mesure de la parasitémie palustre. La mesure du taux d’hématoctrie a été faite pour évaluer l’anémie. L’examen physique et clinique de chaque sujet nous a permis d’évaluer la morbidité palustre.

Results: La prévalence de l’infection palustre obtenue au cours du premier passage, période sèche est de 4.9% soit 66 sujets infectés parmi les 1328 inclus. Les prévalences les plus élevées ont été retrouvées à Ménaka ville avec 12%, suivi de Tagalalt avec 6.3%, de Tin-avab 6.1% et d’Andaramboukane avec 3.2%. Tous ces sites sont situés autour des mares, contrairement aux sites de Tidarémè-Ikadewane (0.9%), Tabangou (0%) et Anuzegreen (0%). La formule parasitaire était la suivante: Plasmodium falciparum 84.9%; P. vivax 7.6%; P. malariae 4.5% et P. ovale 3%. Les mélanodermes étaient beaucoup plus exposés à l’infection par P. falciparum, soit 2 fois plus de risque que les leucodermes (OR = 1.93; IC95% = 1.02–3.65; p = 0.02). La prévalence des porteurs de gamétocytes était de 0.48%. Nous n’avons pas identifié de souches chloroquino-résistantes à P. falciparum. Le taux de morbidité palustre était de 3.46% au cours de ce passage.

Interpretation: Le paludisme est hypo-endémique en saison sèche dans le cercle de Ménaka, où circulent les quatre espèces de Plasmodium. La prévalence assez élevée de l’infection à P. vivax mérite une réflexion pour un schéma thérapeutique adapté.

162C
Paludisme dans une Zone de Savane du Mali: Morbidité et mortalité, prévalence de la réponse des souches de P. falciparum à la chloroquine [MIM-AS-187396]
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Introduction: Le paludisme reste toujours un problème majeur de santé publique, les enfants de moins de cinq ans constituent un des groupes à risque. Au Mali, le paludisme est saisonnier, nous avons initié une étude longitudinale de 7 mois du début de la saison des pluies à la saison sèche froide pour comprendre la distribution des différents types de paludisme et d’identifier la période de forte prévalence.

Methods: Nous avons mis un système de détection pas-sif de cas de paludisme dans le Village afin d’évaluer le taux de morbidité parmi les 355 enfants entre 0 à 9 ans entre Juin et Décembre 2004. A chaque visite d’un enfant au centre de santé, une goutte épaisse pour l’identification et la quantification de la densité parasitaire, un confetti pour la détermination du gènotype des souches de *P. falciparum* ont été faits en plus de l’évaluation du taux d’hémoglobine. Un examen clinique est conduit et consistait à une prise de la température, une palpation de la rate et une interview de la maman pour décéler des événements de coma et de convulsions. La réponse des souches de *P. falciparum* a été évaluée par l’utilisation des critères de tests in vivo de l’OMS de 1996.

Results: Au cours de cette étude, 502 consultations ont été effectuées au niveau du centre de santé du village. Le paludisme représentait 44.22% (222/502) des consultations. Le plus nombre d’enfants se présentant avec le paludisme a été observé pendant le mois d’Août avec 64 cas soit un taux de morbidité de 28.82% durant la saison des pluies. Le plus faible taux était observé dans les mois d’Octobre et Novembre avec 2.95%.

Discussion: Le paludisme représentait 44.22% (222/502) des consultations. Le plus nombre d’enfants se présentant avec le paludisme a été observé pendant le mois d’Août avec 64 cas soit un taux de morbidité de 28.82% durant la saison des pluies. Le plus faible taux était observé dans les mois d’Octobre et Novembre avec 2.95%.

Interpretation: Selon nos résultats, une stratégie de contrôle doit être mise en place en début de l’hivernage pour prévenir la morbidité et la mortalité palustres observées entre les mois d’Août et Septembre dans les zones de savane où le paludisme est saisonnier.

163A Malaria and enteric fever in the slums of Kolkata, India: Data from a prospective, community-based study [MIM-Lv-48836]


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Introduction: Recent research has indicated that the malaria burden in Asia may have been vastly underestimated. We conducted a prospective, community-based study in an impoverished urban site in Kolkata (formerly Calcutta) to estimate the burden of malaria and enteric fever and search for potential risk factors.

Methods: Following a census treatment centre based surveillance for febrile illness was conducted throughout 2004. Clinical data and a blood sample for microscopy and culture were collected from each patient.

Results: In a population of 60,615 we detected 3371 febrile patients over a 12 months period. The blood film of 93 febrile patients contained *P. vivax*. The blood cultures from 95 patients grew *S. typhi* and from 63 patients *S. paratyphi*. A malaria patient was found to be significantly older (mean age 29.2 years) compared to patients with enteric fever (15.8 years; *p* < 0.001). A household member with malaria was a highly significant risk factor for malaria; 9 of 91 (10%) malaria patients compared to 438 of 57,218 (1%) of malaria free residents had a household member with malaria (*p* < 0.001). Having a household member with typhoid fever and no soap for hand washing were highly significant risk factors for typhoid fever. A GIS analysis of the spatial distribution of malaria and enteric fever cases demonstrated different high risk neighbourhoods for each disease.

Interpretation: Vivax malaria is problem of significant proportion as typhoid fever in Kolkata in 2004. Focal interventions such as mosquito control could be an approach to reduce malaria while improved personal hygiene, safe water supply and sanitation as well as vaccination programs could help to reduce the local typhoid fever burden.
**164B**

**Program evaluation of the Togo Integrated National Child Health Campaign: Impact on malaria morbidity in young children at community-level** [MIM- DT-473704]

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**Introduction:** In an effort to scale-up malaria control efforts and achieve a high, equitable country-wide insecticide-treated bed net (ITN) coverage, Togo hosted the first national integrated measles vaccination and ITN distribution campaign. In December 2004 over 900,000 long-lasting ITNs were handed out, targeting all households with children <5 years. As part of a multi-disciplinary evaluation, the impact on prevalence of anaemia, malaria infection and clinical malaria at population level was assessed.

**Methods:** Two community-based cross-sectional surveys were conducted in three districts in South, Central, and North Togo during the rainy season before (September 2004) and after the campaign (September 2005). A stratified two-step sampling method was used to select representative samples of children <5 years living in rural areas (target \( n = 2700 \) /survey, different children/survey). In addition to a structured questionnaire on history of illness and treatment and use of preventive measures, a physical examination was completed and malaria parasitaemia and haemoglobin (Hb) level determined. To estimate the impact of annual variation in malaria transmission, results were linked to retrospective facility-based morbidity surveillance and rainfall data.

**Results:** At the time of submission of this abstract, the post-intervention survey has not taken place. So far, the Baseline 2004 survey indicated a high burden of malaria in the target population. Out of 2532 enrolled children from 1740 households, 23.1% reportedly sought health care for a febrile episode. Only 9 (0.4%) of children were reported to have slept under an ITN the previous night. Overall, 74% were parasitaemic, and 26.8% of them had a documented fever at the time of the survey. Parasitaemia prevalence was significantly higher in the Northern districts Ogou and Tone, compared to Yoto (62.5%, 78.5%, and 78.9%, \( p < 0.0001 \)). Malaria prevalence increased rapidly in infancy from 18.2% at 0–2 months to 43.0% at 3–5 months, increased further until the age of 2 years and remained high thereafter, ranging from 60 to 71%. Peak clinical malaria prevalence occurred at age 12–17 months. The majority of children were anaemic (84.4%, Hb < 11 g/dL). Moderate-to-severe anaemia (<8.0 g/dL) was found in 21.7% with a peak prevalence over 30% in children aged 6–18 months. Malaria and anaemia were strongly correlated; moderate-to-severe anaemia was more likely in parasitaemic than aparasitaemic children (OR = 3.7, 95% CI 2.5–5.3).

**Interpretation:** Since December 2004, Togo is well underway to reach WHO’s childhood ITN coverage target at national level. Preliminary data of the impact on the childhood malaria burden, their interpretation and relevance to future campaigns will be discussed.

**165C**

**Consecutive two-year survey of transmission-blocking activity in natural gametocyte-carriers living in a malaria endemic village of Mali, West Africa** [MIM-AT-44460]

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**Introduction:** Plasmodium falciparum malaria transmitted by Anopheles gambiae s.l. mosquitoes is endemic with seasonal patterns of transmission in Mali. Population living in areas like Bancoumana experience two peaks, one in August (middle of rainy season) and another in October (end of rainy season). They may develop a certain transmission-blocking immunity; thus, we evaluated the potential of blocking activity in different age groups two consecutive transmission seasons.

**Methods:** Consenting gametocyte carriers recruited after positive thick smears for mature stage five P. falci-
parum gametocytes. They were transported to our laboratory in Bamako, where blood taken by vein puncture was mixed with CPD, and then divided in two, and one portion was washed twice with PBS to obtain a washed blood. The unwashed and washed blood, were given simultaneously to mosquitoes through membrane feeders connected to a water jack pump. A week later, mosquitoes from each group were dissected to determine oocyst rates.

Results: Of 50 gametocyte-carriers, 20 infected mosquitoes in either washed or unwashed blood or both. There is no significant difference in oocyst infection rates between washed and unwashed blood ($X^2_{Yates} = 0.21, p = 0.64$). In contrary, mean oocyst densities in unwashed blood were significantly higher than that in washed blood (ANOVA, $F = 4.02, p = 0.04$). Oocyst infection rates were significantly higher in the age group over 18 than in the age group 9–18 ($F_{isher}, p \ll 0.05$). Evidence of transmission-blocking effects is that infection rates and oocyst loads in some of the washed blood groups are at least two-folds higher than rates and loads in the unwashed blood group.

Interpretation: Our results show that population living in malaria endemic areas develop natural TBI to be necessarily assessed for further TBV studies.

9 + 26. Treatment of malaria/rational drug use

Posters 166–244

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

166A

Effect of quinine versus artemether in treatment of Sudanese children with cerebral malaria: Biochemical evaluation [MIM-BA-47572]

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Introduction: Cerebral malaria is a major cause of death of patients suffering from malaria. Of the more than 500,000 African children who develop cerebral malaria each year, 10–20% die and approximately 7% are left with permanent neurological damage.

Methods: Forty one hospitalized patients were randomly selected and enrolled in the study; 21 males 20 females, age range from 1 to 15 years. The objectives of the study were clarified to all patients’ parents to obtain their consent. Twenty-four children were treated with Artemether whereas, 17 patients were treated with Quinine according to WHO regime.

Results: All patients of the two groups completely recovered from cerebral malaria with no recrudescences confirmed by clinical and microscopical examinations. Biochemical investigations, serum creatinine, urea, total protein, albumin, and alkaline phosphatase were found within the normal levels during treatment in the two groups. In Quinine treated group there was significant decrease in the blood glucose level compared to the artemether treated group ($p < 0.01$). However, artemether treated group showed statistically significant increase in the liver enzymes compared to that of children treated with quinine although the two groups were found within the normal values.

Interpretation: On the basis of the biochemical results reflecting normal metabolic profile, artemether is effective in the treatment of cerebral malaria with no serious side effects.

167B

A clinical trial to compare the efficacy of intrarectal versus intravenous quinine in the treatment of childhood cerebral malaria [MIM-JA-277784]

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Introduction: Malaria is responsible for up to 500 million episodes of infection, 2.7 million deaths and remains the major cause of morbidity and mortality in children. Cerebral malaria is the most lethal form of complicated malaria. Quinine remains the most effective treatment for severe malaria, but accessibility is still a problem. Intrarectal quinine can be used as early treatment and could decrease the morbidity and mortality associated with severe malaria.

Methods: Randomized double-blind placebo controlled clinical trial. We recruited children aged between 6 months and 5 years presenting to the Acute
Care Unit during the study period. Patients diagnosed with cerebral malaria were randomized to treatment with either intrarectal or intravenous quinine. All patients were followed up for 7 days. Outcome measures included fever clearance time, parasite clearance time, coma recovery time, time to sit unsupported, time to begin oral intake, time to sit unsupported, time to begin oral intake, time to sit unsupported and duration of intervention and death. Adverse drug events and neurological sequelae were also documented.

Results: A total of 110 children were recruited between September 2003–January 2004. Fifty-six of these received intrarectal quinine and fifty-four received intravenous quinine. The coma recovery time, fever clearance time, parasite clearance time, time to begin oral intake, time to sit unsupported, time to begin oral intake, time to sit unsupported and duration of intervention were similar in the two treatment arms. Overall mortality rate was 8.2% and neurological sequelae were seen in 3.96% of the survivors. There was no statistically significant difference in mortality or occurrence of neurological sequelae in the two treatment arms. Intrarectal quinine was well tolerated and no adverse events were documented in this study.

Interpretation: Intrarectal quinine is as effective and as safe as intravenous quinine so it could be used as a safe and effective alternative to intravenous quinine in the treatment of childhood cerebral malaria.

168C Efficacy of artesunate + amodiaquine, artesunate + sulfadoxine–pyrimethamine, chloroquine + sulfadoxine–pyrimethamine in P. falciparum malaria in Comoros Union [MIM-TA-71550]

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Introduction: Antimalarial resistance is a real public threat in Comoros Union. This study was undertaken to provide nationally relevant information on the antimalarial efficacy of artesunate + amodiaquine (AS + AQ), artesunate + sulfadoxine–pyrimethamine (AS + SP) and chloroquine + sulfadoxine–pyrimethamine (CQ + SP), with a view to updating antimalarial policy in Comoros Union.

Methods: Between March and April 2003, standard WHO methodology for in vivo efficacy assessment was used in the three Comoros Union’s islands to study the therapeutic response of uncomplicated falciparum malaria treated with AS + AQ (n = 110), AS + SP (n = 108) or CQ + SP (n = 108). Follow-up was 14 days with polymerase chain reaction genotyping to distinguish late recrudescences from re-infections.

Results: A total of 1097 out-patients were screened and 463 smears/thick blood films were taken (Grande Comore 118, Anjouan 142, Moheli 203). The inclusion criteria were fulfilled for 326 patients. Overall, 155 subjects reached an analyzable endpoint, treated, respectively by CQ + SP (n = 51), AS + SP (n = 53) or AS + AQ (n = 54). No case of ETF was recorded in any study arm. In subjects treated with artesunate + amodiaquine, ACPR rate was 100% whilst 1.96% (1/51) of late parasitological failure occurred in the arm chloroquine + sulfadoxine and 1.85% (1/53) of late treatment failure were recorded in the arm artesunate + sulfadoxine–pyrimethamine. No death not related to the treatment was observed in the arm AS + AQ.

Interpretation: Whatever the new first-line treatment that will be definitely adopted in Comoros Union, monitoring of acceptability and tolerance in the context of pharmacovigilance is a necessity. Parameters, that may affect compliance should also be considered.


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**Introduction:** Drug combination for the treatment of *P. falciparum* malaria might delay the emergence and spread of resistance. The efficacies of combination regimens – including SP plus artesunate – were superior to that of SP monotherapy.

**Methods:** The efficacies and adverse effects of artesunate plus sulfadoxine–pyrimethamine (SP) versus that of SP alone for the treatment of uncomplicated *P. falciparum* malaria were assessed through an opened randomized clinical trial conducted in New Halfa, eastern Sudan during September and October 2004.

**Results:** Compared with those given the combination, the patients given SP alone were more likely to be febrile (30% versus 3.3%; *P* = 0.006) and parasitaemic (50% versus 6.7%; *P* < 0.0001) on day 1. By day 3, 16.7% of the patients were still febrile and 6.7% were still parasitaemic in SP group versus none of the patients in the combination (*P* = 0.02 and *P* = 0.1, respectively). The adverse effects (nausea, itching and giddiness) were observed in three (10%) versus four patients (13.3%), *P* > 0.05 received the combination and SP, respectively. During the follow-up, 23.3% of the patients who received SP had gametocytaemias, that were not detected in any of the patients who received the combination, *P* = 0.005.

**Interpretation:** Thus, while the adverse effects were similar, rapid fever and parasite clearance time and less gametocytaemia were observed in the combination when compared with SP monotherapy.

**170B**

Disposition kinetics of *P. falciparum* during treatment with chlorpheniramine and chloroquine combination in acute uncomplicated malaria in children

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**Introduction:** Chlorpheniramine has been found to reverse chloroquine resistance in vitro. It has also been observed to improve the clinical efficacy of chloroquine. Evaluation of the kinetics of *Plasmodium falciparum* during treatment with chlorpheniramine plus chloroquine in children with acute, uncomplicated malaria and was correlated with the conventional indices of therapeutic evaluation.

**Methods:** One hundred and six patients with malaria were randomized to one of two treatment regimens of chlorpheniramine plus chloroquine. Outcome of treatment were assessed using both the conventional fever clearance time (FCT), parasite clearance time (PCT), cure rate and parasite kinetics parameters using the area under the parasite density versus time curve (AUCpd), apparent half time of reduction of *parasitaemia* (*t*1/2pd), volume of blood completely cleared of parasite (CLBpd). Parasite clearance was also assessed using the Parasite clearance50 (PC50), the time for the parasite count to fall by 50% of the enrolment pre-treatment value.

**Results:** The FCT was 1.4 ± 0.7 days and 1.3 ± 0.7 days for the high dose CP plus CQ and the very high dose CP plus CQ groups, respectively. The mean PCT was 2.8 ± 0.7 and 2.9 ± 0.7 days, the cure rate was 95.8 and 94.1%, respectively. The parasite kinetics indices AUCpd, *t*1/2 pd and CLBpd were 1.61 ± 3.28 versus 1.51 ± 2.85 ul h−1, 3.65 ± 1.04 versus 4.00 ± 1.79 h and 0.006 ± 0.005 versus 0.008 ± 0.0058 ul h−1 kg−1 in the high dose CP plus CQ and the very high dose, respectively. There was positive and significant correlation between *t*1/2 pd and PC50 *r*2 = 0.049, *p* = 0.047 and between *t*1/2 pd and PC30 *r*2 = 0.073, *p* = 0.015.

**Interpretation:** No therapeutic advantage between the two doses of CP plus CQ combination. Positive and significant correlation between the kinetic and conventional indices suggest the former may find ready application in therapeutic efficacy monitoring.

**171C**

Comparative efficacy and safety of quinine and artemether in the treatment of severe falciparum malaria

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Introduction: Severe malaria is a medical emergency with devastating multisystemic effects that requires prompt treatment with sensitive and safe drugs otherwise death is imminent. Quinine and artemether are favoured drugs for the treatment of severe malaria. There are reports of decreased sensitivity and resistance to quinine. Artemether is known to be a sensitive broad-spectrum antimalarial and its use is gaining popularity in the treatment of severe malaria.

Methods: Thirty-two patients with severe malaria were randomly assigned to receive either artemether or quinine under medical supervision. 16 patients were allocated into each treatment group. Patients in the quinine treatment group were given 10 mg/kg body weight quinine in 5% dextrose/saline intravenously eight hourly till recovery from coma or able to take oral dose, while artemether group were given 1.6 mg/kg body weight intramuscularly for 5 days. The patients were then followed up for 14 days for clinical and parasitological response.

Results: Mean fever clearance time for quinine was significantly lower when compared with artemether, (46.50 ± 20.49 versus 72.00 ± 27.71 h) (P= 0.006). The malaria parasite clearance time was however significantly lower with artemether than with quinine (31.50 ± 14.45 versus 46.50 ± 6.00 h) (P= 0.001). Coma resolution time was 9.00 ± 24.59 h for quinine and 4.50 ± 13.05 h for artemether (P=0.523). Adverse events, such as tinnitus and insomnia encountered in quinine group were generally mild. There was no adverse effect of note observed with artemether.

Interpretation: While quinine cleared fever faster, artemether was better tolerated, showed more rapid malaria parasite clearance, a faster and sustained recovery from anaemia as well as shorter coma and jaundice resolution time.

172A
Assessment of in vitro schizonticidal activity of methylene blue against Plasmodium falciparum from Nigerian children [MIM-CN-134360]
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Introduction: The spread of resistance in Plasmodium falciparum to chloroquine and sulphadoxine-pyrimethamine, the commonly available antimalarial drugs in Africa has placed serious obstacles on the successful chemotherapeutic control of malaria in endemic countries in Africa. Methylene blue, a thiazine dye is basically used in the treatment of various methemoglobinemas. Sporadic reports have shown some antimalarial therapeutic effect when administered to patients with clinical manifestations of malaria.

Methods: Fresh blood isolates were obtained from symptomatic Nigerian children residing in Ibadan, Southwest Nigeria prior to drug administration. Their age ranged from 4.5 to 11.5 years. Inclusion criteria include: presence of signs and symptoms of acute uncomplicated malaria, mono-infection with P. falciparum, initial parasitaemia >4000 asexual parasites/μl of blood and no history of recent antimalarial drug intake within the last 2 weeks. To determine the antimalarial activity of MB and evaluate its efficacy against P. falciparum isolates in vitro, standard WHO microtest technique of schizont inhibition assay was used to culture fresh isolates from recruited patients. Quinine and Chloroquine were used as reference drugs in the assay.

Results: Fourteen out of twenty isolates (70%) were successfully cultivated to mature schizonts within 48 h. The MIC values were in the range of 10 °C 1000 nM for MB, 96.9 °C 969 nM for CQ and ≥638.6 nM for QN. The mean IC50 values were 9.59 nM for MB, 196 nM for CQ and 607 nM for QN. Ten of the fourteen (71.4%) successful isolates were sensitive to MB (IC50<9.59 nM). Ten of the fourteen (71.4%) successful isolates were sensitive to CQ (IC50 <100 nM). Eight of the twelve (66.7%) successful isolates in the plate were sensitive to QN (IC50<500 nM). Four of the fourteen (28.6%) isolates were resistant to CQ (IC50 >100 nM), 33.3% of the isolates were resistant to QN (IC50 >500 nM), and four of the fourteen (28.6%) isolates were resistant to MB (IC50 > 9.59 ± 3.25 nM). Three of the four (75%) CQ-resistant isolates were sensitive to MB while only one (25%) was resistant to MB (IC50 = 26.35 nM). Three of the four (75%) CQ-resistant isolates were also resistant to QN meaning that only one (25%) was sensitive to QN. Two of the four (50%) QN-resistant isolates were sensitive to MB. Two of the three (66.7%) isolates showing cross-resistance between CQ and QN were sensitive to MB in vitro.
The third isolate (lab 024) was resistant to all the three drugs.

**Interpretation:** This study showed that MB has significant schizonticidal activity comparable with CQ andQN. It is also effective against CQ-resistant parasites. However, its safety needs to be assessed in larger populations in different malaria endemic settings.

**173B**

**Effect of Suphadoxine-pyrimethamine on antioxidant defense system and lipid peroxidation [MIM-AA-2001737]**

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**Introduction:** Due to the spread of resistance to chloroquine by *P. falciparum*, Sulphadoxine-pyrimethamine (SP) became the alternative drug of choice for the treatment of malaria in most endemic areas. Production of oxygen radicals form part of the host defense and pathology of malaria. Exacerbation of intra-erythrocytic oxidative stress might contribute to eliminating the parasites. The effect of treatment with SP on the antioxidant defense system was investigated using rabbit as a model.

**Methods:** Ten male rabbits were divided into two groups of five animals each. The first group was administered with normal saline and served as control. The second group received a single dose of SP (26.25 mg/kg body weight). Blood samples were collected before and at 6, 12 and 24 h after saline or drug administration. Activity of cellular enzymic antioxidants, superoxide dismutase (SOD) and catalase (CAT), and reduced glutathione (GSH) were assayed using standard photometric methods. Serum lipid peroxidation was assessed by the formation of malondialdehyde while protein content was assayed by the method of Lowry.

**Results:** SOD activity was observed to increase progressively by 4.9, 63.4 and 120.8% at 6, 12 and 24 h, respectively. Malondialdehyde levels also significantly increased by 45.5, 118.2 and 186.4%. However, the activity of GSH was observed to have decreased by 41.9% by 6 h and remained so till the 12th hour, but by 24 h after drug administration, the activity has increased significantly up to 48.4% above the 0 h level.

**Interpretation:** SP treatment altered the enzymatic antioxidant defense system and lipid peroxidation in blood and therefore could induce oxidative stress. The increase in SOD activity is an indication of generation of reactive oxygen species.

**174C**

**Efficacy of fansidar alone and in combination with chloroquine for malaria treatment in Sudan: Interrelation between resistance, age and gametocytes [MIM-IA-18724]**

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**Introduction:** The combination of anti-malarial therapy for treatment of uncomplicated *P. falciparum* malaria was a policy advised by world health organization, adopted by national malaria control programs, and became a trend in sub-Saharan Africa. The synergism of action, prolongation of therapeutic life span in terms of efficacy of individual drugs, and reduction of parasite transmission, especially the artemisinin based combinations, were the privileges of the deployment of combination therapy.

**Methods:** We enrolled 260 individuals with uncomplicated falciparum malaria in rural Eastern Sudan. In the study area the prevalence of chloroquine (CQ) and fansidar (SP) resistance-associated genes are extremely high (>80%). We modified the 28-days in-vivo drug efficacy protocol of the WHO (1996). Patients were allocated randomly in two treatment regime (CQ plus SP and SP alone) with considerable similarity between the two groups. Treatment outcome had been classified into three categories: early treatment failure (ETF), late treatment failure (LTF) and adequate clinical and parasitological response (ACPR). Patients had parasitemia at day 3 with or without symptoms that resolved by D7, were categorized as having delayed parasitological response (DPR).
Results: Our results revealed a comparable treatment failure (TF) for SP alone (31.7%) or with CQ (36.6%). Benefiting the epidemiological setting, we were able to show that the host age and parasite gametocytogenesis as surrogate markers for immunity and transmissibility, respectively, were significantly associated with SP resistance. Patients achieved ACR, were significantly older than patient had TF (median age, 21 and 12 years, respectively), \( P < 0.001 \). Regarding gametocytogenesis; (a) the gametocyte productivity was significantly higher in patients with TF compared to ACR; 0.72 and 0.45, respectively, \( P < 0.001 \), thus, gametocytemia more associated with younger age (b) gametocyte counts were comparable between TF and ACR groups of patients until Day7 of follow-up, thereafter (D14, D21 and D28), gametocyte count was significantly higher in patients with TF; \( P = 0.024 \), 0.002 and 0.061, respectively. However, the longevity of gametocytes in patients with TF and ACR were comparable. Interpretation: We demonstrated that semi-immunity to malaria contributes in SP resistant parasites clearance. So, fast propagation in SP resistance may attribute to the selection of parasites and expansion of resistant populations by gametocytogenic effect for SP.

175A
Comparative efficacy study of CQ, DHA and DHA plus mefloquine combination in children with acute uncomplicated falciparum malaria [MIM-OA-43473]
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Introduction: Malaria is a major parasitic disease with an estimated annual prevalence of 300–500 million clinical cases. Over 2 million children below the age of 5 years are reported to die from malaria in Africa alone each year. As a result of increasing prevalence of chloroquine resistance to malaria parasite. This study was therefore designed to compared the efficacy of CQ, dihydroartemisinin and combination of dihydroartemisinin plus mefloquine in the treatment of malaria in southwestern Nigeria.

Methods: Seventy-five subjects aged 2–13 years attending the outpatient department of Ijede health center, Ikorodu Ikorodu Local Government Area, Lagos State, Nigeria were screened for malaria parasites and enrolled into the study after informed consent from the parents/guardians. Subjects were allotted to one of the three treatment groups and followed-up for 28 days. One group was treated with standard dosages of chloroquine (CQ) (25 mg/kg body weight), the second group was treated with dihydroartemisinin (DHA) (2 mg/kg body weight for 7 days) and the third group was treated with the combination of dihydroartemisinin plus mefloquine (MQ) (DHA 2 mg/kg and MQ 15 mg/kg body weight).

Results: Therapeutic responses of the subjects to the antimalarial drugs showed that 16% of the children failed treatment with chloroquine. No treatment failure was observed in the other treatment groups. The parasite clearance time (PCT) of patients treated with dihydroartemisinin plus mefloquine combination (PCT = 36.0 ± 16.97 h) was significantly shorter (\( P = 0.003 \)) compared to those treated with chloroquine alone (PCT = 73.5 ± 45.44 h). Fever clearance time (FCT) was significantly shorter (\( P = 0.003 \)) in children treated with dihydroartemisinin plus mefloquine combination (FCT = 20.0 ± 6.93 h) compared to those treated with chloroquine alone. However, neither PCT nor FCT was significantly different (\( P > 0.05 \)) in children treated with either dihydroartemisinin or dihydroartemisinin plus mefloquine combination.

Interpretation: These results suggest that dihydroartemisinin in combination with other antimalarial drugs may be a good alternative for chloroquine in areas of emergence or high chloroquine resistance.

176B
Home treatment practices of childhood malaria by mothers in an urban setting in southwestern Nigeria [MIM-OA-270192]
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Introduction: The WHO/TDR home-based management (HBM) of uncomplicated malaria recommends that prompt and effective treatment for
children with malaria must be carried out within 24 h at the household level. This can only be achieved however by a good working knowledge and practices of the correct therapeutic home management of children with the disease by mothers and caregivers in endemic areas.

Methods: We studied home treatment practices of malaria by mothers of children presenting with fever at the health centre of a university community. A total of 159 child pair were enrolled for the study. Data was collected with structured questionnaires administered to the mothers of the enrolled children. Information on the demographic characteristics, presenting complaints, drug dosages, compliance and duration of the drugs administered at home, were obtained from each child presenting at the health centre. Mothers were interviewed before they were seen by the doctor except when the child was very ill. Children aged 1–144 months with fever and P. falciparum asexual parasitaemia on examination of thick blood film for malaria parasites were recruited.

Results: Most mothers (54%) were either professionals or skilled workers and 92.3% had at least secondary education. Most of the mothers 145 (91.2%) treated their children at home before presenting at the clinic. Chloroquine was the most commonly administered antimalarial used by 42% of the mothers, 6% used sulphadoxine-pyrimethamine (Fansidar) and 2% used camoquine (amodiaquine) while 1% of the mothers gave halofantrine and artemisinin. Drug treatment administered at home by the mothers was mostly with incorrect dosage. 66% of the mothers that administered chloroquine gave sub-therapeutic doses. Gener ally, drug compliance reduced as the number of days of administration of the drugs increased. Paracetamol was the major antipyretic drug used at home by 76% of the mothers. About 62% of mothers obtained drugs from chemists while left over drug from previous purchase was used by 15%. There was no case of severe malaria and only one patient was referred to a tertiary hospital for further care. There was no mortality.

Interpretation: The success of this RBM strategy for malaria reduction depends on the knowledge and practices of mothers and caregivers. There is therefore a need for appropriate and urgent education of mothers about proper drug usage for home management of malaria.

Malaria burden in children living in a stable transmission area of Burkina Faso: Results from 5 years cohort studies [MIM-TA-37440]

Centre National de Recherche et de Formation sur le Paludisme (CNRFP) Burkina Faso

Introduction: In most of the malaria endemic regions, the presence of fever is the major criteria on which is based the decision to treat for malaria episode. In the study area, P. falciparum is the main parasite identified in more than 90% of the malaria infection. To characterized a site for malaria vaccines testing we investigated the part of the fever attributable to P. falciparum positive parasitemia at different threshold; as well as the incidence of clinical malaria episodes.

Methods: During 5 years in the village of Balonguen we conducted yearly three cross-sectional studies at the beginning; the peak and the end of the transmission season. at each visit the body temperature were recorded and a blood slide obtained from all the children. A longitudinal follow-up was also carried out every year during the high transmission season. The children were visited at home twice a week to record their temperature and obtain a blood slide if the T > 37.5 °C.

Results: During 5 years in the village of Balonguen we conducted yearly three cross-sectional studies at the beginning; the peak and the end of the transmission season. at each visit the body temperature were recorded and a blood slide obtained from all the children. A longitudinal follow-up was also carried out every year during the high transmission season. The children were visited at home twice a week to record their temperature and obtain a blood slide if the T > 37.5 °C.

Interpretation: Our findings suggest: (i) that the presence of fever may not be a good indicator for the presumptive diagnosis of malaria infection in children below 1 year and above 5 years and (ii) the presence of fever is not related to the parasites density.
178A
Impact of a pretreatment with Fansidar® or Coartem® on malaria infection and clinical episodes in under five children in Burkina Faso [MIM-OA-58536]
Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina faso

Introduction: The risk of malaria episode vary widely across malaria endemic regions depending on transmission pattern, level of immunity and age. To access the impact of some malaria control tools, a pretreatment of intervention groups may be required. To characterize a site for malaria vaccines trials, we measured the incidence density of clinical malaria and Plasmodium falciparum infection rate during the high transmission season in 2004 in pretreated children with Fansidar® or Coartem®.

Methods: A cohort of 580 children from two villages of the Health District of Saponé was enrolled in a cohort for longitudinal follow-up. At the beginning of the study, randomly selected children received supervised curative therapy with Fansidar® (197 children) or Coartem® (88 children); a third group no treatment (295 children). They were actively followed up by study nurses by home visit twice a week. A blood smear was taken from children with fever (Axillary Temperature ≥ 37.5 °C) or history of fever during the last 48 h. Blood films were also taken from children brought to the nurses with illness. We collected from pretreated children a blood film once every 2 weeks for the active detection of infection until the child was found positive.

Results: The incidence density of malaria episode was significantly higher in the pretreated group with Coartem® than non-treatment group (6.97% CI 95% [5.74–8.20] versus 5.07% with CI 95% [4.46–5.67]). No significant difference were found when comparing the Coartem® and the Fansidar® groups (respectively 6.97% CI95% [5.74–8.20] versus 5.09% CI95% [4.38–5.8]) or the Fansidar® and no treatment group (respectively 5.09% CI 95% [4.38–5.8] versus 5.07% CI 95% [4.46–5.67]). The incidence density of malaria infection was higher in the Coartem® group than the Fansidar® group; however the difference was not statistically significant (9.07% CI 95% [7.66–10.47] versus 7.44% CI 95% [5.58–8.30]).

Interpretation: Our findings suggest that the radical elimination of parasitemia may increase the susceptibility to malaria infection and therefore clinical malaria episode.

179B
Responses to malaria among under five children in rural Ethiopia [MIM-WA-6852]
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Introduction: Malaria is the leading cause of illness and death in sub-Saharan Africa particularly among children less than 5 years old. Early diagnosis and prompt treatment of malaria fever with effective anti-malarial both at health facility and community levels is strongly recommended. However, there is a limitation of information and experience on the early diagnosis and treatment of malaria among under five children in rural areas of Ethiopia. Therefore, the objective of this study was to assess the treatment seeking behaviour of children with malaria fever in a rural community.

Methods: A community-based cross-sectional study was conducted in October–November 2003 in 18 rural Peasant Associations (PAs) of Adami-Tulu district in central Ethiopia. All mothers or caretakers of children less than 5 years of age in all households in the PAs were interviewed, and history of malaria illness and actions taken preceding 2 weeks of the survey was asked for all children.

Results: Out of 3873 children identified in 2372 households, 817 (21.1%) had malaria fever according to the mothers/caretakers. The main symptoms presented included fever (99%), shivering/chills (92.2%), vomiting (55.1%), sleeplessness/restlessness (12.9%), refusal to feed (21.3%) and high grade body temperature/hot body (10%). Of the total febrile children, 27.3% were first taken to a public health facility, 27% taken to private clinics, 5.6% received home treatment, and 13.3% were neither treated at home nor taken to a health facility. About 72% of the febrile children did not get any treatment within 24 h of the onset of ill-
ness. Among 708 children who received antimalarial treatment, 78.8% got it from one source, 19.4% visited two sources of treatment and 1.8% sought treatment from three sources. Multiple visits were more common among children who obtained their first treatment from CHWs with a subsequent visit to public or private health care facilities. A smaller proportion of children who were first seen at public health facilities or private clinics switched to other sources of treatment.

Interpretation: Children were more likely to have received antimalarial treatment from public health facilities, private clinics and CHWs. Home management of malaria or the use of local herbal remedies at home or traditional healers as the first source of treatment was found to be uncommon. Peripheral public and private health care facilities and community-based interventions should be strengthened to address malaria among children in rural areas. Furthermore, effective antimalarials should be available within the reach of the community for malaria treatment.

180C
A comparison between Coartem®gaved at six doses and four doses in the treatment of uncomplicated Plasmodium falciparum malaria in Senegal, West Africa [MIM-FB-89628]
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Introduction: Faced with the spread of chloroquine resistance, the national malaria control program recommends to evaluate the efficacy and safety of other combination treatments. The aim of this study was to compare the efficacy and safety of Coartem six doses versus four doses and formulate recommendations for the malaria treatment policy.

Methods: An open, randomised, descriptive studies were conducted in the districts of Velingara South east Senegal between September and December 2004. A total of 340 patients were included weighting at least 5 kg and suffering from uncomplicated Plasmodium falciparum malaria. (200 patients for Coartem 6* and 140 patients for Coartem 4*). The patients were seen again on D1 and D2 for treatment and evaluation.

Patients were followed up on D3, D7, D14, D21, and D28 for clinical and parasitological control. For 25% of patients, a venous blood sample was taken on D0, D14 and D28 for haemogram and measurement of transaminase and creatinine levels.

Results: Coartem gaved at six doses showed that the adequate clinical and parasitological response (ACPR) at D14 and D 28 was 100%. The ACPR of the four doses was 99.3% at D14 and 83.6% at D28. After PCR correction the ACPR of Coartem four doses at D28 was 96.4%. The difference of efficacy was not statistically significant. The number of patients with anaemia drop during follow-up for all drugs. No severe adverse events and no renal and hepatic disturbance function were observed.

Interpretation: Coartem at four or six doses, demonstrates very good efficacy in Senegal. Due to the increase of drug resistance as it appeared in south Asia with coartem four doses, it will be recommended for the NCP to use the six doses as the therapy for uncomplicated malaria in sub‐saharian Africa.

181A
Endogenous opioids, mu-opiate receptors and chloroquine-induced pruritus: A double-blind comparison of naltrexone and promethazine in malaria fever [MIM-KB-56028]
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Introduction: Chloroquine induces a severe generalised pruritus, in predisposed Black African patients. Chloroquine may interact with micro-opiate receptors in rats, and both histamine and malaria parasite blood density, contribute to the itching severity in malaria fever in humans. The aim of our present study was to assess and compare the antipruritic efficacy of the micro-opiate receptor antagonist, naltrexone, and the antihistamine, promethazine, in chloroquine treated patients with malaria fever.
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Methods: A double-blind, randomized, parallel group comparison of the chloroquine-induced pruritus intensity and time profile in patients with parasitologically proven malaria fever, who were pretreated with a single dose of either naltrexone 50 mg or promethazine 25 mg orally (six patients each). All patients had an established history of severe pruritus following chloroquine treatment of malaria fever. A self-assessed itching severity score was undertaken at 0, 6, 12, 24, 48 and 72 h after initial chloroquine dosing, and the areas under the pruritus-intensity time curve AUCP0–72 h was determined in each patient and correlated to the malaria parasite density in blood.

Results: Both naltrexone and promethazine subjectively reduced itching severity compared with prior historical experience. One patient on naltrexone and two on promethazine never experienced any itching. There was no statistically significant treatment effect, but a significant time effect (P = 0.001, F = 4.77, d.f. = 5) by two-way repeated measures ANOVA. The AUCP for naltrexone was 82 ± 25 units/h, and 57 ± 34 units/h for promethazine [95% confidence interval for the difference being 73–123]. However, the malaria parasite density in the naltrexone group (740 ± 178 microl (-1)) tended to be higher than in the promethazine group (314 ± 69 microl (-1)). Correction of the AUCP for malaria parasite density (parasite pruritogenic index, AUCP . units/h/parasites/microl blood) tended to be lower with naltrexone 9.1 ± 2.6 than with promethazine 12.2 ± 7.0. There was a highly significant and positive correlation between the malaria parasite density and resultant pruritus was significantly different between naltrexone and promethazine.

182B

The impact of home based management of malaria fever among children under 5 years in the displaced communities of Northern Uganda [MIM-162254]

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Introduction: Home based management of fever using pre-packed, unit-dosed malaria treatment has become an important strategy to implement prompt and effective malaria case management. However, data on its impact are scarce. Here we report on results from Uganda.

Methods: Homapak™, a pre-packed combination therapy for young children consisting of chloroquine and SP (current 1st line treatment) was made available through trained community volunteers in the internally displaced persons (IDP) camps in Kitgum District. Pre- and post-intervention cluster household surveys of treatment practices and anaemia among children aged 2–24 months were undertaken in 4 out of 18 IDP camps. A pre-coded questionnaire was administered to the carer of the identified child and haemoglobin estimation was made using a HaemoCue photometer. Data were analysed using Stata™ software. During data analysis rates were adjusted for age, camp, education of mother and number of people per household using multivariate regression models.

Results: A total of 500 records of children 6–24 months of age were available for the baseline and 552 for the follow-up survey (6 months after start of implementation). The 2-week point prevalence of febrile illness decreased slightly from 83.1% at baseline to 74.3% at follow-up. The proportion of children with fever episodes who received treatment on the same day (within 24 h) increased from 21.7% at baseline to 58.6% at follow-up OR = 4.6 (95% CI 3.3–6.5 p < 0.0001) and that of treatment within first 2 days (within 48 h) increased from 58.5 to 87.3%, respectively, OR = 5.6 (3.8–8.2, p < 0.0001). The average haemoglobin level at baseline was 8.98 g/dL.
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(90.2%) versus 83.3%. However, significant reductions were observed for severe (Hb < 7 g/dL) and moderate (Hb 7.0–9.9 g/dL) anaemia which reduced from 9.9 to 4.4% (OR = 0.36, p < 0.0001) and from 60.9 to 51.8% (OR = 0.61, p < 0.0001), respectively.

Interpretation: Our data show that even in the difficult circumstances of conflict situations delivery of anti-malarials at household level significantly improves treatment practices and reduces malaria associated anaemia in young children.

183C
Punica granatum’s dermis indicates anti-malarial therapeutics and prophylaxis [MIM-DB-92763]
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Introduction: Debilitating malaria has become a disease of the home in over populated developing nations. It inflicts heavy burden on a Nation’s plan outlay. A home level therapy comprising of Tropo-Equatorial herbs and salt is attempted to cure and prevent. It is termed OMARIA T and P.

Methods: Sun dried dermis powder of Punica granatum Linn. at chloroplast stage + dried powder of N. arbor-tritis Linn. leaves (15%) + NaCl (20%). Measured v/v or w/w and filled into gelatin capsule of size No 1. 2 g, orally with water eight hourly for 2 days constitutes therapeutic regimen (OMARIA-T). In regular use at a Indian Red Cross Society’s (IRCS), District’s charitable dispensary since 1998. OMARIA-P. Consists of only dried dermis powder of P. granatum as a mono dose of 1 gm/day (filled @500 mg into gelatin capsule of size No 1) for consecutive 7 days: 1/2 for child; 1/4 th for infants. Short booster doses of identical potency given every three months interval.

Results: Seven thousand eight hundred eighty three febrile children were recruited with an overall Plasmodium falciparum prevalence of 33%. Three thousand seven hundred fifty children were enrolled in the AS + AQ treatment arm and 3608 in the SP arm. During the 90 day follow-up period 11 deaths and 93 hospitalizations were reported in the SP arm and 8 deaths and 84 hospitalizations were reported in the AS + AQ arm. No major adverse drug event was reported. (Note: A detailed analysis of the study will

184A
Effectiveness of sulphadoxine-pyrimethamine (SP) or amodiaquine + artesunate (AS + AQ) for the treatment of uncomplicated malaria in children in Zanzibar [MIM-AB-11732]
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Introduction: Zanzibar was among the first places in sub-Saharan Africa to implement artemisinin based combination therapy for the treatment of uncomplicated malaria. An effectiveness study was conducted in Unguja and Pemba islands to determine the risk of death and hospitalization with severe malaria in children between 3 months and 5 years of age over 90 days following treatment of an episode of uncomplicated malaria with either SP or AS + AQ.

Methods: Children 3 months to 5 years of age reporting symptoms of fever during the past 2 days, with a clinical diagnosis of malaria were randomly allocated either into the SP or AS + AQ treatment arm. The recruited children were followed over a period of 90 days.

Results: Seven thousand eight hundred eighty three children were enrolled in the AS + AQ treatment arm and 3608 in the SP arm. During the 90 day follow-up period 11 deaths and 93 hospitalizations were reported in the SP arm and 8 deaths and 84 hospitalizations were reported in the AS + AQ arm. No major adverse drug event was reported. (Note: A detailed analysis of the study will
be presented during the meeting which may supersede the content of this abstract).

**Interpretation:** Preliminary results do not show any difference in the incidence of death or hospitalization for severe malaria within a 90 day follow-up period following administration of either SP or AS + AQ.

**185B**

**Chemoprophylaxis with sulfadoxine–pyrimethamine to prevent recurrence of anaemia in Gambian children treated previously for this condition**

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**Introduction:** Severe anaemia is usually treated by blood transfusion although transfusion is associated with increased risk of transmission of HIV and other infections. Thus, there is a need to explore new strategies to reduce the incidence of severe anaemia in children with suboptimal haemoglobin levels because they are at increased risk of developing severe anaemia if they develop a malaria infection before their haemoglobin level has returned to the normal range.

**Methods:** In August 2003, a trial was started to study whether monthly chemoprophylaxis with sulphadoxine/pyrimethamine (SP) given during the malaria transmission season can protect Gambian children treated for severe anaemia from developing a recurrence of their severe anaemia. Morbidity was monitored throughout the rainy season and volunteers were followed for a maximum of 18 weeks. Malaria smears were collected whenever a volunteer presented to a health centre with symptoms compatible with malaria and study subjects were seen at the end of the malaria transmission season to document morbidity and mortality. The primary end point of the study is the mean haemoglobin level at the end of the malaria transmission season.

**Results:** After receiving treatment from the hospital, 1200 children admitted to hospital with haemoglobin of less than 7 g/dl were randomised to receive either monthly SP or placebo during the rest of the malaria transmission season. The treatment with the trial medication was safe and well tolerated. No serious adverse events related to trial medication occurred.

**Interpretation:** Data from the study will be presented.

**186C**

**Malaria chemoprophylaxis and the serologic response to measles and diphtheria-tetanus-whole-cell pertussis vaccines**


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**Introduction:** To study the effect of malaria chemoprophylaxis on the vaccine response elicited by measles, diphtheria, tetanus and whole cell pertussis (DTP) vaccines.

**Methods:** In 1975, children <74 months of age from Burkina Faso, were divided into those receiving amodiaquine hydrochloride chemoprophylaxis (CH+) every 2 weeks for 7 months and those who did not (CH−). After 5 months, children received either one dose of measles or two doses of DTP vaccines. Any titer 1:20 from an initial titer of <1:10 was considered seroconversion for measles vaccine, while a four-fold rise in titer for D, T, and P antigens was considered a seroresponse for D, T, and P antigens.

**Results:** For recipients of the measles vaccine, the seroconversion in CH+ and CH− children, respectively, was 92 and 96% (P > 0.05). The seroresponse in CH+ and CH− children, respectively, was 83 and 87% for diphtheria (P > 0.05) and 95 and 91% for tetanus toxoid (P > 0.05). While analysis for pertussis showed a 39% (CH+) and 67% (CH−) response (P < 0.05), analyses using logistic regression to control for sex, age, chemoprophylaxis, weight-
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for height Z-score, and pre-vaccination GMT, demonstrated that chemoprophylaxis was not associated with a significantly different seroresponse for any of the vaccines.

Interpretation: Malaria chemoprophylaxis was not associated with the seroresponse to any of the vaccines accessed. Malaria chemoprophylaxis prior to vaccination in malaria endemic settings does not improve or impair immunogenicity of these vaccines.

187A
Efficacy and safety of artemether-lumefantrine versus amodiaquine plus artesunate: Randomised controlled trial in Uganda [MIM-HB-200532]

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Introduction: In 2004, the Ugandan Ministry of Health reviewed data from drug efficacy studies conducted in Uganda in 2002–2004 and provisionally selected artemether–lumefantrine (Coartem®) to replace chloroquine + sulfadoxine–pyrimethamine as first-line therapy for uncomplicated malaria. However, comparative data on Coartem are lacking from Uganda. We are currently comparing Coartem to amodiaquine (AQ) + artesunate (AS) to inform future policy decisions.

Methods: We are conducting a randomised single-blinded trial comparing the efficacy and safety of Coartem and AQ/AS for treating uncomplicated falciparum malaria in Tororo Uganda, a holoendemic area. Eligible children aged 1–10 years with uncomplicated P. falciparum malaria receive Coartem (six doses over 3 days) or amodiaquine (25 mg/kg over 3 days) plus artesunate (4 mg/kg daily for 3 days). All treatment is directly supervised. Clinical and laboratory evaluations, including assessment for adverse events, are performed at each clinic visit over 28 days of follow-up. We assess treatment outcomes using the WHO criteria (2003).

Results: A blinded interim analysis including 121 participants (targeted sample size = 400) is presented here. Of these participants, 95 (79%) are below 5 years of age (mean age 28.5 months). The mean parasite density at enrolment is 35,713 parasites/ul, and mean haemoglobin is 9.8 g/dL. Of 121 participants enrolled in the study, 117 (97%) completed 28-day follow-up and were assigned a treatment outcome. Treatment failure occurred in 51 (43%) participants, including early treatment failure (1%), late clinical failure (27%) and late parasitological failure (15%). An adverse event of moderate or greater severity was reported in 63 (52%) participants. Events were commonly undistinguishable from symptoms of malaria. Only two participants experienced serious adverse events, and a causal association with the study medication was not suspected.

Interpretation: We expect to complete enrolment by July 2005. Genotyping will be performed in Kampala to distinguish recrudescent and new infections, and complete, unblinded results will be presented.

188B
Efficacy and safety of a combination of artesunate/mefloquine, Artequin (TM), in African children and adults with uncomplicated P. falciparum malaria [MIM-NC-204512]

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Introduction: The combination of artesunate and mefloquine is one of the most effective treatments of multidrug resistant P. falciparum. Artequin combines these two antimalarials in a pre-packed single blister for simultaneous co-administration once daily for 3 days. Efficacy and safety of this combination have been demonstrated in Phase III clinical studies elsewhere. The aim of this study was to document the usefulness of Artequin in small children and in adults with an elevated body weight in West Africa.
Methods: This study was an open-label multicenter, clinical trial on patients with uncomplicated *P. falciparum* malaria in four endemic areas of malaria transmission in West Africa. Patients satisfying strict inclusion/exclusion criteria were hospitalised and stratified following their body weight into two different dosing/age groups and treated with a 3-day course of oral artesunate/mefloquine administered simultaneously. 100 children above 5 years of age and a body weight of 15 to 30 kg received Artequin 300/375 while 100 adults with a body weight above 55 kg were to be treated with Artequin 600/750. Patients were followed up on outpatient basis for both 14 and 28 days for clinical and parasitological response.

Results: A total of 203 patients have been included, i.e. 100 children and 103 adults with a mean age of 9.0 and 24.6 years and mean body weights of 25.1 and 63.6 kg, respectively. One hundred ninety seven patients were evaluable (three children and three adults dropped out due to formal reasons). The analysis of the patient who completed the study (197) revealed an overall cure rate of 100% after 14 and 28 days in both groups. The median time to fever clearance was 1 day for both children and adults. There was a complete parasite clearance within 48 h after first drug intake without any recrudescence after 28 days. The parallel and quick fever and parasite reduction rates were perceived by patients as early recovery from their malaria episode, after simple treatment recommendations, which contributed to excellent compliance. In 29% of all patients, mild to moderate neurological and gastrointestinal side effect were reported, all of them compatible with the disease without necessity of treatment interruption or concomitant therapy. Tolerability was significantly better in children than in adults (4% children versus 56% adults with at least one adverse event). No clinically significant changes in haematological, biochemical and ECG parameters were observed.

Interpretation: The study results clearly demonstrated high therapeutic efficacy and safety of both Artequin 300/375 in small children of a body weight between 15 and 30 kg as well as Artequin 600/750 in adults with elevated body weight.
190A
Safety of antimalarial combination therapies and assessment of adverse events in clinical trials in Uganda [MIM-GD-124264]
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Introduction: Data on the safety and tolerability of antimalarial regimens is essential for policy decision-making. However, assessment of safety in antimalarial clinical trials is particularly challenging given the potential overlap between symptoms of malaria and common drug side effects. We systematically evaluated for meaningful differences in the safety and tolerability of antimalarial regimens which might influence drug policy in drug efficacy studies conducted in Uganda in 2002–2004.

Methods: We conducted randomized, single-blinded trials at five sites in Uganda. Patients with uncomplicated malaria were randomized to receive chloroquine plus sulfadoxine–pyrimethamine (CQ + SP), amodiaquine plus SP (AQ + SP), or amodiaquine plus artemisinine (AQ + AS), and were followed for 28 days. Follow-up included prospective evaluation for the occurrence of adverse events. We developed a system for monitoring adverse events using international guidelines, including standard definitions, a detailed grading system for severity, and a standardized approach to assessing relationship of events to the study medications. Adverse events were defined as any untoward medical occurrence which may or may not have had a causal relationship with the study agent.

Results: Of 2560 participants enrolled at the five sites, the median age ranged from 1.3 to 4.0 years. The risk of clinical treatment failure (unadjusted by genotyping) for all treatment groups combined was 23–55% at the different sites. The proportion of participants experiencing any adverse event ranged from 81 to 99%, but severe and life-threatening events occurred uncommonly (1–3%). Comparing the incidence of adverse events between the different treatment groups, participants treated with CQ + SP were more likely to experience fever than those treated with AQ + SP or AQ + AS. When the analysis was restricted by excluding treatment failures, only the comparison for pruritus yielded a significant difference between the treatment groups (20% CQ + SP, 27% AQ + SP, 20% AQ + AS, p = 0.014). Assessment of the relationship of adverse events to the study medications varied widely between the sites, with the proportion of events graded as probably related to study drugs ranging from 0 to 35%. Serious adverse events occurred rarely. Excluding treatment failures and events judged to be unlikely related to the study medication, only five serious events were reported (1 CQ + SP, 2 AQ + SP, 2 AQ + AS).

Interpretation: Treatment failure confounds assessment of adverse events in malaria studies and evaluating relationship of events to administration of study medication is problematic. Standardized guidelines for gathering safety data are needed.

191B
Pragmatic trial of the effectiveness of combination treatment for uncomplicated malaria [MIM-SD-87789]
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Introduction: Poor adherence to antimalarial treatment can reduce treatment efficacy and enhance the development of resistance. Effectiveness trials in operational settings, without supervision of treatment doses, are important because acceptability and adherence may be important factors in discriminating between alternative treatment combinations.

Methods: Children 6 months to 10 years presenting with febrile illness were screened for malaria in three health centres. Those with uncomplicated malaria were enrolled, randomized to receive either AQ/AS, SP/AQ or SP/CQ, treated and followed for 28 days. The first dose was supervised in the health centre, subsequent doses were given to the mother to administer at home. Mothers were visited at home on day 3 to check for left over medication and side effects and ask about compli-
The primary endpoint was clinical cure by day 28. Molecular typing was used to distinguish recrudescent from new infections and to determine the prevalence of DHFR and DHPS mutations. Prior use of SP and CQ was determined from HPLC analysis of day 0 blood samples.

Results: 1813 children were enrolled. Mean duration of symptoms was longer in rural than urban areas (2.7 days and 2.0 days). Three percent of children received no treatment at home and 18% of children received incomplete treatment. Incomplete treatment was not associated with side effects or reported bad taste or treatment group but was associated with an increased risk of clinical failure (RR) in all treatment groups. AS/AQ was associated with a high rate of return to clinic with subsequent malaria episodes (within 28 days, 12% returned with clinical malaria needing re-treatment), and was associated with lower packed cell volume on day 28. 20% of children had had prior treatment with SP. Interactions between the effects of site and treatment group on failure rates were examined as were associations of site and treatment failure rates with prevalence of resistant parasites on day 0. At screening the prevalence of the DHFR triple mutation (codons 51, 59 and 108) was 71%, and of the quadruple (with DHPS 437) 34%. Presence of the quadruple mutation at screening was associated with a higher risk of treatment failure with SP combinations.

Interpretation: Poor adherence is associated with increased risk of treatment failure. Supervision of the first dose is important since doses may not be taken at home. SP/AQ is more effective than AQ/AS, but SP resistance should be carefully monitored.

Antimalarial and immunomodulating activity of some Sudanese herbal medicine [MIM-AE-100716]
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Introduction: The Sudan is being the largest country in Africa, covering an area of one million square miles with the different metrological, polyethnic and a diverse flora. Most people in rural areas rely on traditional medicine where appropriate health services become inaccessible.

Methods: Forty-nine plant parts representing 26 species from 15 families were extracted and screened for their activity on chloroquine sensitive strains 3D7 and Dd2. Plants were collected according to their traditional use and/or to their taxonomical affiliation to their families that had been reported to have antimalarial activity. Authentication was achieved by comparison with herbarium specimens by taxonomist and a voucher specimen from each plant was deposited.

Results: Thirty four methanol extracts (59%) exhibited significant activity against 3D7 with IC50 values 50μg/ml, while 21 extract (57%) showed antimalarial activity on Dd2 with IC50 values 50μg/ml. On the other hand 13 extracts (22%) and ten extracts (18%) only showed an activity with IC50 values 5μg/ml on 3D7 and Dd2, respectively. Human lymphocytes treated with the most of extracts demonstrated a minimum level of toxic inhibitory effect at concentration 100μg, whereas S. cornatus, B. aegyptiaca, A. nilotica and T. indica enhanced lymphocytes proliferation.

Bioassay guided fractionation of two plants namely M. senegalensis (Lam.) Exell. and A. nilotica revealed that the active ingredients were found in dichloromethane and ethyl acetate fraction with IC50 values 2.1 and 1.5μg/ml, respectively. Continuous purification of the bioactive fraction from M. senegalensis using a number of reverse phase medium pressure column chromatography lead to the isolation of a pure compound. Its structure was elucidated using spectral properties and identified as triterpenes pristimerin with IC50 values equal 0.5μg/ml against Dd2, while its effect on lymphocytes was detected at IC50 values of 6.8μg/ml.

Interpretation: The promising response of A. nilotica and M. senegalensis conclude that some Sudanese plants subjected to long-term clinical trials in folk medicine possess a potent antimalarial activity with minor effects on lymphocytes proliferation.
193A
Efficacy and safety of sulfalène–pyriméthamine versus sulfadoxine–pyriméthamine in the treatment of uncomplicated *P. falciparum* malaria in Senegal [MIM-OF-307192]

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Introduction: In Senegal, national malaria control program control recommends Sulfadoxine Pyriméthamine (Fansidar®) in Intermittent Preventive treatment (IPT) for the pregnant woman. Recent studies undertaken in the country showed high rates and increasing prevalence of markers resistance: DHFR and DHPS. We tested the efficacy and the safety of the sulfalène–pyriméthamine (Métakelfin®) which could be an alternative to the SP.

Methods: A randomize study was conducted from October to December 2004 in the medical district of Guédiawaye in the suburb of the capital. Protocol WHO 2003 with 28 days follow-up was used. For all the patients, a venous blood sample was taken on D0 and D7 for haemogram and measurement of transaminase and creatinine levels. The criteria of judgement were the rate of early therapeutic failure, the rate of late failure parasitological and the rate of adequate clinical and parasitological response (ACPR).

Results: Seventy patients were included in each two arms. At day 28, the ACPR of Métakelfin® was 81.4% against 92% for Fansidar®. PCR analysis is currently carried out. 33% of the patients of Métakelfin® group presented adverse events against 22% for the Fansidar® group. No severe clinical and biological adverse events were observed. Interpretation: With the sight of these results, sulfalène–pyriméthamine seems less effective than sulfadoxine–pyriméthamine.

194B
A qualitative study to identify community structures for management of severe malaria: A basis for introducing rectal artesunate in the under 5 years children in Nakonde District of Zambia [MIM-KF-45588]

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Introduction: Due to poor accessibility to modern health facilities, malaria is normally managed at home using indigenous and cosmopolitan medicines. In view of problems and implications associated with management of severe malaria at home, rectal artesunate is being proposed as a first aid drug to slow down multiplication of parasites in children.

Methods: A qualitative study using standardised in-depth and focus group discussions (FGDs) guides to collect information from four villages in Nakonde district, was conducted between March and April 2004. The guides were administered on 29 key informants living in the community and those whose children were admitted in the health facility. Participants in the 12 FGDs came from the four participating villages. Participants and informants were fathers, younger and older mothers including grandmothers and other influential people at household level. Traditional healers, headmen, village secretaries, traditional birth attendants, church leaders and black smiths. FGDs and interview transcriptions were coded to identify common themes that were related to recognition, classification and naming of malaria illness, care-seeking behaviour and community treatment practices for severe malaria.

Results: Parental prior knowledge of the disease was important as the majority of informants (23 out of 29) and participants (69 out of 97) mentioned four combined symptoms that were used to recognise severe malaria. The symptoms were excessive body hotness, convulsions, vomiting yellow things and swelling of the fontanelle. On the other hand, all informants mentioned two or more of symptoms associated with severe malaria. In all 12 FGDs, participants reported that treatment of severe malaria commenced with the family and moved into the community as the illness progressed. Although treatment of severe diarrheal effects, were common among the winamwanga, no rectal medicines to treat severe malaria were identified. Apart from the
anti-malarial fansidar, which was mentioned by 23 in IDIs and 40 in FGDs, participants and informants also frequently mentioned indigenous medicines provided by healers and other respectable herbalists for repelling evil spirits, once a child had severe malaria. Mothers were the important arms for administration of ant-malarial drugs in the villages. Referrals began with healers to CHWs, where no CHWs existed healers referred sick children to the health facility directly.

Interpretation: Our findings suggest that rectal artesunate may be a well-received intervention in Nakonde District. There is a precedent for rectal application of traditional medicine for childhood illness and more importantly, residents already recognize severe malaria as a distinct and highly dangerous condition for which existing treatment options are not always ideal in terms of time delay and referral steps.

195C
The quest for therapy in the event of severe malaria in under five children and its implications for the deployment of rectal artesunate in Ghana [MIM-MG-452564]
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Introduction: Mortality from malaria is on the increase with over 1–2 million deaths each year. Over 90% of these are African children who due to local perceptions and poor access to health facilities do not survive severe complications. A rectal formulation of artesunate, which achieves rapid and substantial (99–100%) reduction of parasitaemia within 24 h of administration, permits emergency treatment of patients who cannot take drugs by mouth has been recommended.

Methods: The study is in three phases. The formative phase involved: Indepth interviews with community members and health workers, Focus group discussions with men and women using problem tree, case studies of children admitted to hospital with severe malaria and a community survey. Issues explored were local terms, recognition of signs and symptoms, treatment and referral practices and the rectal use of drugs. The designing and execution of health education strategies has begun. Posters and flip charts have been prepared and discussions are ongoing with health workers and communities on other educational strategies. A total of 93 communities have been randomised into three arms and community entry has begun in preparation for the deployment.

Results: The results so far indicate that there are local terms for the separate signs and symptoms of severe malaria distinct from simple malaria “asa” or “atridii”. The perception is that “mimi semu” (Dirt in the stomach) if not properly treated can lead to “mimi pa” (sore in the stomach) which if not treated can cause the child to have other severe signs like “vies” (severe vomiting), “Hedola wawee” (very hot body) “Mu ta” (no blood) and “mimi kpo” (lump in the stomach). Treatment for these conditions are normally herbal with pepper and ginger and are given rectally in the form of an enema at home. For loss of blood the treatment ranges from vegetables and beans to a mixture of canned tomatoes with coca cola or a malt drink. Once the condition reaches the stage of “etomu” (loss of consciousness) and “hiowe” (convulsions) there is cause for concern and the child will be rushed to a health facility.

Interpretation: Even though herbal preparations are used rectally (enema) for treating some signs of severe malaria, the use of the rectal artesunate, which is a pellet, will not be a problem.

196A
A reversed-phase high-performance liquid chromatography method for the determination of amodiaquine and the main metabolites in small volumes of blood and plasma [MIM-LH-43888]
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Introduction: Amodiaquine (AQ) are used in malaria combination therapy in Africa with drugs like the artemisinins. Therefore, monitoring of effectiveness, compliance and surveillance of AQ toxicity has high priority. For this purpose we developed a high-performance liquid chromatography (HPLC) method
with UV detection for the analysis of AQ and the main metabolites in blood and plasma. The reliability of the method was tested on plasma samples from Ghanaian children.

**Methods:** One hundred unit liters of sample was added to 90 ul milli-Q water, 200 ul sodium carbonate buffer (1 M, pH 9.5) and 2000 ul diisopropylether (full blood) or tert-butyl methyl ether (plasma). After reciprocal shaking and centrifugation the organic phase was transferred to a new tube and extracted as above for 20 min with 120 ul of phosphate buffer (0.1 M, pH 4). After separation, the organic phase was discarded and 90 ul of the water phase was injected into the chromatograph. Ninty/N9262l was analysed using a Zorbax CN, 4.6 mm × 250 mm, 5 mm, column, protected by a C18 guard column with acetonitril-phosphate buffer (0.1 M, pH 2.3)-sodium perchlorate (1 M) (14:85:1) as mobile phase, flow rate 1.2 ml/min, and UV detection at 237 nm.

**Results:** The calibration curves were obtained using two replicates of five levels of the tested compounds (10, 25, 50, 100 and 250 ng/ml), standard curves were based on area and curve plots were linear (r > 0.9968). The inter day variations were <10 CV% for all drugs measured, and the intra day variation measured at two levels was <10 CV%. LOD was 10 ng/ml and LOQ was 25 ng/ml for the drugs in both full blood and plasma. Linearity was obtained up to 5000 ng/ml. The recovery for the drugs in full blood samples was 42, 76 and 98% for bis-AQ, DEAQ and AQ, respectively for concentrations between 25 and 500 ng/ml. In plasma the recovery was 81, 94 and 96% for DEAQ and AQ, respectively. There was no interference from chloroquine, desethylchloroquine, sulfadoxine, pyrimethamine or mefloquine. The reliability of the method was tested on plasma samples from 10 Ghanaian children (2.0–12.0 years of age), mean weight 18.0 kg (range 10.0–40.0 kg) who were given AQ 10 mg/kg bodyweight for 3 days. AQ was not detected in any of the blood samples, and bis-AQ only in two children at day 3 and 7. DEAQ was measured in all blood samples, at day 3 the range was 103–330 ng/ml, at day 7 the range was 45–162 ng/ml.

**Interpretation:** This method is specific, sensitive and precise enough to measure low drug concentrations in small volumes of full blood and plasma and can be applied in drug utilisation studies as well as in pharmacokinetic studies in small children.

197B Disposition of oral amodiaquine in Papua New Guinean children with falciparum malaria [MIM-FH-99797]


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**Introduction:** Amodiaquine (AQ) has been used in children for malaria treatment in Papua New Guinea for well over three decades, but very little pharmacokinetic data exists. We therefore, assessed the disposition of oral AQ and CYP2C8 polymorphism in 20 children with non-severe falciparum malaria who underwent the standard treatment with AQ recommended by the National Department of Health, Papua New Guinea.

**Methods:** AQ was orally administered at a dose of 30 mg kg⁻¹, divided into three daily doses of 10 mg kg⁻¹ on days 0, 1 and 2. Finger prick blood (75/N9262gl) was collected up to the post-dose day 14 on a filter paper. AQ and desethylamodiaquine (DEAQ) blood concentrations were determined by the validated solid-phase extraction-high performance liquid chromatographic method. The lower limit of quantification was 35.6/gl⁻¹ for AQ (coefficient of variation, <10%; n = 3), and 32.9/gl⁻¹ for DEAQ (CV; 10%; n = 3). Day to day variation during the analysis of samples was 12% for AQ and 13% for DEAQ. Calibration curves were linear for AQ and DEAQ within concentration range of 32.8–1067.6/gl⁻¹ (r² = 0.98, P < 0.001). CYP2C8 (*1, *2 and *3) genotyping was performed using a PCR-RFLP method.

**Results:** The median age and weight for these children were 4.6 (1.10–8.11) years and 15.5 (9.6–25.0) kg, respectively. The mean ± S.D. capil-
lary blood concentrations of AQ on days 1, 2, and 3 were 75.6 ± 47.2, 51.2 ± 14.0, 53.0 ± 11.9 µg l⁻¹, respectively; DEAQ at 2, 4, 12, 24, 36 and 48 h post-dosing were 142.5 ± 88.4, 145.1 ± 53.0, 94.5 ± 41.6, 143.8 ± 88.8 (trough), 214.7 ± 50.8, and 227.5 ± 85.1/µg l⁻¹ (trough), respectively. The mean (95% CI) Cmax of 368.8 (306.6–431.0)/µg l⁻¹ for DEAQ was reached in median time of 3 days. All 20 children were CYP2C8*1 homozygous. No significant correlation between age and DEAQ AUC total or MRT was observed.

Interpretation: Our data is consistent with those previously reported for healthy adult volunteers and malaria patients in Africa. The standard oral dosing regimen of AQ used here seems pharmacokinetically adequate in the absence of CYP2C8 mutations.

198C  
Visual instructions promote malaria treatment  
[AHM-AH-24695]

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Introduction: Malaria treatments are typically based on a rationale of symptom relief, not parasitological cure. Importance of adherence to multidose antimalarial treatments such as currently recommended artemisinin-combination may therefore be poorly understood or accepted. Low cost communication materials to support adherence with a 3-day regime for the recently registered antimalarial Lapdap® (chlorproguanil-dapsone) were tested. Findings inform development of treatment information strategies generally.

Methods: Exploratory action research, visual perception and communication principles were used to develop communication materials, and pretested and piloted with 280 community members and 96 public and private providers in rural and urban Tanzania and Kenya. We explored understanding of visuals for days, malaria, tablets, adults and children of different ages and health stages, and effect of leaflet size. We assessed understanding of sequenced visuals, two-way tables for dosages and linkages between different visuals sequences. Pretesting data were qualitatively analysed throughout data collection to inform ongoing materials development. Understanding and acceptance of final versions were analysed quantitatively by age, sex and educational status.

Results: Leaflets showed dosage by age and link between full courses of treatment and full cure. Provider charts explained need for full treatment. Visuals easily recognised in information leaflet context: mosquito (=malaria), sun, tablets, people. Children’s ages were identified by motor skills depicted. Representations of amount of malaria infections require short textual explanation. Sequences showing reduction of malaria over time were recognized. Sequences of sick people improving over time were well recognized, after careful development of positions and actions during pretesting. Linkages between sequences and two-way dosage chart were understood by most with some reading skills. Results are in concurrence with demonstrated visual perception principles. Reasons for using full course of treatment were better understood by dosage charts depicted reducing levels of parasites within each age band in the chart. Findings support communication principle that explaining why to take a full course may promote adherence to treatment that “kills the disease” rather than treat symptoms. Final piloting: leaflet used accurately by 94% overall and by 70% of low literacy respondents. Materials were said to indicate good quality medicine.

Interpretation: Careful pretesting increases understanding and acceptability of medicine use materials. Visual instructions may need support explanations to increase understanding for low literates. Pretesting materials is an essential step in medicine development.

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Introduction: The increasing resistance of *P. falciparum* to chloroquine (CQ) and sulfadoxine–pyrimethamine (SP) has lead to the increase in malaria morbidity and mortality. This could be disastrous for Africa. In spite of this, CQ and SP continue to be used in some countries. New antimalarial drugs need to be evaluated urgently. This meta-analysis compared the efficacy and tolerance of SP given alone or in combination with one or three doses of artesunate in children with uncomplicated malaria.

Methods: The methodology used was a systematic review and a meta-analysis of four randomised controlled trials that compared SP alone with the combination of SP and artesunate given for either one (SPAS1) or 3 days (SPAS3) in children aged 6 months to 10 years. The primary endpoint was the parasitological cure rate at day 28. Secondary endpoints included the parasitological cure rate at day 14, time to fever and parasite clearance, gametocyte carriage and occurrence of adverse events.

Results: Cure rate at day 28 corrected by PCR was 2.5 times higher in the combination of SPAS3 than in SP alone (pooled OR = 2.55, 95% CI 1.93 to 3.37). There was no difference in cure rate at day 28 corrected by PCR between the combination of SPAS1 and SP alone (p < 0.001). By day 28 all children on the combination therapy were agametocytaemic as opposed to those on SP alone (p < 0.001). All drug regimens were well tolerated and safe.

Interpretation: The combination of SPAS3 is more efficacious than SP alone in treatment of children with uncomplicated *P. falciparum* malaria. The combination is recommended for adoption as a replacement for SP alone in areas where malaria is endemic.

PCR use in diagnosis of recrudescence and reinfections in antimalarial drug efficacy assessment in Pissy sanitary district of Burkina Faso [MIM-SI-175218]

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Introduction: In malaria high endemic area, during the assessment of antimalarial drugs efficacy, new infections may be falsely classified as treatment failures. In order to differentiate the true recrudescence from new infections, we carried out the parasite genotyping by PCR using three *P. falciparum* polymorphism markers (MSP1, MSP2 and GLURP) after the evaluation for 28 days of the efficacy of chloroquine (CQ) and sulfadoxine–pyrimethamine (SP) in children below 5 years with uncomplicated malaria in urban area.

Methods: The study was conducted in Ouagadougou during the high transmission season 2003. The protocol of the study was based on the 2001 WHO guidelines for monitoring the efficacy of antimalarial drugs (CQ and SP). Blood collected on filters papers at day 0 and at any treatment failure day after day 9 was used for the genotyping of parasites by PCR. The *P. falciparum* antigenic genes loci (MSP1, MSP2 and GLURP) were used in order to differentiate between recrudescence and reinfection. The bands profiles of pre-treatment and failure day were compared. Paired samples with different bands profiles were classified as reinfections, whereas a recrudescence was concluded if the bands profiles were similar.

Results: Early Treatment Failure (ETF) was recorded in 26 of 131 (19.9%) children in CQ group and 7 of 123 (5.7%) in SP group. 64 of 131 (49.6%) were classified late treatment failure (LTF) in children treated with CQ and only one child was LTF before day 9. In SP group, 11 patients (8.9%) were LTF after day 9. In total 74 children were classified ETF after day 9 and their filter papers collected at day 0 and treatment failure day were used for genotyping. When combining the three markers, we found 7 (11.1%) and 1 (9.1%) of new infections, 19 (30.2%) and 6 (54.5%) of recrudescences, 37 (58.7%) and 4 (36.4%) mixed infections.
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(recrudescence + new infection), respectively for CQ and SP. The results obtained with only two polymor-
phism markers (MSP1 + MSP2 or MSP1 + GLURP) were similar to that obtained with the three mark-
ers (MSP1 + MSP2 + GLURP). Moreover, the results shown that the average number of clones per carrier
was 3.3 clones in pre-treatment samples. The extent of multiplicity decreased significantly (P = 0.008) in post-
treatment samples.

Interpretation: Our results has clearly shown that PCR could be helpful to allow the correction of the malaria
drug efficacy rate in high transmission area. Only 2 markers (MSP1 + MSP2 or MSP1 + GLURP) could be
used to diagnosis the recrudescence and new infections.

201C

Randomised, double-blind, placebo controlled
study of the antipyretic effect of ibuprofen in chil-
dren with uncomplicated malaria [MIM-SI-209286]
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Introduction: Antipyretic drugs are widely used in chil-
dren with fever, though there is a controversy about
the benefit of reducing fever in children with malaria.
In a double blind randomised placebo controlled trial,
we investigated the effect of ibuprofen in fever in
children with uncomplicated P. falciparum malaria in
Gabon.

Methods: Children aged between 2 and 7 years with
uncomplicated malaria were included in the study.
They were hospitalised until two consecutive thick
blood smears were negative for malaria parasites.
All patients were treated with quinine dihydrochlo-
ride intravenously. For the treatment of fever, all
patients received mechanical treatment when the tem-
perature rose above 37.5°C. In addition to the mechani-
cal treatment, patient were assigned randomly to
receive ibuprofen (7 mg/kg body weight, 3 times/day)
or placebo. Rectal temperature was measured hourly
until discharge.

Results: Fifty children were included in the study.
The fever clearance time was lower in children receiv-
ing ibuprofen in addition to the mechanical treatment
compared to those receiving only mechanical treat-
ment (placebo group): 46.6 h (±24.0) versus 54.2 h
(±25.3), but the difference was not statistically sig-
nificant (P = 0.3). The time spent with fever and the
area under the fever curve were significantly lower in
ibuprofen group compared to placebo group.

Interpretation: Ibuprofen had no significant effect in
reducing fever clearance time in children with P. fal-
ciparum malaria, it did, however lead to a reduction
in fever peaks. The clinical benefit of antipyretics in
malaria remains an open question.

202A

Safety and efficacy of dihydroartemisinin-
piperaquine in Rwandan children with
uncomplicated P. falciparum malaria [MIM-
CK-70620]
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Introduction: Dihydroartemisinin-piperaquine (DHA-
PQP), is a new and co-formulated artemisinin-based
combination treatment (ACT). A clinical trial compar-
ing the efficacy, safety and tolerability of DHA-PQP
to that of AQSP and AQ + artesunate (AQAS) was car-
rried out in Rwandan children with uncomplicated P.
falciparum malaria.

Methods: A randomized, open trial was carried out in
2003–2004 in three sites in Rwanda. Seven hundred
sixty two children aged 12–59 months with uncompli-
cated P. falciparum malaria were randomly allocated
to one of the following treatments: AQAS, AQSP and
DHA-PQP. Patients were followed up to day 28 after
treatment. Adverse events and clinical and parasitolog-
ical outcomes were recorded.

Results: Children treated with DHA-PQP had a signif-
icantly lower risk of failure compared to those treated
with AQSP and AQAS. Parasites clearance was signif-
icantly faster for children treated with DHA-PQP and
AQAS compared to those treated with AQSP. How-
ever, no difference on fever clearance was observed.
Mean PCV increased in all groups, though the increase was lower in the DHA-PQP group as compared to the AQAS and the AQSP. The frequency of adverse events was significantly higher for patients in the AQAS and in the AQSP groups compared that in the DHA-PQP group, asthenia, anorexia and vomiting being the most common.

Interpretation: DHA-PQP is a well tolerated and efficacious drug, and represent a potential alternative to AQSP, the current first line treatment in Rwanda.

203B
Partnership to improve the quality of evidence needed to change policy for anti-malarial treatment in West Africa: The example of the West Africa network for monitoring anti-malarial treatment II [MIM-WK-238630]

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Introduction: Resistance to cheap anti-malarial drugs has forced countries in Africa to revise their anti-malarial drug policy (AMDP). Insufficient evidence on the magnitude of anti-malarial drug resistance has been one of the key factors that have accounted for delays in the adoption of efficient AMDP. Technical support networks have been established with support from WHO and its partners to assist countries in dealing with this issue and to open dialogue for the harmonization of policy within sub regions.

Methods: The West Africa network for monitoring anti-malarial treatment II (WANMAT II) was established in June 2003 by nine countries in West Africa. The Malaria Consortium appointed a Gates Malaria Partnership (GMP) funded policy advisor in May 2004. To assess the current status of the network, the policy advisor visited five out of the nine countries (Benin, Burkina Faso, Côte d’Ivoire, Niger and Nigeria) in the network from August to November 2004. Interviews were conducted with officials within the Ministry of Health (MOH), WHO country office, country team members, key partners and stakeholders as well as sentinel sites personnel. A debriefing meeting with partners was held at the end of each visit.

Results: Overall 35 sentinel sites have been identified for monitoring anti-malarial drug efficacy in these countries, with a range of 3–12 sites per country. Many of them are yet to be equipped, and their personnel need to be trained or re-trained. Only two out of the five countries have developed a database for anti-malarial drug efficacy within their National Malaria Control Programme (NMCP). Approaches for monitoring drug efficacy are variable. In two countries, Research Institutions have been contracted and are conducting this activity on behalf of the NMCP. In the other three, studies are conducted at sentinel sites by staff under direct supervision of the NMCP. All the countries have adopted the WHO in vivo protocol as their monitoring tool and have been reporting results of studies to authorities and partners. However, various modifications to the original protocol may have undermined the comparability of the overall results, even in the same country. In addition, quality assurance/quality control (QA/QC) activities are not systematically conducted.

Interpretation: Based on these findings, countries have agreed to establish a sound uniform monitoring system. Sustained regular high quality tracking of resistance trends and engagement of control programme implementers and national decision makers throughout are essential to ensure that those suffering from malaria have access to effective treatment.

204C
Development of a monoclonal antibody based competitive-ELISA for measuring chloroquine concentration in biological fluids [MIM-IK-23807]

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Introduction: Withdrawal of chloroquine (CQ) treatment in Malawi has led to the re-emergence of CQ sensitivity in P. falciparum, giving the hope of re-introducing the drug for malaria treatment. Research groups working on HIV management are focusing
on CQ as the drug has shown anti-retroviral activity. Controlled trials are needed to discern the potential role of CQ in HIV management. There is a need for development of quick, cheap, sensitive and specific analytical methods that could be used in the field.

Methods: We developed an inhibition ELISA that has been optimised and validated for the determination of CQ in biological fluids. The assay employs monoclonal antibodies raised in mice to 2-amino-5-diethylaminopentane, which corresponds to the N-side-chain of the 4-amino-7-chloro-quinoline moiety of CQ. To test the reliability of the method, the ELISA was used to monitor CQ concentration in four healthy volunteers after ingestion of a single dose of 500 mg CQ. Blood samples were collected before CQ ingestion, after 3 h and at days 1, 3, 7, 14, 21 and 28. The level of CQ concentrations determined by the ELISA were compared with values obtained by a HPLC method.

Results: A linear standard curve using 50 µl whole blood was obtained over a concentration range of 2000–0.1 ng/ml ($r = 0.97$). The monoclonal antibodies were specific for CQ with no cross-reactivity to the related aminoquinolines. The ELISA was sensitive enough to be used for drug utilisation and pharmacokinetic studies with a detection limit of 2.0 ng/ml. The analytical recovery was >92% at a high, middle and low concentration. Inter and Intra assay coefficient of variation were <10% at a high, middle and low concentrations. The cut-off for the ELISA (35 ng/ml) was determined from the mean inhibition value plus the 95% confidence interval for the 1:2 dilution of blood samples collected 28 days after CQ ingestion. The ELISA and HPLC results were highly correlated ($r = 0.98$). The washout of CQ from the blood of the 4 volunteers was calculated by applying the concentrations obtained at the follow-up days in CQ kinetic equation at a detection limit of 2.5 ng/ml. Subsequently a hypothetical cut-off point has been suggested to test for the previous usage of CQ.

Interpretation: The ELISA could be used as a screening tool and in pharmacokinetic studies with acceptable sensitivity, precision and recovery. It has the advantage of being quick, easy, requires small volume and suitable for large scale field screening.

205A Prompt and effective malaria treatment for febrile illness: A comparison between two autonomous health administrations in Tanzania [MIM-RK-166255]

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Introduction: Mainland Tanzania and the Zanzibar Archipelago operate autonomous health administrations and delivery systems. The former is vast and sparsely inhabited while the later is small but densely populated. These differences make geographic access to health facilities better in Zanzibar than on the mainland. In 2002 and 2003 we conducted household surveys in these 2 independent parts of the country to assess treatment utilisation for febrile illnesses at household level.

Methods: We randomly selected 1182 and 2377 households from Zanzibar and mainland Tanzania, respectively. This sample was drawn from two districts in Zanzibar and three on the mainland. Every available resident who was willing to participate was asked to answer questions about febrile illnesses, medicine use, and types of health providers they had visited for care, as well as to provide other individual and household information. The survey identified 410 individuals in Zanzibar and 1035 on the mainland who had experienced fever or malaria in the 14 days prior to the survey. Analyses have been corrected for clustering both between individuals in the same household and within study sites and weighted to adjust for population sampling fractions.

Results: Zanzibaris were more likely to have used antimalarial drugs within 24 h of the onset of fever (30%) compared with mainland residents (20%). The difference was statistically significant. Prompt and effective antimalarial drug use was significantly higher among residents of mainland Tanzania (18%) than Zanzibar (7%). During the time of the survey in Zanzibar routine
treatment for uncomplicated malaria was still chloroquine even though it had already been singled out as ineffective. On the mainland, chloroquine had been replaced with sulfadoxine–pyrimethamine. Respondents from mainland districts reported seeking treatment for febrile illness from retail shops at 58% and from health facilities at 42%. Residents of Zanzibar utilised health facilities in the majority of cases, 60%, compared with 39% who got medicines from retail sources. We examined educational level of household head, age of patient, and location of residence as potential predictors of care utilisation patterns. All three factors appeared to strengthen respondents’ likelihood of using antimalarial treatment promptly. These factors were also independently associated with prompt use of effective drugs in both mainland Tanzania and Zanzibar.

Interpretation: Zanzibaris were more likely to seek early treatment at health facilities than residents of mainland Tanzania. Efforts to improve malaria treatment in the two settings can be tailored to documented differences in patterns of access to care.

Comparing chloroquine and amodiaquine for treatment of uncomplicated malaria in children in Guinea-Bissau [MIM-PK-121990]


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Introduction: We have previously shown that the chloroquine dose recommended by the national malaria programme is insufficient for treatment of uncomplicated malaria. This study compares treatment of uncomplicated malaria in children in Guinea-Bissau as recommended by the national malaria programme (chloroquine in a total dose of 25 mg/kg), either with a total dose of 50 mg/kg chloroquine or with a total dose of 15 or 30 mg of amodiaquine.

Methods: Seven hundred twenty nine children with mono-infection with *Plasmodium falciparum* were selected for treatment with chloroquine 25 mg/kg (group I), chloroquine 50 mg/kg (group II), amodiaquine 15 mg/kg (group III), or amodiaquine 30 mg/kg (group IV) by block randomisation. The children were visited and malaria films obtained once weekly until day 35. On day 7, 100 microliter of capillary blood was drawn for analyses of chloroquine or amodiaquine concentrations in whole blood. Whenever a child had recurrent parasitaemia, a filter-paper blood-sample was collected for later PCR analysis.

Results: Children who were admitted to hospital were considered as treatment failures. On day 35, the non-PCR adjusted cumulative risks of treatment failure were 29, 14, 14, and 10%, respectively. The cumulative relative risks of treatment failure, using chloroquine 25 mg/kg as reference, were 0.46 (0.26–0.79), 0.43 (0.25–0.75), and 0.31 (0.16–0.56), respectively. When amodiaquine 30 mg/kg was compared with 15 mg/kg the cumulative relative risk of treatment failure was 0.70 (0.34–1.40). No differences in adverse events were observed during the three days of treatment or follow-up. PCR-analyses in order to distinguish between recrudescence and re-infection are ongoing. The results will be presented at the meeting.

Interpretation: Re-infection rate is 2–4% per week. Mono-therapy with a higher dose of chloroquine or with amodiaquine might be effective. If ACT is going to be introduced amodiaquine should be considered as the partner drug—probably in the higher dose.

Does injection use influence choice of and satisfaction with private health clinics? A user perspective [MIM-FK-216766]

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Introduction: Injections are widely used at all levels of health care systems by private and public providers. Although its use is a worldwide phenomenon little has been done to understand users views of the practice in Kenya. A study conducted with private practitioners in Kilifi Kenya suggested that practitioners believed the use of injections increased carer’s satisfaction with
the outcome of a consultation. However, community perceptions of injections were not explored. This study set to fill this gap.

Methods: Qualitative methods; in-depth interviews and focus group discussions, were used to collect data from carers and users of private clinics.

Results: Clients perceived that private practitioners prescribe injections in most consultations. They believed that there are specific illnesses, and stages of illnesses, that are suitable or unsuitable for treatment by injection. The need to use injections was therefore perceived to vary with the type and stage of illness. They also believed that injection use was sometimes driven by profit motives and expressed negative perceptions about the potential side effects of injections, such as exposure to HIV/AIDS infection.

Interpretation: There is a conflict of belief between clients and practitioners regarding injection use. These findings have an implication for access to prompt treatment of childhood illnesses through private clinics and compliance with treatment prescribed.

208A


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Introduction: Le paludisme, de par son ampleur et sa gravité constitue toujours un problème majeur de santé publique dans les pays tropicaux. La pharma-corésistance de Plasmodium falciparum est l’obstacle le plus important dans la prise en charge de la maladie. La chimiothérapie ne bénéficie aujourd’hui que de l’association sulfadoxine/pyriméthamine. Les taux d’échecs relativement élevés observés pour cette molécule impose l’orientation vers d’autres alternatives.

Methods: Les souches plasmidiales non-soumises à la pression de l’association sulfalène-pyriméthamine, pourraient présenter une meilleure efficacité et constituer une bonne alternative. La présente étude s’inscrit dans ce cadre. Il s’agit d’un essai ouvert, comparatif, randomisé en simple aveugle sur 2 bras, sans insu pour le traitement. Le site d’étude a été la Formation Sanitaire à Base Communautaire d’Anonkoua-Kouté, dans la périphérie d’Abidjan et l’étude s’est déroulée du 2 janvier au 9 mars 2005. Au total, 152 patients âgés de 2 à 53 ans ont été enrôlés dans cette étude. Un effectif de 75 patients a été inclus dans le groupe sulfadoxine/pyriméthamine et 77 dans le groupe sulfalène/pyriméthamine.

Results: L’évaluation à J28 montre pour le groupe sulfadoxine/pyriméthamine un taux de Réponses Clinique et Parasitologique Adéquate (RCPA) de 92% (69/75) et un taux d’Échecs Thérapeutiques Précoce (ETP) de 8% (6/75). Dans ce groupe, la tolérance biologique et générale a été jugée bonne. Le portage de gamétocytes a varié entre 2 et 20% selon les périodes de suivi. Dans le groupe sulfalène/pyriméthamine, le taux de RCPA à J28 a été de 98.7% (76/77) et le taux d’ETP a été de 1.3% (1/77). La tolérance tant au niveau général qu’au niveau des organes a été jugée bonne. Le portage de gamétocytes dans ce groupe a varié entre 1.3 et 9% selon la période de suivi.

Interpretation: La chimiothérapie ne bénéficie aujourd’hui que de l’association sulfadoxine/pyriméthamine. Les taux d’échecs relativement élevés observés pour cette molécule impose l’orientation vers d’autres alternatives comme le sulfalène/pyriméthamine.

209B

New TLC method for detection and semi-quantitative determination of levels and metabolites of anti-malarial drugs in human urine [MIM-LL-19018]

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Introduction: The available methods of antimalarial drug detection in urine have limited use due to the fact that most of them are based on qualitative analysis as well as there is no single method which can be used to identify all the antimalarials in clinical use, such use of a combination of detection methods is a strong limitation to large scale field studies.
Abstracts / Acta Tropica 95S (2005) S1–S506

Methods: Urine from research subjects was collected in 40 mL sample vials and stored at 4°C until analysis. Drug levels in aliquots of urine were determined by using thin layer chromatography (TLC), which were developed using different solvent systems on 10 cm x 20 cm aluminium-backed silica gel 60 F254 TLC plates. Plates were sprayed with 20% ethanolic-AlCl3 before visualization at 254 and 366 nm. Thereafter, plates were sprayed with Dragendorff or methanolic-H2SO4/Cerium IV ammonium sulphate/P-anisaldehyde and then heated to 110°C for 15 min.

Results: Chloroquine, amodiaquine and quinine and their metabolites were easily detected in all mid and polar solvent systems whereas; sulfadoxine, pyrimethamine and sulfadoxine–pyrimethamine combination were not detected in all the mid and polar solvent systems. The solvent system diethylamine-toluene-isopropanol (1:4:5 v/v/v) enabled the detection of all antimalarial drugs in sampled human urine. Toluene–isopropanol–dimethylformamide (5.5:3.0:1.5 v/v/v) solvent system selectively differentiated and confirmed the presence of quinine in urine. Drugs and metabolites shown different RF values and reaction patterns under UV–vis at 254 and 366 nm. Furthermore, different color reactions observed with spray reagents helped to detect and confirm their presence.

Interpretation: Our TLC method is a new tool to help address the role of drug absorption and excretion in the chemotherapy of malaria as well as detect types of antimalarial drugs commonly used by people in the community.

210C
Bilan de 11 ans de suivi de l’efficacité thérapeutique in vivo de la chloroquine pour le traitement des accès palustres simples chez des enfants de moins de 5 ans, dans une zone d’holoendémie avec transmission pérenne: Diélmo, Sénégal, (1990–2000) [MIM-AL-221952]
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Introduction: Première endémie tropicale mondiale, le paludisme en ce début de XXe siècle, reste encore un grand fléau pour l’économie des pays en développement. Aujourd’hui, face à l’accroissement continu de la résistance de P. falciparum aux antipaludéens, la définition de nouvelles stratégies thérapeutiques basées sur des données scientifiques d’évaluation des molécules est une nécessité. 

Methods: Dans le cadre d’un suivi étroit, permanent depuis 1989, d’une cohorte exposée à une transmission anophéline pérenne, des traitements d’accès palustré ont été évalués en rétrospectif sur une période de 11 ans chez des enfants de 6 mois à 5 ans. Le but était de fournir aux autorités sénégalaises des informations sur l’efficacité des antipaludiques utilisés au Sénégal en particulier sur la chloroquine dans un contexte de diagnostic fiable, de traitement précoce supervisé et de suivi de l’efficacité thérapeutique.

Results: Sur 2560 accès palustres traités, 1357 répondaient aux critères d’évaluation de l’OMS. Cent cinquante sept traitements (12%) étaient des échecs thérapeutiques précoce, 351 (26%) étaient des échecs thérapeutiques tardifs, et 849 (63%) étaient des réponses cliniques et parasitologiques adéquates. Parmi les 351 cas d’échec thérapeutique tardif, les taux d’échec clinique tardif et d’échec parasitologique tardif étaient respectivement de 4 et 22%.

Interpretation: Cette étude vient appuyer les décisions de la réunion de consensus organisée par les autorités sanitaires sénégalaises en juin 2003, d’abandonner l’usage de la chloroquine utilisée seule dans le traitement des accès palustres simples en faveur des traitements combinés à base d’artémisinine.

211A
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Introduction: In line with the current WHO recommendation for the use of artemisinin-based combination therapies (ACT), many countries have changed their antimalarial drug policies to conform to this trend. This
A study intends to derive data on the efficacy and safety of this drug when used in the treatment of uncomplicated *P. falciparum* infections in Kenya, with a view to making recommendations for its widespread use as an alternative first line treatment of uncomplicated *P. falciparum* malaria.

**Methods:** This study was an open-label clinical trial on patients with uncomplicated *P. falciparum* malaria in Bungoma District of Kenya, an area with an endemic malaria transmission pattern. 200 patients satisfying strict inclusion/exclusion criteria were enrolled in the study and treated with a 3-day course of oral artesunate/mefloquine administered simultaneously. Patients were followed up on out-patient basis for 28 days for clinical and parasitological response. Artequin was administered in the dosage of approx. 4–5 mg/kg/day of artesunate and 8 mg/kg/day of mefloquine. Based on weight, 150 patients weighing 35 to 55 kg received Artequin 600/750 while another group of 50 patients weighing more than 55 kg received Artequin 600/1500.

**Results:** The day 28 cure rate was 98.4%, while day 14 and day 7 cure rates were 98.4 and 99.2%, respectively. The mean parasite reduction rates from baseline on day 1, 2 and 3 were 52.11, 90.45 and 99.88%, respectively. There was rapid relief of symptoms by 82, 89 and 97% on days 1, 2 and 3, respectively. The median time to fever clearance was 1 day with a range of 1 to 2 days both for the aggregate and individual treatment groups. The initial symptoms of headache, joint pains abdominal pains and dizziness were all cleared after the second day of treatment while malaise persisted on until the fifth day of visit. The commonest side effects of mild to moderate degrees of headache, dizziness and asthenia occurred in less than half of the patients. There were three cases of severe side-effects, i.e. one with dizziness and vomiting whiles two with headache without necessity of treatment interruption. Three patients discontinued prematurely, two due to severe vomiting and malaise, one with psychic disturbances. There were no clinically significant changes in the haematological, biochemical, and ECG parameters.

**Interpretation:** In changing antimalarial treatment policies for countries, factors like rapid cure rates, tolerable profile of side effects and low propensity to develop resistance are to be considered especially when initiating malaria control programmes.
213C
Surveillance de la résistance de *P. falciparum* à la sulfadoxine-pyriméthamine et à l’amodiaquine en zone forestière au Cameroun (Kribi) [MIM-TM-21472]

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Introduction: Des gènes de résistance à la sulfadoxine-pyriméthamine (SP), dihydrofolate réductase (dhfr) et dihydroptéroate synthase (dhps), ont été retrouvés chez les souches de *P. falciparum* entretenues au laboratoire. Il est nécessaire de savoir si la présence de mutations ponctuelles sur ces gènes serait synonyme de l’échec thérapeutique. Nous nous sommes donc orientés vers l’étude de la corrélation entre les marqueurs moléculaires et la réponse clinique au cours du paludisme non-compliqué.

Methods: Dans le but de mettre sur pied un système de surveillance de l’efficacité et héritabilité des antipaludiques disponibles, des enquêtes de l’efficacité thérapeutique ont été effectuées chez les enfants âgés de moins de 5 ans en 2001 et 2004 dans le district de Ny’eté.

Results: En 2001, les taux d’échec étaient de 13.6% et 10.2% pour la SP et l’AMQ, respectivement. Parmi les isolats collectés en 2001, 85% portaient les triple mutations sur le gène dhfr (Ile 51/Arg-59/Asn-108), 7.4% de double mutations et 7.4% de séquences sauvages. En 2004, 10% d’échecs thérapeutiques ont été effectuées chez les enfants âgés de moins de 5 ans en 2001 et 2004 dans le district de Ny’eté.

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Interpretation: Notre étude signale l’intensification du degré de résistance à la SP à Hévécam (Kribi) et souligne l’urgence à y instaurer un système de surveillance pour apprécier son évolution.

214A
Assessment of community attitudes and perceptions toward sulfadoxine-pyrimethamine (SP) and SP plus artesunate in rural Tanzania [MIM-EM-22269]

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Introduction: Antimalarial drug resistance is a major challenge to malaria control efforts in endemic countries. In the face of high-level chloroquine resistance, Tanzania changed malaria treatment guidelines in 2001, recommending sulfadoxine-pyrimethamine (SP). As part of an implementation evaluation project SP plus artesunate (AS) was introduced in one district in 2003. We evaluated community attitudes and preferences for malaria treatment before, during and after these changes.

Methods: We collected qualitative data in three phases through 64 focus group discussions, 62 illness narrative interviews and 40 in-depth interviews in 6-districts. Participants included caretakers of children under 5 years, adult men, women, youths, pregnant women, and health care providers. Interviewers transcribed each data collection activity from audio tape recordings and/or handwritten field notes. Through content analysis investigators identified and coded common themes. This analysis focuses on specific comments concerning SP and SP + AS over time. In particular, we compared normative attitudes and perceptions reported in the
abstract or in group settings with more diverse opinions drawn from participants’ individual experiences.

**Results:** Prior to its widespread introduction as first-line treatment, SP was commonly regarded as effective against malaria and even preferred over chloroquine by some community members. Within a year, many individuals expressed that they would rather return to using chloroquine. Focus group participants attributed this shift to concerns about SP-related side effects. In describing these, most participants concentrated on illness symptoms which they commonly attributed to the drug. They seldom mentioned severe cutaneous reactions, expressing only second-hand knowledge of this risk through media reports and hearsay. The data also demonstrate widespread confusion between generic and commercially branded SP-containing products. At the same time, SP was widely accepted for intermittent preventive treatment of malaria in pregnant women. Moreover, data from individual illness narrative interviews indicate that nearly all people who took SP for malaria were satisfied with the results. Perceptions of SP + AS in the intervention district were similar but generally more positive. A small number of participants interpreted the combination as an innovation to mitigate side effects from SP.

**Interpretation:** SP and SP + AS were well accepted by individual patients despite less favourable attitudes prevalent in the community. Experience and anticipatory guidance from health officials may influence local perceptions when new malaria treatments are deployed.

215B

A study to implement guidelines for the management of malaria at health centre level by using prescribing indicator standards in Ndola, Zambia

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**Introduction:** Malaria is transmitted by female Anopheles mosquito when they take up a blood meal. Malaria is caused by four species of the Plasmodium parasite: *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Control of malaria requires understanding the transmission dynamics. Malaria kills 750,000 children per year below the age of 5 years. Anemia and cerebral malaria are the major complications that cause death. The objective was to evaluate the management of malaria at health centre.

**Methods:** The design of the study was cross-sectional. Two methods were followed, retrospectively and prospectively. A study population of 20 health facilities, each with at least 30 malaria encounters recorded in each health facility was chosen. Sixteen health centres out of Health Centres were randomly sampled. At each one of these health facilities a total of 30 medical records with a diagnosis of malaria were studied from each health centre. In total 479 records were examined. The sample size with the power of 80 and 95% Con
d\ncidence interval was calculated. Prospective study was carried out. In this approach structured observations and in-depth exit interviews were carried with patients to generate the data.

**Results:** A total of 393 records were for the years 1998 and 1999 and 86 were for the year 2000. Results showed that chloroquine tablet, syrup or injectable was the drug of choice for uncomplicated malaria. Antibiotics were also given in addition to anti-malarials in some cases. sulphadoxine–pyrimethamine (SP) and quinine were also given in a few cases. The records showed that in about 80% of the cases, temperature was not recorded. Most of the records indicated that blood smears were not taken most probably due to the non-availability of functional laboratories. Only about 7% did parasite count as confirmation for malaria. A great number of the prescribers were Clinical Officers. Registered Nurses and Enrolled Nurses prescribed in some health centers that did not have Clinical Officers. Medical officers were the main prescribers in health centers managed by missionaries. Different combinations of drugs were prescribed by Clinical Officers in the treatment of uncomplicated malaria. In some situations uncomplicated malaria was treated with Halofantrine (1%) and Quinine (1%).

**Interpretation:** Existing malaria treatment guidelines were not adhered to by prescribers. Antibiotics were given even in situations where they were not indicated. Prescriptions differed considerably between different categories of Health.
216C
Economic and other contextual determinants of the acceptability and viability of malaria intermittent preventive treatment during pregnancy in Tanzania [MIM-GM-6048]
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Introduction: Intermittent preventive treatment of during pregnancy (IPTp) using sulfadoxine–pyrimethamine (SP) is currently recommended by WHO to be considered by all malaria endemic countries. Tanzania is among a few African countries implementing IPTp guidelines. IPTp guidelines specify that pregnant women attending antenatal care (ANC) clinics should take at least two doses of SP under direct supervision of health workers. However, little is known about the influence of economic and other factors on the acceptability and viability of IPTp in different country settings.

Methods: Two districts were selected on the following criteria: their location in different regions with variations in malaria transmission intensities and records on drug resistance levels, residents’ socio-cultural and economic statuses and their access to information on malaria and exposure to various malaria preventive services. Quantitative and qualitative data collection techniques have been applied. The study populations include eligible users of ANC services, health care providers both in public and private organizations, community health representatives, district health management team members, and officers responsible for preventive health services. Additional data were obtained from a review of official health information documents.

Results: Key findings from pilot study and analysis of data from the main study in one of the four selected districts will be presented and discussed. Keywords: malaria; intermittent treatment; pregnancy; health systems.

Interpretation: None.

217A
HIV-seropositivity (HIV-SP) is associated with severe complications and poor responses to malaria treatment and chemoprophylaxis under battlefield conditions [MIM-SM-143608]
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Introduction: HIV-seropositivity is likely to cause severe complications and poor responses to malaria treatment and chemoprophylaxis under battlefield conditions. The aim of the study was to investigate effects of HIV-SP on responses to treatment and chemoprophylaxis under battlefield conditions.

Methods: Subjects were 62 sero-negative (HIV-SN) and 44 (HIV-SP) male Zimbabwean soldiers operating in the Congolese Jungle. This study was part of the drug efficacy study carried out by Mudambo et al. according to the WHO manual for drug efficacy studies and guidelines from the Ministry of Health Zimbabwe. We compared parasitemia, temperature, hematocrit, complications, number of evacuees to Zimbabwe, ETF, LTF, mortality and chemoprophylaxis failure. In the military, the drug policy was: first line chloroquine (CQ); second line, sulfadoxine–pyremethamine (SP) or Fansidar; third line, quinine (QN) and Malasone was used as the weekly chemoprophylactic measure.

Results: Results showed that on day 3 temperature was significantly (P < 0.01) lower in HIV-SN (37.1 (0.9) OC and HCT higher 44.3% compared to HIV-SP 38.4 0C and 37.9 (0.9)%. Hemoglobin was 11.7 and 9.8 g/dl, respectively and parasitemia was significantly (P < 0.0001) higher in HIV-SP than HIV-SN. Clinical failures were 17.7% (HIV-SN) and 72.7% (HIV-SP) and of these, evacuees were 6/62 (9.7%) and 15/44 (34%), respectively Odds ratio = 4.83, RR = 3.52 (P < 0.002). Mortality: 3/62 (4.8%) (HIV-SN) and 12/44 (27.9%) HIV-SP odds ratio = 7.38, RR = 5.64 (P < 0.001) and HIV-SP had a 2.8 chance of getting malaria compared to HIV-SN. Complications included: meningitis, cerebral malaria, Ebola like symptoms, convulsions, neurological symptoms including loss of memory and disorientation, renal failure, bronchop-
neumonia, dehydration, severe anemia, persistent diarrhoea and vomiting and hypoglycaemia. ETF were 4 and 24 and LTF 7 and 8, respectively. The total Malasone (prophylactic drug) failure was 41%.

Interpretation: It was concluded that HIV-SP is associated with severe malaria complications, high mortality rate, ETF and therefore, a risk factor for malaria under battlefield conditions. New drug policy required to replace CQ, SP and Malasone with artemisin based combinations.

218B

Higher risk of antimalarial treatment failure in HIV positive than in HIV negative individuals with clinical malaria [MIM-LM-234960]

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Introduction: The risk of clinical malaria is higher in HIV+ adults and increases with falling CD4-cell count. Outcome of antimalarial treatment depends on both the drug’s action and the host’s immune response. Therefore, HIV infected individuals with varying degrees of immunological impairment might respond differently to antimalarial treatment. Interaction between HIV and malaria could have implications in areas where malaria is prevalent.

Methods: We studied the effect of HIV-1 infection on the safety and efficacy of antimalarial treatment. We collected additional HIV related data in a randomized controlled clinical trial comparing sulphadoxine-pyrimethamine (SP) versus arteether-lumefantrine (AL) in adults with uncomplicated P. falciparum malaria in Ndola, Zambia. HIV status and CD4 count was assessed with a anonymous and unlinked procedure. Time to PCR corrected treatment failure at day 45 was the primary outcome. Data of the patients excluded or lost to follow-up were censored at the time of the last recorded visit. The relative risk of treatment failure was calculated by Cox regression and results were adjusted.

Results: HIV infected patients represented 33.4% (185/554) of our study population; 58.8% had a CD4 count lower than 300/mm3. SP and AL were generally well tolerated. Serious adverse events were observed in 2 HIV infected individual with CD4 counts <300/mm3, one treated with SP and the other treated with AL. Treatment outcome could be determined in 83.9% (465/554) of the eligible patients. PCR corrected treatment failure was inversely related with CD4 count (adjusted relative risk, 1.50; 95% confidence interval, 1.02–2.21; P = 0.03). HIV infected individuals with a CD4 count <300/mm3 had a higher risk of treatment failure (adjusted relative risk, 1.85; 95% confidence interval, 1.05–3.27; P = 0.03) and new infections (adjusted odds ratio, 4.07; 95% confidence interval, 1.19–13.81; P = 0.02). SP treatment was essentially a risk factor for early treatment failure (adjusted odds ratio, 9.38; 95% CI, 2.11–41.65; P = 0.003) while HIV infection with CD4 <300 for late treatment failure (adjusted odds ratio, 2.63; 95% CI, 1.40–4.93; P = 0.003).

Interpretation: These findings indicate that a large proportion of HIV+ individuals might fail adequate antimalarial treatment. Furthermore, when treatment is successful, these patients are at increased risk for a new patent infection. The analysis of preliminary data showed, after stratification by HIV status, an efficacy of AL of 89% in HIV negative, 76% for HIV positive with 300 CD4/μl or less, 100% for HIV positive with over 300 CD4/μl (p = 0.02510). Similarly SP efficacy was 79, 57.5, 86% (p = 0.028) in the three groups of patients. Multivariate analysis showed that the odds of treatment failure in HIV+ individuals increased 2.3 with falling CD4 count (per 100/μl) (p = 0.002; odds for trend: p = 0.0009). HIV infected individuals with 300 CD4/μl or less had 2.97 more risk for treatment failure than the rest of the population (P = 0.001). However, HIV infected individuals with over 300 CD4/μl tended to have lower parasitaemia at enrolment and to respond better to antimalarial treatment than HIV negative patients. At time of enrolment, the proportion of patients with 300 CD4/μl or less was higher then expected (59% of the HIV infected). These findings indicate that a large proportion of HIV+ individuals might fail adequate antimalarial treatment, spread of malaria resistance might be influenced by the HIV epidemic and HAART might be needed to be started at 300 CD4/μl.
Community management of uncomplicated malaria using rapid diagnostic test and artemisin combination therapies in Senegal (west Africa) [MIM-JN-46970]

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Introduction: Each year, up to two million deaths due to malaria occur throughout the world, with Africa having more than 90% of this burden. More of 80% of malaria morbidity and mortality occurs at community level and do not arrive at the health center. Artemisin-based combination therapy (ACT) for malaria is rapidly gaining acceptance as an effective approach to limit the widespread of Plasmodium falciparum resistance to commonly used antimalarial drugs.

Methods: The objectives of this study were to evaluate the feasibility of a correct and adequate use of ACT by the community health workers and evaluate the adherence and the compliance of this treatment at community level. We conduct this effectiveness trial in a rural area at 200 km in the south east of Dakar. The clinical inclusion criteria was the WHO 2003 in a moderate transmission area. The community health workers randomly allocated treatment and enrolled patients after positive rapid diagnostic test (dipsticks). The first dose (in clinic) was supervised by drug dispenser and the subsequent doses were given at home. The clinical and parasitological follow-up after treatment was done at day 3, 7, 14 and 28.

Results: The community health workers treated 101 patients with Coartem® and 102 with Arsucam®. The means of age were 5 and 6 years for Arsucam® and Coartem®, respectively. The predictive positive value were 85% with the HRP2 malaria test and 100% with the pLDH test. We excluded eight patients and four were lost follow-up. The administration were correct in 94% of cases with Arsucam versus 90% with Coartem. The adequate clinical and parasitological response at day 28 were 99% for patients treated with Arsucam and 98% for patients treated with Coartem without PCR correction. We observed a good compliance in both groups for 97% of patients. However, 22% felt a bad taste when they taken Arsucam versus 11% for Coartem (p = 0.04). We observed 8% of nausea and vomiting with Arsucam versus 4% with Coartem patients (p = 0.23) and no severe adverse effects.

Interpretation: The success of home treatment requires an educated public with an access to efficacious drugs. As a key issue for ACT deployment, community health workers should be better supervised and motivated mainly during the rainy season.

Efficacité et tolérance de l’association Artésunate plus Amodiaquine (Amonate®) versus Arthéméth- thor plus luméfantrine (Coartem®) six doses dans le traitement des accès palustres simples à Plasmodi- ium falciparum au Sénégal [MIM-PN-459728]

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Introduction: Face à l’avancée de la résistance de Plasmodium falciparum aux antipaludiques usuels en monothérapie, l’OMS a préconisé l’utilisation des combinaisons thérapeutiques pour le traitement des accès palustres simples. Parmi ces combinaisons, celles à base de dérivés de l’artémisinine sont privilégiées. Ces combinaisons thérapeutiques d’antipaludiques (CTA) sont considérées à l’heure actuelle comme les plus efficaces pour le traitement du paludisme non-compliqué. Aussi a-t-il été recommandé d’évaluer leur efficacité et leur tolérance de manière à permettre aux décideurs de faire un choix éclairé pour la mise en œuvre des nouvelles stratégies thérapeutiques nationales.

Methods: Il s’agit d’une étude ouverte, comparative, randomisée qui s’est déroulée entre Octobre-Novembre 2004 dont l’objectif est de comparer, l’efficacité, la tolérance clinique et biologique de l’Amonate® (Artésunate plus Amodiaquine) versus Coartem® (Arthéméther plus luméfantrine) six doses dans le traitement des accès palustres simples à P. falciparum au Sénégal. Le protocole OMS 2003 a été utilisé avec un suivi de 28 jours. La créatinémie, les transaminases et l’hémoglobine ont été mesurées à J0 et J14 chez 25% des malades têtus au sort. Un prélèvement sur papier filtre pour génotypage a été fait à J0, J14 ou J28 chez les patients porteurs parasites Les
critères de jugement étaient : l’absence de parasites et de signes cliniques aux jours 7, 14 et 28 et la tolérance biologique et clinique.

Results: 145 patients ont été inclus. 70 patients ont reçu le Coartem® six doses et 75 patients ont été traités à l’Amonate®. Le taux de réponse clinique et parasitologique adéquat (RCPA) à J7, J14 et J28 est de 100% pour l’Amonate® et le Coartem®. Aucun effet secondaire clinique ou biologique n’a été observé. Les patients traités par le Coartem® ont eu un meilleur confort clinique, avec plus de patients présentant une réduction de la perturbation biologique.

Interpretation: Les deux associations Amonate et Coartem six doses ont démontré leur efficacité dans le traitement des accès palustres simples à P. falciparum au Sénégal.

221B Pharmacokinetics and clinical efficacy of midazolam and lorazepam in Kenyan children with severe malaria and convulsions [MIM-MN-175440]


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Introduction: Convulsions are a common complication of severe malaria (SM), and are associated with poor outcome. Midazolam is a water-soluble benzodiazepine which can be administered via several routes, including intravenous, buccal and intranasal routes for termination of convulsions. Lorazepam is an alternative benzodiazepine with a longer duration of action; it would be suitable for seizure prophylaxis in hospitalized patients. However, their suitability needs to be formally evaluated in children with SM.

Methods: Thirty two children with SM and convulsions lasting 5 min or longer were treated with midazolam or lorazepam. Eleven children received a single dose of midazolam (0.3 mg/kg) intravenously (IV) and eight a similar dose intramuscularly (IM). Thirteen children received a single IV dose of lorazepam (0.1 mg/kg). Blood samples were collected over 6 and 72 h, following midazolam and lorazepam administration, respectively. Vital signs (blood pressure, respiration rate, heart rate) were monitored at every blood sampling point. Midazolam and 1'-hydroxymidazolam concentrations were analyzed by high-performance liquid chromatography (HPLC)/mass spectrometry, while unconjugated lorazepam concentrations were analyzed by HPLC with UV detection.

Results: The mean ± S.D. plasma midazolam concentrations of 474.4 ± 146.4 ng/mL and 272.1 ± 136.9 ng/mL were achieved at 5 and 25 min following IV and IM administration, respectively. Plasma lorazepam concentrations of 60.2 ± 40.4 ng/mL were achieved within 20 min, and were maintained above therapeutic levels (30 ng/mL) for at least 24 h. A single dose of midazolam terminated convulsions in all children and six out of eight (75%) children following IV and IM administration, respectively, while a lorazepam IV single dose was effective in rapid (within 10 min) termination of convulsions in all children, and also prevention of seizure recurrence in nine out of 13 (69%) children for over 72 h. There were no significant cardio-respiratory adverse effects following administration of either drug.

Interpretation: Intramuscular administration of midazolam may be more practical in most rural health facilities in Africa, where venous access may be difficult and/or impractical, while IV lorazepam may be useful for seizure prophylaxis in hospitalized patients.
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222C
Randomized trial of the effectiveness of Lapdap [MIM-FN-13390]
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Introduction: Many West African countries have delayed changing antimalarial treatment policy because of cost. Lapdap is a cheap alternative, but its effectiveness in operational settings has been little studied.

Methods: Patients <10 years with uncomplicated malaria were recruited in three health centres in The Gambia, randomized to receive Lapdap or Coartem and followed for 28 days. The first dose was supervised, subsequent doses were given to the mother to administer unsupervised at home. Mothers were visited on day 3 to check left over medication, ask about side effects and compliance, and measure Hb. Patients who returned with malaria after day 28 were treated with study drugs according to the original randomization to enable assessment of safety of repeated treatments. Children were re-visited later to test for G6PD enzymatic activity and genotype. The primary endpoints are clinical treatment failure by day 28 and incidence of adverse events.

Results: One thousand and two hundred children were enrolled from October to December 2004 and followed up to the end of January 2005. Adherence to treatment regimen will be compared between treatment groups, factors associated with poor compliance identified, and association between compliance and treatment failure assessed. Since patients with G6PD A-deficiency are more susceptible to the haemolytic effects of the dapsone component of Lapdap, we will determine the effect of Lapdap on Hb at day 3 in G6PD deficient patients through a test of interaction between treatment group and G6PD status. Lapdap appears effective against some SP-resistant parasites and is likely to exert less selection pressure than SP because of its shorter half-life. It is therefore of interest to examine possible association of DHFR/DHPS mutations with Lapdap treatment failure and also to measure the frequency of these genotypes after Lapdap treatment.

Interpretation: This trial incorporates compliance and acceptability in the evaluation of two alternative treatments, and also evaluates whether risks associated with Lapdap in settings without G6PD screening outweigh the benefits to malaria treatment.

223A
Case management of malaria in under-fives at primary health care facilities in a Tanzanian district [MIM-SN-87269]
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Introduction: Malaria kills over one million people each year, about 3000 a day and the majority of victims are underfive children and pregnant women. This disease continue spreading in sub-Saharan Africa, and the cheapest antimalarial drug chloroquine has lost its effectiveness in almost all endemic countries. Thus, prompt, appropriate and effective therapy of suspected malaria fever cases can reduce malaria death rates even more if the treatment can be administered in the home.

Methods: A random sample of 652 mothers/guardians with sick underfives were selected in the 10 facilities in Kibaha district. Interviews and observations were used. Blood samples for determination of chloroquine levels and thick smears for detection of malaria parasites were taken from the children who were prescribed chloroquine. Information on diagnoses and prescriptions were collected from recording books. The aim was to study case management of malaria in children at public primary health care facilities and to evaluate the accuracy of self-reported mothers/guardians’ information on chloroquine use in children.

Results: Fever (75%) and respiratory (46%) problems were the most common complaints. There was a significant higher use of antipyretics among home treated children was observed when compared to those pre-
Previously visited health facilities \((p < 0.001)\). Use of antibiotics was higher among those who were taken to health facilities previously \((p < 0.0001)\). The average consultation time was 3.8 min. Physical examination was performed in 39% of the children. Malaria was diagnosed in 71% of the children and respiratory problems was the leading overlapping condition (29%). Malaria parasites were found in 38% of the children given malaria diagnosis. Of the 529 blood samples successfully analysed for chloroquine, 97% of the children without history of prior chloroquine home treatment had detectable drug levels in the blood, 11% had high levels \(>1000 \text{ nmol/L}\). Of those prescribed chloroquine, 16% already had high blood concentrations of the drug.

Interpretation: There is low quality of care in these facilities in terms of presumptive malaria diagnosis, consultation time and inadequate physical examination. Thus, interventions targeting both healthcare providers and mother’s/guardian’s are needed to improve the situation.

**224B**

**Formulations, combinations and their effects on the pharmacokinetics of chloroquine and sulfadoxine-pyrimethamine in healthy volunteers**

[MIM-CO-259413]


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**Introduction:** Despite the widespread resistance of \(P. falciparum\) to chloroquine (CQ) and sulfadoxine–pyrimethamine (S/P), both drugs are still widely used. While the recommended treatment for malaria in Uganda has been CQ + S/P and lately to Co-Artem, CQ and S/P are still the only drugs available for malaria. Different formulations of CQ and S/P are often combined. Using Homapak a locally produced generic CQ + S/P versus GMP products, the possibility of pharmacokinetic interactions of the formulations was explored.

**Methods:** Thirty-two healthy adult Ugandan volunteers were divided in four equal study groups and given single oral doses of either Homapak or S/P (Fansidar), CQ (Pharco), and or S/P + CQ combination. Blood sampling was done on the first day at 0, 1/2, 1, 2, 3, 4, 6, 10, 24 h, then for the following 21 days. Plasma was obtained and stored at \(-20\) °C prior to analysis. Sensitive and specific HPLC methods (UV and RF) detectors were used for drug analyses in plasma. Pharmacokinetic calculations using one-compartment modeling for S/P and a two-compartment modeling for CQ was done using the WinNonlin ver 4.1. Data are presented as median values. Non-parametric Kruskal–Wallis test was used to compare between treatment groups using Statistica ver 6.1.

**Results:** Sulfadoxine was more rapidly absorbed in the Homapak group with median \(T_{\text{max}} = 4\) h, compared to \(10\) h and \(6\) h in the S/P + CQ and S/P groups, respectively, while this was evidently slow when the different formulations were combined. The slow absorption of Sulfadoxine was further confirmed when the absorption rates \((k_a)\) were calculated for Fansidar combined with CQ of different formulation in S/P + CQ group, where \(k_a = 0.27\ \text{h}^{-1}\), \(p = 0.004\) and \(p = 0.047\) against Homapak and Fansidar alone, respectively. There were no significant differences in Cmax, AUC, \(t_{1/2}\), C1, and C2. The median chloroquine \(k_a = 1.8, 1.2\) and \(0.47\ \text{h}^{-1}\) in CQ, Homapak and S/P + CQ groups, respectively, indicating that absorption was also delayed in the CQ + S/P group. Other CQ pharmacokinetic parameters did not differ between the groups. Pyrimethamine parameters could only be determined during the first 24 h, during which no differences in Cmax, Tmax and AUC were found.

**Interpretation:** Absorption kinetic interaction was demonstrated when formulations of S/P and CQ from different manufacturers were combined. Homapak, a locally produced generic CQ and S/P is bioequivalent to the branded products.
225C
Prescription patterns of antimalarial drugs among medical practitioners in Osogbo metropolis South-west Nigeria [MIM-TO-75580]
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Introduction: Objective: With increasing prevalence of chloroquine resistance and the recent WHO pressure on change in malaria drug policy to the use of ACTs in Africa, we sought to assess the prescription pattern and level of knowledge in the use of antimalarial drugs including ACTs among medical practitioners in Osogbo metropolis, southwest Nigeria, an endemic area of Plasmodium falciparum infection.

Methods: Questionnaires were administered to all the medical practitioners working in all the health facilities existing in the metropolis. There are: a teaching hospital, a general hospital, a mission hospital, a comprehensive health centre and twenty privately owned health facilities. A total of 100 questionnaires were sent out and had 96 respondents, the remaining 4 were not returned. The questionnaires were self administered.

Results: Sixty-seven percent of the respondents work in the teaching hospital, while the remaining 33% either work in the general hospital or in private medical practice. 82.4% of the respondents prescribed chloroquine despite the widespread resistance. It still remains the most widely prescribed antimalarial drug. 45.7% of the respondents got the dosage regimen correctly (p<0.005). 66.7% will prefer the use of chloroquine injection. 85.6% will give anti-histamine chlorpheniramine for pruritus along with chloroquine, 12.1% will give it because of its synergistic effect and 2.1% will give it because of its reversal mechanism. Other commonly prescribed drugs include: Sulphadoxine-pyrimethamine (71.1%), Halofantrine (53.6%), Amodiaquine and Quinine (51.1%), Mefloquine (20.6%), Artemisinin or ACTs (18.6%) and Septrin (17.5%). Out of all the respondents less than 31% got the dosage regimen correctly for all these antimalarial drugs. Sulphadoxine-pyrimethamine (30.9%), Halofantrine (12.8%), while the rest are less than 5%, Amodiaquine (3.2%), Septrin (2.1%), ACTs, quinine and artemisinin monotherapy (1.1%). About 40% of the respondents will prefer the use of combination therapy in the future.

Interpretation: It is obvious that there is paucity of knowledge in the prescription of antimalarial drugs among medical practitioners. With continued medical education the use of combination therapy especially ACTs will easily be accepted.

226A
Malaria and HIV co-infection in southern region. Status and need for strong collaboration between programmes [MIM-JP-415360]
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Introduction: Malaria and HIV co-infection is a big problem in southern Africa. The burden of both diseases is high. Southern Africa is currently one regions greatly affected by the HIV pandemic. HIV burden in the sub region lies between 10 and 20%. The malaria risk in most of the countries is 100% except for low malaria transmission countries where the risk is less than 50%. Current strategies are failing to adequately respond to this problem as the impact of co-infection was not fully appreciated earlier on.
Methods: Aim of paper is to highlight the burden of malaria and HIV disease in the sub-region looking at the impact of the two diseases on the vulnerable groups and possible interventions aimed at reducing morbidity and mortality in people with HIV-malaria co-infection. Current studies are showing that malaria and HIV co-infection are major factors for faster HIV disease progression and that those with HIV have frequent malaria episodes and the severity of these episodes is more intense. This interlinking impact of the diseases has negative impact on the life of the individual. There is increased morbidity and likelihood of mortality. Overall it lowers the quality of productive life of the individual.

Results: The current interventions for malaria are so directed at the disease such that all fevers in malaria endemic countries are treated as malaria. The increase in HIV fevers in patients with HIV disease implies an increase in the level of malaria drug consumption. As HIV cuts across all age groups and sex, the vulnerable groups in endemic areas with high HIV prevalence should now include people with HIV/AIDS. The issue of joint management is critical. Malaria drug and ARV drug interaction should be looked at as a matter of priority. Malaria prevention strategies such as the use of ITNs and mosquito repellents should be encouraged in people with HIV/AIDS in endemic countries. The malaria units should form strong collaboration with HIV unit to ensure this issue is addressed in a holistic manner. Special training modules for malaria in HIV patients should be looked at to take into account the special and difficult nature of the drugs being used. HIV/AIDS care packages for home based management should include ITNs and emphasis should be on integrated management. The impact on the vulnerable groups of HIV/Malaria co-infection is great. The current interventions for malaria/HIV co-infection need to be reviewed and strengthened.

Interpretation: The issue of case management especially in the wake of ACTs requires urgent attention especially as regards treatment of HIV related fevers. An integrated approach in the management for HIV/malaria co-infection is the best way forward.

Efficacy, safety and pharmaco-vigilance of artesunate/amodiaquine combination for treatment of uncomplicated malaria in Casamance, Senegal (MIM-BP-133770)

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Introduction: High rates of chloroquine treatment failure have been documented in the Oussouye district in Casamance, Senegal. In 1999, a randomised, double blinded, placebo controlled trial conducted in children under 10 years showed that amodiaquine and artesunate-amodiaquine were highly efficacious in treating uncomplicated falciparum malaria in Casamance. Based on these results the combination was deployed in the Oussouye district with a control of efficacy, safety and a pharmaco-vigilance survey.

Methods: Patients with fever and a P. falciparum positive blood smear were recruited in the 4 main health centre in the Oussouye district from 2000 to 2004. They were treated for 3 days with artesunate (4 mg/kg/day)/amodiaquine (10 mg/kg/day) combination under supervision and clinically and parasitologically followed up for 28 days. Biological data were collected in percent of them (bilirubine, ASAT, ALAT, hematocrit, white blood cell count) at day 0, 7 and 28. A card for pharmaco-vigilance survey was establish for each patient to note any serious adverse drug reaction or side effect observed.

Results: From 2000 to 2004, a total of 2691 patients were followed up for 28 days. The percentages of cure rate at day 28 using ITT analysis varied from 89.6 to 95.8%. In the group of patients treated with the combination 2.9% have had a second malaria attack during the rainy season versus 9.5% in the group treated with quinine. No serious adverse drug reaction has been recorded. Bilirubin, creatinin, ASAT, ALAT, hematocrit and white blood cell counts performed in
298 patients did not reveal any significant difference between day 0, day 7 and day 28. Among side effects, asthenia, vomiting, pruritus were recorded in less than 7% of the patients.

**Interpretation:** The combination appears efficacious and safe and the survey of pharmaco-vigilance should be continued.

### 228C

**Efficacy of artemisinin derivatives in treating severe malaria in children: a systematic review and meta-analysis** [MIM-GP-165144]

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**Introduction:** Severe malaria kills about one million children worldwide most of them in Sub-Saharan Africa. Evidence shows that the efficacy of intravenous quinine, which is the mainstay of treatment is decreasing in South-east Asia and Africa. Artemisinin derivatives have the potential to replace quinine in treating severe malaria in children. We reviewed the evidence on the efficacy of parenteral artemisinin derivatives versus quinine in treatment of severe malaria in children.

**Methods:** Using systematic review and meta-analysis design, we searched literature for published and unpublished randomized trials in all major databases and then two reviewers independently selected the trials and assessed their methodological quality using predetermined criteria. Data were independently extracted and analyzed using STATA. Effect measures were calculated using pooled relative risks (RR) and weighted mean difference (WMD). Data reported as medians were not included in pooled analysis. Mortality was the primary efficacy outcome where as parasite clearance time, fever clearance time, coma resolution time, 28th day cure rate, incidence of neurological sequelae and incidence of adverse effects were the secondary efficacy outcomes.

**Results:** Eleven trials were selected (1455 subjects), nine of them from Africa and the rest from Asia. Allocation concealment was adequate in seven trials (1238 subjects). Overall there was no difference in mortality between treatment with artemisinin derivatives and quinine (RR = 0.89, 95% CI 0.71–1.1), the sensitivity analysis gave similar results for adequately concealed and un Concealed/ inadequately concealed trials (RR = 0.93, 95% CI 0.74–1.16) and (RR = 0.66, 95% CI 0.36–1.22). Also the findings showed that parasite clearance time (PCT), tended to be shorter in artemisinin derivatives compared to quinine (Pooled WMD among studies which reported PCT as mean was 4.76 with 95% CI 9.68–0.17 and all three studies which reported PCT as median showed that artemisinin derivatives cleared parasites faster than quinine, each had p < 0.001). All other secondary outcomes were not conclusively more efficacious in artemisinin derivatives.

**Interpretation:** The available evidence suggests that parenteral artemisinin derivatives are as efficacious as quinine in preventing death from severe malaria in children.

### 229A

**Treatment practices of private practitioners for malaria in Chennai, South India** [MIM-ER-774909]

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**Introduction:** Malaria is a major public health problem today. The control of malaria is a twofold approach: (i) to control vector and (ii) to control parasite. Now a day, people prefer private doctors for health services suiting their health care needs. So it becomes necessary to assess whether the private practitioners provide treatment according to the Government guidelines for treating malaria. This study aims at finding out the treatment practices of allopathic, urban private practitioners for malaria.

**Methods:** This study was a qualitative study design, using in-depth interview technique. The study was carried out in Chennai city, India. Eleven divisions (API more than or equal to 11) out of 155 divisions were selected for the study. The guide was designed to capture as much detail as possible on all core aspects like history, laboratory investigations, treatment, patient expectations and national anti malaria program guidelines. The Private practitioners were informed in details on the purpose of the study and the consent was obtained. Care was taken to include a wide range of Private Practitioners with varied years of experience.
so as to capture a wide range of perspectives and treatment practices.

Results: A purposive sample of 44 among 90 private practitioners were recruited and interviewed. Two thirds of them were full time practitioners receiving about 150 patients/week, most of them being males. Half of them were MBBS. For diagnosis, most practitioners advised the patient to undergo QBC test but finally started treatment based on clinical signs and symptoms. For treatment, most practitioners used chloroquine for 3 days and if fever subsides, primaquine is given for 14 days whereas others use primaquine for 5–7 days or 10 days. The Other drugs prescribed after chloroquine was: (i) again chloroquine—300 mg twice per week for 8 weeks; (ii) Malarid DS 1 for 21 days and (iii) Malarid for 14 days, etc. The treatment practices for different age group; different types of malaria were elicited. In the case of follow-up of patients, though almost all practitioners advised the patients to come for follow-up, most of them did not turn back. Private practitioners said that they update their knowledge through practice, peer influence, medical representative, reading books, journals, etc. However, practitioners strongly feel that malaria is not discussed in the Medical Society/Association meetings.

Interpretation: Practitioners prescribe antipyretics, not chloroquine, till they get the lab results while others start antimalarials drugs based on the Clinical diagnosis. Practitioner differs in their treatment modalities and rarely followed NMEP guidelines.

230B
Artesunate-clindamycin versus quinine-clindamycin in the treatment of Plasmodium falciparum malaria: A randomized controlled trial

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Introduction: Artemisinin based drug combinations are the mainstay in the fight against drug resistant malaria in Africa. Currently available antimalarial drug combinations including artemisinins are pharmacokinetically unmatched and are therefore potentially increasing the risk of selecting resistant mutants in areas of high malaria transmission. We aimed to test the potential value of short half-life artemisinin based combination therapy for uncomplicated falciparum malaria in sub-Saharan Africa.

Methods: We conducted an open label randomized controlled clinical trial to evaluate the efficacy and tolerability of 3 days, twice daily, oral artesunate-clindamycin therapy (2 and 7 mg/kg per dose) compared to a standard 3 days quinine-clindamycin regimen (15 and 7 mg/kg per dose for six doses) for treating uncomplicated falciparum malaria in 100 Gabonese children aged 3–12 years. Primary endpoint was the PCR corrected cure rate for the per protocol population.

Results: Artesunate-clindamycin showed comparable activity to quinine-clindamycin in the per-protocol analysis of day-28 cure rates (87% versus 94%). No serious adverse events were reported and tolerability was good and similar in both groups. Fever and parasite clearance times were significantly shorter in the artesunate clindamycin group.

Interpretation: Artesunate-clindamycin and other matching short plasma half-life combinations of artemisinins merit further attention in regions with high malaria transmission.

231C
Monitoring susceptibility to sulfadoxine–pyrimethamine among cases of uncomplicated, Plasmodium falciparum malaria in Madagascar

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Introduction: At the end of 2002, the use of SP for the intermittent preventive treatment (IPT) of pregnant women in Madagascar – to decrease the prevalence and severity of malaria-associated severe anaemia in the
women and the frequency of low birthweight in their babies – was proposed. The routine use of SP-based IPT throughout the country could only be justified, however, if SP remained effective against the parasites causing human malaria.

Methods: To be enrolled in the study, a villager had to be aged >1 year, have fever or a history of fever in the previous 48 h, have an infection with \textit{P. falciparum} but no other \textit{Plasmodium} species, have a parasitaemia of >1000 asexual parasites/\mu l, have taken no antimalarial medication in the previous 14 days, have no eczema, and provide informed consent (or that of a parent/guardian).

Results: In a study, carried out in 2003–2004 in Moramanga district, 104 uncomplicated cases were each treated with a standard dose of SP and with paracetamol and then followed up for 28 days. No case of therapeutic failure occurred and all the asexual parasitaemias cleared by day 3. It therefore, appears that SP is effective against \textit{P. falciparum} in Moramanga. Also, there was no dhfr S108N mutant \textit{P. falciparum} among examined samples.

Interpretation: This is an encouraging observation to make before IPT is initiated throughout the country in Madagascar. And there is a need to extend the monitoring of SP efficacy to other districts.

232A  

Taux de prévalence et expression de l'infection palustre à \textit{P. falciparum} dans la consultation en milieu urbain au Congo [MIM-TR-202592]

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Results: Notre étude a permis d’enregistrer 108 personnes en deux mois. Plus de 60% des GE étaient infectées par \textit{P. falciparum}. Plus de 55% de ces infections étaient asymptomatiques, 20% des sujets avaient un accès paluste simple et 2.7% (3/108) avaient un accès paluste grave (selon les critères de gravité de l’OMS, 2000). 3 accès palustres graves ont été enregistrés: deux filles âgées, respectivement, de de 4 ans et 10 ans; une femme de 69 ans décédée. La léthalité a 1/3 a été observée. Tous les cas d’accès palustres graves enregistrés avaient une anémie de gravité variable.

Interpretation: l’infection à \textit{P. falciparum} prévaut en saison sèche. La baisse des piqûres d’anophèles ne préserve pas, en milieu urbain, des infections palustres qui sont asymptomatique, simple et grave. La léthalité est élevée, l’anémie en est le corollaire.

233B  

Randomized controlled trial comparing artesunate/mefloquine versus artemether/lumefantrine in treatment of uncomplicated falciparum malaria in Mali [MIM-IS-108717]


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Introduction: Artemisin based combination therapy (ACT) is increasingly being adopted as first line antimalarial therapy in sub-Saharan Africa. The choice of the appropriate ACT depends on several factors
including cost, efficacy, side effects and simplicity of administration. In this study we tested the hypothesis that artesunate/mefloquine (Artequin®) is as efficacious and safe as artemether/lumefantrine (Coartem®) in the treatment of uncomplicated *P. falciparum* malaria in Mali.

**Methods:** The study was carried out from August 2004 to February 2005 in Kambila; a rural area of Kati, Mali where malaria is hyper-endemic and transmission highly seasonal. Treatment efficacy was assessed using 28 days WHO 2003 protocol. Treatment safety was assessed clinically and by measuring Complete Blood Count, creatinine and liver enzymes. MSP1, MSP2 and GLURP were assessed as molecular markers of parasite polymorphism to distinguish recrudescent from new infection. The study included patients with weight 10 kg and with uncomplicated malaria. The sample size was computed on efficacy equivalency assumption, 470 patients were enrolled. Artequin was manufactured by Mepha, Switzerland. The study was supported by Mepha and the University of Bamako.

**Results:** Baseline characteristics of patients in the two treatment groups were comparable. No serious adverse events or significant laboratory abnormalities occurred. Using interim data (n = 360), adverse events such as vomiting, diarrhea, abdominal pain, headache, dizziness, fatigue and anorexia were similar in the two groups. No early treatment failure occurred. Non-PCR corrected 14 days cure rate for interim data was 99.4% (n = 177) for Artequin as compared to 98.3 for Coartem (n = 177); p = 0.31. Parasite and temperature clearances were similar in the two groups excepting day 1 where Artequin (95.6%) cleared fever faster than Coartem (83.3%), p < 0.0001. All parasites were cleared by day 3 in both treatment groups. Gametocyte carriage rate was similar in both treatments groups. We will present full data compared by treatment groups including adverse events, non-corrected 28 days cure rate as well as PCR corrected 28 days cure rate, parasite and temperature clearances, and gametocyte carriage rate.

**Interpretation:** Interim data have shown that a three-day course of Artequin is as effective on *P. falciparum* malaria and well tolerated as Coartem. Artequin cleared fever faster than Coartem by day 1. Full data is awaited by end of April 2005.

**234C**

Efficacy and safety of sulfalene–pyrimethamine + amodiaquine versus Coartem* in the treatment of uncomplicated *Plasmodium falciparum* malaria in Senegal [MIM-AS-269685]


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**Introduction:** Malaria is a major problem of public health in the tropical countries. Senegal choose bitherapy for the treatment of uncomplicated malaria in June 2003. The objective of this study, undertaken, was to compare the efficacy and safety of a 3 days regimen of non-artemisinin combination sulfalene–pyrimethamine + amodiaquine (SPA) versus artemether–lumefantrine gave at six doses (C6).

**Methods:** We conducted from November 2004 to January 2005 a prospective randomised study on two parallel groups without knowledge on the treatments, conceived to test the assumption of non-inferiority between these two combinations. The WHO 2003 protocol was used within a 28 days follow-up. A haematological and biochemical assessment (BNF, glycaemia, transaminases, bilirubine and creatinin) was carried out at D0 and D7. The molecular markers of resistance were evaluated by PCR at D0, D7, D14 and D28.

**Results:** At the end of this study, we enrolled 233 patients and analysed 229, 4 patients were lost of follow-up. We treated 113 by sulfalene–pyrimethamine–amodiaquine and 116 by artemether–lumefantrine. We obtained an adequate clinical and parasitological response of 100% with two combinations from D3 until D28. We observed no impaired of glycaemia and kidney function. The percentage of patients with anaemia fell between D0 and D7 from 43 to 28% for patients treated with C6 and from 35 to 25% for the SPA group. The percentage of patients with an increase of transaminases at D0 fell from 23 to 5% for the group C6 and from 26% to 7% (p = 0.54) for SPA. Also bilirubine disturbance decrease quickly from 46.5 to 11.2% for the C6 group and 50.4 to 10.6% for SPA group between D0 to D7. No severe adverse events were observed. The side effects noted were abdominal pain (9.3% with SPA and 4.9% with
C6), itching (2.7% all with SPA) and vertigo (2.6% with SPA and 0.7% with C6).

Interpretation: This comparative study between sulfalene-pyrimethamine + amodiaquine and Coartem® demonstrated a very good clinical and parasitological efficacy in Senegal and both seem well tolerated. It did not show a difference on efficacy and safety.

235A
Feasibility of the use of an emergency pediatrics kit for rectal administration of quinimax® by the community health staff in Senegal [MIM-RT-192454]
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Introduction: Quinine constitutes the reference treatment of non-per os malaria attacks. Since one decade, experiments of its use in intra rectal way are undertaken in Africa. We study the feasibility of using pre-transfer kit of quinimax intra rectal in health community staff in northern Sénégal.

Methods: We did a prospective open randomised case control study in six villages in children between 0 and 10 years old. 20 mg/kg dose of quinimax was administered on the case by way will intra rectal before their transfer to the health post. Thick smear were carried out at day 0, 3 and 7. At the health post, the treatment followed the recommendations of National Malaria Control Program.

Results: 131 patients (79 case/52 control) were enrolled from November 2003 to May 2004. The two groups of patients were comparable. The time of oral recovery was 14 h for the cases and 36 h for the control (p < 0.05). The fever disappear within 45 h for the cases and 55 h for the control (p = 0.0001). The parasitological clearance was obtained among all our patients at day 7. Acceptability was good and 80% of the health community staff found kit’s use easy.

Interpretation: The emergency paediatric kit, represents a considerable contribution in the management of non-per os malaria attacks. Its use is simple, easy, feasible in rural area and peripheral health care.

236B
Randomized clinical trial of artesunate/sulfamethopyrazine-pyrimethamine versus artemether/lumefantrine in the treatment of uncomplicated malaria in Mali [MIM-MS-316122]
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Introduction: Artemisinine based combination therapy (ACT) is increasingly being adopted as first line antimalarial therapy in sub-Saharan Africa. The choice of appropriate ACT depends on several factors including cost, efficacy, side effects and simplicity of administration. In this study we tested the hypothesis that (artesunate/sulfamethopyrazine-pyrimethamine) Coarinate® is as efficacious and safe as artemether/lumefantrine (Coartem®) in the treatment of uncomplicated P. falciparum malaria in Mali.

Methods: The study was carried out from September 2003 to November 2004 in Sotuba; a peri-urban area of Bamako where malaria is meso-endemic and highly seasonal. Treatment efficacy was assessed using 28 days WHO 2003 protocol. Treatment safety was assessed clinically as well as by measuring Complete Blood Count, creatinine and liver enzymes. MSP1, MSP2, HRP2 and GLURP were assessed as molecular markers of parasite polymorphism to distinguish recrudescents from new infections. The study included subjects aged ≥6 months with uncomplicated P. falciparum malaria. The sample size was computed on efficacy equivalency assumption of the two treatments, and 606 patients (303 in each arm) were enrolled over two malaria transmission seasons.

Results: Baseline characteristics of the two treatment arms were similar. No early treatment failure occurred. The cure rate by day 28 was higher in the Coarinate® group (98.7%) than the Coartem® group (89.6%); p < 0.0001. After PCR correction the cure rate by day 28 was 100% in Coarinate® group compared to 99.0% in Coartem® group; p = 0.3. Parasite and temperature clearances were similar in the two treatments except by
day 1 where Coarinate (90.8%) cleared fever faster than Coartem (77.6%); \( p < 0.001 \). All parasites were cleared by day 7 in both treatment groups. Gametocyte carriage rate was similar in both treatments. No serious adverse or significant laboratory abnormalities event occurred. Two out of fifty subjects had a mild and transient liver enzyme elevation on day 14 in Coarinate® group, compared to 0 of 35 in Coartem® group. The liver enzyme for these patients fitted within normal ranges about 2 weeks later.

Interpretation: Administered in a three-day course, Coarinate® is as effective on *P. falciparum* malaria and well tolerated as Coartem®. Coarinate® cleared fever faster than Coartem by day 1. Coarinate® showed an additional benefit in the prevention of new infections.

237C Evaluation de l’efficacité et de la tolérance de la dihydroartémisinine (Cotecxin®) dans le traitement du Paludisme simple à *Plasmodium falciparum* au Sénégal [MIM-AS-339080]


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Introduction: En Afrique Occidentale, la chloroquine présente de plus en plus de résistance et dans certains pays, son taux d’efficacité est inférieur à 20%. Les autres molécules comme l’aminodiaquine ou la sulfadoxine–pyriméthamine présentent également une efficacité limitée dans les territoires où la chloroquinorésistance est présente. Les dérivés de l’artémisinine peuvent constituer la réponse à ces multi résistances.

Methods: Cette étude menée dans le district de Guédiawaye du 1er Décembre 2004 au 04 Janvier 2005 a pour objectif d’évaluer l’efficacité et la tolérance du plus récent de ces dérivés, la dihydroartémisinine (Cotecxin®) qui a été testée principalement en Asie sur des populations et sur des souches de *Plasmodium* différentes de celles rencontrées sur le continent africain. Le protocole OMS 2003 avec un suivi de 28 jours a été utilisé avec un bilan biologique (NFS, créatinine et transaminases) à J0 et J28. Un prélèvement sur papier filtre pour génotypage a été fait à J0, J14 ou J28 chez les patients porteurs parasites. Les critères de jugement principaux étaient le taux de réponse clinique et parasitologique adéquat (RCPA) à J28, la tolérance clinique et biologique.

Results: 65 patients âgés au moins de 2 ans ont été inclus et ont reçu une dose quotidienne de dihydroartémisinine de J0 à J6. Un seul cas d’échec thérapeutique a été noté soit un taux de RCPA à J28 de 98.5%. Après correction par PCR la RCPA était de 100%. La tolérance clinique était bonne avec des effets secondaires mineurs disparaisant au bout quelques jours. Aucun effet secondaire grave n’a été signalé. La tolérance biologique était bonne avec une amélioration de l’anémie. La créatinine et les transaminases n’étaient pas perturbées.

Interpretation: La dihydroartémisinine (Cotecxin®) peut constituer une alternative à la multi résistance de *Plasmodium falciparum* au Sénégal. Cependant son association avec un autre antipaludique permettrait de pérenniser son efficacité.

238A Antimalarial treatment practices for management of fever in children in urban Kampala [MIM-SS-15232]


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Introduction: Prompt treatment with effective anti-malarial drugs is the cornerstone of malaria control in Africa. At the Roll Back Malaria summit in Abuja in 2000, African leaders committed to ensure that 60% of malaria cases were treated with appropriate antimalarials within 24 h of onset of symptoms by 2005. We investigated antimalarial treatment practices for management of fever in a representative sample of children in Kampala, Uganda and evaluated whether the Abuja target is currently being met.

Methods: We conducted a census of a parish in Kampala identifying 16,172 residents living in 4931 house-
holds. Households with at least one child aged 1–10 years were randomly selected from the census for recruitment into a longitudinal study of combination antimalarial therapies. At the time of enrollment, a questionnaire on household demographics and treatment seeking practices was administered at home. Primary caregivers who reported that a child under their care experienced an episode of fever within the past 2 weeks were asked about the actions they took in response to the illness. Information on drugs given, time to treatment, and expenditure on management of the illness was obtained.

Results: An interim analysis including 274 households is presented here. Primary caregivers of 114 (42%) households reported that a child under their care had experienced fever in the past 2 weeks. Of these, 89 (78%) reported giving any antimalarial treatment in the management of their child’s illness, including chloroquine (CQ, N = 61), sulfadoxine–pyrimethamine (SP, N = 35), quinine (N = 24), amodiaquine (N = 5), and an artemisinin (N = 1). Only 13 (11%) febrile children were treated with CQ + SP, the current first-line therapy recommended for uncomplicated malaria in Uganda. Considering the first action taken, children treated at a clinic or hospital were more likely to receive an antimalarial other than CQ monotherapy, compared to children treated with medications obtained at a drug shop (61% versus 18%, \( p < 0.001 \)). Administration of antimalarial treatment within 24 h was reported by 17 (15%), but only 5 (4%) reported giving an antimalarial other than CQ monotherapy within 24 h. The proportion of patients receiving antimalarial treatment within 24 h was not significantly different between households that first sought treatment from a clinic or hospital and those that went to a drug shop (22% versus 15%, \( p = 0.51 \)).

Interpretation: In Kampala, prompt treatment of febrile children with effective antimalarial drugs occurs uncommonly. Even in this urban area, where health care facilities and medication are widely available, the Abuja targets are not being met in 2005.

**239B**

Composition and in vivo antimalarial activities of essential oils from three Piper species grown in Cameroon [MIM-FT-363874]

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Introduction: In the West province, Cameroon, plants which claim to have antimalarial properties are administered to patients in various forms. Fumigation, mostly used by traditional practitioners in the treatment of malaria is a process whereby the patient inhales the vapor of a concoction of boiled leaves. In this study, we determined the oil content, chemical composition and evaluated antimalarial properties of essential oils obtained from three Piper species used traditionally to treat febrile conditions.

Methods: Essential oils extracted from fruits by hydrodistillation were dried over anhydrous sodium sulfate column. These oils were then analyzed by gas chromatography and mass spectrometry. Their antimalarial activities were evaluated in mice infected with Plasmodium berghei (ANKA). The classical 4-day suppressive test was employed as described by Peters (1970).

Results: Results showed that the yield of extraction were 1.2, 1.4 and 3.6%, respectively for Piper nigrum, Piper capense and Piper guineense. Monoterpenes (61.0–88.8%) represent the predominant class of identified constituents with f\(\text{O}-\text{pinene} (13.1–65.0\%) as the main constituent common to the three oils. These oils show relative inhibition of the growth of Plasmodium berghei (ANKA) in mice. At respective doses of 200, 300 and 500 mg/kg of body weight of mouse per day, oil of Piper nigrum demonstrated the suppression of parasitemia (65.6, 56.9 and 22.6%) followed by Piper guineense (61.0, 54.0 and 43.8%). The corresponding values for Piper capense were 48.8, 16.5 and 15.0%. Chloroquine used as a standard drug (10 mg/kg) present the percentage inhibition of 100%.
Interpretation: These results clearly showed that these essential oils had some effect in reducing *P. berghei* growth in vivo in animal models thus validating their use against malaria induced febrile conditions in West Cameroon.

240C

Antiplasmodial activity of aqueous extracts of “Saye”, a traditional antimalarial remedy used in Burkina Faso [MIM-MT-68126]


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Introduction: The cost of western drugs makes 75–80% of the population in developing countries like Burkina Faso rely on traditional medicine for treatment of malaria. In Burkina Faso with the development of traditional medicine improved remedies are more and more proposed in the treatment of diseases and malaria notably. “Saye” is one of these remedies proposed by a pharmacist and widely used in Burkina and neighboring countries for the treatment of malaria.

Methods: A preliminary antiplasmodial screening of “Saye” was carried out. “Saye” is an association of 3 plants, which are Phyllanthus amarus Schum and Thonn, (Euphorbiaceae), Cochlospermum planchonii Hook, (Cochlospermaceae) and Cassia alata Linn. (Caesalpiniaceae). The aqueous extracts of “Saye” were tested for in vivo antiplasmodial activity in vitro against *Plasmodium falciparum* and in vivo against *Plasmodium berghei* in mice.

Results: First results gave in vitro a IC50 around 777.93 μg/ml on wild strain of *P. falciparum* and 2724.33 μg/ml on the strain 3D7; indicating a very low antiplasmodial activity in vitro. On the other hand we obtained in vivo a lethal dose (LD50) around 1660.55 mg/kg and an effective dose (ED50) equal to 95.78 mg/kg.

Interpretation: In vivo “Saye” extracts showed a promising antiplasmodial activity and an acceptable safety in mice. The difference between the activity in vivo and in vitro could be due to the metabolism of the extract compounds. According to the antiplasmodial activity observed in vivo these results could justify the use of “Saye” as alternative treatment for uncomplicated malaria.
(52.5%), followed by the children aged 6–10 years (35.7%).

**Interpretation:** Each year, millions of children die from severe falciparum malaria. The bacteria isolated cause septicemia with symptoms resembling that of severe malaria. Thus, persistence of symptoms after treatment and deaths may not be due to malaria alone.

242B

**Erythropoietin (Epo) and metallothionein (MT) treatment during cerebral malaria (CM) in mice**

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**Introduction:** CM is an acute encephalopathy with increased proinflammatory cytokines, sequestration of parasitised erythrocytes and localised ischaemia. CM induces cognitive impairment in about 10% of the survivors. The neuroprotectants Epo and MT have significant anti-inflammatory, antioxidant and anti-apoptotic effects during various brain diseases. However, their roles in CM remain to be elucidated, and therefore we examined the neurobiological responses to exogenously injected Epo and MT during murine CM.

**Methods:** C57BL/6j mice, infected with *P. berghei* ANKA and treated with either recombinant human Epo i.p. (5000 U/kg/OD) or MT (750 mg MT-II/kg/BD) were studied on day 7, day 9, and when presenting signs of CM (typically by 10–15 days after infection). Physiological saline was used as control.

**Brain pathology was investigated by immunohistochemistry, immunofluorescence and TUNEL (Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-digoxigenin nick end labelling) as a marker of apoptosis.**

**Results:**

A. Localised neuronal apoptosis indicating irreversible pathology
B. Epo treated mice with CM showed significantly reduced apoptotic cell death
C. MT-treatment in the acute, post-infectious phase did not affect the CM course clearly.

**Interpretation:** This is the first report of the neuroprotective effect of EPO in CM. Its possible therapeutic potential needs to be further examined. MT failed as neuroprotectant in this setting, the pathophysiological mechanisms have to be further elucidated.

243C

**Re-thinking first-line treatments for malaria in areas of intense transmission**

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**Introduction:** Artemisinin-based Combination Therapies (ACTs) are widely being proposed as the first-line treatment for uncomplicated malaria in many sub-saharan African countries. Many countries have decided to change their official malaria treatment policies. Yet these decisions are based on trials which do not necessarily apply to the majority of patients treated for malaria in primary health care in Africa.

**Methods:** We compared the inclusion criteria for antimalarial drug efficacy studies (WHO 2003 protocol) with the characteristics of patients treated with antimalarials over the course of 12 months in a health post in a rural area of Mali, and with patients presenting with malarial symptoms in a rural village over the period of 2 months.

**Results:**

The WHO (2003) protocol stipulates that subjects should be aged 6 months 5 years; have a measured axillary temperature of at least 37.5 °C; and have a pure *P. falciparum* parasitaemia of >2000 per mcl. Over a 12 month period (October 2003–October 2004) in the rural health post of Finkolo (Sikasso district, Mali), 713 patients were treated for presumptive malaria, but only 198 of these (27.8%) were aged less than 5 years. Temperature was not recorded, and there were no diagnostic facilities available to determine parasitaemia. In the course of a 2 month study in the village of Missidougou, 245 patients presented with presumptive malaria, but only 54 of these (22%) fulfilled all the inclusion criteria for the WHO protocol.
Interpretation: Findings of studies on a small sub-population cannot be extrapolated to the entire population of patients with presumed malaria. ACTs are scarce and expensive, and should be targeted to those who will most benefit from them.

244A

Effects of microscopy on outpatient malaria case management in Kenya [MIM-DZ-8658]

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Introduction: Use of malaria microscopy in older children and adults is a proposed strategy to overcome the low specificity of presumptive malaria diagnosis and reduce unnecessary wastage of antimalarial drugs and associated cost. However, under routine conditions of rural facilities in Africa, limited information is available on the effects of microscopy on malaria case management.

Methods: In 2002, we assessed clinical practices and accuracy of routine reading of malaria blood smears in government health facilities with functional microscopy in Kisii and Kwale districts, Kenya. All patients over 5 years old presenting to outpatient departments of study facilities were enrolled. Information on fever, results of routine microscopy, diagnosis made and drugs prescribed were collected from patient-held records before they left facility. Two expert microscopists re-examined routine malaria slides during the post-survey work.

Results: We evaluated 436 consultations performed by 31 clinicians at 17 facilities. Overall, 74.1% patients had a blood slide performed, commonly among febrile (79.4%) but also among patients without fever (52.9%). Among febrile patients with routinely reported positive and negative malaria smear results, 95.7 and 77.6% had antimalarial drug prescribed, respectively. Nearly half (51.4%) of febrile patients without blood smear performed were treated for malaria. Sensitivity, specificity, positive and negative predictive value of routine blood smear readings was 67.4, 60.7, 22.5 and 91.7%, respectively. We estimated that in the two surveyed districts a single change of clinical practice such as the respect of negative smear results in older children and adults would create annual savings on antimalarial drugs of 6338 USD under current sulfadoxine-pyrimethamine treatment policy but 78,848 USD under new artemether-lumefantrine policy.

Interpretation: Potential benefits of microscopy are not realised; use and interpretation is irrational; accuracy is sub-optimal, however, clinicians should be confident in results of negative slides. Ambiguities in clinical guidelines may explain current practices.

10. Capacity building

Posters 245–257

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

245B

What partnerships are really about: Challenges and achievements of institutional collaboration—an example from the Gates Malaria Partnership [MIM-PB-209580]

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Introduction: The Gates Malaria Partnership (GMP) was formed in 2001 by a group of nine European and African institutions with long-standing experience in malaria research, control and training and an interest in intensifying their collaboration on the identification and testing of innovative approaches to the control of malaria in endemic countries, particularly those in Sub-Saharan Africa. The partnership is supported by the Bill and Melinda Gates Foundation through a 5 year US 40 million grant.

Methods: The GMP has three structural components: (1) promotes research into new interventions for malaria control; (2) develops mechanisms and systems for transferring malaria related knowledge into practice and (3) supports capacity strengthening and addresses: (a) research capacity building through financial and scientific support to PhD students and (b)
institutional and human capacity strengthening through the establishment of, and provision of financial and technical support to, training centres in the southern partner countries. Although divided as above, there has been a real opportunity for institutional interaction between and within the components. This presentation will address the experience gained within the capacity strengthening arm.

Results: Developing models of best practice in delivering intermittent preventive treatment during pregnancy (IPTp) in Tanzania and The Gambia will serve as a recent case for discussing north–south and south–south interactions, research and training synergies, involvement of PhD students in malaria control and measures taken by the training centres to adapt to the future. The IPTp projects in both Tanzania and The Gambia attempt to adopt a holistic approach to service delivery in which the quality of supportive mechanisms in the district health system and the perceptions of providers and users about service quality are considered as important as the correctness and magnitude of service delivery itself. The projects were not designed to benefit from the capacity of the partner institutions at large but their complexity and multidisciplinary nature called for broad involvement in order to answer specific questions and resolve specific problems in the process of defining important programmatic components. The innovative natures of the project designs are considered a direct result of the broad involvement of the capacities of the partnership and form the basis for the planning of the future strategic direction of the centres.

Interpretation: Using the IPTp case study, the presentation analyses whether complex institutional collaboration has advantages over bilateral models. Whilst the start was challenging, it is argued that the partnership has matured and identified ways to benefit.

246C
Afro-immuno assay multi-centre network project and capacity building for malaria vaccine development in Africa [MIM-DD-3468]


(1) Noguchi Memorial Institute Ghana; (2) Medical Research Unit, Lambarene, Gabon; (3) Muhimbili University College of Health Sciences, Tanzania; (6) Institut Pasteur, Dakar, Senegal; (5) Centre National de Latte contre le Paludisme, Burkina Faso; (6) Blair Research Institute, Zimbabwe; (7) Biomedical Primate Research Centre, Netherlands; (8) Dept. of infectious Disease Immunology, State Serum Institute, Denmark; (9) Dept. of Clinical Biochemistry, State Serum Institute, Denmark

Introduction: To minimise differences in comparable studies conducted to assess antibody mediated immunity to malaria vaccine candidate in different areas of Africa, the Afro-immuno assay (AIA) project was initiated. AIA develops standardized assays using the same study designs, reagents and statistical tools to assess the relationship between malaria specific antibody responses to 4 potential malaria vaccine candidate antigens and protection from clinical malaria. The AIA network is sponsored by AMANET.

Methods: Archived plasma samples were obtained from studies with similar longitudinal cohort designs in six geographical and epidemiological settings comprising low to holo-malaria endemicity including Ghana, Burkina Faso, Senegal, Gabon, Tanzania, Zambia. Quality assurance was done by partners from Serum Institute, Denmark and the Biomedical Research Centre, Netherlands Standardised ELISA assays for measuring IgG, IgM, and IgG subclasses were developed. Excel based ELISA curve fitting program that converts ELISA OD to concentrations were developed in addition to a program used for managing ELISA data and morbidity surveys. Workshops for hands on training on the use AIA ELISA SOPs, curve fitting programs, statistics and data management were organized.

Results: Subsequent to training workshops, the same plasma samples and reagents were sent to partner sites for ELISA measurements and quality assurance, prior to testing of site specific samples. Analysed ELISA data originating from site quality assurance tests indicated comparability for inter-site and intra-site measurements, thus indicating the possibility of standardizing studies and assays across Africa that ensures comparability and builds capacity. Site specific samples have been tested at all sites and manuscripts are being prepared for publication.

Interpretation: AIA has helped build capacity and will provide more conclusive data regarding antibody mediated immunity to four malaria vaccine candidate anti-
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gens. Standardized ELISA assays are available for use in vaccine trials in Africa to ensure comparability.

247A

Utilization of malaria primary prevention programs in Cameroon: Cultural determinants of participation in rural and urban settings [MIM-SF-142240]

Fankem S.

Fondation Tchewé-Minteu and The Mel and Enid Zuckerman Arizona College of Public Health, University of Arizona

Introduction: Malaria is still a major problem in Africa. The recent surge in malaria prevention and control underscores the need for capacity building, which requires a thorough understanding of populations' beliefs. A successful program will involve understanding the socio-cultural motivations governing people’s choice to participate in primary prevention programs. We hypothesize that, beliefs of populations living in different settings, urban versus rural, affect use of primary malaria prevention programs.

Methods: We propose a study that will employ questionnaires developed from focus group data to discern study participants’ beliefs and perceptions of primary malaria prevention programs. To have a better representation of the population, subjects in urban and rural settings will be randomly approached in daily life situations for participation in this study. Once consented, the participant will complete a questionnaire designed to assess his/her baseline knowledge of malaria primary prevention, beliefs and knowledge towards malaria prevention programs. Because Cameroon is bilingual (French and English), we will have two surveys, one in each of the national languages. When necessary, translators will administer the survey using local dialects.

Results: We expect that due to varying socio-cultural beliefs, there will be a difference in barriers to use of malaria primary prevention programs in urban and rural settings.

Interpretation: Knowledge of cultural barriers to successful malaria prevention programs acquired from the proposed study may be used to design culturally competent education and prevention programs throughout Cameroon to curtail the damages due to malaria.

248B

A virtual learning environment (VLE) as a repository for development and sharing of malaria related learning materials [MIM-HH-111150]

H. Howden-Leach,

Gates Malaria Partnership Liverpool School of Tropical Medicine Liverpool England, UK

Introduction: The Gates Malaria Partnership, an innovative capacity development programme, has funded 4 training centres in sub-Saharan Africa: Gambia (CIAM), Tanzania (CEEMI), Ghana (GMC), and Malawi (MAC). This initiative provides technological and educational support offering alternatives to the established blend of learner centred and didactic pedagogy with face-to-face tutorial contact and largely paper based materials.

Methods: The introduction to the concept and use of a VLE is proposed. Each centre is supported to establish a repository of collaboratively developed, malaria related resources for dissemination. Support in updating centre websites to encourage an interactive approach and promotion of VLE use is ongoing. A range of inexpensive, effective software packages, to aid curriculum planning and materials development, have been introduced. To enhance existing learner-centred training approaches and support the transition from a didactic to a more constructive pedagogy and, a series of workshops are being facilitated in distance learning and the concepts and design of re-useable learning objects.

Results: Accomplishments include: CIAM’s wish to review and revise their current Community Health Nurses learning materials as the basis for their first distance-learning course and further develop distance-learning materials for health communicators in order to produce a ‘malaria skit’ for behaviour change purposes. GMC are considering designing a course for the training of trainers in community advocacy for malaria control through distance learning in the District Health systems in Ghana. MAC and CEEMI have also identified areas in which they wish to develop. CEEMI has an excellent opportunity to support their current District Malaria Focal Persons Course throughout Tanzania and MAC are planning to assume responsibility for housing, running and supporting the VLE in the future.
Interpretation: There is positive feedback to incorporate the concept of a VLE to support current training programmes and to collaboratively develop, store and share malaria related resources.

249C
Community based malaria control plan experience in Malawi [MIM-TL-82305]
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Plan International In Malawi Population Service International Ministry of Health, Malawi

Introduction: Plan Malawi in partnership with other stakeholders is facilitating the implementation of Community Based Malaria Prevention and Control in Lilongwe, Kasungu and Mzuzu Programme Units.

Methods: Drug revolving Fund, ITN and Community Resource People (CORP), Child to child groups established. ITN committees—Sell ITNs, disseminate Information and monitor. Drug Revolving Fund (DRF) groups—Administer malaria drugs, recognize danger signs and make timely referrals. And Health Surveillance Assistants train and supervise the Corps. Advocacy is carried out at all levels. Plan supports the training of all actors and in partnership with PSI purchase cheaper nets. Bartering for those who do not have cash is encouraged. Male involvement to support programme is facilitated. Nets are sold through Door-to-door and at the Community Based Child Care Centers. Plan purchase the initial stock at 0.9 US$ per net and sold at 1.28 US$.

Results: Letting communities prioritize their issues empowers the community and instills absence of ownership. Communities are motivated when they see the results of their own efforts. The idea of getting 10% of the sale of nets encourages the members to sell more. Male involvement provides understanding and support for pregnant mothers and Children to sleep under the net. Demand for nets has been created and the momentum must be sustained.

Interpretation: Communities are motivated when they see the results of their own efforts. The idea of getting 10% of the sale of nets encourages the members to sell more. Conducting regular visits encourages the communities to do more and better. Male involvement provides understanding and support for pregnant mothers and Children to sleep under the net. Demand for nets has been created and the momentum must be sustained.

250A
Improving malaria case management at Ntcheu district hospital [MIM-DM-96492]
(1) Malaria Alert Centre, College of Medicine, Blantyre, Malawi; (2) Malaria Project, College of Medicine, Blantyre, Malawi; (3) Ntcheu District Hospital, Malawi; (4) University of North Carolina, USA

Introduction: Effective case management is a major strategy for reducing malaria mortality. Management of severe malaria has been extensively researched at Malawi’s Queen Elizabeth Central Hospital. Geographical and organizational limitations often compound human and financial resource constraints at a district. To improve care of severely sick children a simple management tool, the critical care pathways, was introduced by on site capacity building for the whole health care team at Ntcheu district hospital.

Methods: In order to establish best malaria management process within the resources and constraints of the district hospital, capacity building was approached from the perspective of the whole management and health care team at Ntcheu district hospital. The team identified factors affecting performance, gaps impeding implementation of suitable interventions and solutions to address these. Through on site training, visits to the research and regular wards at QECH and local consensus building meetings, the Ntcheu team was exposed to best care practices and has adapted and adopted use of critical care pathways for monitoring of sick children.

Results: Pre-and post-intervention assessment identified a committed and hard working team of technical and management personnel, constrained by heavy case load, insufficient knowledge about best care practices and limited incentive schemes, including regular shortages of supplies. The team identified and prioritized required interventions. They adjusted the nursing rota in order to create bank nurse system, which improves nursing levels on the wards. While the value of a bigger budget cannot be disputed, by reorganizing patient flow and making malaria diagnostic services accessible in OPD, they have reduced patient waiting
time there. Critical care pathways are a standard of care for monitoring of very sick children on the ward, and the management team locally produces these. Daily review of ward statistics is done through morning handover meetings attended by all staff.

**Interpretation:** Budgetary constraints are a feature of health care delivery systems in Africa and innovations in capacity building should focus on building teams. The teams can identify problems and work towards solutions.

### 251B

**Innovation and change in capacity building for malaria control** [MIM-GM-16300]


(1) Ghana Malaria Centre, School of Public Health, Accra, Ghana; (2) Centre for Innovation against Malaria, Banjul, The Gambia; (3) Ghana Malaria Centre, School of Public Health, Accra, Ghana; (4) Malaria Alert Centre, College of Medicine, Blantyre, Malawi; (5) Centre for Enhancement of Effective Malaria Interventions, Dar es Salaam, Tanzania; (6) Malaria Alert Centre, College of Medicine, Blantyre, Malawi; (7) Centre for Innovation against Malaria, Banjul, The Gambia

**Introduction:** There is increasing recognition that to be effective, capacity strengthening in malaria control must of necessity move outside the health sector to engage and empower other sectors, individuals and communities. Under the Gates Malaria Partnership (GMP), four African training centres were established with a mandate to improve the skills, knowledge and attitudes of those involved in malaria advocacy, control, prevention and management by assuming a catalytic role in spearheading innovative projects.

**Methods:** The need to emphasise malaria as a developmental problem focussed on the vulnerable child has guided project design. A holistic approach has been taken specifically to reach policy and decision makers, opinion makers, families, youths, and non-health as well as health sectors. Building the capacity of these groups is seen as a prerequisite to achieving the goal of fewer malaria related deaths. Expanded monitoring and evaluation plans have been developed to assess the effectiveness of the capacities that have been built. A variety of approaches, outcomes and indicative impact indicators are illustrated by three projects, representing a range of target groups.

**Results:** To assist district health programming, EPI registers in Malawi were expanded to capture data on ‘fevers presumed to be malaria’ and their outcome. The performance of community, health centre and District teams influences the degree of success and was tackled by joint capacity building efforts. Preliminary analysis of the newly collected data shows good recording accuracy across 5 health centres. In the Gambia, 21 media practitioners participated in a three-armed media training project: tutorials on writing accurate, relevant, entertaining and educational malaria stories; developing an exchange forum for health and media personnel and active participation in a pan-African website on malaria reporting for long-term support and mentoring. In a preliminary impact analysis, reporting on malaria issues increased by 50% for three out of four major local newspapers. Behaviour change through empowerment is the focus of a Mother/Caregiver Advocates (MCAs) project operating within the health systems of 2 districts in Ghana. Using an adapted cascade training model, 33202 people were sensitised in malaria prevention, home-based care and referral by 159 MCAs. Preliminary data show a significant decline in severe malaria reporting at OPDs.

**Interpretation:** Capacity strengthening approaches designed to have an impact on malaria control are dependent on the presence of key elements. These elements and the innovation in the approaches used above will be highlighted and models for replication presented.

### 252C

**Capacity strengthening for the accelerating development and deployment of malaria vaccines in Africa** [MIM-CM-22782]

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African Malaria Network Trust (AMANET) Dar es Salaam, Tanzania

**Introduction:** Malaria is a leading cause of morbidity and mortality in sub-Saharan Africa, especially among children and in pregnancy. Current control measures are failing due to poor healthcare systems and emergence of parasite and drug resistance, calling for an
accelerated development of malaria vaccines. This in Africa is, however, hampered by many reasons including poor financial returns to investments on R&D and inadequacies in personnel, infrastructure, political will and advocacy.

Methods: To overcome this, AMANET has introduced several pull mechanisms through capacity strengthening of African malaria institutions, which include characterisation and development of potential vaccine trial sites, personnel training, advocacy, networking, immunological evaluation of malaria candidate vaccines through the Afro-immunoassay (AIA) initiative and sponsorship of vaccine trials. AMANET supports both short- and long-term training; the latter includes sponsorships for masters and doctorate programmes to fill personnel gaps in institutions selected to conduct vaccines trials. Short-term training is in the form of training workshops for capacity building in the development, testing and deployment of malaria vaccines, covering topics on bioethics to the conducting of vaccine trials.

Results: AMANET has financially supported the characterisation of two sites in readiness for malaria vaccine trials of which one, Balonguen, in Burkina Faso has already successfully conducted a phase 1b malaria vaccine trial. Two more sites in Amani, Tanzania and Ndola, Zambia will soon be supported in a similar manner. The current 2005/2006 AMANET vaccine trial portfolio includes phase 1b trials for AMA-1 in Mali, GLURP in Gabon, GMZ2 in Ghana and phase 1b/2b trial of MSP3 in Burkina Faso. AMANET has set up and supports the AIA initiative, a network that aspires to develop common assays for evaluating immune responses to candidate malaria vaccines and identify more possible candidates. The network which initially involves six African countries from endemic areas of different transmission intensities, from hypo- to holo-endemic has to date optimised and introduced SOPs for ELISA tests on GLURP, MSP3, MSP1-19 and AMA-1 and conducted three workshops on GLP and data management. It will expand to include more countries and antigens. AMANET has conducted 25 workshops and trained around 500 malaria workers. Plans are underway to integrate the workshops into a modular course that will lead to accreditation and certification.

Interpretation: Through the pull mechanisms of increased advocacy, networking, training and south-south mentorship, AMANET is creating a critical mass of African malaria workers and strengthening institutional capacity to conduct malaria vaccine trials. It is hoped that this will speed up the development and deployment of malaria vaccines in Africa.

253A
MIMPAC network. Workshops, training and capacity building in support of malaria immunology and Pathogenesis in Africa [MIM-FN-253770]
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(1) Medical Research Unit, Albert Schweitzer Hospital, Libreville, Gabon; (2) University of Buea; (3) NOGUCHI Memorial Institute for Medical Research; (4) University of Ibadan, Nigeria; (5) Centre National de Formation et de Recherche sur le Paludisme, Ouagadougou, Burkina Faso; (6) University of Karthoum, Sudan

Introduction: MIMPAC is a network of scientists with interest in immunology and pathogenesis of malaria in Africa. It includes research on natural resistance or immunity, malaria vaccines, pathogenesis of malaria, human and parasite genetics. MIMPAC was formed in Accra, 2000, in response both to the difficulty of comparing data across sites/ecological zones and to the lack of exchanges between African scientists sharing the same interests in immunology and pathogenesis of malaria.

Methods: We took advantage of the MIM/TDR immunological studies being carried out in sub-Saharan Africa under WHO/TDR/MIM sponsorship to bring African scientists out of isolation and to address questions, relevant to malaria together. The following objectives were then considered:

- Promotion of multidisciplinary research and training in the immunology and pathogenesis of malaria in Africa.
- Promotion and strengthening of intra-African and international collaboration in malaria research.
- Encouraging the sharing of research capability and resources.
- Building sustainable and affordable research capacity in Africa.
Results: Since its creation, various studies have been carried out by MIMPAC investigators (publications AND post-graduate training), and workshops such as data management workshop in Ibadan (Nigeria) and workshop on field and clinical studies on malaria in Ouagadougou (Burkina Faso) were organized. The students exchange-training programme between institutions within the network started in January 2005 is very successful. The purpose of this activity is to share resources and utilize capacity, which exist in the African laboratories in the academic training of students in immunology and pathogenesis of malaria. Trainees will visit a laboratory in the network and work under supervision of the responsible principal investigator at the host institution. The training will not exceed a maximum period of 3 months. During the period of the visit, the trainee will engage in research towards the completion of an MSc, MPhil or PhD training in the home institution.

Interpretation: Challenge 1 is to explain to health programme managers the importance of our work in the laboratory. Challenge 2 is to use in African laboratories the most appropriate and new technologies for evaluating the impact of malaria control interventions.

254B
Peer health education for promoting knowledge and practice of malaria prevention among school children and their families [MIM-AP-59778]

(1) Centre for Innovation Against Malaria, Banjul, The Gambia; (2) Liverpool School of Tropical Medicine, Liverpool, UK; (3) Nova Scotia Gambia Association, Banjul, The Gambia; (4) Medical Research Council Laboratories, Banjul, The Gambia; (5) London School of Hygiene and Tropical Medicine, London, UK

Introduction: Health promotion in schools aims to improve the health and well being of students by empowering them with the knowledge, skills and confidence to take responsibility for their own health. We incorporated a malaria component to an established peer health education programme in schools in The Gambia, and evaluated its impact on knowledge attitudes and practice of school students and their families.

Methods: The intervention comprised training of peer health educators, teacher coordinators, and members of drama troupes, in key malaria messages and principles of health education, over a 10-week period. The peer educators then conducted a rolling programme of presentations to children and youth in their own schools using drama, puppetry, small group and in-class presentations about malaria, as well as community outreach programs targeting out-of-school youth and the general public. The programme was evaluated by KAP surveys of school children and mothers/carers of children under 5 in the community in a randomized design. Twelve communities including 18 schools were randomized to receive the programme immediately or after a delay of 18 months.

Results: The intervention and evaluation methods were piloted in 10 schools. The value of a pre-intervention interview was investigated to determine whether this would influence the person’s test score when interviewed for the second time. Methods to assess schoolchildren’s comprehension of malaria messages that did not rely on literacy were developed using photographs to prompt students explanations of their knowledge and experience of malaria, while the interviewer checks the key malaria messages stated.

The repeatability and inter-interviewer agreement of these assessment methods was investigated by repeat interviews. Twelve school communities were paired on urban/rural location and school type, and one community in each pair randomly selected to receive the intervention. In each school a systematic sample of 75 students were interviewed 10 weeks and 6 months after the start of the programme, and in each school catchment area a sample of 80 women were interviewed at the same time, and bednet use by children under 5 years in their care was recorded.

Interpretation: From the interviews scores were derived for knowledge about malaria treatment and prevention and knowledge scores and net coverage in children compared between intervention and control schools. The results will be presented at the meeting.
255C
Building district ownership for malaria control: A button-up approach [MIM-MP-32112]
S. Al-hussein, M. Awuah, K. Opoku-mensah, M. Pappoe, I. Quakyi
(1) School of Public Health, College of Health Sciences, University of Ghana, Legon, Ghana; (2) Noguchi Memorial Institute for Medical Research; (3) Ghana Health Service/Ministry of Health, Ghana; (4) Ministry of Local Government and Community Development, Ghana; (5) Gates Malaria Partnerships, London School of Hygiene and Tropical Medicine, London.

Introduction: Malaria is endemic in Ghana, posing major health and developmental concerns. The NMCP/RBM initiated four main strategies to deal with the problem, including advocacy and community participation. GMC/SPH developed community advocacy training for malaria control to contribute to reduction of morbidity and mortality from malaria, through training of Mothers/Caregivers in management and control of malaria, using integrated strategies of Advocacy, Empowerment and Community Mobilization.

Methods: Thirty-four trainers trained mother/caregiver advocates (MCAs) for the implementing areas. Adult learner-centred approaches, emphasizing experiential and activities learning were the main methods used. Materials derived from official malaria control documents and IEC were used for the training of MCAs. The MCAs were trained to support mothers, caregivers and other community members to prevent malaria, manage simple malaria, and refer severe cases to clinics. They were also oriented on how to share the information acquired with community members and household. The training focused on ITN use, malaria prevention, management of simple malaria, community mobilization and sanitation, referral of severe malaria.

Results: One hundred and fifty-six mother/caregiver advocates (MCAs) from the two districts, consisting of 87 from Asante Akim North District with 132 communities and 69 from Shama Ahanta East Metropolitan Area, with 129 communities were trained. They are conducting community-based trainings through group or house-to-house sessions and community durbars to sensitize communities on malaria control and prevention especially in children and pregnant women. Political/administrative support for the MCA programme is strengthened at all levels in the implementing areas. Commercial enterprises have been involved to bring in necessary logistics and support for malaria control. Plans to integrate the programme into district health systems and development programmes in the implementing areas established with signing of memorandum of understanding and development of sustainability plan. Output from the MCAs activities in the districts include: Community members sensitized on malaria prevention, management and seeking prompt care for fever. Reducing rates of severe malaria cases reporting to OPDs of health institutions. Improved community mobilization and involvement in malaria control activities including environmental management and sanitation.

Interpretation: The MCAs training focusing on advocacy and community mobilization can help control malaria at community levels. Anecdotal records to be confirmed indicate reducing trends in severe malaria cases reporting to the OPDs in these areas of implementation.

256A
Behaviour change for improved malaria prevention and treatment in White Nile State (WNS), Sudan [MIM-SS-99450]
S. Sah, R. South, R. Ali, F. Kebede
(1) Plan International UK, London, UK; (2) GlaxoSmithKline, London, UK; (3) National Malaria Administration, Khartoum, Sudan; (4) Plan International Sudan, Khartoum, Sudan

Introduction: Behavioural deficiencies at all levels contribute to the 7.5 million cases and 35,000 deaths due to malaria in Sudan annually. With support from the GlaxoSmithKline African Malaria Partnership, Plan Sudan, the National Malaria Administration and WNS Ministry of Health are implementing a behaviour change promotion programme to contribute to a 50% reduction in morbidity and mortality by 2010.

Methods: Since April 2003 a behaviour programme is supporting the use of appropriate technologies such as insecticide-treated nets (ITNs), intermittent presumptive treatment (IPT) and improved case management. Training is being provided for health workers, laboratory technicians, medical assistants, nurses and doc-
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Support for health facility information systems aims to improve case recording and reporting. In 372 communities a health committee and local volunteers are being trained to engage with residents in planning malaria control activities and education sessions are being organised for pregnant women and mothers of young children. Teachers and school children participate through classroom activities and songs and dramas about malaria.

Results: An interim evaluation currently in progress will provide early indicators of the effectiveness and impact of the various interventions. It is anticipated that community participation in the overall approach, including the involvement of schools and health committees, will create a sense of ownership and responsibility for practicing and promoting key behaviours and will assist in sustaining the efforts in the long-term. Diagnosis and case management will be improved and health service planning should benefit from improved health information. Morbidity and mortality related to malaria should decline due to improved prevention, treatment and vector control behaviours and practices linked to demand for effective technologies such as appropriate medicines, ITNs and IPT.

Interpretation: Sustainable behaviour change involving health professionals, volunteers, community leaders, schools, pregnant women, mothers and the wider community can make an important contribution to reducing malaria morbidity and mortality.

257B Microfinance against malaria [MIM-EV-257842]

E. Bruegge, C. Dunford, B. Gray, R. Davis, R. South, M. Armah-Klemesu, K. Dearden
(1) Freedom from Hunger, Davis, USA; (2) GlaxoSmithKline, London, UK; (3) Noguchi Memorial Institute for Medical Research, Legon, Ghana; (4) Brigham Young University, Provo, USA

Introduction: Microfinance Institutions (MFIs) are not normally involved in the fight against malaria, yet have a strong incentive since it is a major reason for poor loan repayment. With support from the GSK African Malaria Partnership, MFIs in West Africa are offering financial services combined with malaria education to the very poor to: (1) improve prevention, detection and treatment in the home, and (2) stimulate demand for better treatment and prevention services from local providers—private and public.

Methods: Freedom from Hunger prepared five credit union federations in Benin, Burkina Faso, Mali and Togo and ten Rural Banks in Ghana to train its field staff to offer malaria education. During village bank meetings where financial services are delivered by the credit union or rural bank, the same field staff facilitate non-formal, dialogue-based learning sessions in which the group members discuss ways to prevent, detect and treat malaria. In addition, the innovative approach links local financial institutions and their clients with National Malaria Control Programs, health professionals, and private sector suppliers of medicines and insecticide-treated nets (ITNs) through a variety of distribution systems for anti-malarial drugs and ITNs.

Results: The benefits of the integrated service occur at multiple levels: (1) Client and Community: Reduction in malarial incidence, duration and mortality through improvement in early detection, treatment and prevention practices. Households have greater economic capacity to purchase ITNs and effective drugs. There is increased earning for the client due to reduced incidence of malaria; (2) Institutional: MFIs improve client responsiveness and value of the integrated services with the addition of the malaria education. Staff capacity in nonformal, dialogue education techniques, training systems, and facilitation skills are strengthened. Linkages with health-oriented organizations are more effectively coordinated to combat malaria and (3) National Malarial Community and broader development community: The program offers learning opportunities about how to combat malaria through: (a) dialogue-based community education, (b) financially sustainable integration of health and financial services and (c) innovative partnerships between financial and health-oriented organizations. The presentation will report study data on the changes resulting from the malaria education and the dissemination of information with the wider community.

Interpretation: Anticipated are significant increases in the number of clients who have greater knowledge of malaria, change their behaviors of prevention and treatment, and have more financial resources to purchase ITNs and effective drugs compared to non-clients.
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11 + 23: *Plasmodium/human/Anopheles* genomics

**Posters 258–272**

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

**258C**

La PCR en Temps Réel: Etude des allotypes de la MSP-1 de *P. falciparum* sur la splénomégalie et l’anémie à Missira, au Mali [MIM-MB-374762]

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**Introduction:** La PCR quantitative peut estimer le nombre de copies de gènes présents dans un systeme donné. Nos études ont montré que 90% des enfants portent 5 à 8 génotypes de *P. falciparum*. La relation entre les événements cliniques tels que la splénomégalie, l’anémie, la fièvre et la présence d’un génotype dominant pourrait être à la base de l’événement clinique observé.

**Methods:** Nous avons d’une manière randomisée, inclus 198 enfants d’âge (1–9 ans) et sexe confondu qui se sont présentés au Centre de Santé du Village durant les 4 jours d’étude. Tous les sujets ont subi un examen clinique (prise de température, palpation de la rate, observation des conjonctives) et des examens biologiques (goutte épaisse, Polymerase Chain Reaction Quantitative, détermination du taux d’hémoglobine). Chaque sujet a été informé du protocole et un consentement éclairé a été obtenu des parents des enfants. Le test de Chi 2 et le test de corrélation de Pearson ont été utilisés.

**Results:** Notre population d’étude était composée de 48% de sujets masculin (95/198) et 52% de sexe féminin (103/198) avec une moyenne d’âge de 4 ans. La prévalence de l’infection palustre était de 55.6% dans la population d’étude. La splénomégalie prévalait chez 72.5% des enfants. La mesure de l’hémoglobine faite chez 154 enfants de notre effectif donnait 44.80% (69/154) de sujets avaient un taux d’hémoglobine inférieure à 10 g/dl de sang. La variation du taux d’hémoglobine entre les tranches d’âge [1–4] et [5–9] était significative. La PCR classique montrait que la splénomégalie était associée avec les allotypes MAD20 et RO33 respectivement. La PCR quantitative a confirmé que l’allotype Mad20 variant dans le même sens avec la splénomégalie (t = 2.843; p = 0.006) et avait une corrélation avec la fièvre (Pearson corrélation = 0.265; p = 0.043). Par contre nous n’avions pas trouvé de corrélation entre le nombre de copies d’aucun des 3 allotypes avec un taux d’hémoglobine inférieur à 10 g/dl.

**Interpretation:** Un nombre élevé de copies des allotypes MAD20 de MSP-1 était associé à la splénomégalie et à la fièvre. Le spectre large des événements cliniques serait dû à la multiplicité des allotypes ayant différents répertoires antigèniques.

**259A**

Transposable elements insertion polymorphism in natural populations of *Anopheles gambiae* s.s. from Cameroon [MIM-MB-61940]

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**Introduction:** Both M and S molecular forms of *Anopheles gambiae* ss are present in Cameroon where they frequently occur together at the same place. However, no MS hybrids are found and microsatellite data revealed higher levels of genetic differentiation between sympatric populations belonging to different molecular forms than between allopatric populations of the same molecular form. We assessed transposable elements (TEs) distribution within and between these populations.
Methods: Six transposable elements were chosen because of their known occurrence at low copy number in *An. gambiae*, and were cloned from the Kisumu laboratory strain. Natural populations of *An. gambiae* were sampled in three localities from South Cameroon: Simbock, Ipono, and Mfou. Genomic DNA was extracted from single females, their molecular form was determined, and individual Southern blots were performed using the TEs cloned from the Kisumu strain as probes. This allowed assessment of copy number for a given TE family in each specimen. Insertion polymorphism was assessed by scoring each individual insertion for its presence or absence.

Results: Insertion polymorphism was high for most of the TE families. No evidence was found for different TE dynamics with regard to molecular form of *An. gambiae*: M and S populations displayed similar number of insertions and level of polymorphism. However, the TE family Aara8, which displayed the lowest insertion polymorphism, proved to be a very efficient marker to distinguish between M and S sympatric populations, as some insertions appeared to be fixed in one form and absent from the other one. Among five fixed insertions, one was shared by both molecular forms, one was found only in S individuals and three were present exclusively in the M individuals.

Interpretation: TEs allow to distinguish between molecular forms of *An. gambiae* in Cameroon. Future work should aim at cloning fixed insertions of Aara8, localizing them in the genome, and testing if they can discriminate the M and S forms in other parts of Africa.

260B
In vivo transcriptomes of *P. falciparum* in distinct patient cohorts from Senegal [MIM:jD-64995]

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Introduction: Patients infected with *P. falciparum* present with a range of outcomes from asymptomatic parasitemia to severe disease determined by host and parasite factors. To study the host pathogen interaction we analyzed parasite whole genome expression from isolates taken directly from patient blood samples. These isolates were collected from patients who vary by geography, age, disease severity and treatment.

Methods: A custom-made oligonucleotide array with probes based on the *P. falciparum* 3D7 sequence was used to detect in vivo *P. falciparum* transcripts from total RNA derived from 5 to 15 ml of blood from malaria infected patients with parasitemia >1% (range = 1.4–11) from an outpatient clinic in Pikine, Senegal (EIR = 1) which is hypoendemic for malaria and Velingara, a hyperendemic region (EIR = 100). The match only integral distribution algorithm was used to assess the expression level for each transcript and Spearman rank sum correlation coefficient was used for comparison between samples and with the 3D7 strain cell cycle transcriptome. RT-PCR was used to confirm expression levels for select transcripts.

Results: We found that in vivo transcriptomes derived from patients with mild malarial infection in the hypoendemic region, showed good correlation (coefficient 0.8–0.93) to the ring stage 3D7 transcriptome. However, rifins and stevors, likely involved in immune evasion, demonstrate a higher transcription level in the in vivo samples. PF14_0752 was over-expressed in 4/5 in vivo samples from this region. This gene had homology to nine other genes, all with unknown functions, which display characteristics consistent with a new surface antigen family. [Daily et al., 2005. J. Infect. Dis. 191, 1196–1203; Daily et al., 2004. Malar. J. 3, 30]. We will present comparative data of in vivo transcriptomes derived from a cohort of patients residing in a hyperendemic region of Senegal who vary by age, parasitemia, disease severity and a matched sample pre and post-antimalarial treatment.

Interpretation: The comparative analysis of in vivo transcriptomes of field isolates may identify important pathogen strategies towards survival in the human host and parasite biology related to disease outcome.
Analysis of frequency TNF-alpha, IL-2 and IL-6 promoter in Senegalese adults with cerebral and uncomplicated Malaria [MIM-NK-37530]

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Introduction: Cytokine unbalance has previously been associated with particular acute manifestations of infectious and non-infectious diseases, and tumor necrosis factor alpha (TNF alpha), among other cytokines, plays a central role.

Methods: To investigate the host genetic factors affecting the clinical course of Plasmodium falciparum malaria, polymorphisms of TNF-alpha promoter regions (mutations-308 G/A, and -863 C/A) and interleukins (IL)-2 (mutation-114 G/T) and 6 (mutation-597 G/A) were analyzed in patients with uncomplicated acute malaria (UM) or cerebral malaria (CM). Two hundred and eighty-three patients were included in this study: 120 were uncomplicated (patients consulting in the Medical Biology Laboratory) and 163 had confirmed cerebral malaria (hospitalized in the intensive care unit in Principal hospital in Dakar). Genetic Polymorphism analysis was performed by PCR-RFLP.

Results: The difference in genotype frequencies was analysed using the chi-square test and fischer exact test. Probabilities values ($p$) < 0.05 were considered as significant. We found that the frequency of TNF mutation at position 863 gene promoter (CA genotype) was significantly higher in CM than in UM patients ($P$= 0.04): the genotype frequencies were 9.8 and 3.1%, respectively, whereas the TNF alpha mutations frequency at position 308 A (GA genotype; CM: $f=25\%$, UM: $f=29\%$) and interleukin 2–114 (GT genotype) did not significantly differ between the two groups of patients. The Interleukin 6 mutation at position 597 (GA genotype) was found only in cerebral malaria ($f=8.2\%$) and absent in the uncomplicated malaria (CM versus UM: $P=0.01$).

Interpretation: These preliminary body of data showed some differential genetic mutations in cytokines genes promoter underlining a potential critical role on malaria clinical outcome. Further investigations of the role of these cytokines may contribute to better understanding the pathogenesis of malaria.

Seasonal dynamics of P. falciparum prevalence and high parasite diversity of first infections in early childhood from central Ghana, West Africa [MIM-RK-195605]

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Introduction: Infants are the target group of intermittent preventive treatment (IPTi), a potential control measure in areas of high malaria endemicity. In 1070, 3-month-old children enrolled in an IPTi study on sulfadoxine–pyrimethamine, the prevalence and multiplicity of P. falciparum infection (MOI) was assessed over a period of 1 year in nine neighbouring villages in a holoendemic area. Variants of the merozoite surface proteins (msp) 1 and 2 where typed to determine parasite diversity and MOI.

Methods: Blood films were Giemsa stained and microscopically examined. Hemoglobin (Hb) was measured by photometer and DNA was isolated from filter papers. Genus- and species-specific nested-PCR was performed on all samples. P. falciparum positive samples were genotyped for msp-1 and msp-2. Lengths polymorphisms and clone differentiation were assessed with family-specific PCR assays and fluorescence marked primers. Alleles were identified by length in base-pairs (bp) using an Applied Biosystems/Hitachi PRISM® 3100 Genetic Analyzer and GeneMapper™ Software. The mean multiplicity of infection (mean MOI) was estimated by adding the individual values of MOI of all isolates and dividing them by the number of P. falciparum positive samples.

Results: 144 (13.5%) samples were P. falciparum positive by microscopy and 158 (14.8%) revealed positive results for P. falciparum-DNA and msp-1/msp-2 gene fragments. 76 msp-1 and 81 msp-2 alleles were identified. K1 type allele variability was 46%,
Mad20 with 41% second in terms of genetic diversity while the number of Ro33 alleles (10%) was limited. When grouped into the two msp-2 families 59% were 3D7 and 41% FC27 specific. Fragment sizes were unequally distributed in the host population as clones clustered around certain lengths. Prevalence (monthly range 5.5–25.8%) was lowest at the end of the dry season and peaked at the end of rainy season. Hb levels (average 10.3 g/dl (monthly range 9.5–10.9 g/dl)) were also varying according to rainfall and were associated with prevalence. Geometric mean parasite density was 803 parasites/ml (monthly range 529–1945 parasites/ml). Between 1 and 14 different clones per individual were found and the mean MOI was 3.7 (monthly range 2.9–5.4). Of all P. falciparum positive samples 9.5% (15/158) were due to monoinfections. The presented malariometric indices considerably differed when values between the nine neighbouring villages were compared.

Interpretation: In areas of holoendemic endemicity most early P. falciparum childhood infections are caused by multiple parasite strains throughout the season.

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Hyperreactive malarial splenomegaly in Kumasi, Ghana [MIM-RM-201120]

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Introduction: A common cause of massive splenomegaly in malaria endemic countries is hyperreactive malarial splenomegaly, HMS, defined as persistent splenomegaly occurring in the absence of demonstrable underlying disease. HMS has a tribal predilection in Papua New Guinea, Nigeria and Uganda, and strong familial association in Uganda and Papua New Guinea. This study aimed to determine the extent of familial association of splenomegaly in Kumasi, and the segregational patterns of this association.

Methods: 22 HMS patients with 99 relatives, and 15 population controls of similar socio-economic background with 51 relatives participated in a case controlled study. Clinical and laboratory data, as well as the presence and degree of splenomegaly were collected on all participants. The pedigree of each family was also recorded.

Results: Splenomegaly was identified in 27% of relatives of HMS patients and 6.1% of population controls (p = 0.04). Immunoglobulin levels were significantly higher (p = 0.005) and haemoglobin levels significantly lower (p = 0.009) in the patients than in the population controls.

Interpretation: In Ghana, relatives of HMS patients are more likely to have splenomegaly than population controls, but with no obvious pattern of Mendelian segregation. HMS may have a complex aetiology involving multiple genetic and environmental factors in Ghana.

264C

Plasmodium falciparum genetic diversity in children with severe and acute uncomplicated malaria during the raining season in benin republic [MIM-TM-5910]

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Introduction: In order to investigate the impact of genetic diversity of Plasmodium falciparum on severe and acute uncomplicated malaria in Beninese children, we analyzed the distribution of allelic families of msp1 (K1, MAD20, RO33) and msp2 (FC27 and IC1) in patients isolates.

Methods: One hundred and ninety six patients aged 02 months to 12 years suffering from acute uncomplicated malaria and 137 others presenting symptoms of severe malaria were enrolled into this study. Para-
site DNA was extracted using the methanol and heat extraction method, and parasite genotyping was performed by PCR.

Results: Three hundred and ten fragments from msp1 gene and 280 others from msp2 gene were obtained on the whole in samples obtained from severe malaria patients. MAD20 allelic family was much more frequent than K1 or RO33 allelic families of msp1 gene and FC27 or IC1 of msp2 gene in these patients \((P = 0.043)\). However, K1, RO33 and FC27 allelic families were more frequent in samples obtained from patients with acute uncomplicated malaria. FC27 dominated other msp1 and msp2 allelic families, and was often associated with RO33 \((r^2 = 0.81; P = 0.02)\). A positive correlation between parasite density and K1 and IC1 allelic families was also observed \((r^2 = 0.83; P = 0.042)\) in severe malaria, while only K1 was positively correlated with parasitaemia in patients with acute uncomplicated malaria \((r^2 = 0.87; P = 0.02)\). No correlation between age of patients and parasitaemia was observed in either severe or acute uncomplicated malaria. However, clonality (as observed with msp1) decreased significantly \((P = 0.02)\) with age in patients with acute uncomplicated malaria.

Interpretation: Results suggest that clone multiplicity may be involved in the development of severe malaria in non-immune individuals. The products of expression of mad20 may be strongly involved in host-parasite interactions in children living in endemic areas.

265A
Interspecific nucleotide variability within the Anopheles gambiae species complex [MIM-IM611512]
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Introduction: The Fc gamma Receptors IIa (FcγRIIa) are known to mediate immune phagocytosis. Polymorphism in the FcγRIIa, which may alter the relative affinity of the receptor expressed on effector cells involved in antibody-mediated protection, may ultimately influence the outcome of the disease. A recent malaria study showed that the FcγRIIa R/R131 may have an association with protection against the disease.

Methods: A total of 256 individuals were included in this study, 115 patients with severe Plasmodium falciparum malaria (84 with cerebral malaria and 31 with non-cerebral severe malaria), 85 with mild malaria and 56 healthy controls living in the same study area. Genotyping of FcγRIIa was done using gene-specific poly-
merase chain reaction (PCR) amplification, followed by an allele-specific restriction enzyme (BstUI) digestion of the PCR product.

Results: The results showed that the FcγRIIa-R/R131 genotype was at higher frequency in those with severe malaria as compared to those with mild malaria (37.4% versus 22.4%; \( P = 0.023 \)) and the FcγRIIa-H/H131 genotype was more frequently seen among patients with mild malaria as compared to those with severe malaria (21.1% versus 10.4%; \( P = 0.036 \)).

Interpretation: This study revealed that the FcγRIIa-R/R131 genotype is associated with susceptibility to severe malaria and the FcγRIIa-H/H131 genotype is associated with resistance to severe malaria in Sudanese patients.

267C
Genes coding for tryptophan-rich proteins are transcribed throughout the asexual cycle of *Plasmodium falciparum* [MIM-FN-160787]

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Introduction: *Plasmodium falciparum* infection leads to host cell modification due to molecules released by the parasite. Among them is a novel antigen termed Tryptophan-Threonine-rich Antigen. Orthologues are found in other plasmodial species and shown to be successful vaccine candidates in mice. Five members of this gene family have been identified in *P. falciparum* in silico. This study aims to characterize this gene family with this unusual trp-rich domain found only in plasmodia species.

Methods: A series of laboratory adapted isolates and field isolates from malaria patients in Lambaré, Gabon, were used in this work. The laboratory strains were maintained in continuous culture using standard methods. Genomic DNA and total RNA were isolated from these isolates. PCR products were generated from each gene and sequenced to check for polymorphisms. RT-PCR was performed to identify intron/exon boundaries. Fragments of each gene were used to express recombinant GST fusion proteins in *E. coli* BL-21. Antibodies were raised against the recombinant proteins which were used to determine the size and stage specific expression by western blot using parasite extracts and also to determine the localization of the proteins by IFA.

Results: Out of the five members of the TyThrA gene family, four have been characterized in more detailed. One consists entirely of a tryptophan-rich domain and a signal peptide, and is expressed on the surface of merozoites (Merozoite associated Tryptophan-rich Antigen). All others also have a signal peptide and an additional repetitive domain. All contain a small intron region which is spliced out of the mature messages. All four genes are present in all parasite strains sequenced so far and show only limited polymorphisms. Gene expression profiles indicate that TryThrA is expressed very early in the asexual life cycle. Two others, MaTrA and TrpA-5 are expressed very late. TrypA-3 messenger is found in the middle of the asexual cycle of *P. falciparum*. LysTrpA contains two stop codons which interrupts the ORF and is considered a pseudogene. This gene family has not been detected in any other species except plasmodia. Whereas most species contain only a limited number of family members, in *P. vivax* and *P. knowlesi* the number seems to extent 20.

Interpretation: The conservation and uniqueness of trp-rich domains points to an essential function for the parasite. Due to limited SNPs, location of MaTrA on merozoites, properties of murine homologues, trp-rich proteins may be considered vaccine candidates.

268A
Development of a malaria repository at the London School of Hygiene and Tropical Medicine (LSHTM) [MIM-AR-244209]

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Introduction: Many malaria studies have been conducted under the auspices of the LSHTM, both cross-sectional and longitudinal. Consequently a large number of parasite, mosquito and human blood samples are stored at the School. The Repository aims to provide designated storage for such samples, with a systematic
labelling system linking each sample to a database providing baseline demographic and clinical information. This will facilitate the identification of suitable samples for novel research.

**Methods:** Stage 1 focused on determining the most suitable storage system with respect to long-term management and facilitating the retrieval of samples upon request, as well as ensuring the security of the samples and data with appropriate backup and alarm systems. Stage 2 involved designing a master database to facilitate the extraction of sets of working samples from the different studies stored in the Repository, based on the characteristics of the samples and of the subjects providing those samples. Stage 3 is ongoing. Additional sample sets with their associated databases are being incorporated into the Repository. These include bloodspots as well as sera and plasma samples.

**Results:** This poster reports on the progress of the Repository to date, outlining the roles of the Advisory and Management Committees, describing the development of the master database, and detailing the various sample sets that have been deposited into the Repository. These include bloodspots as well as sera and plasma samples.

**Interpretation:** We have shown that it is possible with careful planning to maximise the usefulness of stored samples by setting up a systematic repository.

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**269B Genetic polymorphism of the serine rich antigen N-terminal region in Plasmodium falciparum field isolates from Brazil [MIM-ER-24208]**

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**Introduction:** Different antigens of the malaria parasite have been characterized. SERA ranks as a candidate antigen for inclusion as a subunit in a malaria vaccine. Although SERA showed a quite conserved sequence in *P. falciparum* isolates, two regions of polymorphism have been observed in different *P. falciparum* laboratory samples. Here we have investigated the frequency of polymorphism in exon II of SERA gene, which encodes most of the amino-terminal region of the antigen in *P. falciparum* field samples.

**Methods:** Blood samples were collected from *P. falciparum* infected individuals in three areas of the Brazilian Amazon: Rondônia (*n* = 29), Pará (*n* = 8), Amazonas (*n* = 15). Venous blood sample was collected from each individual. Packed red blood cells (RBC) were separated for phenol chloroform DNA extraction and PCR analysis, and all samples were cryopreserved in glycerol (w/v) in duplicate and stored in liquid nitrogen tank. DNA samples were amplified by double or nested PCR.

**Results:** Two fragments have been characterized by polymerase chain reaction: one of 175 bp corresponding to the repeat region with five octamer units (allele I) and one other of 199 bp related to the 6 repetitive octamer units (allele II) of SERA protein. The 199 bp fragment was the predominant one in all the studied areas – Rondônia and Amazonas (93%) and Pará (62%).
– and only one fragment was amplified in any of the studied isolates. The higher frequency of this fragment has not been described before and could be explained by an immunological selection of the plasmodial population in the infected individuals under study.

Interpretation: If the here reported sequence polymorphism affects the immune recognition of SERA, the analysis of the polymorphism of \( P. falciparum \) isolates is a fundamental stage before the final drawing of an universal vaccine against malaria.

### 270C

**High prevalence of \( P. falciparum \) gametocytes—a new view on the human reservoir for \( P. falciparum \) transmission [MIM-ps-57609]**

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**Introduction:** Malaria transmission depends on the presence and infectiousness of gametocytes in the circulation. Gametocyte detection by microscopy is laborious and of limited sensitivity. Therefore, gametocyte measurements are frequently not included in field studies that evaluate interventions and decisions on control are mostly based on asexual parasite dynamics only. We present a sensitive, high-throughput gametocyte-quantification assay that could be used in large-scale epidemiological studies.

**Methods:** Real-time quantitative nucleic acid sequence-based amplification assay (QT-NASBA) was developed for quantification of \( P. falciparum \) gametocytes with a detection limit of 20–100 gametocytes/ml of blood. QT-NASBA was used for total parasite and for gametocyte quantification during a drug efficacy trial in Western Kenya, testing efficacy of sulfadoxine–pyrimethamine (SP) and SP + artesunate in children aged 0.5–10 years with uncomplicated \( P. falciparum \) following the standard 28-day protocol (\( n = 119 \)).

**Results:** Gametocyte prevalence was much higher than expected with 85% by QT-NASBA, compared to 24% by microscopy at the day of treatment. In total, 97% of the children had gametocytes during the 1-month follow-up and 33% had gametocytes at all sampling points during follow-up. Similar gametocyte prevalences were found during a cross-sectional study in a low transmission-area in Burkina Faso. To test the potential epidemiological importance of low gametocyte densities, gametocytes from in vitro culture were diluted well below the detection limit of microscopy, quantified by QT-NASBA and fed to mosquitoes. Even densities as low as 400 gametocytes/ml of blood were capable of infecting mosquitoes, suggesting that previously undetected sub-microscopical gametocytes might be an important determinant of the transmission potential of \( P. falciparum \). Studies on the infectiousness of microscopically gametocyte negative populations in endemic areas are in progress.

**Interpretation:** The almost universal presence of gametocytes and the infectiousness of low-density gametocyteaemia changes the scope of our current knowledge of the infectious reservoir and the implication for control needs to be further investigated.

### 271A

**Prevalence of glucose 6-phosphate dehydrogenase deficiency and sickle cell trait in high and moderate malaria transmission areas of Muheza, Tanzania [MIM-MS-49320]**


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**Introduction:** Sickle cell trait and glucose 6-phosphate dehydrogenase (G6PD) deficiency have been shown to be protective against malaria. Protection conferred by these genetic factors in malaria endemic areas is more evident in childhood prior to the development of acquired immunity. This study was conducted in high (lowlands) and moderate (highlands) malaria transmission areas of Muheza, Tanzania, to determine prevalence of G6PD deficiency and sickle cell trait and their association with malaria infection.

**Methods:** A cross-sectional malariometric survey was conducted during short rains in November/December 2003 involving four villages from highlands and four from lowlands. Finger prick blood was collected into
EDTA tubes from 1930 individuals aged 6 months to 45 years. Thick and thin blood smears were prepared for malaria diagnosis, and haemoglobin level was estimated using Haemocue. Twenty percent and 50% of the collected blood samples were randomly selected for G6PD and haemoglobin S (Hb S) analyses, respectively. Fluorescent screening test was used for G6PD analysis and Hb S was done by sickling test.

Results: Overall prevalence of malaria parasites in the study population was 29.8%, and stratum specific prevalence was 42.1% in lowlands and 19.0% in highlands. Prevalence of G6PD deficiency and sickle cell trait were significantly higher in lowland compared to highland areas (G6PD = 10.1% versus 4.6%, \( p = 0.03 \), and Hb S = 16% versus 5.8%, \( p = 0.0001 \)). The odds ratio of having G6PD deficiency or sickle cell trait in the lowlands compared to highlands was 2.85, 95% CI (1.149, 7.143), \( p = 0.011 \) and 2.86, 95% CI (1.163, 7.143), \( p = 0.011 \), respectively. Mean haemoglobin levels were significantly higher in the highlands (11.93 g/dl) than in the lowlands (11.33 g/dl, \( p = 0.044 \)). Neither G6PD nor sickle cell trait had any significant effect on Hb level or parasite density.

Interpretation: High frequencies of G6PD deficiency and Hb S observed in lowland areas where malaria transmission is high may be due to high selection pressure exerted on human population.

272B
An integrated genetic and physical map for the malaria vector Anopheles funestus [MIM-CW-320378]

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Introduction: Anopheles funestus is one of the two major vectors of malaria parasites in Africa with An. gambiae, but so far, its genetics is less studied. The construction of a genetic map of this species is an important step towards the identification of genes involved in traits such as resistance to insecticides or vectorial capacity.

Methods: A number of 174 An. funestus F2 progeny from three families were scored with microsatellite, single nucleotide polymorphism (SNP) and RFLP markers. Microsatellites loci were genotyped using fragment analysis method of Beckman CEQ8000 machine, while SNPs loci were scored using the heated oligonucleotide ligation assay (HOLA) and the SNPs analysis method of Beckman CEQ 8000. A restriction reaction was carried out to score the RFLP marker.

Results: We have constructed a genetic map consisting of 26 microsatellite markers, a single RFLP and 9 single nucleotide polymorphisms (SNP) loci that we identified during this study. Three linkage groups were identified, corresponding to the three chromosomes. Chromosome 3 was split in two sub-groups corresponding to 3R and 3L chromosomal arms. We mapped four markers to the X chromosome, 15 to the second and 17 to the third chromosome. The chromosomal location of many of these markers has been determined and there is a strong coherence between the genetic and physical map positions.

Interpretation: This integrated map will be a valuable tool for future studies in this important malaria vector. In our case especially, it is a step forward to identify genes involved in insecticide resistance using the quantitative trait loci (QTL) approach.

12: Priorities in social and economic aspects of malaria research and control

Posters 273–287

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

273C
Current obstacles to the control of malaria in Chennai: An appraisal by anti-malaria program experts [MIM-DB-61400]

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Introduction: Chennai a major metropolitan city in Southern India is highly endemic for malaria since 3–4 decades. Local upsurge is seen though the incidence declined in 1958 and 1984 in response to National programs, which lost its sustainability in bringing down the cases further. Mosquito resistance and drug resistance was not as important a factor in Chennai for the high incidence as in some other places. This study was carried out among senior Health officials involved in control of malaria locally.

Methods: A descriptive study was conducted among senior public health experts in Chennai during the period December 2004–February 2005. 17 health officers and entomologists completed a pre tested semi-structured questionnaire on obstacles and remedial action to malaria control. Also questionnaire had open-ended segment for expression of their experience and opinion.

Results: Most respondents (94%) wanted more stringent surveillance program to control malaria. They felt the National anti malaria program in India was not reaching the private practitioners most of whom do not follow the guide lines in the program. 89% opined that available anti malaria drugs can be effective. However, 65% agreed that there was large unregulated drug distribution and this required urgent attention. Similarly the non-availability of a vaccine or drug resistance was not felt as major barrier by 77 and 82% for effective malaria control. Ninety-four percent wanted stringent public health acts which can be very effective. The need for international training was felt by 94% of them and 47% agreed more International funding for Bed nets which can contribute to control malaria locally. There was no statistical difference of opinion between medical experts and entomologists. Unanimously almost 100% felt that strong Community participation and effective health education is required and ranked it as the most effective way to control the dreadful disease. Hence formulation of appropriate education package should be an important priority for Stakeholders while planning and implementing National programs.

Interpretation: Effective malaria control largely requires active community participation. Education package should be an important priority in National Programs. The need for vaccine or alternative drugs may not be a priority for cities like Chennai.
official guidelines for treatment of malaria in Zomba. Speed of response was the most desirable characteristic of anti-malarial drugs. Acceptable speed of symptom resolution was usually described as occurring within 24 h of taking an anti-malarial drug. Taste was the last characteristic for consideration, but it was highly debated with regard to anti-malarial drugs for children. Cost was not usually considered as an important factor but some drugs were never purchased because they were too expensive.

Interpretation: The findings suggest an urgent need for more studies to explore ways of improving health seeking practices and knowledge of anti-malarial drug treatment in rural and peri-urban communities. Similar problems are likely to prevail in Malawi.

275B Willingness to pay for insecticide treated mosquito bednets in the Kainji Lake Area, Niger state, Nigeria [MIM-TI-401850]
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Introduction: Currently advocated malaria control strategies under the roll back malaria (RBM) initiative prioritize prompt diagnosis, early treatment and use of insecticide treated bednets (ITNs). Presently, there are little information on the level of people’s willingness to pay for ITN, knowledge, perception and acceptability of (ITNs) in Nigeria. There is therefore the need to provide data on the usage and social marketing of ITNs as well as their re-impregnating potential.

Methods: A total of 198 household heads of four villages in the Kainji lake area of Niger State were studied. Semi-structured questionnaires were used to elicit information from the respondents in addition to use of qualitative methods of focus group discussion and in-depth interviews among adult population in the communities. A bidding format was used to elicit willingness to pay (WTP) values using two different starting bids. The scenario was constructed in a way to reduce the possibility of respondents acting strategically.

Results: Average monthly income of respondents was N5916 (US$46.20) and a median of N5000 (US$39.00). None of the respondents had ever used a treated bednet prior to the baseline phase of the study. Respondents’ major sources of information about ITN were: friends/neighbors (39.1%), relations (25.0%) and radio (6.3%). Over 90.0% of the respondents had positive perception about the use of ITN. 98.5% compared to 89.6% of the respondents were willing to pay for ITNs at a cost in the pre and post-intervention period respectively. The mean WTP at N 500.00 and N 350.00 were N700.00 and N383.00, respectively. Similarly, 96.0% against 84.0% indicated their willingness to personally re-treat their nets in the pre and post-intervention at an average cost of N67 (US$ 0.52) and N12.00–17.00 (US$ 0.10/0.13).

Interpretation: The income level of the respondents suggests that there may be need for subsidy to enable them buy nets, the preferred mode of payment is largely on installment basis. Furthermore, health education needs to be focused on pregnant women on (ITNs) usage.

276C “Quand la fièvre”; À propos des accès fébriles chez les jeunes enfants à Maroua (Province de l’Extrême, Nord Cameroun) [MIM-EK-215316]
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Introduction: A Maroua chef-lieu de la Province de l’Extrême-Nord du Cameroun, les recours thérapeutiques des familles lors des épisodes de fièvre chez les jeunes enfants sont tributaires de leur perception de la gravité de celles-ci. D’un point de vue populaire, la fièvre est répertoriée comme une maladie à part entière et non-comme le symptôme d’une maladie. Avec un climat soudano-sahélien, Maroua est une zone de transmission saisonnière. Les enfants acquièrent leur immunité entre dix et douze ans.

Methods: Notre recherche s’est essentiellement déroulée dans le service de pédiatrie de l’hôpital provincial de Maroua. Mais au préalable, nous avons fait un sondage dans les quartiers de Maroua sur les représentations populaires du paludisme, notamment, ses causes, ses manifestations aînés que les thérapies utilisées dans les familles pour combattre la fièvre. L’approche des familles à l’hôpital Provincial
nous a permis de confronter le savoir à l’agir. Ainsi, nous avons effectué une centaine d’entretiens semi-directifs auprès des mères d’enfants hospitalisés pour “paludisme” dans ce service et auprès des personnels de santé. En outre, nous avons recouru à l’observation participante.

Résultats : L’étude montre l’existence de savoirs populaires composites autour du paludisme ; il y a une véritable logique pratique des mères face aux accès fébriles chez leurs enfants. Dans l’acceptation populaire, les principaux symptômes du paludisme sont : l’hyperthermie, les céphalées. Le diminutif “palu” en français, ou encore “fabbodje” en fulfulde, principale langue de communication et d’échanges est connu des mères. Mais il recouvre un sens beaucoup plus complexe. La fièvre chez le jeune enfant est souvent liée à son développement. Elle est aussi assimilée à la pathologie du “lait gâté”, maladie socialement répertoriée. Les mères ont un savoir particulier pour la prise en charge des fièvres (préparations de décoctions). Cette démarche thérapeutique n’est pas orientée vers une maladie particulière – le paludisme – mais vers ces maladies infantiles socialement répertoriées. Dans la quête de son les attitudes de nos interlocutrices ne se posent pas en rejet d’une médecine au profit d’une autre. Quand les symptômes persistent malgré les médicaments administrés ou lorsqu’ils se compliquent (convulsions), un diagnostic alternatif est établi, ce qui donne lieu à une thérapie nouvelle.

Interprétation : Même si ces nosologies populaires sont souvent inconnues du savoir médical, elles ne favorisent pas la prise en charge adéquate et précoce du paludisme.

277A
Taking insecticide treated nets to scale: The Tanzania experience [MIM-KK-363636]
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Introduction: The National Insecticide Treated Nets (NATNETS) programme is a long-term multi-donor, multi-partner initiative to promote the national use of ITNs by making nets affordable, accessible and acceptable. NATNETS is made up of complementary interventions aimed at: (i) changing behaviour through mass promotion campaigns; (ii) supporting rapid development of a commercial distribution system for ITNs and insecticide re-treatment kits and (iii) providing a targeted subsidy for nets to pregnant women.

Méthodes: The main interventions are: (1) SMARTNET implemented by PSI, which focuses on assisting the Tanzanian net manufacturers to expand their wholesaling and retailing network of different net products in the country and increasing demand for ITNs through the Malaria Haikubaliki (Malaria is Not Acceptable) campaign; (2) Tanzania National Voucher Scheme (TNVS), which enables pregnant women and their infants to have access to an ITN at very low cost through the retail network; and, in addition, provides free insecticide for the retreatment of nets and (3) the ITN cell in the National Malaria Control Programme responsible for coordinating all ITN activities in the country, for managing the TNVS and ensuring an enabling environment.

Résultats: In 2004 nearly 2 million ITNs were distributed in Tanzania and this number will further increase in 2005 as a result of the TNVS. In addition, over 2 million insecticide treatment kits were sold in 2004. Further results will be presented on: effects of the behaviour change campaigns on ITN uptake and coverage; effects of the voucher scheme on the overall demand for ITNs; the expansion of the commercial ITN distribution networks throughout the country; spin-off effects in terms of use of public health facilities (especially ante-natal services) as a result of the voucher scheme; the introduction of long-lasting insecticidal nets onto the Tanzanian market.

Interprétation: NATNETS represents a successful long-term coordinated national initiative to reach Abuja targets for ITNs in one of the most highly endemic countries in sub-Saharan Africa with many useful lessons learned.
Factors influencing compliance to intermittent preventive treatment with SP for malaria during pregnancy in rural Malawi [MIM-AL-32835]

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Introduction: Malaria in pregnancy is a major public health problem. In Malawi the malaria policy aims to reduce gestational malaria infection through intermittent preventive treatment with sulphadoxine-pyrimethamine (IPT-SP). Currently it is estimated that 65% of women in rural areas received one dose of SP and less than 30% two doses as per the policy (MDHS 2000). This social scientific research investigated what factors influence low and inconsistent compliance to IPT-SP in rural Malawi.

Methods: Both qualitative and quantitative methods were used. At first phase, focus group discussions (FGD, \( n = 8 \)) with women (\( n = 7 \)) and men (\( n = 1 \)); in-depth interviews (IDI) with women in reproductive age (\( n = 34 \)) in eight villages; and observations at antenatal clinic (ANC) were carried out on perceptions, knowledge and practices regarding treatment and prevention of malaria in pregnancy. FGDs and IDIs were translated and transcribed, and about 15% of the transcriptions were checked for quality of translation. At second phase, knowledge, attitudes and practices (KAP) survey (\( n = 248 \)) was carried out concerning treatment and prevention of malaria, perceptions and use of ANC services, knowledge and practices regarding pregnancy and adverse outcomes.

Results: Main factors influencing compliance were health system based factors, e.g. poor communication, quality of ANC services, lack of SP, etc. Personal, patient-dependant factors were less important. Observations showed that content of health education on malaria prevention were superficial and concentrated on selling insecticide treated nets, and in different language than spoken in the area. Communication during direct observed therapy (DOT) was minimal. KAP survey and IDIs revealed that women perceive SP as first line treatment and SP received during ANC visit was interpreted as diagnosis of malaria, not as IPT. Drug sorting exercise showed that many women did not recognize SP and other common drugs used in ANC. Women said that names of pills are not important because nurses know what medication to give and what for. Compliance per se was not a problem for women. Those women who attend ANC received SP through DOT. Women considered SP a drug with little side-effects suitable for pregnant women. Central problem was late show up at ANC (average 24 weeks). IDIs revealed that main motivation to attend ANC is to get ANC card which would allow attendance to clinic in case of problems whereas ANC drugs were not considered important.

Interpretation: National malaria policy aims that at least 60% of pregnant women receive full two doses of SP by 2010. This is not achieved unless health system based factors such as accessibility and quality of services are first significantly improved.

Local knowledge, attitude, practice and behaviour on malaria among pastoral communities of Ngorongoro crater, northern Tanzania [MIM-PM-80344]

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Introduction: Tanzania is endowed with dynamic cultures and customs, all begetting varied ways of seeking health care. It is within this complexity, studies which aim to assess the knowledge, attitude, practice and behaviour towards malaria become significant since patterns of seeking health care tend to differ from one culture to the other.

Methods: Four villages were selected based on reports of previous malaria outbreaks. The villages included Osinoni (1720 m), Olbalbal (1780 m), Endulen (1800 m) and Oloirobi (2100 m). A total of 295 heads of households were selected and interviewed using a convenient sampling design. Observation methods were also employed to collect relevant information.

Results: Malaria was considered the most important public health problem in all villages. However, the knowledge of malaria as a disease was relatively poor among residents of Oloirobi (2000 m). 72.6% could mention the symptoms of malaria (as fever, chills, sweating and headache). Poverty, ignorance and ineffectiveness were attributes to the disease. However, the majority of people believed that malaria was spread through the bite of mosquitoes. People were relatively much more aware that malaria can be prevented through the regular use of mosquito nets. While a majority of respondents knew that anti-malarial drugs can be obtained from health centers and dispensaries, knowledge of the correct dosage was poor.
fectiveness of antimalarial drugs were perceived as major contributing factors to the frequent occurrence of malaria epidemics. Most (67.8%) of them preferred chemoprophylaxis to utilization of mosquito nets (5.8%). A significant segment of the respondents (44%) in Olbalbal would do nothing to prevent themselves from malaria. Women and children were considered less susceptible to malaria as compared with other age groups. Although it was observed that most of the respondents (76%) would seek treatment for malaria from health facilities, a significant proportion (19%) preferred alternative medicine. The long distance to the nearest health facility was considered as an important constraining factor in seeking treatment for malaria.

Interpretation: Findings indicate a mismatch between knowledge and practice in malaria prevention and treatment. Appropriate health education and the use of ITNs must be part of the immediate malaria control strategies in the highlands and lowlands of Ngorongoro.

280A
Barriers to seeking treatment for malaria: Evidence from a low-income area in Tanzania [MIM-FM-131195]
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Introduction: Understanding the health care seeking-behavior of households is important in designing appropriate services for the treatment and prevention of malaria. This paper highlights findings on the barriers to malaria treatment at the household level in Tanga district, North-eastern Tanzania. Reasons behind choice of health care provider when a malaria/fever episode occurs is one of the key research questions explored.

Methods: A sample of 1600 households from both rural and urban settings was interviewed about their consumption and expenditure on malaria treatment during the 2 weeks period prior to the survey. In addition, a sub-sample of 250 households kept weekly diaries on their consumption and expenditure on malaria/fevers, other illnesses, and general consumption of primary household goods for 1 year.

Results: Preliminary analysis of choice of provider indicates that the most important factors determining where treatment is sought include: proximity to health facility (79%), availability of drugs (38%), inexpensive drugs/services (25%), and good personal experience with the health facility (18%). All data has now been entered and cleaned. Final analysis is underway and results relating to: (i) expenditure patterns across different providers and (ii) the determinants of demand for different providers will be available by the time of the conference.

Interpretation: Understanding the form and extent of barriers to treatment, even where the physical infrastructure exist, is important in providing insights on how to make malaria treatment more accessible to consumers.

281B
Social marketing for improved understanding of and access to effective malaria treatment in rural Tanzania (ACCESS) [MIM-HM-92112]
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Introduction: Prompt access to appropriate and effective treatment of suspected malaria/fever cases could significantly reduce malaria death rates. In high malaria risk areas various household/individual and health system factors act as “barriers” to successful treatment and care seeking.

Methods: The ACCESS programme will address these.

Results: ACCESS social marketing activities were well accepted and supported by community leaders. Their main concerns were unfriendliness of health care providers, lack of diagnostics in health facilities, treatment malpractices, untrained shopkeepers, expired drugs, confusion due to multiplicity of SP brands and the potential side-effects of SP. Social marketing events were held in 30 villages. The public appreciated the campaigns and suggested that they should be implemented at least twice a year in every village. Mobile clinic services held during village events were very well attended. In one village 99 children were diagnosed
within a single day and 44% were found to be malaria positive. Six of them (13%) had symptoms of severe malaria. One child was referred to a health centre. A total of 85 health facility staff received refresher training on fever management. About 500 shopkeepers of drug and general shops selling antimalarials received a one-day training on the prescription of anti-malarials. 

**Interpretation:** Failure to receive appropriate malaria treatment has many causes. Only a broad set of interventions is likely to improve the situation. Public interest highlighted the importance of such work and first results indicate the situation is improving.

282C

**Development and implementation of malaria communication strategies—A tool for scaling up malaria control activities [MIM-NN-343122]**

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**Introduction:** Awareness on the burden of malaria needs to be raised, particularly among national and international partners. Behaviour change communication is key in the development and implementation of information, education and communication interventions for malaria control. Behaviour change is not merely a product of knowledge or availability of information; it depends on such factors as prevailing social norms, availability of resources and perception of priorities.

**Methods:** To address the challenges in the implementation and delivery of information, education and communication strategies technical support in the development of the communication strategy for malaria was provided to Zambia and Zanzibar. To facilitate the development of the malaria communication strategy in the two selected countries, a task force was established with about 10–15 people. A 5 days meeting was conducted with the members of the task force. The members in the group reviewed relevant documents in preparation for the development of the strategy. A framework of the malaria strategy defining the goals, objectives, definition, principles and approaches and steps of the strategy was developed.

**Results:** By the end of 5 days a communication strategy outlining a logical framework, objectives, activities, indicators and methods of monitoring and evaluation was developed. Experience in developing a communication strategy shows that countries that have developed malaria communication strategies have increased and scaled up IEC malaria control interventions. Appreciation of IEC/advocacy interventions in some of the NMCP teams at higher level is on the increase.

**Interpretation:** The process of developing a malaria communication strategy is time consuming; however the outcome of this process has shown positive results in the implementation of advocacy and IEC activities for malaria control.

283A

**Management of fever due to malaria among children 0–18 months by mothers/care givers in Enugu, Nigeria [MIM-On-36988]**

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**Introduction:** Malaria represents one of the major causes of mortality and morbidity in sub-Saharan Africa. In Nigeria it is the most common cause of patient visit to hospital and ranks among the five most common causes of death for all ages. It is responsible for 8–12% of childhood deaths among children less than 5 years old. It is responsible for 150–200 births per thousands of children below age of 5 years.

**Methods:** 300 mothers/care-givers between 20 and 50 years old were interviewed using an already pre-tested questionnaire on their practices for management of malaria in their children 0–18 months, their attitude and level of knowledge on malaria.

**Results:** 31.7% of care-givers reported that mosquito bite is the cause of malaria while only 11.7% of the respondents knew that Plasmodium parasite is the major cause of malaria. 17.5% reported fever as the most appropriate sign and symptom of malaria infection while about 40% reported high fever as the most appropriate cause of childhood convulsion. Some of the preventive measures mentioned included shellbox spray (20.1%), window netting (12.6%), mosquito coil (9.4%), stop eating oily food (8.5%), ITN (3.0%) among others. With regard to drugs for treatment, chloroquine and multivitamin scored highest (14.1 and 14.6%, respectively), paracetamol (13.7%), fansidar
Among others. Most (67.7%) reported purchase of these drugs from patent medicine stores and other from clinic (26.7%) and the rest from market (7.7%).

**Interpretation:** Most care givers do not have good knowledge about malaria disease and its management. Very few know about use bed net for prevention. Need to refocus on prevention measures thru awareness creation at various levels if the Abuja targets will be achieved.

**284B**
The socioeconomic dimension of cerebral malaria in The Gambia: Implication for research and control

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**Introduction:** Studies from The Gambia have shown that irrespective of secondary level care, long-term morbidity and case fatality rates associated with childhood cerebral malaria (CM) remain unacceptably high. The success of the current malaria control strategy relies on early detection and treatment of mild cases, which in turn is heavily dependent on caregivers’ participation and understanding of important features of malaria infection.

**Methods:** To examine factors that may potentially influence knowledge, attitudes and practices pertaining to the important features of malaria, its complications and the existing national malaria control programme. A descriptive study was conducted between June and December 2001 among 73 primary caregivers of children admitted for cerebral malaria in Bansang hospital Bansang, Central river division (CRD), The Gambia. The study was designed as a pilot to describe background characteristics of caregivers in a rural Gambian setting in order to general hypotheses that could inform a major epidemiological assessment of factors influencing the control and prevention of cerebral malaria deaths and sequelae in the study area.

**Results:** The mean age of caregivers interviewed was 37.8 years (95% CI, 34.7–40.9 years). A total of 59 (81.1%) caregivers earned less than 1000 Dalasis (£20.00) a month and only 2.9% (2/69) had some western education; the remaining ‘educated’ caregivers (17.4%) having had some Koranic lessons. The eldest person in the compound or the father was responsible for taking decision for when hospital treatment was necessary in 85.2% of the cases. Mosquitoes and/or malaria were recognised as causes of febrile illness complicated by convulsions and coma (cerebral malaria) by only 7 (10.8%) caregivers. Educated caregivers were less likely to be ignorant of the aetiology of malaria infection or delay commencement of treatment of mild cases at home than the uneducated (OR: 0.12; 95% CI: 0.02–0.89; p = 0.02). In addition, those who slept outside in the first 6 h of the night (used as proxy for quality of housing) were less likely to be engaged in malaria preventive measures (OR, 0.16 [95% 0.03–0.83], p = 0.004). But there was no significant relationship between educated and uneducated and early presentation of cerebral malaria cases (OR: 0.70 [95% CI 0.14–2.86]; p = 0.76).

**Interpretation:** It is hypothesised that these caregivers’ cultural and socioeconomic backgrounds are atypical and may differ from other population groups in The Gambia. This is a priority area for further research.

**285C**
Tanzania national voucher scheme. Distribution of insecticide treated nets using vouchers—Lessons from Tanzania

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**Introduction:** The Tanzania National Voucher Scheme (TNVS) project represents a unique and sustainable approach towards achievement of the Abuja goal to protect 60% of pregnant women and children in Tanzania from Malaria by ensuring that they sleep under an ITN. This approach provides targeted subsidies (vouchers) through the public health system, yet relies on the private sector and retail shops to make the ITNs available, not only throughout major cities, but also in remote and rural areas of the country.
Methods: The TNVS, funded by the GFAFM, is comprised of three main components: (i) "training and promotion" implemented by World Vision and CARE designed to train health clinic staff; sensitize communities; and to promote the health benefits of ITNs; (ii) "logistics" managed by MEDA which facilitates the delivery of vouchers and retreatment kits to health clinics, ensures availability of ITNs through a network of wholesales and at retail shops, maintains records of voucher distribution and redemption and ensures repayment to wholesalers and manufactures in exchange for vouchers returned and (iii) an M and E component undertaken by IHRDC in association with LSHTM to inform and report back to managers at the Ministry of Health and the Global Fund.

Results: Results (7 months into the project) include: (1) 926 health care workers at 720 clinics have been trained; (2) 428,155 vouchers have been circulated. ("circulated" means vouchers provided to district medical officials for further distribution to individual clinics); (3) 1022 approved retailers participating, of which 80% are rural based and approximately 80% have never sold an ITN before; (4) 86,000 vouchers redeemed for an ITN at a participating retail shop. ("redeemed" means the number of vouchers returned by retailers through wholesalers and manufacturers. Vouchers "distributed" means the number of voucher "stubs" collected back by the program from the health clinics. These stubs are proof that the voucher was presented to a woman attending a clinic. The "redemption rate" is to be calculated by dividing the number of redeemed vouchers by the number of vouchers distributed. The MIS system is still being populated with these numbers, but indications from the first project areas – Dar es Salaam and Dodoma – suggest redemption rates of 75% to 90%); (5) reimbursement to wholesalers and manufactures for vouchers returned is accomplished, on average, within 5 days and (6) operations in 7 of the 21 regions in Tanzania.

Interpretation: The TNVS is the first, national level, program that achieves the twin goals of targeting ITNs to specific populations, while at the same time promoting a sustainable, far-reaching and long-term commercial network for distribution of ITNs.

286A
A Study to increase net ownership using local mechanisms in Mpongwe district, Zambia [MIM-VS-149760]

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Introduction: Challenges on ITNs expansion has been affordability and sustainable re-impregnation demot- ing ITNs acquisition on rural community priority lists. At 27% ITNs coverage is too low for Zambia for appreciable impact on malaria. The Situation is exacerbated by ITNs not being afforded by the populace due to poverty. To address these challenges local fund raising mechanisms were sought in a rural community.

Methods: Consenting members of the community participated through FGD, PRA and KI interviews on the existence of systems in their community used to aid each other acquire items of value and/or achieve tasks quickly. Items of most value in the community were identified and reasons why these were so highly ranked. Using drama groups, personal testimonies of ITNs users and IEC materials, the research team made feedback presentations and taught villagers on cause of malaria, self-protection from mosquito bites and ITNs benefits.

Results: In Mpongwe rural district, systems for aiding each other acquire items of value or achieve tasks quicker were found to exist. The most widely used being the “Imbile” whereby villagers pool their labour to finish agricultural tasks quicker. Youth groups do fund-raising through sinoya whereby they pool labour for cash for farmers with money. The proceeds are used for group benefit for acquiring items on priority lists. Not ITNs. For men items valued are bicycles and farming implements. Women value kitchen utensils. Sinoya was found to be more “robust” and identified by the communities as a method through which they could acquire items of value including insecticide treated bednets (ITNs).

Interpretation: With consistent use of advocacy materials, rural communities could be encouraged to assist each other acquire ITNs through pooling their labour to achieve the high coverage required to see positive impact on malaria.
287B Predicting the cost-effectiveness of introducing a pre-erythrocytic malaria vaccine into the EPI schedule in Tanzania [MIM-FT-392500]

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Introduction: A Phase 2b clinical trial reported promising results for the RTS.S/AS02A vaccine, showing that an effective vaccine against malaria is feasible. Economic evaluation is an essential part of the appraisal of candidate malaria vaccines. We report a model of the cost-effectiveness of pre-erythrocytic malaria vaccines. Our objective is to assess the cost-effectiveness of introducing this type of malaria vaccine into the EPI under a range of scenarios, conditions, and assumptions.

Methods: We use a dynamic stochastic simulation model of the epidemiology of *P. falciparum* in endemic areas and of case-management for a typical meso-endemic malaria setting in Tanzania. We consider a range of vaccine profiles and a range of epidemiological settings. The study is a cost-effectiveness analysis adopting a societal perspective for both costs and effects. The cost-effectiveness ratios are presented as cost per clinical and severe episode averted, cost per death prevented, cost per year of life saved and cost per disability adjusted life year (DALY) averted. The cost-effectiveness ratios (CERs) are presented discounted and undiscounted and according to different ways of computing DALYs.

Results: The cost-effectiveness of such malaria vaccines compares to that of other established preventative and curative interventions against malaria. The CERs increase rapidly and approximately linearly with vaccine cost-per dose. Discounting costs and effects increase CERs. When DALY’s are computed excluding the age weighting, to capture the public health impact of the vaccine, the cost per DALY averted reduces back towards undiscounted levels. The inclusion of indirect costs related to the productive time lost due to illness reduces significantly the CERs. The approach used can be adopted and further developed for comparative analyses of the cost-effectiveness of different vaccines and other intervention strategies in different health systems.

Interpretation: CERs can be generated from this model for pre-erythrocytic malaria vaccines, helping countries and donors to begin early consideration of the role of such a vaccine.

13: Clinical presentation and diagnosis of malaria in children

Posters 288–309

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

288C The epidemiology of malaria in Bolifamba, a rural setting of Mount Cameroon: Seasonal variations in parasitological indices of transmission [MIM-TA-141660]

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Introduction: There are several clinical features of malaria, which could be used as indicators of infections namely fever, splenomegaly and anaemia. We have in this study investigated the prevalence of the different parasitological indices and determined the influence of season on prevalence rates as well as established whether or not an association exists between parasitaemia and the above indicators.

Methods: The study was carried out on 2157 subjects in Bolifamba a rural village on the east slope of Mount Cameroon during June–August of 2001 (rainy period) and January–May of 2002 (dry season). Blood was collected for detection of parasites and estimation of PCV. Anaemia was defined as PCV < 31%. Fever was defined as axillary or rectal temperature >37.5°C. Clinical examination included palpation of the spleen, graded according to the classification of Hackett. A multiple logistic regression model was used to determine the relationship between presence of parasitaemia and age group, gender, anaemia, fever, and the month of examination.

Results: Parasite prevalence was 55.9 and 49.5% in the wet and dry season, respectively (*p* < 0.0001). Rainfall was significantly associated with parasite density (*p* = 0.001). The fever rate was 40.31 and 19.6% in the wet and dry seasons, respectively (*p* < 0.0001). Spleen
rate was 37.35% in the wet season and 3.98% in the dry season \((p<0.0001)\) with Hackett scores \(\geq 2\) also more frequently recorded in the wet (25.8%) than in the dry (2.44%) season \((p<0.0001)\). No seasonal difference was observed for the anemia rates. All the above parasitological indices were significantly higher in subjects less than 5 years old compared with those older. A multiple logistic regression model showed that age group, anemia, fever and month of examination were significantly associated with the presence of parasitaemia.

**Interpretation:** This large-scale study confirms intense transmission of malaria in rural Bolifamba with the under fives at higher risk. The findings have provided base line data for an ongoing immunological study to assess the immune status of subjects.

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**289A**

L’altération de la fonction renale au cours de la fièvre bilieuse hémoglobinurique de l’enfant aux cliniques Universitaires de Kinshasa (CUK) [MIM-MA-38166]

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**Introduction:** Le processus de changement de politiques de médicaments antipaludiques en Afrique a coïncidé avec la réapparition de la bilieuse hémoglobinurique dans les populations autochtones. L’atteinte de la fonction rénale constitue l’une des complications majeures de ce syndrome jusqu’alors peu décrit chez l’enfant en zone d’endémie. A travers cette étude, nous avons évalué la fonction rénale de 26 enfants suivis pour fièvre hémoglobinurique.

**Methods:** Une étude documentaire a été menée auprès d’enfants de moins de 12 ans, admis aux Soins intensifs pédiatriques pour un paludisme grave en Afrique et la réapparition de la bilieuse hémoglobinurique dans les populations autochtones. L’atteinte de la fonction rénale est constituée par les complications majeures de ce syndrome jusqu’alors peu décrit chez l’enfant en zone d’endémie. A travers cette étude, nous avons évalué la fonction rénale de 26 enfants suivis pour fièvre hémoglobinurique.

**Results:** Vingt six dossiers sur 453 enfants suivis pour paludisme grave pendant ces 4 années ont été retenus (5.7%). Tous ces enfants (100%) ont présenté une fièvre, un icère ainsi qu’une hémoglobinurie. Vingt trois enfants sur 26 inclus ont reçu la quinine. En ce qui concerne la NFS, 21 cas ont une hyperleucocytose \((>22.400/mm^3)\) et ce à prédominance neutrophilique \((80.8\%)\). L’anémie sèvère \((Hb < 5 g/dl)\) a été retrouvée chez 15 enfants soit 57.7\%. L’altération de la fonction rénale était relevée chez 18 enfants soit 69.3\% de cas avec une clairance de la créatinine <50 ml/min/1.73 m². L’oligo anurie a été retrouvée dans 61.5\% de cas et 9 enfants (34.7\%) ont reçu une épuration extrarénale par dialyse péritonéale. Trois enfants soit 11.6\% sont décédés.

**Interpretation:** L'utilisation des amino-alcools en remplacement de la chloroquine expose les enfants en zone d'endémie à des complications rénales liées à la bilieuse hémoglobinurique.

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**290B**

Medullogramme au cours de l’anémie associée au paludisme grave de l’enfant à Kinshasa [MIM-MB-1752]


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**Introduction:** La destruction directe des globules rouges par le Plasmodium falciparum à travers divers mécanismes pathologiques ne suffit pas à elle seule pour expliquer l’anémie sèvre. D’autres mécanismes sont évoqués et, l’étouffement des progéniteurs érythroïdes est décrit comme l’un des phénomènes pathologiques qui s’associent à l’hémolyse massive. Au cours de notre étude menée chez les enfants souffrant d’anémie palustre sèvre, nous avons cherché à vérifier l’existence de cette dysérythropoïèse.
Methods: Notre étude a été menée dans deux hôpitaux pédiatiques de Kinshasa de mai 2002 à juin 2003. 48 enfants réunissant les critères suivants ont été retenus: être âgé de < 12 ans; présenter une anémie sévère avec fièvre et ayant nécessité une transfusion (Hb < 5 g/dl); avoir une goutte épaisse positive à Plasmodium falciparum et n’avoir que ce seul diagnostic. Nous avons réalisé la numération de la formule sanguine, la goutte épaisse et les réticulocytes. Les ponctions médullaires ont été faites à la crête iliaque. Le séchage du frottis a été réalisé à l’air libre, la fixation au Maygrunwald et la coloration au Giemsa. Les lames ont été lues par deux hématologistes. Les tests statistiques usuels ont été utilisés.

Results: L’anémie observée était normochrome dans 70% de cas (CGMH: 28–35%), avec 50% de cas de microcytose (VGM<80μm3) et 48% de normocytose (VGM = 80–100μm3). Dans 83, 3% des cas le taux de réticulocytes était bas (<0.5% de globules rouges). Le médiulogramme obtenu après ponction médullaire auprès de ces enfants est caractérisé par les images suivantes: un cytoplasme feuilleté dans 44% de cas; des mitoses multiples et inégales dans 44% de cas; un noyau en roseau ou polylobé dans 33% de cas; un asynchronisme de maturation nucléocytoplasmique dans 22% de cas. Les métamyélocytes géants (16%), la bi et multinucléarité (13%), les macrophages hyperactifs (5%) et le cytoplasme avec vacuoles (2%) ont été aussi retrouvés. Cependant nous n’avons pas trouvé des corrélations entre les constantes globulaires et ces images ($x^2 = 0.86; p=0.168$).

Interpretation: La normochromie observée prouverait que le paludisme constitue la cause de cette anémie. Le caractère aergénératif de l’anémie suggère que les images obtenues sont liées à une atteinte centrale qui serait la conséquence de cytokines sur la moelle.

291C

Malaria diagnosis in Southern Africa: Strengthening the role of rapid diagnostic tests [MIM-LC-227784]


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Introduction: Clinical diagnosis has remained as the primary method of diagnosis for malaria disease in endemic countries in Africa. However, a lot of problems especially regarding over diagnosis and over treatment remain. Clinical diagnosis was felt to be adequate when chloroquine and sulphadoxine-pyrimethamine were effective antimalarial drugs. The current situation of high CQ and SP parasite resistance is rendering their use as drugs for management of uncomplicated malaria ineffective.

Methods: Aim of the paper is to discuss the role of Rapid Diagnostic Tests (RDTs) in Southern African countries as they move towards artemisinin based combination therapy (ACTs) for the management of uncomplicated malaria. Countries of the sub-region are moving towards combination therapy using artemisinin based compounds. Clinical diagnosis in the public sector in countries changing to expensive drugs is becoming unjustifiable especially in view of the high cost of the ACTs and relatively restricted availability of these drugs. The need for confirmatory diagnosis has become critical. Due to inadequate microscopic services in most countries rapid diagnostic tests (RDTs) are showing potential to fill in this gap and complement for microscopy.

Results: RDTs possess a number of advantages for use in different settings though their major disadvantage outside cost is the lack of stability especially in areas of high humidity and temperature. The advent of the global fund for AIDS/TB and Malaria is supporting countries in the procurement of RDTs to com-
plement the current available microscopic services for malaria case management. Partnership with producers and distributors of RDTs is increasing. Initially, a number of evaluations were done in Zambia and Zimbabwe and subsequently phased implementation initiated in these countries. In Southern African countries with unstable malaria transmission have more experience in the use of RDTs in both the private and public sector. All countries moving towards new policies using ACTs are looking at RDTs as an alternative intervention to complement microscopy. RDTs have a major role in countries of southern Africa as countries move to more expensive drugs and the health system regards confirmatory diagnosis as a standard of care for managing malaria even at lower health facilities. The challenge remains whether the current specifications of RDTs including cost will be robust enough for diagnosis of malaria in the adverse African conditions.

**Interpretation:** RDTs will not replace microscopy their role will be to complement microscopy where there is no laboratory.

**292A**

*Plasmodium vivax* is associated with severe malaria in Papua New Guinean children [MIM-VD-213122]

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**Introduction:** Severe malaria (SM) is known to be associated with *Plasmodium falciparum* (Pf) infection. Little information is available on the contribution of *P. vivax* (Pv) to severe disease. Few data suggest that mixed Pf+Pv infections may protect against SM. A morbidity surveillance has been ongoing in the East Sepik Province of Papua New Guinea, which allows long-term assessment of clinical and laboratory features of malaria, including severe disease, in an area endemic for Pf, Pv and *P. malariae* (Pm).

**Methods:** The present analysis is based on data collected during an 7-year period. All presumptive malaria cases, whether uncomplicated or severe, were investigated in a rural health center in the context of intermittent trials against malaria. A detailed clinical exam was performed followed by haemoglobin measurement and microscopical examination for parasite species and density. The case definition of SM was derived from the WHO’s guide to clinical practice, i.e. asexual blood stage parasitaemia + recent history of fits or coma or respiratory distress or anaemia <5 g/dl. No measurement of blood pressure, glycaemia, or renal function was performed.

**Results:** 8961 children aged <5 years were recruited as presumptive malaria cases. Among those, 53.1% were parasitaemic, 34.8% with Pf, 14.5% with Pv, 1.3% with Pm, 0.1% with *P. ovale* and 2.4% with mixed infections. Among parasitaemic children with all data available (3829), 10.4% (95% CI 9.4–11.4) fulfilled the case definition of SM. The proportion of SM was 11% (9.8–12.3) for Pf, 8.4% (6.9–10.3) for Pv, 6.4% (2.6–13.9) for Pm, and 15.7% (10.8–22.2) for mixed infections. 22% of the Pf SM had fits versus 26% of the Pv SM and 11% of the mixed SM; 2% of the Pf SM had coma versus 3% of the Pv SM and 0% of the mixed SM; 40% of the Pf SM had respiratory distress versus 58% of the Pv SM and 67% of the mixed SM; 42% of the Pf SM had anaemia versus 18% of the Pv SM and 26% of the mixed SM. Patients with mixed infections had higher total parasite density than those with single Pf infection. When children with cough or diarrhoea were excluded (more specific definition of SM), there were 6.3% (5.4–7.4) of Pf SM, 4% (2.6–5.0) of Pv SM, 2.1% (0.4–8.2) of Pm SM, and 8.7% (5.1–14.2) of mixed SM.

**Interpretation:** *P. vivax* alone is associated with SM in children. There is no indication that mixed infections did protect against SM. Interventions targeted towards Pf only might be insufficient to reduce the overall burden of SM in areas where Pf and Pv coexist.
Malaria in children admitted to two rural district African hospitals: Age patterns and implications for control [MIM-CG-111481]


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Introduction: Available effective malaria control tools are to be implemented on a large scale, especially targeting the groups most at risk. Hospital data, despite their limitations, are a good and often unique source of data that can be used as sentinel data on the morbidity and mortality of the surrounding population. We present a comparison of data on malaria admissions from two hospitals, that adds further evidence on the relationship between intensity of transmission and malaria morbidity.

Methods: The rural district hospitals at Ifakara, Tanzania, and Manhiça, Mozambique, run very similar hospital surveillance systems. Routine surveillance data on pediatric admissions to these two hospitals were analyzed to describe the age pattern of children admitted with clinical malaria (clinical diagnosis of malaria + asexual P. falciparum parasitemia) and severe malarial anemia (PCV < 15% + parasitemia) and to calculate the minimum community incidences for these conditions. Data were analyzed by different periods of surveillance (Ifakara in 1995 and 2000 and Manhiça, with moderate transmission intensities, was shifted to the right, with infants accounting for <20% of malaria admissions and children aged 1–2 and 2–3 years accounting for around 25% of cases each. The minimum community incidences of clinical malaria ranged from 25 to 224 episodes per 1000 infants per year in Manhiça and from 71 to 172 in Ifakara, in children aged 1–4 years the incidences ranged from 19 to 229 episodes per 1000 in Manhiça and from 61 to 74 in Ifakara. The clinical presentation of severe malaria (malaria with impaired consciousness and severe anemia) followed no clear cut variations according to the transmission level.

Interpretation: Children younger than 2 years carry the burden of disease at both sites. Control strategies should thus target this age group. Clinical presentation of severe malaria does not seem to follow clear cut variations according to the transmission level.

The “simplified” multi-organ-dysfunction score (MODS) is a tool to early evaluate the severity of children with Plasmodium falciparum malaria [MIM-RH-15751]


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Introduction: Malarial disease has a substantial social and economic impact in most tropical countries. Recently we could show that the MODS, a quantitative approach for severity, is a useful tool to discriminate different levels of severity in patients with P. falciparum malaria on admission. In this survey we prospectively evaluate the “simplified MODS” in African children, based on clinical findings mainly, to assess severity on admission and to find a correlation with outcome variables.

Methods: The study is conducted at the Albert Schweitzer Hospital in Lambaréné, Gabun, since 15th...
August 2003. The primary analysis comprises 192 consecutive patients admitted to the hospital with the diagnosis of *P. falciparum* malaria. The score was evaluated by a trained physician on admission, demographic and clinical data were recorded according to a structured study protocol. Informed consent was obtained from all patients. Outcome was evaluated by the ability to walk unaided, sit unaided and to eat and drink expressed in hours after admission.

**Results:** Of the 192 consecutive patients 167 patients were old enough to walk before the onset of *P. falciparum* disease (87%) (cohort 1), 22 patients were only able to sit because of young age (11.5%) (cohort 2), 3 patients were even not able to sit (1.6%). The mean age was 45 months (range, 4–192 months). The simplified MODS on admission (mean 15.3, b3.5; range 10–36) was highly correlated to the duration of symptoms after admission: cohort 1: patients with a high score on admission were significantly longer not able to walk (Spearman’s $r = 0.73$, $P < 0.001$), to sit ($r = 0.68$, $P < 0.001$) and to take oral food ($r = 0.60$, $P < 0.001$); cohort 2: ability to sit unaided (Spearman’s $r = 0.54$, $P = 0.01$) taking oral food ($r = 0.51$, $P = 0.02$). A score of 16 and more was associated with a higher morbidity in all groups (log rank Test, $P < 0.001$). Four patients died within 15 h after admission with a score of 26, 27, 29 and 36, respectively.

**Interpretation:** The simplified MODS can be used in children with *P. falciparum* malaria to assess severity on admission. This score is easy to apply and can therefore be used independently from financial resources in all malaria clinics in tropical countries.

**295A**

**Anaemia in young children with malaria at a hospital in northern Cameroon** [MIM-PJ-123550]

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**Introduction:** The aims of the study were to determine the clinical, social and socio-economic indicators of anaemia in young children with malaria approaching a hospital in northern Cameroon.

**Methods:** The authors have conducted a hospital-based cross-sectional study of 91 consecutive children. Patients below the age of 60 months in whom malaria was diagnosed by microscopic examination and whose parents had given informed oral consent were included in the study.

**Results:** Anaemia (haemoglobin <110 g/L) was detected in 82% of the patients, while 26% of the patients presented with a high parasite load (>100 *Plasmodium* per 100 high-power fields). There was no significant association between the levels of haemoglobin and of parasitemia in the study population. Anaemia was found significantly more often in children between the ages of 12 and 24 months and in patients born at home. Severe anaemia (haemoglobin <50 g/L) was found significantly more often in patients who had not adhered to the national vaccination programme. Moreover, maternal education was associated with the patients’ levels of haemoglobin. Most clinical findings suggestive of pathology were associated with haemoglobin, rather than parasite load.

**Interpretation:** Our findings suggest that other reasons for anaemia were more important than the current malarial episode in causing anaemia in this hospital-based study.

**296B**

**Pigmented white blood cell number as a negative correlate of clinical immunity in Ugandan children with severe complicated or uncomplicated malaria** [MIM-BK-99000]

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**Introduction:** Previous studies have documented the usefulness of pigmented white blood cells (WBC) as an index of malaria disease severity in African children and Thai adults. Our studies have confirmed and extended these findings to demonstrate that measurements of pigmented WBC might be useful for predicting clinical immunity in children with severe and complicated, or mild and asymptomatic malaria.

**Methods:** Children 6–60 months old with severe malaria (cases) and age-matched controls with mild uncomplicated malaria were enrolled in Apac District
in Northern Uganda. Blood samples were used to make smears and quantification of pigmented white blood cells (WBC) and parasite density was by microscopy. A total of 500 WBC were examined for the presence of pigment. Children were divided into two groups: those with and those without pigmented WBC in blood smears. Correlations between pigment numbers and putative measures of morbidity (temperature, parasite density, hemoglobin levels and PCV) or immunity (IgG-anti-*P. falciparum* lysate, IgG anti-SERA5 and EBA175) were assessed by the Spearman’s rank correlation.

**Results:** There was a significant association between the presence of pigmented WBC and the clinical outcome ($c^2 = 34.60; P < 0.0001$). Measures of pigmented WBC were significantly higher in severe malaria by comparison with those in uncomplicated malaria. Children with severe malaria with no pigmented WBC had significantly higher haemoglobin, packed cell volumes (PCV), and IgG anti-SERA5 by comparison with their counterparts with pigmented WBC. When the numbers of pigmented WBC were considered, children with severe malaria with 1–9 pigmented WBC had significantly elevated hemoglobin and PCV by comparison with those with 10 or more pigmented WBC. In severe malaria children there was a significant inverse correlation between numbers of pigmented WBC and IgG anti-SERA5. By contrast, in children with uncomplicated malaria, there was no significant difference between all measures of morbidity or immunity and the presence or absence of pigmented WBC. In children with uncomplicated malaria there was a significant negative correlation between numbers of pigmented WBC, and age, IgG anti-EBA175, and IgG anti-*P. falciparum* lysate. In these children, titers of IgG anti-SERA5 and EBA175 are significantly associated with protection against severe malaria.

**Interpretation:** In addition to being markers of severe malaria, pigmented WBC were negative correlates of clinical immunity in Ugandan children under 5 years old. Measurements of pigmented WBC as end-points in malaria vaccine trials in children need to be evaluated.

**297C**

**Cerebral malaria and psychiatric manifestations in India adult: A prospective study [MIM-DK-22556]**

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**Introduction:** As per WHO (1993) the assessment and analysis of local problems and an appropriate epidemiological information system is an essential part of a control programme before embarking any control activity.

**Methods:** 881 adults of strictly defined admitted cerebral malaria patients were studied. Detailed clinical/neurological examination was done at the time of admission, daily thereafter, at the time of regaining consciousness, at the time of discharge and at weekly intervals in those having neurological sequelae. All patients were guidelines.

**Results:** Apart from fever and unconsciousness in all the patients, other features were convulsion (21.31%), trismus (1.31%), abnormal behaviour (3.6%), decorticate rigidity (1.13%) and decerebrate rigidity (0.90%). One hundred forty five (32.87%) patients expired and mortality was highest in pregnant ladies (30.28%). The important neurological sequelae in survivors were psychosis in 15 (5.06%), cerebellar ataxia in 14 (4.72%), hemiplegia in 5 (1.68%), peripheral neuropathy in 3 (1.01%), EPR with trismus in one (0.33%) and isolated sixth nerve palsy in one (0.33%) patients and all showed complete recovery in further follow-up.

**Interpretation:** Increased incidence of haemoglobinuria and jaundice, presence of neck rigidity, psychiatric behaviour no prognostic relation to fundus abnormalities and high incidence of cerebellar ataxia and psychosis as neurological sequelae in survivors.

**298A**

**Microscopy for clinical trials [MIM-KK-38600]**

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Introduction: Microscopy remains the accepted “gold standard” for malaria diagnosis and monitoring anti-malarial drug and vaccine efficacy. This is a subjective method hence dependant on the microscopist’s capabilities. There are no accepted international standards on the performance of microscopy from sample collection to result interpretation. More often studies do not report in detail how the microscopy was performed in publications.

Methods: A comprehensive evaluation and training is not commonly practiced. Of the methods use in the laboratory, microscopy has not been subjected to rigorous of quality control and quality assurance program. We know that false positive slides can ruin promising products in developmental stages. To avert the problems of microscopy in clinical trials, we have developed a series of training, proficiency testing of microscopists, quality control and quality assurance microscopy techniques at our field clinical trial unit.

Results: This is a preamble for the development of microscopy centres of excellence. Results of this training program and proficiency testing with their impact on clinical trial endpoints will be presented.

Interpretation: The development of centres of excellence in microscopy will go a long way in the establishment of accepted and approved standards in microscopy.

Spatial ecological study of prevalence of fever in relationship to malaria endemicity in Malawi [MIM-LK-89726]

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Introduction: In malaria endemic countries like Malawi, fever, a cardinal symptom of malaria, is often equated with malaria for prompt and early treatment due to non-availability of parasitological confirmation in many places. However, studies have shown that fever, as an indicator of malaria, has low specificity generating many false positives. Conversely, the absence of fever can not be interpreted as absence of infection in populations with high levels of immunity, that is, it also has low sensitivity.

Methods: We investigated the prevalence of fever in children in Malawi in relation to malaria endemicity, after adjusting for socio-economic factors and spatial effects. We used survey data from the Malawi demographic health survey of 2000, and geostatistically modelled malaria prevalence rates at sub-district level, to fit a spatial logistic regression model on the presence of fever in under-five children with malaria prevalence in an area as an explanatory variable, adjusting for demographic and socio-economic variables.

Results: Our results showed that fever prevalence was positively related to malaria prevalence in the baseline model (which did not account for spatial variation). However, when spatial effects were accounted for in the model, malaria prevalence was no longer important at explaining fever. The model showed significant unexplained spatial effects in some areas. Importantly, demographic and socio-economic factors such as age of the child, owning a bednet, mothers with secondary education were inversely related to presence of fever, while rural residence and low education were positively related to fever.

Interpretation: These results confirm that not all fever is malaria in malaria endemic countries, but can be a result of other environmental factors, for example competing diseases such as pneumonia, that remain unobserved but vary in space.

Efficacité de la prise en charge à base communautaire du paludisme chez les enfants de 0 à 5 ans en zone rurale du Burkina Faso [MIM-DM-47292]

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Introduction: La prise en charge des cas de paludisme chez les enfants de moins de 5 ans est confrontée aujourd’hui au problème d’accès aux soins. L'utilisation de relais communautaires pour améliorer la couverture en soins n’est pas toujours acceptée des professionnels de santé. Ce constat a rendu pertinente
une étude d’intervention en zone rurale du Burkina Faso, qui avait pour but d’évaluer l’efficacité d’un système de prise en charge du paludisme à base communautaire.

Methods: Dans chacun des 18 villages d’intervention, un Agent de Santé Communautaire (ASC) recruté et formé à la prise en charge des cas et la gestion des médicaments, visite à la demande et plus systématiquement une fois par semaine chaque ménage, y recense les cas de paludisme chez les enfants de 0 à 5 ans, confectionne les GE/FM, délivre les médicaments sous forme d’unités thérapeutiques et réfère les cas graves au niveau des centres de santé. Dans 15 villages contrôles, aucune organisation particulière n’a été organisée concernant la prise en charge des cas de paludisme.

Results: D’octobre 2003 à septembre 2004, 2654 cas présumés de paludisme ont été recensés par les ASC dans les 18 villages d’intervention, dont environ 60% ont été confirmés par les examens parasitologiques. L’incidence cumulée des cas présumés de paludisme recensés dans les villages d’intervention était de 123 pour mille, la gestion des antipaludiques n’a connu aucune rupture majeure de stock. La proportion des cas de paludisme référés dans les centres de santé parmi la population des enfants de moins de 5 ans provenant des villages d’intervention (96/21637) est significativement plus faible que celle des villages contrôles (182/20078), p < 1/1000. La proportion des cas justement référés pour raison de paludisme grave est significativement plus élevée dans les villages d’intervention (64/96) que dans les villages contrôles (49/182), p < 1/1000. Les coûts de la prise en charge, induits par les références abusives étaient 4 fois moins élevés pour l’ensemble des villages d’intervention (158,5 D.) comparés à ceux des villages contrôles (658,9 D.).

Interpretation: Les relais communautaires permettent d’assurer une prise en charge correcte des cas de paludisme, soulagent les services de santé des cas pouvant être traités à domicile et enfin réduisent les surcoûts imputables à la faible couverture en soins.

301A
Evaluation of the hexagon malaria rapid diagnostic test kit in five communities on the copperbelt province of Zambia [MIM-SM-12355]
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Introduction: Malaria in Zambia is still the number one cause of morbidity and mortality. Most rural health facilities that are not electrified and some urban health facilities that cannot afford microscopes cannot use microscopy for the diagnosis of malaria. Such health facilities usually depend on clinical diagnosis. As such, a more feasible, cost effective and quick method for malaria diagnosis would be more appropriate. Rapid diagnostic tests (RDTs) attempt to achieve prompt and accurate diagnosis of malaria. The Hexagon Malaria kit was used in this evaluation exercise for the detection of Plasmodium falciparum (pf). This kit is a one step test for the quantitative detection of the Histidin rich protein (HRP-II) released from Plasmodium falciparum. The pf HRP-II is detected by means of an immunochromatographic method using colloidal gold particles coated with monoclonal antibodies against pf HRP-II. These react with pf HRP-II present in the specimen resulting in immunocomplexes which migrate along the membrane and are bound by a second monoclonal anti-pf HRP-II antibody fixed in the test zone to form the test line. Excess immunocomplexes migrate further and are bound in a second line by anti-mouse Ig Immunoglobulins, forming the control line.

Objective: To evaluate the performance of the Hexagon malaria test kit against the standard (gold) microscopy method.

Methods: Study design This was an exploratory study. Study setting and population The study was carried out in selected townships of Ndola and Kitwe, and covered a population of about 100,000 people. The sample population was the people above 60 months attending clinic and who had fever and the sample size was 119. Methodology >from these people, a thin blood smear was taken for microscopic detection of plasmodium falciparum, and some blood was drawn into a capillary tube for the detection of histidin rich protein-II of plasmodium falciparum on the RDT according to the outlined procedure.
Results: Of the 119 people in the study, 32 (26.9%) were true positives or were positive to both microscopy and the RDT test; 57 (49.9%) were true negatives or were negative to both microscopy and the RDT; 30 (25.2%) were false positives, that is, negative to microscopy and positive to the RDT. None (0) of the study participants were false negatives, that is, negative to both microscopy and RDT. The RDT test kit had a specificity of 65.5% and its sensitivity was 100%.

Interpretation: The specificity and sensitivity of 65.6 and 100%, respectively. With a sensitivity of 65.5%, the Hexagon malaria test kit was found not to be suitable for the detection of Plasmodium falciparum species in this setting.

302B
Randomised trial of educational interventions to improve management of uncomplicated malaria in children: Impact on prescriptions of drugs in Tanzania [MIM-BN-25626]
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Introduction: Access to early diagnosis and prompt treatment is the basis for effective malaria control. But the quality of malaria diagnosis and case management is poor because of inadequate clinical examination, lack of diagnostic facilities such as microscopes and non-observance of standard malaria treatment guidelines. We aimed to assess the effectiveness of a multi-component intervention package designed to improve quality of malaria case management in underfive children.

Methods: We conducted a cluster randomised trial in 16 primary health care facilities in Kibaha and Bagamoyo districts. Five facilities received clinical plus microscopy training, clinical training only (n = 5) and control group (n = 6). We enrolled 3131 children with fever from June to September 2003, 2721 patients completed day 7 follow-up. The main components of interventions were health workers educational materials, supply of microscopy and reagents, and interactive group training for health workers. Main outcome measure was reduction in rate of prescriptions of antimalarials.

Results: Patients in the clinical plus microscopy group were less likely to receive antimalarials (59.6%) compared to the clinical group (95.4%) the control group (99.2%) (p < 0.003). No significant differences were found between groups for the other outcomes.

Interpretation: Our findings identify that additional training of health workers; provision, training and use of microscopes for malaria diagnosis improved the treatment of clinical episodes of fever in children while reducing the amount and costs of drugs.

303C
Increasing malaria morbidity and mortality in children admitted to Mulago Hospital, Uganda, 2002–2004 [MIM-RO-456115]
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Introduction: Malaria is a leading cause of morbidity and mortality amongst Uganda’s paediatric population. With the advent of the Roll Back Malaria Initiative and the Abuja Declaration, there have been increased efforts aimed at control, prompt and effective treatment in the community. Few studies have assessed the effects of such interventions on inpatient severe malaria morbidity and mortality. We describe changes in the inpatient burden and mortality from paediatric severe malaria in Mulago Hospital.

Methods: We undertook a retrospective chart review of all paediatric (age 0–12 years) admissions with a diagnosis of malaria to Mulago National Referral Hospital, in Kampala, Uganda, over a 3-year period (between January 2002 and December 2004). Data was extracted on the type of malaria complication, associated co-morbidity and outcome of treatment and used to determine the frequency of malaria admissions and mortality.
Results: There were 19,047 malaria admissions over the 3-year period. The frequency of malaria as an admitting diagnosis was 295 per 1000 children admitted, and the mortality was 13 per 1000 children admitted. From 2002 to 2004, an increase was seen in the frequency of malaria as an admitting diagnosis (237 versus 332 per 1000 admissions) and mortality related to malaria (10 versus 16 deaths per 1000 admissions). Severe malaria anaemia was the commonest manifestation of severe malaria (50.2% of cases), and had a case fatality rate of 4.9%. Cerebral malaria, though accounting for only 14% of severe malaria cases, was associated with a higher case fatality rate (16.6%). Non-malaria co-morbidity was documented in 28% of patients and was associated with a two-fold increase in severe malaria mortality.

Interpretation: The burden of severe malaria morbidity and mortality in children in the Kampala area of Uganda appears to be increasing despite increased community-based control activities. Future research will explore the potential reasons for this increase.

304A Predictive symptoms in the diagnosis of malaria [MIM-AO-406156]
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Introduction: Falciparum malaria is responsible for high incidence of morbidity and mortality in endemic areas. Malaria infections may be symptom-less or fatal, between these two extremes, illness may take a variety of clinical forms differing in pattern and severity. Misdiagnosis and delayed may lead to mortality especially among the high risk group. In this study, we investigated the predictive values and association of clinical presentations and the severity of the malaria infection in children.

Methods: The study was carried out in Ibadan, a city in southwest of Nigeria, a hyper-endemic area for malaria. Ethical approval was obtained from hospital and an informed consent was obtained from care-givers prior to recruitment. Demographic information, clinical history and clinical examination done were obtained from a total number of 957 children (705 acute uncomplicated malaria (UM) and 235 severe malaria (SM)).

Results: The study population comprised of 555 males (58%) and 402 (42%) females with a median age of 26.0 months. Fever was the most common complaint at presentation (95%) while others were chills (9%), loss of appetite (23%), vomiting (32%), diarrhea (12%), jaundice (5%), Pallor (5%), cough/catarh (38%), breathlessness (8%), lethargy/weakness (15%), convulsion (16%), irritability (5%) and clouded consciousness (8%). There was a significant difference in the frequencies of nine of the 13 common symptoms at presentation between the 2 groups of children. The risk of having uncomplicated malaria was higher among patients presenting with loss of appetite (OR = 1.5), diarrhea (OR = 2.2), cough and catarh (OR = 2.6), lethargy (OR = 1.6) and irritability (OR = 2.1) while the risk of the malaria infection developing into the severe disease was higher among children presenting with jaundice (OR = 7.3), pallor (OR = 2.6), convulsion (OR = 9.3) and clouded consciousness (OR = 18.1).

Interpretation: Prompt and correct diagnosis is essential in treatment of malaria in children. This data would be useful in rural areas, at the primary level of care where early presumptive diagnosis of malaria is imperative to reduce mortality due to misdiagnosis.

305B Malaria and asymptomatic parasitemia in Gabonese infants [MIM-o-178756]
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Introduction: Lambaréné, Gabon is an area situated south of the equator, where malaria is hyperendemic and perennial with an entomological inoculation rate estimated at an average 50 infective bites per person per year. Here the burden of malaria still falls heaviest on children below the age of five. However, little information is available for infants.

Methods: All children born in hospitals and at home in Lambaréné, Gabon, between December 2002 and July 2004 were eligible for study inclusion. A monthly follow-up with a thick blood smear was made for every child. In case of parasitemia without symptoms, the mother was urged to return in case of illness. We determined the incidence of both malaria and asymptomatic parasitemia in infants under the age of 3 months.

Results: Of 878 infants who were included at birth, we identified malaria in three infants and, additionally, asymptomatic parasitemia in six infants. The malaria incidence density was 1.1/1000 person-months (risk: 0.34%). The incidence of parasitemia was 3.4/1000 person-months (risk: 1.0%).

Interpretation: Malaria and asymptomatic parasitemia in infants aged below 3 months in Lambaréné are very low. Public health measures aimed against malaria such as intermittent preventive treatment are not necessary in this age group.

306C
Iron deficiency: Challenges in a malaria endemic area [MIM-KP-373112]
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Introduction: Most epidemiological studies on iron deficiency and iron supplementation have based their findings on iron measures that are unreliable in areas with high malaria transmission and inflammation. Additionally, the so-called ‘gold standard’ of iron deficiency diagnosis, namely bone marrow iron microscopy, has been shown to be inconsistent, even in non-malarious settings. The broad objective of the study is to evaluate the diagnosis of iron deficiency among children living in a malaria endemic area.

Methods: The study involves a detailed descriptive analysis of children presenting to hospital with severe anaemia requiring blood transfusion and children going for elective surgical procedures in theatre. Samples of venous blood, bone marrow, urine and stool are collected. Samples will be analysed at Wellcome Trust Research laboratories in Blantyre, Malawi and Liverpool School of Tropical Medicine laboratories in United Kingdom. A sample size of 250 severely anaemic and 100 non-severely anaemic (surgical) children will be recruited. The study is being carried out in Blantyre and Chikwawa districts, Malawi.

Results: Bone marrow iron assessment will be improved by comparing 3 methods: (1) bone marrow iron microscopy (using newly developed iron cytological grading system); (2) flow cytometry (assessment of CD71 expression on erythroid progenitor cells); and (3) atomic absorption spectrophotometry (precise estimation of the total elemental iron). Secondly, performance of different biochemical measures of iron (sensitivity, specificity) in the presence of malaria will be determined against bone marrow iron. Lastly, an algorithm for diagnosis of iron deficiency in malaria endemic areas in health centres, district and central hospitals, which can influence policy, will be formulated.

Interpretation: Preliminary results to be presented.

307A
Acute renal failure in malaria in Northwest region of India—A histopathological and clinical correlation [MIM-VS-396750]
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Introduction: Acute renal failure complicates approximately 5% of hospital admission and up to 30% intensive care units, oliguria, (urine output <400 ml/day) in frequent but not invariably clinical feature. Most of ARF are reversible including of severe malaria. The kidney being relatively unique among major organs with ability to recover from almost complete loss of function.

Methods: Patient admitted in tertiary care hospital with malaria (positive P. falciparum/P. vivax) with renal failure as per WHO guidelines and subjected to PBF (thick and thin film), Optimal test, PCR and renal biopsy. The exclusion criteria were setup to rule
out other causes of renal disease before and during illness.

**Results:** Out of 933 patient of malaria during year 2002–2003. Acute renal failure was observed in 219 patients of malaria. (PV – 45 and PF – 174). Oliguric renal failure was in 54 (24.65%) (PV – 12, PF – 19, mixed – 23) while non-oliguric renal failure was in 165 (75.34%) (PV – 24, PF – 123, mixed – 18). Maximum patient of renal failure were in age group of 20–40 years. Total death of malaria with acute renal failure was 63. Death of patient of malaria of oliguric renal failure 42 (65.62%) (PV – 9, PF – 24, mixed – 9) and of non-oliguric renal failure 21 (33.33%)(PV – 21, PF – nil, mixed – nil). Histopathological findings of 39 patients were suggestive of acute tubular necrosis (ATN) and mesangioproliferative glomerulonephritis (MPGN) (oliguric RF – 30 (76.92%), non-oliguric – 9 (23.07%)) and out of that ATN was in 21 (53.84%) cases (3 oliguric and 18 non-oliguric) and MPGN in 18 (46.15%). The incidence of renal failure was 2.07% in 1994, 6.16% in 2001 and 23.47% in 2003 in this region of Northwest India. Though renal failure was common with PF malaria and rare with PV malaria but we confirmed *P. vivax* with acute renal failure by PCR in four cases.

**Interpretation:** Renal failure prevalence of renal failure in malaria in hospitalized patient is 16.43%, oliguric renal failure was having poor prognosis. Major histopathological finding in malarial ARF are ATN and mesangioproliferative glomerulonephritis.

**308B**

A clinical and histopathological study of renal dysfunction in malaria in India [MIM-VS-49616]

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**Introduction:** Acute renal failure (ARF) is a fatal complication of falciparum malaria, involving interplay of hemodynamic alterations, direct nephrotoxicity and immune responses leading to cellular injury manifesting as acute tubular necrosis (ATN). We attempted to evaluate the clinical and histopathological profile in renal dysfunction.

**Methods:** A prospective study carried out in a tertiary care Hospital in India. Patients of both sexes age >14 years with positive peripheral blood smear were selected for biochemical and histopathological analysis. 20 patients of ARF were subjected to renal biopsy/necropsy.

**Results:** Out of 622 patients of malaria admitted in malaria ward 146 (23.47%) developed acute renal failure. Out of 104 patients were *P. falciparum* positive, 24 patients were positive for *P. vivax* and 18 patients were positive for both Pf and PV parasite. 42 (28.7%) patients died due to acute renal failure in which 28 (66.7%) patients died due to oliguric acute renal failure while 14 (33.3%) patients died due to non-oliguric acute renal failure. 104 (71.23) patients survived after treatment which included haemodialysis. Severe malaria with acute renal failure causes structural changes in renal tissue, which manifest as ATN with or without mesangioproliferative Glomerulonephritis. Haemodialysis can bring a significant improvement and should be initiated as early as possible. 28 (66.6%) patients of non-oliguric acute renal failure had acute tubular necrosis while 90% of oliguric acute renal failure had mesangioproliferative GN and acute tubular necrosis (ATN).

**Interpretation:** The increasing rate of complications in malarial illness may be due to increasing resistance to chloroquine, the first line drug. We feel that the changing virulence and pathogenesis of parasite is important contribution to increasing renal complications. This fact is further strengthen by renal biopsy/necropsy results which showed mesangioproliferative glomerulonephritis.

**309C**

Ability of adult patients, physicians and hospital laboratories to diagnose malaria [MIM-AT-62226]

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**Introduction:** The proper diagnosis and treatment of malaria is important to control the disease. The study was carried out at five health services in the Buea sub-division to evaluate the correlation between adult patients, clinical and laboratory diagnosis of malaria.

**Methods:** One hundred and twenty-six volunteer febrile patients were interviewed before consultation on their
ability to diagnose malaria. The Physician’s provisional diagnosis, hospital laboratory results and antimalarial drugs prescribed were recorded. Duplicate thick blood films from patients were made in the hospital laboratories and examined later as reference laboratory results. The Patient’s, clinical and hospital laboratory diagnosis of malaria were compared with the reference laboratory diagnosis.

Results: The frequency of correct malaria diagnosis of patients, Physicians and hospital laboratories was 69.1, 73.8 and 63.9%, respectively and were significantly higher than wrong diagnosis (P < 0.005, P < 0.005, P < 0.05, respectively). Gender, age educational status and occupation did not influence the ability of patients to correctly diagnose malaria. 64.7% of the patients took self medications before consultation and was mostly antimalarial drugs, predominantly (78.7%) quinine sulphate. There was no significant difference between prior antimalarial drug therapy and the prevalence nor density of parasitaemia (P > 0.996). The most prescribed antimalarial drug was quinine sulphate (84.5%). 70.9% of patients did not employ any of the available malaria control methods.

Interpretation: Our results suggest that the majority of patients can correctly diagnose malaria in our community. Clinical diagnosis of malaria is more reliable than hospital diagnosis in the study area.

14. Vaccine development

Posters 310–325
Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

310A
An interferon gamma elispot assay for quantitation of T-cell responses against pre-erythrocytic malaria vaccine candidate antigens [MIM-DA-143360]

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Introduction: Cell-mediated immunity appears to be crucial for the prevention and control of diseases. Immune responses against pre-erythrocytic stage malaria can be assessed largely by T-cells and related interferon-gamma release, in addition to humoral immune responses. We used an Elispot Assay to characterize responses to CSP, SSP2, EXP1, LSA1 and LSA3 vaccine candidate antigens and to establish baseline immune responses of semi-immune Ghanaian volunteers prior to a malaria vaccine trial.

Methods: We enrolled 41 volunteers aged 18–55 years, eligibility requirements being male or non-pregnant and non-nursing female, haemoglobin greater than 10 g/dl, CD4+ T-cell counts greater than 400 and negative hepatitis B and C serology. Venous blood was collected in heparinised tubes and peripheral blood mononuclear cells (PBMCs) were separated by centrifugation, washed and counted. We used the in vitro IFN-gamma Elispot Assay to measure naturally acquired T-cell responses to peptides from CSP, SSP2, EXP1, LSA1 and LSA3 antigens that are expressed in the sporozoite or hepatic stages of the Plasmodium falciparum. PBMCs were stimulated with 33 short peptides and 25 long peptides. Positive and negative controls were run in parallel.

Results: For assays with class I binding peptides, 8/96 assays were positive with Elispot frequencies of 73–258 IFN-gamma spot forming cells (SFC)/106 PBMC. With the longer (15–34 mer) peptides 33/169 assays were positive with Elispot frequencies of 70–430 SFC/106 PBMC. Overall, 17 of the 30 evaluable volunteers had detectable IFN-gamma responses to at least one of the Plasmodium falciparum peptides.

Interpretation: For assays with class I binding peptides, 8/96 assays were positive with Elispot frequencies of 73–258 IFN-gamma spot forming cells (SFC)/106 PBMC. With the longer (15–34 mer) peptides 33/169 assays were positive with Elispot frequencies of 70–430 SFC/106 PBMC. Overall, 17 of the 30 evaluable volunteers had detectable IFN-gamma responses to at least one of the Plasmodium falciparum peptides.
Correlation of MSP-1 processing inhibitory and blocking antibodies with age and parasitaemia in individuals naturally exposed to malaria in Igbodra (MIM-HA-320005)

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Introduction: Considerable evidence that antibodies to MSP-119 can confer protection against malaria has been reported and monoclonal antibodies that inhibit MSP1 secondary processing also prevent invasion suggesting that this is their mechanism of action.

Methods: The ability of antibodies in the sera to block the binding of processing-inhibitory monoclonal Abs 12.8 and 12.10 to MSP-119 was examined with competitive ELISA. The binding of biotinylated mAbs 12.8 and 12.10 to recombinant MSP-119 in the presence of saturating concentrations of polyclonal antibodies was analyzed by competitive ELISA. Competitive ELISA was used to delineate blocking antibodies in polyclonal serum samples and also define the binding specificities of the polyclonal antibodies produced within the age groups in natural malaria infection. Blocking antibodies are operationally defined as antibodies which lack MSP-1 secondary processing inhibitory activity but which compete with mAbs 12.8 and 12.10.

Results: The proportion of processing inhibitory antibodies was highest (22%) in children 5–12 years. This age group had the lowest mean parasite density for children below 12 years old and the lowest non-specific neutral MSP-119 antibodies within the entire population studied. Only one subject (3%) over 40 years old produced antibodies that completely abrogated processing of MSP-142. None of the children below 6 months had antibodies that completely inhibited MSP-142 processing. Processing inhibitory antibodies increased with age. The competition between polyclonal sera and mAbs 12.8 and 12.10 for the binding sites on MSP-119 showed that the percentage of individual polyclonal serum samples that reduced the binding of biotinylated mAbs 12.8 and 12.10 to recombinant MSP-119 varied between age groups. The level of competition between mAb 12.8 and 12.10 varied within individual serum samples and also within age groups.

The percentage of serum samples from each age group that reduced the binding of mAbs 12.8 and 12.10 to less than 20–65% varied within and between age groups. Interpretation: The marked competitive effect of these serum samples suggests the presence and importance of antibodies to the first epidermal-like growth factor domain of MSP-119 within the entire population studied.

312C
Processing of Plasmodium berghei sporozoite antigens by mouse primary hepatocytes and presentation to specific CD8+ T cells (MIM-SB-185031)

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Introduction: Immunity to the liver stage of malaria is dependent on malaria-specific CD4+ and CD8+ T cells. Very little is known about how antigens derived from live sporozoites are processed and presented to CD8+ T cells. We therefore studied the ability of primary hepatocytes to process and present a circumsporozoite protein (CSP) derived peptide to specific CD8+ T cells in vitro.

Methods: Primary hepatocytes from Balb/c mice (H-2Kd) were isolated by perfusion of the liver with collagenase. They were either infected with P. berghei sporozoites or pulsed with the P. berghei CTL epitope peptide (PbCS (252–260)), then co-cultured with H-2Kd-restricted CD8+ T cells specific for the PbCS (252–260) epitope. Production of IFN-γ by the CD8+ T cells was determined by intracellular cytokine staining and FACS analysis. Unpulsed hepatocytes and hepatocytes incubated with salivary gland material from uninfected mosquitoes were used as negative controls. For inhibition studies, hepatocytes were first incubated with different inhibitors of the processing pathways before pulsing with the peptide or infection with sporozoites.

Results: We observed that the CSP is optimally processed and presented by primary hepatocytes 8–16 h after infection with sporozoites. It is processed by the proteasome, since processing and presentation were strongly inhibited by proteasome inhibitors, as well as by the golgi transport inhibitor, brefeldin A. On the other hand, no inhibition was observed with the pro-
tease inhibitors leupeptin and pepstatin. These results suggest that sporozoite antigens are processed and presented in vitro and possibly in vivo via a cross-presentation pathway.

Interpretation: Knowledge of how malaria antigens are processed for presentation to CD8+ T cells could open new avenues for vaccine design where effective stimulation of CD8+ T cells is needed.

313A
Improved cross-strain recognition of polymorphic Plasmodium falciparum PlEMPI CIDR1-as induced by shuffled PlEMPI CIDR1-a vaccine antigens

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Introduction: The PlEMPI CIDR-1a domain of P. falciparum is a prime target for the development of a malaria vaccine because it is exposed on the surface of the infected erythrocyte, antibodies to variant surface antigens are associated with resistance to disease, and immunization of Aotus monkeys provides protection in a strain-specific manner. Cross-strain protection by immunization with CIDR-1as is currently limited by the antigenic polymorphism of CIDR-1a from different P. falciparum strains.

Methods: We have used DNA shuffling to generate libraries of chimeric CIDR-1a antigens in order to develop a novel PlEMP-1 vaccine that induces cross-strain protection. After using multigene and synthetic shuffling strategies, chimeras were selected from recombinant libraries for improved protein expression, CD36 receptor binding, and improved cross-strain immunogenicity. We immunized mice with shuffled CIDR-1as and parental CIDR-1as using both protein immunization and DNA immunization procedures. Sera from immunized mice were tested for reactivity with CIDR-1as from various P. falciparum strains displayed on the surface of CHO cells using a whole cell ELISA approach.

Results: DNA immunization alone resulted in a significant cross-reactive antibody response, but this response was lower than that produced by a DNA prime-protein boost format, which yielded higher titers and also recognized parasitized erythrocytes. Several shuffled CIDR-1a vaccine antigens induce antibodies with extended cross-strain recognition of CIDR-1as compared to parental CIDR-1a vaccine antigens. The improved cross-reactivity of chimeric, CIDR-based variant antigens was achieved using both multigene DNA shuffling and synthetic shuffling. Multi-gene DNA shuffling uses multiple parental genes that have multiple regions of sequence similarity and allows these genes to recombine in vitro in ways that mimic homologous recombination. The second approach of synthetic shuffling captures amino acid diversity of a family of genes and provides a means to incorporate predetermined levels of diversity into specifically defined domains of the polypeptide. Iterative shuffling and screening of chimeric CIDR-1a vaccine antigens is being done to further improve immunity across P. falciparum strains.

Interpretation: These results indicate that DNA shuffling strategies can be important tools in the development of new vaccines against pathogens that have multiple serotypes or undergo antigenic variation.

314B
Analysis of an LSA-1 DCTag adjuvanted vaccine

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Introduction: We test an adjuvant, DCTag, believed to have widespread applicability for Ag presentation. Immunological attack against some Ags needs to be antibody driven, while others require CD4 or CD8 dependent cellular or cytokine associated help. A protein expressed during the P. falciparum liver-stage development, liver stage antigen-1 (LSA-1), has been identified as a potential target for protective immunity against malaria. We will evaluate DCTag with a new LSA-1 based vaccine.
Abstracts / Acta Tropica 95S (2005) S1–S506

**S311**

Methods: To use DCTag, the antigen is conjugated onto nano-beads of defined size and surface chemistry that aid in their targeting to dendritic cells. We used ELISpot assays and ICS for detection of INF-gamma and other lymphokines in A/J, Balb/c and C57BL/6 mice in response to a GMP produced LSA-1 vaccine construct, LSA-NRC. Our study assesses the immunogenicity (humoral and cellular) of nano-bead-LSA-NRC conjugates in mice in a side-by-side comparison with Montanide ISA 720. Spleenocytes from immunized mice were primed in vitro with full length LSA-NRC protein, pools of overlapping peptides or individual 15-mer LSA-NRC peptides. Anti-CD4 or anti-CD8 antibodies were used to determine cell type dependence on cytokine responses.

Results: Initial results show that LSA-NRC protein in either Montanide or DCTag adjuvants produced high antibody titers and CD4 T-cell dependent high INF-gamma production. We have mapped the responses to 3–5 specific peptides within the protein. Interestingly, C57BL/6 mice were completely non-responsive to LSA-NRC, when it was delivered with Montanide ISA 720 resulting in no antibody or INF-gamma production. The results of the DCTag-LSA-NRC vaccine in C57BL/6 mice are being evaluated.

Interpretation: The results will aid us in determining the usefulness of the DCTag nano-bead approach and its applicability in a multi-stage vaccine formulation.

**315C**

Developing a deployable vaccine for Africa [MIM-TL-0]

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Introduction: There is a need for an effective vaccine that reduces the incidence of severe malaria in African children and so impact malaria’s burden on public health. Alongside the complexities of the science there are several fundamental process and strategic factors that must be considered by those developing malaria vaccines. These need to be built into programmes in order that any resultant vaccine is appropriate for deployment in Africa.

Methods: The final recipient of the vaccine, children under 5, must be the focus of the entire programme. To justify the introduction of the vaccine into vaccination programmes efficacy would need to be high enough and be long lasting. The regime would also need to fit within current childhood vaccination programmes. New vaccines have complex technologies, and thus will be expensive and require purchasing by public funds which would need to be secured and sustainable. A malaria vaccine would be the first new vaccine to be introduced straight into Africa. This brings several implications from licensing issues through to the requirements of a phase IV programme.

Results: Several different types of malaria vaccines are being developed and better and longer lasting efficacy might be achieved by combining two or more of these approaches than might be obtained by any single theory. It is likely that many African authorities would want to see a European or American licence approval before they granted their own, so careful consideration of regulatory strategy would be needed in conjunction with an early application for orphan drug status for the vaccine. Prior to the introduction of a new vaccine into widespread community use data on effectiveness and long-term safety would be needed via a comprehensive phase IV programme. Technology in vaccine development is expensive so a new vaccine is likely to cost several tens of dollars. Advanced purchase schemes will be needed to ensure that the vaccine completes the later stages of development and manufacture, and for African government to be able to afford to use it. There are initiatives for such funding schemes and these would need to be put in place well before a government wishes to deploy a vaccine.

Interpretation: These issues are fundamental elements of development plans. Vaccines are being developed by industry, public agencies and in public private partnerships. Consideration of the benefits of each model should assess best practice and then lessons shared.
**316A**
Differential var gene group transcription using real time PCR [MIM-TL-0]

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Introduction: The var gene encoded variant surface antigen family Plasmodium falciparum membrane protein-1 (PfEMP1) is an important target for protective immunity and implicated in parasite sequestration. The newly sequenced parasite genomes indicate that all parasite var gene repertoires are similarly structured by sequence similarities in both upstream and coding regions. We exploited this to design real time primers for quantification of var genes groups A, B and C in field isolates.

Methods: The 3D7 genome was used as template for all primer design. Primers for the inter-parasitic conserved var genes (var1, var2) were designed to target conserved regions in their coding sequences. The majority of var genes are flanked by one of the conserved upstream regions upsA, upsB or upsC. Primers were designed to target conserved regions of the upstream regions upsB and upsC, whereas attempts to design usable primer pairs targeting upsA were unsuccessful. Instead we exploited coding sequence similarities specific for group A var genes to design primers targeting these genes. Quantitative real-time PCR was performed using seryl-tRNA synthetase and fructose-bisphosphate aldolase as endogenous controls.

Results: All primers were tested on 3D7 gDNA to determine amplification bias and efficiencies. Determination of primer target bias in 3D7 was not performed, however, all primer pairs yielded fragments of the expected size and their expected identities could be confirmed by sequencing. No primer pair designed to amplify more that one gene were found to have the predicted primer bias. However, all primers had amplification efficiencies around 100%. Primers were then tested on gDNA from 100 Tanzanian field isolates. All primers had similar amplification bias and efficiencies and all primers amplified fragments with the expected melting temperature and size. Thus, the primers can not quantify the absolute the expression levels of A, B and C var genes within a sample but are able to show differential transcription between two or more samples. Primers were then tested on isogenic but phenotypically distinct 3D7/NF54 parasite pairs with known differential var gene transcription patterns. Differential transcription measured by the group specific primers was compared to the differential transcription measured by var gene specific primers. There was a good correlation between the differential transcriptions measured by the two approaches.

Interpretation: Our work demonstrates that differential transcription analysis of group A, B and C var genes in field isolates is possible and thus enables future studies on the association between var gene expression and severity of malaria disease.

**317B**
Development, standardization, and application of an in vitro Plasmodium falciparum growth inhibition assay [MIM-CL-245672]


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Introduction: An in vitro growth inhibition assay (GIA) is frequently used to evaluate the biological function of antibodies to the erythrocytic stage antigens of Plasmodium falciparum. This usually involves incubation of synchronized late-stage parasites with antisera followed by quantitation of newly invaded erythrocytes to measure their capacity to inhibit parasite invasion or growth. We have developed and standardized a method for performing GIA by measuring parasite lactate dehydrogenase (pLDH) activity.

Methods: Measurements of pLDH activity and/or antibodies that recognize pLDH specifically have been used for diagnosis of Plasmodium infection and for drug sensitivity testing. We have applied a biochemical assay for pLDH assay to GIA of blood-stage malaria parasites in a standardized fashion, and also characterized important variables related to this assay to obtain reproducible results. To limit non-specific toxic effects of sera from various species, IgG fractions were purified from animal and human sera. Subsequently we
have used this assay to test numerous sera from pre-clinical animal studies and human clinical trials with AMA1, MSP1 and other parasite proteins.

**Results:** A number of the variables involved in the GIA were assessed, including intra-assay variability and the relationship between the percentage parasitemia and biochemical measurement of pLDH. The percentage CV of OD650 in triplicate wells was less than 5%. For parasitemia ranging from 0 to 4.3%, pLDH (OD650) correlated linearly with parasitemia by microscopy ($p < 0.05$, $R^2 = 0.998$). Specificity of the inhibition was established by reversal of in vitro growth inhibition with specific parasite proteins. Using this assay antisera or IgG fractions obtained from Aotus, rhesus, mice, rabbits, guinea pigs, rats and humans demonstrated inhibitory activity in vitro. This assay has also been used to study the GIA activity of human sera and IgGs from residents of malaria endemic areas and to explore mechanisms of antibody-mediated parasite invasion/growth inhibition. With support from the Malaria Vaccine Initiative/PATH, progress has been made in the establishment of a growth inhibition assay (GIA) Reference Center which provides protocols, trains visiting investigators, and tests selected human and animal sera for GIA activity. Modifications of the GIA to allow high throughput of samples being are being evaluated.

**Interpretation:** This GIA method provides an important tool to collect significant amounts of data with animal and human sera (or IgGs) from clinical trials in a standardized fashion and to allow comparison of results from different laboratories.

318C

**Antibody responses to glutamate rich protein (GLURP) and merozoite surface protein (MSP3), in Ghanaian children [MIM-HN-19740]**


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**Introduction:** GLURP and MSP3 are targets for antibodies involved in antibody dependent cellular inhibition that may lead to protective immunity against malaria. Comparative assessment of IgG and subclass responses to GLURP and MSP3 in relation to immunity against malaria will provide relevant information that will be useful in future malaria vaccine development.

**Methods:** The levels of plasma antibodies to GLURP and MSP3 were measured by Enzyme-linked immunosorbent assay (ELISA) in samples obtained from a cohort of 300 children, 3–15 years of age. The plasma samples came from a previous longitudinal morbidity survey carried out over a period of 18 months (1994–1995) covering two malaria transmission seasons, in which children were classified as susceptible or resistant to malaria.

**Results:** The prevalence of IgG responses to GLURP and MSP3 in the cohort were 48 and 41%, respectively. The pattern of IgG subclass responses to both antigens were similar, indicating higher prevalence for cytophilic antibodies than non-cytophilic antibodies. The prevalence of IgG response to either GLURP or MSP3 was 60%. Spearman’s rank-order correlation coefficient analysis revealed that IgG and subclass responses to both GLURP and MSP3 increased with age (0.54 > $r$ > 0.35, $p < 0.001$), for all subclasses compared. The association between antibody levels and protection was statistically significant for GLURP IgG ($p = <0.001$) and MSP3 IgG ($p = <0.001$), and for cytophilic IgG1 and IgG3 (0.009 > $p$ > 0.001) for both GLURP and MSP3 (Mann-Whitney Rank Sum Test).

**Interpretation:** Naturally acquired IgG, especially cytophilic antibodies against GLURP and MSP3 correlate with protection from clinical malaria. The complementarity of antibodies against both antigens supports, their use as a hybrid in a future malaria vaccine.
319A
Cytophilic antibodies against AMA1, MSP119, MSP3 and GLURP protect Ghanaian children from clinical malaria [MIM-AO-25320]
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Introduction: Antibodies play an important role in the age and exposure-dependent acquisition of immunity against malaria. Although most studies have measured antibodies against several antigens, few have assessed the antibodies against several antigens collectively, using the same standardized protocols. We used the same standardized ELISA protocols to assess the role of IgM, IgG and IgG subclasses to four malaria vaccine candidate antigens and to relate the responses to protection from clinical malaria.

Methods: We measured IgM, IgG and IgG subclasses’ levels in pre-malaria transmission season plasma samples from a cohort of 352 Ghanaian children aged 3–10 years, against the apical merozoite antigen 1(AMA1), Glutamate-rich protein (GLURP), the merozoite surface proteins, MSP119 and MSP3 and crude malaria schizont antigen using the same ELISA procedure. Antibody levels for children who were protected against clinical malaria were compared with those of susceptible children, based on morbidity data collected during the study.

Results: Except for MSP3 where the IgM prevalence was twice that of IgG, IgM responses to AMA1, MSP119 and GLURP were comparable to the prevalence of the corresponding IgG responses in all the children. The prevalences of the IgG1 and IgG3 for MSP3, MSP119 and GLURP in all children were always higher than non-cytophilic IgG2 and IgG4. IgG subclass response to AMA1 was predominantly IgG1 (>95%). Levels of both IgM and IgG against all recombinant antigens tested were significantly correlated with protection from clinical malaria (p < 0.011).

Interpretation: Combined IgG responses, especially cytophilic IgG, strongly correlate with protection against malaria in Ghanaian children.

320B
Analysis of the genetic polymorphism of the P126 N-terminal region in Plasmodium falciparum field isolates from the Brazilian Amazon [MIM-LP-27860]
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Introduction: The amino-terminal portion of the Plasmodium falciparum p126 protein, containing 6-octamer repeats, has been shown to be involved in the induction of protection against challenge in monkeys. However, was observed a polymorphism in some isolates that contained 5- instead of 6-octamer repeats. We evaluated the genetic polymorphism of N-terminal region of the P126 protein in P. falciparum isolates from Brazilian endemic areas and its possible role in development of specific immune response.

Methods: The study was carried out in Candeias do Jamari, a rural area situated about 70 km from Porto Velho, the capital of Rondonia State, in the southwestern part of the Brazilian Amazon and in Peixoto de Azevedo village, which is localized in the Mato Grosso State in the southern Brazilian Amazon Forest. Blood samples were taken from 93 individuals from Peixoto de Azevedo and from 104 individuals from Candeias do Jamari. The repetitive region of the gene encoding the P126 antigen was amplified in a Nested PCR method using specific primers and each amplified fragment was analyzed by SSCP. The humoral response
was evaluated by ELISA using the Nt47 synthetic peptide corresponding to the repeat region of the amino terminus of P126.

**Results:** We only detected two different allelic fragments in both area studied: one of 175 bp corresponding to the 5-octamer repeat region (allele I) and the other one of 199 bp corresponding to 6-octamer repeat region (allele II). In Candeias do Jamari, the allele II was detected in a higher frequency (92.6%) than allele I (7.4%). In Peixoto de Azevedo while the frequency of the allele II was higher in Candeias do Jamari. Analysis by PCR-SSCP does not revealed DNA microheterogeneities of sequences between fragments with same size and only one SSCP pattern was observed for each fragment identified. We observed a high frequency of IgG and IgM antibodies in Candeias do Jamari and in Peixoto de Azevedo. The frequency of individuals presenting IgG1 antibodies was higher in Candeias do Jamari. Not only the frequency of responders but Candeias do Jamari also showed higher levels of IgG1 antibodies than did Peixoto de Azevedo. However, no association was observed between allelic fragments and the humoral immune response against Nt47.

**Interpretation:** The data presented here show that the limited genetic polymorphism of the P126 observed in *P. falciparum* isolates from Porto Velho and Peixoto de Azevedo does not seem to influence the development of specific immune response in infected individuals.

**321C**

### Adjuvant formulations for recombinant malaria vaccines [MIM-AS-77610]


**Malaria Vaccine Development Branch, NIAID, National Institutes of Health, Rockville, MD, USA**

**Introduction:** Proteins vaccines rarely work optimally without the addition of adjuvants. Malaria vaccines may represent extreme cases: we need high level, long duration immune responses, in formulations that contain multiple antigens, with major limits on cost. The pressing need for effective vaccines and the high incidence rate of malaria in people living in endemic areas has meant that malaria vaccines have often been used as test beds for adjuvant/formulation development.

**Methods:** A major limitation of malaria vaccine development, especially in the public sector, has been the development of assays to adequately characterize vaccine formulations. Assays for measuring binding and dissociation of proteins and immunostimulating agents such as CpG to particulate adjuvants (e.g. Alum), for droplet sizes for emulsion and for chemical stability of proteins and other components are critical to enable meaningful analysis of immunogenicity in animal models and in humans. The complexity of QC required increases rapidly with multiple immunostimulating components and multiple antigens.

**Results:** Optimizing the formulation is critical to achieve both good immunogenicity and in some cases, safety. For example, poor binding of proteins to alum leads to poor immunogenicity, but crucially, poor binding is a high risk factor for generating systemic hypersensitivity reactions. Binding of protein to alum is modified by the presence of CpG and vice versa, and both are critically dependent on the presence of buffer components. Water-in-oil formulations frequently give high antibody responses, but as these are at least in part dependent on generating long-term antigen depots post-vaccination, antigen stability in these formulations both during storage and post-injection may be critical. Assessing the impact of changes in formulations on immunogenicity in humans often depends on extrapolations from animal models and this remains an area of controversy.

**Interpretation:** Reliable assessment of the relative merits of different formulations requires investment in the QC of formulations. There is a pressing need for access to expertise in formulation especially in the public sector.

**322A**

### Analysis of the parasitophorous vacuole proteome by one- and two-dimensional electrophoresis [MIM-MT-71802]

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**Introduction:** Most blood stage proteome studies focus on merozoites. However, important blood stage proteins may be secreted into the parasitophorous vacuole (PV)
as is the case for the glutamate rich protein (GLURP). Therefore, characterization of the protein composition of the PV is important both for understanding the molecular biology of the blood stage and for vaccine candidate identification. In this study we compare the protein profile of purified PV to that of free merozoites.

**Methods:** A synchronized *P. falciparum* F32 culture was allowed to develop into middle stage schizonts and after treatment with the protease inhibitor E64 a high number of PV membrane enclosed merozoite structures (PEMS) was obtained. The preparation of PEMS by protease inhibitor treatment was originally described by Salmon and co-workers who found that, in opposition to purified merozoites, merozoites from PEMS are infectious. The PEMS preparation was analyzed and compared to merozoite preparations by one- and two-dimensional SDS PAGE and western blotting using human immune serum as primary antibodies and IgG subclass specific secondary antibodies.

**Results:** By an improved purification method we obtained more than 95% pure PEMS. As the protein composition of the PEMS has never been investigated we compared the protein profile of PEMS preparations to that of merozoites purified from culture supernatants by one- and two-dimensional SDS–PAGE. It was evident that several proteins were present in PEMS, which were absent from merozoites. Furthermore, when protein preparations were reacted with serum from malaria immune adult Liberians in Western blotting several protein bands were recognized in PEMS, which were not recognized in merozoites. The reaction pattern of different IgG subclasses differed greatly and distinct bands were detected which were recognized by one subclass exclusively for both IgG1, IgG2 and IgG3. Some of these bands were PEMS specific. The reaction pattern of different IgG subclasses differed greatly and distinct bands were detected which were recognized by one subclass exclusively for both IgG1, IgG2 and IgG3. Some of these bands were PEMS specific. We speculate that the difference in infectivity of purified merozoites and merozoites from PEMS may be caused by some of the proteins present in the PEMS preparation that are absent from purified merozoites. These may be merozoites surface proteins lost during purification or soluble factors of the parasitophorous vacuole. Identification of these proteins is currently being pursued by two-dimensional electrophoresis and mass spectrometry.

**Interpretation:** Immunogenic proteins were found to be present in preparations of parasitophorous vacuoles, which were not detected in merozoites. These molecules may carry out important function during the blood stage and may be vaccine targets.

**323B Isolation of Plasmodium falciparum antigens preferentially recognised by partially immune adult humans in a high transmission malaria endemic area [MIM-VT-31388]**

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**Introduction:** Numerous studies have shown that partial protective immunity does develop slowly in adult residents of malaria endemic zones. Given that the plethora of known antigens have so far not been able to yield highly protective vaccines, we postulated that a comparison of the immune responses of susceptible children and immune adults may help identify additional protective antigens.

**Methods:** Using pooled sera from susceptible children and immune adults, we differentially screened a Lambda-zap cDNA expression library constructed from *Plasmodium falciparum* blood stage mRNA. Identified clones were amplified and subcloned into TOPO-pBAD and sequenced. The sequences were analysed using bioinformatics methods. The open reading frames of interesting clones were amplified, subcloned in pBAD/THIO-TOPO and expressed in *E. coli* nova Blue cells. Antibodies specific to the clones were plaque purified and used to identify native proteins by Western blotting on crude *P. falciparum* antigens.

**Results:** We identified nine clones that were preferentially recognised by immune adults. Sequence analysis revealed these nine clones constituted three different genes arbitrarily named UB5, UB7 and UB9. UB7 open reading frame translated into a hypothetical malaria protein, which is homologous to the apicomplexan small protein of Cryptosporidium parvum. UB 9 showed 97% homology with a circumsporozoite related antigen and malaria export protein-1 (EXP-1) gene. The UB5 open reading frame was 100% homologous to a region of *P. falciparum* chromosome 10 coding for a 117 amino acid long hypothetical protein with an N-terminal signal peptide of 25 residues and two transmembrane helices. It expressed as a 40,000 recombinant fusion protein with thioredoxine. The recombin-
nant constructs were highly unstable. Plaque purified antibodies specific to UB5 identified a 30,000 native protein on P. falciparum crude antigen.

Interpretation: We demonstrated the potential of differential screening to identify vaccine candidates like the circumporozoite protein and the export Protein-1 (i.e. UB 9). The same approach was employed to select other vaccine candidates such as by UB5 and UB7.

324C
Genomic approach for Plasmodium falciparum antigen selection of erythrocytic malaria vaccine candidates [MIM-VV-283347]

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Introduction: To date no systematic genome wide protein identification process has been applied to Plasmodium falciparum (Pf). Such a process associated with a rationale down-stream analysis could identify novel malaria vaccine candidates. By taking advantage of the genomic data, high throughput peptide synthesis, bio-informatics and malaria immune sera, it is, in principle, possible to identify novel malaria proteins expressed at the erythrocytic stage in a very short time.

Methods: The identification of Pf parasite proteins, which contain “natively unstructured” or coiled-coil regions, which do not present a folding problem, was performed using “SelectSeq” and sequence profiles tools, respectively. To identify proteins that are expressed in the asexual parasite blood stage we used the proteomic and transcriptomic data available in the literature. The selected antigens were synthesized and characterized. Antibody recognition of peptides was assessed by ELISA screening with 37 sera from adults living in endemic area (Burkina Faso). 22/30 peptides (73%) were recognized by human adult sera. The ELISA results showed that 11 peptides were well-recognized with end-point titers between 1350 and 109 350. Several peptides were recognized by 40–50% immune donors. Mice immunized with these peptides showed variable humoral response and some sera were positive in immunofluorescence assays on infected red blood cells. One identified protein of about 140 amino acids was synthesized and found to be recognized by 80% of immune sera.

Interpretation: These results showed the power of this simple, low cost approach to select potential vaccine candidates and to assist the recombinant DNA technology approach, which is still costly and time consuming.

325A
Aiming for sustained high-level antibody responses—Formulation of Pf25 and Pvs25 transmission blocking vaccines [MIM-YW-345345]

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Introduction: The mode of action of mosquito stage transmission blocking vaccines is through antibody-dependent inhibition of the parasite development. To be effective, the vaccine-induced antibody responses should last at least throughout one transmission season. The current undertaking is to search for a formulation for Pf25 and Pvs25 that is capable of inducing sustained high-level antibody responses, and is capable of completely blocking the parasite transmission by mosquitoes.

Methods: Recombinant Pf25 and Pvs25 were formulated with various adjuvants. The formulated vaccines were given to mice, rabbits, and rhesus monkeys. Serum antibody levels in immunized animals
were determined by ELISA. The transmission blocking activity of these sera was measured by membrane feeding assays.

Results: Despite the promising evidence that the Pvs25/Alhydrogel® induced antibodies can significantly inhibit the parasite development in the mosquito midgut, the formulation was not sufficiently potent to induce consistently high-level antibody responses for a complete transmission blockade. Nine additional adjuvants/formulations were tested for their ability to enhance the immunogenicity of Pfs25 and Pvs25. The water-in-oil emulsions Montanide® ISA720 and Montanide® ISA51 are among the most potent adjuvants for Pfs25 and Pvs25. The specific antibody levels induced by Pfs25/ISA51 and Pvs25/ISA51 were consistently high, sufficient to block the oocyst production in mosquitoes. Moreover, the specific antibody levels in rabbits immunized with the ISA51 formulation persisted for over a year, likely due to strong depot effect common to the water-in-oil formulations. The vaccines were well tolerated in rabbits and rhesus monkeys.

Interpretation: The Pfs25/ISA51 and Pvs25/ISA51 formulations are sufficiently potent to induce lasting high-level antibody responses required for an effective transmission blocking vaccine. A Phase 1 clinical trial on these formulations is currently underway.

15. Operational research

Posters 326–334

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

326B Factors that facilitate infant survival in a low socio-economic malaria-endemic community in Lagos state, Nigeria [MIM-CA-416885]

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Introduction: Infants are delicate, relying mostly on caregivers for their survival. The survival of infants in developing countries is plagued by diseases, poverty, behavioural habits and cultural beliefs of the caregivers. For strategies to improve infant survival in a resource-poor setting, the health seeking behaviour and home care practices of caregivers during infant illness episodes were studied.

Methods: A total of 742 caregivers in 5 out of 11 health districts of Ajeromi/Ifeodom Local Government areas of Lagos state whose infants were ill 4 weeks preceding the survey were interviewed in this cross-sectional study. Quantitative data were collected using semi-structured questionnaire. Their demographic characteristics, health seeking behaviour, home practices and perception of childhood diseases were taken with the instrument. Qualitative data were obtained through focus group discussions and in-depth interviews with caregivers, health workers and traditional healers. The data were analyzed using EpiInfo 6.04 and Textbase Beta statistical software.

Results: Sixty-two (8.4%) of those interviewed lost their infants 4 weeks preceding the study, 51.6% of infants who died did so in hospitals, 35.5% at home and 12.9% at unspecified places. More than half of the infants, 484 (67.1%) had fever of which 39 (8.1%) died. The notable causes of death were malaria (22.6%), acute respiratory infections (14.5%) and diarrhoea (12.9%). Majority of the caregivers (60.0%) whose infants died did not seek external help until 24 h or more after onset of illness signs. This shows that timing of appropriate intervention could be very crucial. For most of the caregivers (41.0%), the first line of treatment within the first 24 h of onset of illness signs is home management. Infant’s tendency to survive an illness episode was significantly associated with treatment sources ($P < 0.05$) and infant complementary feeding methods ($P < 0.05$), full term delivery ($P < 0.01$), and birth weight ($P < 0.01$). The association ($P < 0.01$) between the parity of mothers and infants’ tendency to fall ill suggests that the mothers acquire significant knowledge of the management of childhood illness as they have more children.

Interpretation: Education of caregivers on the prompt and appropriate home management of infant’s illnesses will significantly reduce deaths attributable to malaria. This can be done particularly at antenatal and postnatal clinics.
A sociological perspective of malaria complications this 21st century among children within the Buea health district, 2004 [MIM-MB-217845]

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Introduction: Despite scientific advances made at targeting the malaria parasites in terms of diagnosis, treatment and control measures, knowledge, attitudes and practices of preventing, controlling and treating malaria in children are yet to change in certain parts of our towns and villages. Majority of the population is deficit in knowledge on the severity of malaria which sometimes according to Adams et al (1984) the patient is severely ill having a heavy titer of parasites in blood yet afebrile.

Methods: A convenient sampling technique was used to select a total of 50 respondents who were either parents or guardians of severely ill children attending the health centres within the Buea health district between the months of May and June 2004. A structured questionnaire was administered on a face-to-face value to elicit information on the objectives of the study viz: health seeking behaviours, cultural belief patterns, and constrains in seeking care particularly at the early stages of the disease.

Results: One hundred percent of respondents accepted that their child(ren) had had attacks of malaria with the last attacks ranging between 1–2 weeks ago (18%) and 8 weeks ago (25%). It was but normal for a parent or guardian to start seeking care only after a few days after the attack and a few attempts at home before seeking modern health care. The home remedies were oral mixtures of herbs and medications like paracetamol or aspirin from the medicine stores or hawkers (64%) to oral mixtures of herbs and topical applications of herbs before automedication from the medicine store or hawker (26%). Three respondents (18%) would go straight to hospital when the child does not sleep well at night. Respondents hold that there exist practices on malaria attacks as well as other diseases particular when it becomes too frequent (82%), while 18% had no cultural views on malaria. The cultural views were simply related to the use of herbs that have been believed to be use over years by fore fathers. No other specific cultural barriers were identified by respondents.

The main constrains and problems associated malaria treatment were frequency of attacks (31%), financial difficulties (25%) and difficulties in the administration of drugs (2%).

Interpretation: The frequency of attacks could act as a demotivator to populations. The health seeking behaviours need sensitization. These very simple parameters could waste scientific research efforts that target diagnostic, therapeutic and control strategies.

Enhancing malaria control using a computerised management system in southern Africa [MIM-MB-35428]

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Introduction: Malaria control programmes utilising indoor residual spraying are only effective if a high coverage of targeted structures is achieved and an insecticide that is effective against the specific mosquito vector is correctly applied. Ongoing monitoring of spraying operations is essential to assure optimal programme performance and early corrective action, where indicated.

Methods: Successful development and application of a computerised spraying operations management system in Mpumalanga Province, South Africa during 1998 resulted in its adaptation and introduction in neighbouring Maputo Province, southern Mozambique during 2000. The structure and components of this computerised management system are described, and its operational benefit in southern Mozambique, where community-based spray operators apply intradomiciliary insecticide, are reviewed.
Results: The ongoing monitoring of individual spray operator’s productivity has facilitated early detection of operational problems, leading to prompt investigation and supportive corrective action. A standardized aid for evaluating insecticide application rates (grams per square meter) are constantly monitored for under- and over-application. Where sub-optimal application rates were detected, investigation included scrutiny of insecticide preparation, nozzle condition, application pressure, distance of nozzle tip from sprayed surface, and spraying rhythm. Spraying coverage exceeded 90% of 222,000 structures in southern Mozambique. In areas where the management system repeatedly identified lower coverage than expected, investigations were conducted to determine whether this was the result of diminished community support, absenteeism or poor motivation of the specific spray operator. Maps depicting the coverage achieved by each team proved useful to malaria control programme management for tracking teams’ progress, providing ongoing updates to senior health management and encouraging spray personnel to attain their target.

Interpretation: The computerised management system allowed programme management and field supervisors to monitor spraying coverage, insecticide consumption and application rates on an ongoing basis and supported a successful transition to community-based spraying.

329B
Entre discours et pratiques: Le paludisme dans une plantation au Sud Cameroun [MIM-MJ-78976]
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Introduction: Afin de mieux comprendre les raisons des échecs thérapeutiques et médicamenteux liés aux crises de paludisme subies régulièrement par les populations camerounaises qui vivent dans les zones forestières où le paludisme est persistant il est utile d’étudier, avec une approche anthropologique, les discours et pratiques des populations et des soignants qui vivent et gèrent le paludisme au quotidien.

Methods: Nous avons mené une enquête durant le 1er semestre 2002 dans une plantation du Sud Cameroun (Hévecam) à partir d’entretiens semi-directifs auprès des patients et du personnel médical et d’une observation des consultations. 272 personnes ont été ainsi enquêtées dont 19 membres du personnel soignant. L’enquête a été réalisée principalement à l’hôpital de la plantation et complétée par des entretiens avec les infirmiers des dispensaires, les cadres de l’entreprise et des discussions avec les vendeurs ambulants de médicaments. Elle s’inscrit dans le cadre du projet PaL+ Représentations, comportements et gestion du paludisme dans une plantation en forêt tropicale (Sud Cameroun), Ministère de la Recherche, France.

Result: Notre enquête révèle que le recours à une formation sanitaire est aléatoire, même pour traiter les maladies pour lesquelles les capacités de la médecine moderne sont universellement reconnues. Ce recours dépend de facteurs exogènes et endogènes à cette maladie: difficultés d’accessibilité aux soins médicaux, impact du fonctionnement hiérarchique du système sanitaire, des rapports soignants-soignés, multiplicité des lieux d’approvisionnement en médicaments et de prestations médicales, hors système qui constituent un éventail de choix et parfois des raccourcis pour les personnes en quête de soins. Les facteurs endogènes sont dépendants des convictions thérapeutiques du malade et du personnel soignant et notamment le sentiment d’accoutumance créé par la récurrence de la maladie favorise l’automedication chez les malades et entraîne, chez le personnel soignant, la prescription systématique des antipaludiques disponibles dans le secteur du médicament, qui induit une multitude de posologies et d’effets thérapeutiques inhérents à chacune d’elle, crée une diversion dans la prise en charge de cette maladie.

Interpretation: Il faut souligner que l’absence de discours de valorisation du médicament générique confère à cette catégorie de produit un statut de “substitut inefficace des spécialités”, une “médication aléatoire” tant pour les malades que pour les soignants.


**330C**

L’insertion de la moustiquaire dans le système des objets au sein de l’habitation: Approche anthropologique [MIM-MM-153329]

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**Introduction:** La moustiquaire imprégnée d’insecticide est le moyen de prévention préconisé par les institutions internationales pour lutter contre le paludisme. Pour apprécier l’efficacité de ces politiques il nous a paru intéressant d’étudier comment cet objet est introduit parmi les objets usuels et prend place dans l’espace d’habitat. L’étude s’inscrit dans le cadre du projet PAL+ Représentations, comportements et gestion du paludisme dans une plantation du Sud Cameroun du Ministère de la Recherche, France.

**Methods:** L’enquête s’est déroulée dans une plantation d’hévéas située au sud du Cameroun (HEVECAM) où résident 25 000 personnes. Elle a duré 3 mois, organisés en plusieurs séjours de terrain au cours de 2003. La collecte des données s’est faite par des entretiens semi directs sur la base d’un guide d’entretien, par l’observation directe, des échanges spontanés, notamment avec les enfants, et des entretiens de groupe. 40 ménages possédant au moins une moustiquaire, et 10 ménages n’ayant pas de moustiquaire ont été enquêtés. Dans chaque ménage le chef de ménage et son épouse ont été interrogés, et parfois d’autres membres du foyer.

Nous avons réalisé deux focus groups: un groupe de femmes (06) et un groupe d’hommes (07).

**Results:** L’approche anthropologique de la culture matérielle que nous avons réalisée à partir de l’usage de la moustiquaire nous a permis d’étudier le rapport homme/objet et la relation entre les objets, la moustiquaire étant ici traitée comme un objet parmi d’autres. L’étude montre que la moustiquaire est un objet exogène et qu’il n’a pas été prévu de place pour celle-ci. La structuration des éléments matériels qui permettent une commodité d’usage de la moustiquaire dans la chambre à coucher rend difficile son introduction dans cet espace. L’encombrement, l’exiguité, la surpopulation des pièces et des lits vont générer le déploiement de cet objet dans l’habitation. L’emplacement ou la position de certains objets comme par exemple l’interrupteur va rendre difficile le maniement de la moustiquaire dans la nuit. Par ailleurs, l’usage commun de la moustiquaire dans une même couche va provoquer des conflits d’usage mais également des distributions de rôle notamment quant à son achat et son entretien. On peut noter également qu’elle va entrer en compétition avec d’autres objets, tel le ventilateur ou les bombes d’insecticides considérés comme mieux adaptés au contexte climatique et efficaces contre les moustiques.

**Interpretation:** La description de l’habitation, de l’acquisition et de l’usage de la moustiquaire montre que son usage est soumis à des contraintes culturelle, sociale, économique et matérielle qui ne sont pas assez prises en compte par les programmes de prévention.

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**331A**

The dynamics of endemic malaria in growing populations [MIM-GN-30053]

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**Introduction:** Control measures either reduce contact between mosquitoes and humans or reduce the infectivity of the human host. Drug coverage can be effective only when permanent prophylaxis is employed across an entire endemic human population. This is costly. With global warming, temperate regions where malaria had been eradicated risk being re-colonised by malaria if the mosquito vector is still present there. Mathematical modelling provides a window to study the dynamics of malaria transmission.

**Methods:** We derive a suitable mathematical model that captures the dynamics and transmission of endemic malaria in growing populations. Using a perturbation analysis, we obtain an approximation to the solution of the differential equation model in the important cases where disease related death rates are significant and the population is not constant. Using a diffusion approximation coupled with numerical simulations, we study how stochastic variability affects the transmission dynamics of malaria.

**Results:** A mathematical model for endemic malaria involving variable human and mosquito populations is developed and analysed. A threshold parameter $R_0$ exists and the disease can persist if and only if $R_0$
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exceeds 1. $R_0$ is seen to be a generalisation of the basic reproduction ratio associated with the Ross-Macdonald model for malaria transmission. The disease free equilibrium always exists and is globally stable when $R_0$ is below 1. A perturbation analysis is used to approximate the endemic equilibrium in the important case where the disease related death rate is nonzero, small but significant. A diffusion approximation is used to approximate the quasi-stationary distribution of the associated stochastic model. Numerical simulations show that when $R_0$ is distinctly greater than 1, the endemic deterministic equilibrium is globally stable. Furthermore, in quasi-stationarity, the stochastic process undergoes oscillations about a mean population whose size can be approximated by the stable endemic deterministic equilibrium. The number of fatalities due to malaria can be determined.

Interpretation: Asymptomatic infective carriers complicate control. Abruptly lowering vector numbers has little effect on malaria prevalence. The human population should be examined considering the phenomenon of incomplete immunity permitting disease transmission.

332B

Prevalence and clinical profile of malaria among settled Fulani pastoralists in southwest Nigeria [MIM-380950]

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Introduction: The Fulani are often described as the largest nomadic group in the world and are found in most parts of semi arid and arid West Africa. They are nomadic because of the need to provide fodder and water for their cattle. In the last couple of decades however a large number of them are voluntarily settling down in the humid and sub-humid zones of Nigeria. This paper investigates their health with specific focus on malaria.

Methods: The study was carried out between March 2002 and December 2004. The study used a combination of participatory epidemiological survey, geographical information system (GIS) and laboratory tests of serum samples to determine the prevalence, spatial distribution of malaria parasites and clinical profile of malaria, respectively, in three states: Oyo, Ogun and Kwara, in southwest Nigeria.

Results: Out of the 527 serum samples screened for malaria parasite infection 177 (33.6%) were positive. Comparing across the States, Kwara had the highest prevalence of 69 (39.0%) in Oyo State it was 53 (29.9%) while in Ogun State 55 (31.1%). There was a significant difference in prevalence between the three states ($X^2 = 10.964; d.f. = 2; P = 0.004$). Out of the positive cases, 42.9% were male, while 57.1% were female. Female were significantly more infected than male ($X^2 = 8.031, d.f. = 1; P = 0.005$). With regards to age groups 54.8% were children while 45.2% were adult. Children were significantly more infected than adult ($X^2 = 29.091, d.f. = 1; P = 0.0005$). Out of the malaria positive cases, 88.1% was due to *Plasmodium falciparum* while 11.9% were caused by *Plasmodium malariae*. The overall PCV ranges from 13.0 to 51.0 with a mean of 36.3%, SE of mean $= 0.2$, while that of Hb ranged from 4.4 to 19.8, with a mean of 12.2 g/dl, SE of mean $= 0.1$. The results show that the pastoral Fulani had low PCV and Hb as the mean were below the normal range.

Interpretation: The high levels of malaria infection among settled Fulani women and children could be attributed to their settlements close to rivers, unprotected dwellings, exposure to mosquito bites and lack of access to insecticide treated nets.

333C

Assessment of the therapeutic efficacy of artemether–lumefantrine (Coartem®) and sulphadoxine–pyrimethamine (SP)–artesunate in Zambian Children [MIM-NS-19550]


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Introduction: Zambia has since 2003 adopted artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated malaria. The decision was based on the increase in chloroquine treatment failures as high as 52% in 2002 and sulphadoxine-pyrimethamine was between 7.5 and 32.6% in 2003 in the sentinel districts. The drug has been reported to be highly effective against *P. falciparum* in areas of multi-drug resistance.

Methods: The study is a simple, one-arm, prospective evaluation of the clinical and parasitological response to directly observed treatment for uncomplicated malaria. The study was conducted in five sentinel sites of Zambia using the WHO standardized protocol (WHO, 2001). Coartem® was given twice a day for 3 days according to the standard treatment guidelines. SP-artesunate was given once a day for 3 days in line with the recommended treatment schedule. In 2003, 14-day adequate clinical and parasitological responses (ACPRs) were determined. 28-day ACPRs were established in 2004 for each drug. Additionally, MSP1 and MSP2 genotyping was performed to differentiate recrudescence from reinfection.

Results: In all the sites where the study was conducted there were no reports of severe adverse events reported. Of the 183 children on Coartem, only one had parasitaemia on day 14. It was however not established if this was a new infection or recrudescence. SP-artesunate registered lower treatment failures in the sites where SP treatment failures in the country were the highest.

Interpretation: Acts are a long-lasting solution for multi-drug resistance. SP-artesunate can not rely upon given the high treatment failure rates to SP which is supposed to act as a longer acting component of the drug. Parasite clearance was good in both drugs.

334A
How do patients use antimalarial drugs combination therapy? Curative treatment for children suffering uncomplicated malaria in rural Senegal [MIM-AS-19340]
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Introduction: Increased *Plasmodium falciparum* resistance to chloroquine has prompted national malaria programmes in several African countries to develop new policies. Less than a year after the introduction of amodiaquine/sulfadoxine-pyrimethamine (AQ/SP), as first-line treatment in Senegal we examined health staff and public adherence to therapy and its efficacy among children.

Methods: The study was conducted in five rural dispensaries in Mbour, a district 70 km south from Dakar, Senegal. Children, aged between 2 and 10, who presented fever (>38 C) were prescribed AQ/SP. Those with parasite density above 2500/μL had blood and urine samples collected at D3 for drug level measurements; thick blood film analyses were carried out at D3, D7, D14 and D28. For all children, the principal caretakers were questioned on the compliance with the treatment and pill counting was performed. Interviews were conducted with 32 families for more information about their perception of the new therapy. A follow-up over a week was organized in each dispensary regarding the explanations of the AQ/SP prescription delivered by health staff.

Results: Three hundred and fifty-eight children were recruited. Among them, 143 with a confirmed diagnosis of uncomplicated malaria were included for the subsequent biological analysis. The results demonstrated a markedly good efficacy of the treatment as no detectable parasitaemia was observed at D3 and D7 for 87 and 99% of the children, respectively. However, we noticed that 12.8% of patients did not take SP and non-adherence to the full therapeutic dose required was also observed (37%) did not respect the drug regimen and
49.3% of the people we interviewed thought it was unnecessary to continue treatment until the end; 13.4% declared that one can stop when the child did not appear sick anymore. Ten percent thought the treatment was difficult to administer to their child in particular due to bitterness of AQ. Caretakers said they were not provided with enough information neither during the consultation nor at the pharmacy. At the end of the consultation most of them could not mention the correct daily home-administered dose for each day to be given at home.

Interpretation: Despite good clinical and biological efficacy, adherence to therapeutic scheme was poor. Strategies to promote patient adherence would improve drug performance and thus prevent rapid drug resistance emergence.

16. Insecticide resistance
Posters 335–351

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

335B
Knockdown resistance-type pyrethroid resistance in Anopheles gambiae s.s. from south-eastern Ghana

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Introduction: Pyrethroid resistance due to a knockdown resistance (kdr) type mechanism is widespread in West African Anopheles gambiae s.s. However, in Ghana the insecticide resistance status of this vector had not been investigated. As pyrethroid-impregnated bednets are the main focus of future malaria control programmes in Ghana, it was necessary to determine the vulnerability of this malaria vector to three of the pyrethroids that are recommended by the WHO for bednet impregnation.

Methods: Anopheline larvae were collected from georeferenced sites in south-eastern Ghana. Larvae were reared to adults and identified morphologically as Anopheles gambiae s.l. More than 1400 female mosquitoes from these collections were used in WHO pyrethroid susceptibility tests. PCR-RFLP assays for species identification and diagnostic PCR detection of the target-site insecticide resistance kdr alleles were also carried out on 527 specimens randomly selected from the mosquitoes that were used for the susceptibility tests.

Results: All 527 specimens were identified by PCR-RFLP as An. gambiae s.s., with the following distribution of molecular forms: S = 95.45% (503/527); M–S hybrids 0.95% (5/527); and M = 3.60% (19/527). The forms, as anticipated, were not in Hardy-Weinberg equilibrium. The bioassay results showed that there was two- to nine-fold pyrethroid resistance in the field-collected mosquitoes compared with a pyrethroid susceptible laboratory strain, An. gambiae Kisumu. The frequency of the kdr mechanism was very high (0.894) and occurred predominantly in the S form but was not detected in the M form.

Interpretation: Although kdr may not necessarily affect the efficacy of pyrethroid-impregnated bednets it is important that its long-term impact on malaria vector control programmes is closely monitored.

336C
Rapid field applicable microarray-based method for monitoring of all single nucleotide polymorphisms associated with resistance to antimalarial drugs

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Introduction: Drug resistance in malaria has been considered to be of major public health concern in malaria endemic areas. Rational drug policy and deployment of available drugs become key issues to avert the complete loss of efficacy of the available antimalarial drugs. Therefore, monitoring of drug efficacy is of crucial importance in endemic areas, but alternatives are urgently needed for the current 28 day follow-up protocol which poses major problems in terms of recruitment, costs, and compliance.

Methods: We have designed a micro array containing features of all known single nucleotide poly-
morphisms (SNPs) in the drug resistance associated genes pfdhfr, pfdhps, pfcrt, pfmdr1, and pfATPase. After evaluation with sequenced and defined material, field samples both from in vivo and community based studies from Tanzania have been analysed for all implicated SNPs. This is done by PCR amplification of target genes followed by a simple one tube mini-sequencing reaction using commercially available fluorochromes. Subsequent hybridization on multi-well microarrays and scanning unequivocally calls the respective nucleotide. A custom made computer program assists in generating the data output.

Results: pfdhfr, pfdhps, pfcrt, pfmdr1, and pfATPase have been amplified and analysed with the microarray from 12 defined culture strains. There was no discrepancy within over 30 SNPs. In addition, two sets of field samples from Tanzania and from Papua New Guinea have been analysed using microarrays and compared to data obtained by classical RFLP typing and/or by sequencing. Here we found overall consistency of >90%, but were able to show that most discrepancies were due to false determination in mixed infections by RFLP. We showed that the SNP analysis by microarray can be done with less than 10 parasites, and that the microarray can handle mixed infection up to a ratio of 1:1000. Hands on time is limited, and the system can be adapted to a high through put system. Currently we can generate approximately 90 data points each for 300 samples per day, yet with costs far below any other applied system. This system is currently used for epidemiological drug resistance monitoring in three sites in Tanzania, three sites in Papua New Guinea, and in sites in Cambodia (in collaboration with the Institute Pasteur Cambodia).

Interpretation: Such fast and cost effective monitoring system for drug resistance associated SNPs allows large monitoring surveys to predict spread of drug resistance but also to identify re-selection of drug susceptible parasites once a drug has been withdrawn.

337A Occurrence of carbamate resistance in Anopheles gambiae s.s in coastal Cameroon [MIM-JB-96992]

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Introduction: Increasing resistance in An. gambiae mosquitoes to insecticide is a major cause for concern in malaria control operations in many African countries. This is attributed to uncontrolled usage in agriculture and households. To improve on the production scale, the Cameroon Development Corporation is reputed for pesticide use in its plantations. We have for the first time characterized the response of An. gambiae s.s and An. melas to deltametrin, permethrin, DDT and carbosulfan in coastal SW Cameroon.

Methods: Wild populations of Anopheles gambiae s.s and An. melas bred from larval populations in Tiko, Limbe and Idenau, in the coastal area of Southwest Cameroon, were assessed for susceptibility to deltametrin (0.05%), permethrin (1%), carbosulfan (0.5%) and DDT (4.0%) according to the World Health Organization’s standard protocol for testing adult mosquitoes. Siblings of the An. gambiae complex and the molecular forms of An. gambiae sensu stricto were identified using the polymerase chain reaction (PCR).

Results: The knockdown time (KDT) was very rapid following treatment with permethrin and deltametrin. The mosquitoes were completely knocked down within the first 30 min of exposure in all three localities. A reduction in the knockdown time was observed with DDT in Tiko, with 95% of the mosquitoes knocked down only after 60 min of exposure. With carbosulfan, KDT50 and KDT95 were 2–3 folds longer than other tested insecticides, with a near complete loss of knock down effect especially in Limbe and Idenau. The mor-
tality rates (mr) showed that *Anopheles gambiae* s.s and *An. melas* were fully (100%) susceptible to deltametrin and permethrin. DDT resistance was observed in Tiko (mr = 97.3), but remains hypothetical. The mortality rates were very much reduced with carbosulfan in Tiko (90%), Limbe (55%) and Idenau (71.2%)%, indicating resistance to this carbamate class of insecticide. PCR analysis showed that all surviving mosquitoes were *An. gambiae* s.s and all were of the M-molecular form.

**Interpretation:** There is convincing evidence that deltametrin and permethrin are the insecticides of choice to combat the malaria vectors in the area, while the use of DDT and carbamate insecticides should be avoided.

**338B**

Mosquito nets for crops may reduce the insecticide pollution in peri-urban areas of Africa [MIM-FC-350632]

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**Introduction:** To reduce the insecticide contamination of environment which is strongly suspected to select for insecticide resistance in mosquitoes from peri-urban areas, we experienced to replace sprays on vegetables by using mosquito nettings. The protection of cabbages using a net was investigated in a field trial in Benin during the dry season.

**Methods:** The trial was implemented in November in the Research Centre of Agonkanmey. Four treatments were compared in a Fisher block design: insecticide treated net, untreated net, local insecticide protection with 10 sprays of deltamethrin at 12 g/ha and a control without any protection. Nets from local market were in knitted polyester, 30 g/m² and 25 holes/cm². Treated nets were impregnated 2 days before use by dipping with deltamethrin at 50 mg/m². Four wood pickets were placed at each corner to maintained nets at 50 cm height in the nurseries. As a bed-net, the net was removed during the day when the flight activities of pests were reduced to suppress the problem of overheating and excessive shade.

**Results:** The results showed the very good efficacy of the net to protect cabbages from caterpillar attacks when compared with a standard insecticide protection. Moreover an insecticide treated net used on nursery allowed a very efficient protection against small pest such as aphids when compared with an untreated net (0.8 and 52.5% of infested plants, respectively). Before transplanting the percentage of damaged plants was significantly lower with the insecticide treated net (2%) than with the untreated net (6.6%) or the local insecticide protection (21%). At harvest time, the production of marketable cabbages with an untreated net (383 units/100 m²) was significantly better than the standard protection (156 units/100 m²), showing that this method is an economical and valuable method for crop protection.

**Interpretation:** The use of mosquito net on crops reduce drastically the use of insecticide hence pollution. This technique could benefit from the large scale implementation of bednets in Africa by the national malaria programmes and conversely.

**339C**

Evaluation of insecticidal activity of deltamethrin (k-otab) on treated nets, one month after retreatment [MIM-JC-144864]


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**Introduction:** Malawi it is estimated that under five children and adults get 10 and 6 episodes of malaria, respectively each year. Recent attempts to control malaria are focusing on prompt and correct treatment, intermittent preventive treatment and use of insecticide treated nets. Malawi adopted the use of ITNs as a strategy for malaria control and to date the coverage is estimated at 35 and 33% for under five children and pregnant women respectively. Retreatment coverage is estimated at 61%.

**Methods:** In Malawi, much is not known about bionomics of malaria vectors; it was against this background that a study was organized. To check insecticidal activity of deltamethrin on treated nets. To
check susceptibility/resistance status of malaria vectors mosquitoes to deltamethrin. Test was done according to WHO bioassay for adult mosquitoes. A total of 289 anopheles mosquitoes, which were identified morphologically as members Anopheles gambiae and Anopheles funestus were collected. Wild caught mosquitoes were used because the there is no insectary in Malawi. The wild mosquitoes were not found in abundant despite that it was during rainy season when previously mosquitoes, in the same area were found in abundant.

Results: All the anophelines were exposed to treated nets and mortality was 100% after 24 h post-exposure. A total of 289 anopheles mosquitoes, which were identified morphologically as members Anopheles gambiae and Anopheles funestus were collected. Wild caught mosquitoes were used because the there is no insectary in Malawi. The wild mosquitoes were not found in abundant despite that it was during rainy season when previously mosquitoes, in the same area were found in abundant. All the anophelines were exposed to treated nets and mortality was 100% after 24 h post-exposure. From the findings of this study, we write to conclude that the insecticide (K Otab) that was used for treating nets was working very well as evidenced by the 100% mortality of the exposed mosquitoes.

Interpretation: The program therefore needs to develop messages informing members of the community that the mosquitoes they see flying around inside houses, even after treating mosquito nets do not transmit malaria.

340A

The spatial and temporal distribution of insecticide resistance in Anopheles in Africa [MIM-MC-10989]

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Introduction: Malaria is the most serious vector-borne disease in Africa, affecting millions annually and causing more than a million deaths per year. Despite control efforts, malaria has increased in Africa in the last 30 years. This may in part be due to rising levels of insecticide resistance in Anopheles mosquitoes that transmit the disease. The two major vector control methods, indoor residual spraying and impregnation of materials, both rely on insecticides. Increases in insecticide resistance could ultimately lead to failure of malaria control programs based on either of these methods.

Methods: A literature search for Anopheles and insecticide resistance in Africa was initially undertaken via the US National Library of Medicine (http://www.ncbi.nlm.nih.gov), supplemented with information available from the reference collection within the insecticide resistance group at the Liverpool School of Tropical Medicine. A database of references was constructed using Access 2000 and relevant data was extracted from the publications.

Results: Traditionally, an insecticide was used until resistance to it became a limiting factor by the malaria vector. This, coupled to the slow rate of introduction of new public health insecticides, has eroded the number of insecticides suitable for malaria control. Due to time and cost implications the development of new insecticides or novel technologies such as transgenic mosquitoes are long-term solutions. In the short to medium term malaria control will rely on currently available insecticides, and to this end there is an urgent need to prolong their effective lifespan in Africa. This requires information on the current resistance status of Anopheles vectors. We have created a database of insecticide resistance in Africa based on all published Anopheles literature. This allows us to determine the documented spatial and temporal distribution of insecticide resistance on a continental scale. This is currently being expanded to incorporate information in the grey literature.

Interpretation: The same database can be used to monitor resistance at the malaria control program scale generating information essential to an effective insecticide policy.

341B

Susceptibility to insecticides and molecular characterization of the complex Anopheles gambiae in Banambani and Pimperena, Mali (West Africa) [MIM-GD-403850]

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Introduction: Vector control is an important component of the WHO strategy to fight against malaria. Insecticide impregnated bed nets are widely used for the control of malaria vectors in Africa; unfortunately, An. gambiae s.l. mosquitoes’ resistance to most of these insecticides has already described in Africa. Given the reliance of the control strategy on ITNs, it is necessary to assess the susceptibility of vectors to insecticides in different environmental conditions.

Methods: The local vector populations were assayed against the four main groups of insecticides used in malaria control: organochlorines (DDT), and pyrethroids (Permethrin, Deltamethrin, Lambda-cyhalothrin). In this study, our objectives were three-folds: (1) to determine the current level of insecticide resistance; (2) to characterize the tested mosquito populations; and (3) to identify Kdr gene in the same mosquito populations. The study was carried out in Banambani and Pimperena, two localities where vegetables and cotton are cultivated, respectively. The method used to test An. gambiae susceptibility to insecticides was the standard W.H.O test (WHO/VBC/81.806). The kdr diagnostic was performed on all samples.

Results: In Banambani, mosquito populations showed resistance to Lambda-cyhalothrin (78.75%), whereas they were only suspected to be resistant to DDT, Permethrin, and Deltamethrin with, respectively, 83.75, 88.75 and 90% of the observed mortality. In Pimperena, mosquito-vectors were resistant to DDT and Permethrin with, respectively, 24 and 59% of the observed mortality, whereas they were only suspected to be resistant to Lambda-cyhalothrin (80%) and to Deltamethrin (95%). Analyses of specimens collected in the Banambani and Pimperena show that the kdr allele was already present in the Savanna population.

Interpretation: Comparing the samples collected in Banambani and Pimperena, the kdr frequency was significantly higher in Pimperena (98.7%) than in the Banambani (72.41%). The kdr allele is associated only with the Savanna form population.
both *An. arabiensis* and *An. gambiae* s.s. Concerning the latter species, the molecular forms S and M were shown to be “allopatric” in the savanna tropical zone and the coastal plain, respectively, but co-exist in the forest zone. No M/S hybrid was found. A great increase in KDT to DDT (4–5-fold) was noted of the coastal population of Bonassama-Douala. The kdr mutation was detected in the M-form population of *An. gambiae* from this site.

**Interpretation:** Resistance to pyrethroids would be linked to the over use in agriculture and domestic hygiene. This resistance would be principally metabolic but there is a risk that kdr mutation distribution spread beyond the coastal plain.

### 343A
**Evaluation of insecticide susceptibility in malaria vector mosquitoes and their role in malaria transmission in Central Malawi [MIM-SK-118871]**

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**Introduction:** Few studies have been carried out on the biology and bionomics of malaria vectors in Malawi in recent years. The objectives of this study were: (1) to evaluate the insecticide susceptibility status of malaria vectors and (2) to determine their role in malaria transmission. It was carried out in Nkhotakota district along the lakeshore in central Malawi.

**Methods:** Wild caught mosquitoes were exposed to WHO discriminating concentrations of DDT 4%, deltamethrine 0.05%, malathion 5% and bendiocarb 0.01%. After exposure, the specimens were preserved on dry silica gel for species identification by polymerase chain reaction (PCR) assay, ELISA for *Plasmodium falciparum* circumsporozoite protein to detect *Plasmodium falciparum* and analysis of host blood meals for biting preference.

**Results:** Species identification by PCR showed that *Anopheles funestus* was the most abundant species. *Anopheles Arabians* and *An. Gambia* occurred almost in equal numbers and all three species occurred sympatrically. Enzyme Linked Immuno-Sorbent Assay (ELISA) for human blood meal analysis was 99.1% for *An. funestus*, 97.5% for *An. gambiae*, and 82.6% for *An. arabiensis*. ELISA for *Plasmodium falciparum* circumsporozoite protein showed the highest infection rate in *An. gambiae* (6.3%), with *An. funestus* (5.2%) and *An. arabiensis* (4.5%) also playing major roles in transmission. The high sporozoite indices reflect the seriousness of malaria transmission in this holoendemic area of central Malawi. The insecticide susceptibility results, however are encouraging as they indicate that vector control interventions that use insecticides, such as indoor residual house spraying and insecticide treated bed nets, would be successful in decreasing the mosquito densities and longevity if applied correctly.

**Interpretation:** N/A.

### 344B
**Resistance in Anopheles arabiensis in South Africa [MIM-LK-51144]**


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**Introduction:** Anopheles arabiensis is a major vector of malaria in South Africa in addition to *An. funestus* and is responsible for seasonal malaria transmission. DDT resistance in a population of *An. arabiensis* in the Mamfene region of KwaZulu/Natal was recorded in 2003. There was no record of pyrethroid resistance in this population.

**Methods:** *Anopheles arabiensis* originating from Mamfene was colonized and a DDT resistant line selected. Wild females were collected during 2005 using exit traps in sprayed houses. Two-day-old adults were exposed to permethrin, bendiocarb and deltamethrin according to standard WHO bioassay procedure.
Detoxifying enzyme activity (non-specific esterases, monoxygenases and glutathione-S-transferase) was assayed on colonized unselected, resistance selected and familial samples. PCR analyses designed to detect specific mutations associated with pyrethroid/DDT resistance (kdr) and acetylcholinesterase insensitivity were conducted on specimens from Mamfene.

Results: Selection for a DDT resistant An. arabiensis colony: A DDT resistant strain of An. arabiensis is in its 26th generation and shows 25% mortality after 1 h exposure to 4% DDT. This colony is in the 26th generation and shows high levels of resistance to DDT (25% mortality after 1 h exposure). Susceptibility assays on Field collected material: 1–3 day old An. arabiensis were exposed to permethrin and showed 63–73% mortality 24 h post-exposure. Deltamethrin exposure resulted in a mortality of 95–96%. All families tested showed full susceptibility to bendiocarb. Biochemical analysis: Biochemical analysis on individual families reared from wild caught material showed increased levels of non-specific esterase and GST activity. However, there was a lack of correlation between bioassay and biochemical data by family assayed for resistance to DDT. A direct comparison between the DDT resistance selected and unselected colony material revealed increased GST activity in the selected strain, particularly in females. Molecular analysis on target site insensitivity: Molecular analysis on the sodium channel and acetylcholinesterase genes revealed no mutations characteristic of target site insensitivity to insecticide.

Interpretation: Biochemical analyses suggest that insecticide resistance found in An. arabiensis from South Africa is most likely metabolic with no assistance from target site insensitivity mutations.

Introduction: A cross-resistance to carbamates and organophosphates has been recently detected in some Anopheles gambiae populations from West Africa. This resistance results from a qualitative change of acetylcholinesterase, the target site of these insecticides (G119S mutation of the Ace-1 gene). Our investigations were done to characterize Ace-1 resistant allele (Ace-1R) in terms of resistance level, dominance and fitness cost in An. gambiae.

Methods: In a first step our objective was to obtain two strains with the same genetic background except for the insensitive acetylcholinesterase allele. The genome of a wild population of An. gambiae resistant to carbamates and organophosphates was introgressed by that of An. gambiae Kisumu susceptible reference strain through repeated backcrossing (20 generations). At each generation, a discriminating insecticide concentration was applied on larvae to select for resistant heterozygotes. Finally, homozygosity for the resistant allele of this new strain (named Acer-kis) was achieved after 2 generations without backcross and by keeping the offspring whose both parents were homozygotes for Ace-1R.

Results: Larval bioassays with several insecticides were compared between Kisumu (homozygote susceptible) and Acer-Kis (homozygote for Ace-1R). Ace-1R conferred a very high resistance level to carbamates, 500 and 6000 fold for carbosulfan and propoxur, respectively. This mutation confers also a strong resistance to organophosphates with resistance ratios ranging from 40 to 50. As both strains shared the same genetic background except for insensitive Ace-1, we did not observed any significant differences between their susceptibility to deltamethrin and DDT. Preliminary data suggest a strong fitness cost associated with Ace-1R mutation as homozygotes are very rare in wild populations even with a high frequency of resistant individuals. To confirm these findings laboratory studies are currently done to evaluate Ace-1R effects on various fitness components by manipulating larval densities. Bioassays are also done on hybrids between Acer-Kis and Kisumu to evaluate the dominance level of Ace-1 mutation.

Interpretation: The advantage to have two strains with the same genetic background except for Ace1-R allows us to evaluate precisely the effect of this mutation on the main phenotypic traits that are implied in the evolution of resistance in the field.
Preserving the effectiveness of insecticide treated nets and other vector control tools: alternative insecticides and products for overcoming insecticide resistance [MIM-VM-209313]

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Introduction: Insecticide treated nets (ITNs) are the primary method of malaria prevention. The effectiveness of ITNs is threatened by the development of pyrethroid resistant mosquitoes and low coverage of insecticide treatments. For complex emergency situations, alternative control methods need to be devised. It is therefore essential to develop alternative insecticides, long lasting insecticidal nets, and new substrates for insecticide application appropriate to the wide variety of political, cultural and epidemiological situations existing in Africa.

Methods: Development of new vector control products requires partnership between industry, international public health organisations, and northern and southern research institutes. The National Institute for Medical Research and the Kilimanjaro Christian Medical Centre in Tanzania are collaborating with the Gates Malaria Partnership led by the London School of Hygiene and Tropical Medicine, and the World Health Organisation, to develop and evaluate new insecticides, formulations and substrates for malaria control. The development process requires testing against insecticide resistant and susceptible species in the laboratory, followed by field testing in controlled but realistic environments.

Results: Field sites with experimental huts, platforms, and insectaries have been constructed in Tanga and Kilimanjaro regions of Tanzania where resistant and susceptible An. gambiae, An. arabiensis, An. funestus, and Culex quinquefasciatus are found. In addition to new insecticides, some members of the earlier generation of insecticides, the organophosphates and carbamates, were developed primarily for indoor residual spraying are showing potential as net treatments. A variety of long-lasting insecticidal materials are also being developed or under evaluation. An offshoot of this technology – insecticide treated plastic sheeting – has potential for emergency and non-emergency use. The results of recent evaluations will be described.

Interpretation: Partnership between public, private sectors and academia is essential for the development of new vector control tools. Such partnerships are yielding promising technologies which offer prospects of circumventing significant technical and operational problems in malaria vector control.

Efficacy of Bifenthrin-impregnated bed nets against Anopheles funestus and pyrethroid-resistant Anopheles gambiae in North Cameroon [MIM-CM-29118]

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Introduction: Recent field studies indicated that pyrethroid-treated bednets maintain their efficacy despite a high frequency of the knock-down resistance (Kdr) gene in vector populations. These findings need to be evaluated in areas where other resistance mechanisms are involved. In this study, we assessed efficacy of bifenthrin-treated nets against natural malaria vector populations in an area where Anopheles gambiae displays metabolic resistance to pyrethroids, and An. funestus is also present.

Methods: Bifenthrin, a non-alpha-cyano pyrethroid insecticide, was used for this experiment as it is considered to be a promising candidate for impregnation of ITNs, inducing high mortality and significant blood feeding inhibition in Kdr mosquitoes at a dose of 50 mg/m2. Mosquito nets were treated at this dose and at 5 mg/m2, a dose that kills 95% of susceptible mosquitoes under laboratory conditions. Bednets were holed to mimic physical barrier impairment. Ethical clearance was granted and the trial was carried out for 120 nights in three experimental huts from Pitoa, Cameroon. Each treatment was assessed relative to...
the untreated control to evaluate deterrency, induced exophily, blood-feeding inhibition and mortality.

**Results:** Both *An. gambiae* and *An. funestus* female mosquitoes were collected in the huts. *Anopheles gambiae* was predominant in the early rainy season and *An. funestus* gradually increased in abundance at the end of the survey. Treating bednet with bifenthrin at 50 mg/m² significantly reduced anophelines entry rate (>80%) suggesting strong deterrent effect. This was not observed at 5 mg/m². There was evidence for induced exophily with both treatments in *An. gambiae*, and, to a lower extent, in *An. funestus*. When treated with bifenthrin at 50 mg/m², high blood feeding inhibition (>60% reduction) and high overall mortality rates (75–90%) were observed for both vectors. Most of the specimens died within the night of entering the hut, and most blood-fed specimens were found dead. Despite presence of holes, few mosquitoes entered the treated net: only a single *An. gambiae* and two *An. funestus* females were recovered inside the treated nets, and all were found dead. The same trends were observed when bifenthrin was used at 5 mg/m², also in most cases, no significant differences were revealed with the untreated control net.

**Interpretation:** Our results show that bifenthrin-impregnated bednets at a dose of 50 mg/m² are efficient in the reduction of human-vector contact in areas of metabolic pyrethroid resistance within the *An. gambiae* population.

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**Evaluation of malaria vector susceptibility status to insecticides in Zambia [MIM-MM-55748]**

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**Introduction:** There were reports of pyrethroid resistance from West, East and South Africa in the major malaria vectors. WHO/AFRO was prompted to set up a Resistance Monitoring Network in Southern Africa (ANVR) in Dec. 2000 to which Zambia is a member. Zambia identified 10 sentinel sites for monitoring vector susceptibility status to the insecticides of choice in view of expanded ITN use and Indoor Residual Spraying countrywide.

**Methods:** In Chibombo, Chingola and Mwinilunga districts mosquitoes were collected using aspirators and exposed to insecticide treated paper using WHO standard test kits and scored for mortality over a period of 60 min and 24 h. Initial species identification was done using morphological keys of Gillies and Coetzee. All those found to be either *Anopheles gambiae* or *Anopheles funestus* were placed on desiccant in individual ependorf tubes for subsequent species confirmation using PCR at a reference laboratory in South Africa.

**Results:** Chibombo and Chingola mosquitoes showed 100% susceptibility to DDT and to pyrethroids. Mwinilunga mosquitoes had a susceptibility of 99.8% on DDT the only chemical that was used for the test. These results provide the first susceptibility data for the *Anopheles gambiae*, *An. arabiensis* and *An. funestus* group in these sites. They will serve as baseline data against which the future work on insecticide resistance will be monitored in the three districts.

**Interpretation:** DDT and pyrethroids should still be used to control the Vectors in three districts. The number of surviving mosquitoes in Mwinilunga district was insignificant to register resistance therefore more work is needed in tat district.

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**Insecticide resistance and the Kdr mutation in the malaria vector *Anopheles gambiae* from South Cameroon [MIM-NP-401679]**

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**Introduction:** Insecticide resistance in the malaria vector *Anopheles gambiae* is a growing threat for the efficacy of conventional means to control malaria transmission in Africa based on the widespread use of insecticide impregnated materials. Monitoring of the susceptibility to insecticides in natural vector populations and identification of its genetic basis are therefore critical to optimize vector control efforts in endemic countries.

**Methods:** Susceptibility to DDT and pyrethroids was assessed in *An. gambiae* populations from two sites...
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in South Cameroon. Mosquito larvae were collected in garden fields in Foumbot (tropical mountain) and in timber yards in Campo (coastal equatorial forest). Both sites were chosen because high insecticide use in the area might promote inadvertent selection for resistance in local mosquito populations. Larvae were reared to adults and 2–3-day-old females were assayed for susceptibility to DDT (4%), permethrin (1%) and deltamethrin (0.05%), using WHO test kits. Survivors to the susceptibility tests were identified to species and molecular forms using PCR-based diagnostic tests and their Kdr Leu-Phe genotype was determined.

Results: Mortality rates to DDT were 30–35% in Foumbot and 78–87% in Campo, with KdT50 >60 min and 25–30 min, respectively. Mortality rates to pyrethroids ranged from 80 to 98% in Foumbot and from 95 to 100% in Campo, with KdT50 of 15–45 min and 8–12 min, respectively. These results demonstrate moderate to high levels of resistance to DDT and pyrethroids in these natural vector populations. In both locations, An. gambiae s.s. was the only member of the An. gambiae complex recorded among survivors to susceptibility tests (N = 27 in Foumbot; N = 24 in Campo). Only the S molecular form was observed in our samples from Foumbot, while both M and S molecular forms survived exposure to insecticides in Campo (S form = 21/24; M form = 3/24). The Kdr mutation was detected at the homozygous state (RR) in 21 out of 27 survivors in Foumbot, and at the heterozygous state (RS), the remaining being homozygous for the susceptible allele (SS). Presence of the Kdr mutation was only observed within the S molecular form of An. gambiae.

Interpretation: Our results demonstrate the presence of Kdr Leu-Phe mutation in the S molecular form of An. gambiae from South Cameroon. Additional resistance mechanisms are obviously involved, as advocated by our susceptibility assays outcomes.

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Genetic and biochemical studies of insecticide resistance in the malaria mosquito Anopheles stephensi Liston [MIM-Nd-155709]

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Introduction: Anopheles stephensi is one of the important malaria vectors in the Indian subcontinent. The said species has been extensively used in our laboratory for conducting genetic, cytogenetic, insecticide resistance, genetic sexing strains, refractory strains for plasmodium transmission and synthesis of transgenic strain/s in the genetic control programme/s of mosquito. Insecticides are extensively used for the control of malaria vector all over the world.

Methods: The homozygous resistant and susceptible strains of each one of the insecticides mentioned above were synthesized according to the procedure of WHO. Crosses were conducted between resistant and susceptible strains of each one of the insecticides to establish the genetic basis of resistance. The biochemical studies involving proteins, α- and β-esterases, acid and alkaline phosphatases, lactate dehydrogenase, acetyl choline esterases, phosphoglucomutase, alcohol dehydrogenase, glucose-6-phosphate isozymes in different insecticide resistant and susceptible strains of above said insecticides during the developmental stages and compared the same among the resistant strain in A. stephensi by using polyacrylamide gel electrophoresis (PAGE).

Results: The studies clearly showed that the insecticide resistant for all the above said insecticides were autosomal and incompletely dominant. The data showed that there is a marked difference in the banding pattern, intensity and mobility for the above said biochemical parameters between the susceptible and resistant strains of each one of the insecticide studied and also among the resistant strains. However, a few bands were common among the resistant strains. Further, it was observed that each insecticide resistant strain/s had specific band/s, which can be used as a diagnostic marker/s for identifying the same in the field population.

Interpretation: The insecticide resistant genes are exclusively used to conduct basic and applied research including synthesis of transgenic strains, which can be used in the control of An. stephensi. The Biotechnical data generated from the study can be used to develop a rapid test system/s to detect resistance individuals in a natural population for the insecticides included in the present study.
Impact of impregnated bed nets and house spraying on insecticide resistance of Anopheles gambiae s.s. from western Ivory Coast [MIM-MT-105756]

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Introduction: During 2001–2002, a large scale study was performed in forested area of western Côte d’Ivoire, to evaluate the impact of different vector control methods on the resistance evolution of An. gambiae populations. This area was chosen because external selection pressure by agricultural treatments was very low, just as the kdr allelic frequency (F(kdr) < 0.1) in An. gambiae populations. These studies were done in 12 villages, randomly allocated to obtain four groups of 3 villages.

Methods: Long lasting treated bednets (LLNs Permanet ® , deltamethrin 50 mg/m²) were distributed in three villages, while three other ones received untreated nets. House spraying (HS) with deltamethrin (20 mg/m²) were done in three villages. Finally, three villages without any vector control were followed as control. Resistance evaluation was done each term, for 15 months from may 2001 to july 2002. Mosquitoes from each village group were tested using WHO test kits with deltamethrin 0.05%. Genotypes of individuals for kdr mutation and molecular forms M/S of An. gambiae s.s were determined by PCR. Mechanisms of metabolic resistance to insecticides (esterases, oxidases, GST) were evaluated using biochemical assays.

Results: Both molecular M and S forms of An. gambiae s.s were found in sympatry in all village groups. The mean percentage of S form was around 26% of the total population analysed. The frequencies of kdr mutation and molecular forms M/S of An. gambiae s.s were determined by PCR. Mechanisms of metabolic resistance to insecticides (esterases, oxidases, GST) were evaluated using biochemical assays. The mean percentage of S form was around 26% of the total population analysed. The frequencies of kdr mutation ranged from 0.0 to 0.16 and was only detected in An. gambiae belonging to the S form. We did not observed any significant difference between kdr allelic frequencies in villages with LLNs and those with untreated nets until 9th month. However, at 12 and 15 months, the kdr frequencies was significantly increased in villages with LLNs suggesting that they induced a significant selection pressure on mosquito population after 1 year of use. Inversely, in villages with HS a significant increase of resistant individuals compared to control villages was observed until the 9th month. At 12 and 15 months, kdr frequency became similar in both village groups, likely because there was no more residual effect of deltamethrin on the walls. There was no difference between mortalities (95–100%) to WHO tests kits as all the kdr frequencies were low and most individuals with kdr mutation (recessive allele) were heterozygotes. Variations of enzyme activities were observed all along study but were not correlated to a specific treatment.

Interpretation: In area where kdr allelic frequency is low and An. gambiae is not submitted to an external selection pressure such as agricultural insecticides, impregnated nets or house spraying can increase kdr frequency but in a different way along time.

High frequency of Pfcrt and Pfmdr1 polymorphism among chloroquine treated malaria patients in Central Sudan [MIM-MA-186081]

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Introduction: Malaria parasite resistant to chloroquine poses a severe and increasing health problems in tropical countries, implementing molecular markers for monitoring the drug resistance may be essential to overcome the problem.

Methods: Two thousand one hundred eighty five (2185) febrile patients were examined parasitologically. One hundred and seventy six (176) were confirmed positive, only forty patients were completed the follow-up. In vivo and in vitro sensitivity were assay. The prevalence of mutations of Chloroquine resistance P.
Efficacy of chloroquine, sulfadoxine–pyrimethamine in treating uncomplicated malaria in Ghana [MIM-BA-275805]
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Introduction: Antimalarial drug resistance has, for the past few years, been a major challenge to malaria control in Africa, and has impacted negatively on malaria morbidity and mortality in the continent. We undertook a study of the efficacy of some antimalarial drugs in 2003 with the view of providing evidence to the National Malaria Control Programme to help in the review of the antimalarial drug treatment policy in Ghana.

Methods: Children aged 6–59 months with signs/symptoms of uncomplicated malaria including axillary temperature ≥ 37.5°C, mono infection with Plasmodium falciparum; absence of signs/symptoms of severe malaria; and parent/guardian’s willingness to give consent, were randomized into four different treatment groups and followed up for a minimum of 14 days and a maximum of 28 days. Thirty-six children were enrolled into the chloroquine (CHQ) group; 27 into the sulfadoxine–pyrimethamine (SP) group; 54 into the amodiaquine + artemether (ADQ + ART) group; and 51 into the artemether + lumefantrine (COARTEM®) group. Clinical responses for each treatment group were assessed based on the WHO 2003 criteria.

Results: Clinical evaluation of 168 children studied showed that Cure rate (adequate clinical and parasitological response) on day 14 was 100% for the 2 artemisinin-based combinations and 84% for SP and 50% for CHQ. Cumulative cure rates on day 28 were 100% for ADQ + ART, 97.5% for coartem, 60% for SP and 25% for CHQ. The artemisinin-based combinations effected rapid fever and parasite clearance and decreased prevalence of gametocytaemia during the follow-up period. Prevalence of gametocytaemia was highest in the SP group whilst CHQ showed the least mean change of haemoglobin level.

Interpretation: The findings are in agreement with current recommendations for using artemisinin-based combinations for treating uncomplicated malaria in areas of high CHQ failure such as Ghana.
monitoring the efficacy of antimalarial drugs. Eligible children were allocated following block randomisation procedure to one of the two antimalarial drug treatment regimens. Children were followed up during 28 days and clinical examinations were done on days 0, 1, 2, 3, 7, 14, 21, 28. Thick and thin blood smears were taken at days 0, 2, 3, 7, 14, 21 and 28. Blood collected on filters papers at day 0 and at any treatment failure day after day 9 for the genotyping of parasites by PCR in order to differentiate recrudescence and new infections. Haemoglobin levels were measured by the method of Hemocue® with capillary blood at days 0, 14 and 28.

Results: We have screened 556 children. A total of 137 children were assigned to chloroquine group and 125 to sulfadoxine–pyrimethamine group. Early Treatment Failure were 26 of 131 (19.9%) in chloroquine group and 7 of 123 (5.7%) in sulfadoxine–pyrimethamine group. The Late Treatment Failure were 65 of 131 (49.6%) in children treated with chloroquine, 58 were Late Clinical Failure and 7 were Late Parasitological Failure. In sulfadoxine–pyrimethamine group the LTF rate was 8.9% (7 LCF and 4 LPF). After adjusting the results by PCR to differentiate between recrudescence and reinfections, 8 LTF were recorded as new infections in chloroquine group and one new infection in LTF of sulfadoxine–pyrimethamine group giving treatment failure rates of 63.4 and 13.8%, respectively for chloroquine and sulfadoxine–pyrimethamine. The haemoglobin levels increased on average of $0.3 \pm 1.4$ g/dL and $0.9 \pm 1.4$ g/dL in the children treated by chloroquine and on average of $0.5 \pm 0.5$ g/dL and $1.4 \pm 1.8$ g/dL in those treated by sulfadoxine–pyrimethamine.

Interpretation: These results indicate that chloroquine could no longer be used as first line drug in Burkina Faso. The use of SP as alternative treatment could not be the option. The long-term option may to consider combination therapy with artemisinin derivative.

**Antimalaria drug therapeutic efficacy test in Nigeria: Towards a change of policy to artemisinin-based combination therapies [MIM-BA-28506]**

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Introduction: Global malaria control is being threatened on an unprecedented scale by rapidly growing resistance of \( P. falciparum \) to conventional monotherapies such as chloroquine. Worried about the widespread resistance of \( P. falciparum \) to commonly administered antimalarials, Nigeria conducted an antimalaria drug therapeutic efficacy test on chloroquine and sulfadoxine-pyrimethamine in its six geopolitical zones in 2002.

Methods: Prior to the commencement of the study, a workshop was held for all Principal Investigators. In accordance with the protocol, all febrile children aged 6 months to 5 years attending the study facility were examined by a clinician to exclude non-malarial causes. For those with a presumptive diagnosis of malaria a blood film was taken to confirm the diagnosis. Those that were parasite positive were then enrolled into the study if they met all other inclusion criteria. WHO 14-day protocol was followed.

Results: These indicate that, for example, in South-south zone (Calabar), Early Treatment Failure (ETF), Late Treatment Failure (LTF) and Late Parasitological Failure (LPF) were 22.7, 27.3 and 40.9%, respectively, giving a combined total of 90.9% thus leaving an Adequate Clinical and Parasitological Response (ACPR) of merely 9.1% to chloroquine. These figures indicate that an efficacy of chloroquine in South-south zone of the country is much less than 75%. Likewise, the total combination of ETF (21.3%), LTF (12.8%) and LPF (57.4%) leaves an ACPR of only 8.5% to sulfadoxine-pyrimethamine in the same zone. Similar results were obtained from other zones.

Interpretation: There is widespread failure of response to chloroquine and sulfadoxine-pyrimethamine in the country and their continued use might not be to the benefit of the patients. There is need for malaria treatment policy change.
Evolution du paludisme simple à P. falciparum sous 3 traitements chez les enfants d’une cohorte en milieu côtier lagunaire au Bénin [MIM-NA-38313]
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Introduction:

Methods:

Results:
De Décembre 2003 à Avril 2004, nous avons enregistré 161 cas de fièvre dont 77 cas d’accès palustre ont été sélectionnés conformément aux critères retenus. Les groupes d’enfants étudiés sont homogènes. Sur le plan clinique, nous avons enregistré un taux de réponse clinique adéquate égal à 68.4% tout traitement confondu – Ce taux tombe à 31% dans le groupe des enfants soumis à la sulfadoxine–pyriméthamine – Il ne représente cependant que 8% chez ceux traités avec la combinaison sulfadoxine–pyriméthamine-artésunate.

Interpretation:
Les taux d’échec avec la CQ et la SP résultent probablement de l’utilisation inappropriée de ces dernières. La combinaison SP/AS semble efficace mais la résistance élevée à la SP pourrait compromettre son utilisation à long terme.

Therapeutic efficacy of sulphadoxine–pyrimethamine (Fansidar®) and mutations rates to anti-folate genes in different regions of Cameroon [MIM-IA-52456]
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Introduction:
Attributable drug-resistant falciparum malaria burden remains a major public health challenge. Sulfadoxine–pyrimethamine was suggested as the second line drug to amodiaquine following widespread failure of chloroquine in Cameroon in 2002. We therefore, investigated the efficacy to S–P and determined the baseline mutations on marker genes for anti-folate resistance (dhfr and dhps) in the forest (Limbe) and Guinea-Savanna (Nkambe) ecozones of Cameroon.

Methods:
Patients aged between 6 and 120 months were included in the study carried out in 2003 in Limbe (n=108) and in Nkambe (n=103). Clinical and parasitological assessment of patients were conducted according to the 14 days WHO 2002 protocol. Filter paper blood samples were collected prior to treatment and on clinical failure days to determine molecular markers of resistance. Filter paper samples from other towns (Fontem and Dschang) were used to assess the mutation rates on the anti-folate genes. Samples were chelex extracted for parasite DNA, PCR amplified and used in dot-blot assays with 32-P labelled mutation specific probes. Sequencing using the dideoxy-chain
termination method by PCR was conducted to confirm doubtful cases.

Results: Fansidar® is no longer efficacious in Limbe and Nkambe for treating uncomplicated malaria in children below 10 years. Mutation rates are lower in other towns but stay high in Nkambe and Limbe. Combinations with Fansidar are not recommended. 2res In Nkambe, adequate clinical and parasitological response stood at 46.6% while it was 43.5% in Limbe. Late parasitological failure was higher in Limbe (30.6%) compared to Nkambe 10.3%. The prevalence of the 437-Gly mutation was lower in Nkambe, (57.6%), than in Limbe (70%). The serine to asparagine mutation at position 108 of *P. falciparum*’s DHFR present in the investigated sites had varying occurrence rates of 4% in Dschang (Savanna), 14% in Fontem (Upland Forest), 44% in Limbe (Littoral) and 46% in Nkambe (Guinea-Savanna). Analysis demonstrate that all genotypes that carried the 108N mutation also carried the 51-Ile and 59-Arg mutations similar to the Dd2 resistant strain as opposed to the Thailand strain K1 with mutations 51-Asn and 59-Arg. The sensitive alleles (S108) were 51-Asn and 59-Cys.

Interpretation: Fansidar® is no longer efficacious in Limbe and Nkambe for treating uncomplicated malaria in children below 10 years. Mutation rates are lower in other towns but stay high in Nkambe and Limbe. Combinations with Fansidar are not recommended.

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Antimalarial prescription pattern and in vivo sensitivity of quinine sulphate in the treatment of uncomplicated *Plasmodium falciparum* malaria in South Western Cameroon [MIM-AA-157412]

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Introduction: The development and spread of resistance to most front-line antimalarial compounds used in the prevention and treatment of human malaria has given cause for grave clinical concern. With the increasing resistance to commonly used antimalarial drugs, it is possible that different untested ‘local’ treatment regimens will arise. We conducted this study to identify the first-line antimalarial drug of choice prescribed by health care providers in South Western Cameroon and to evaluate its in vivo efficacy in the treatment of uncomplicated falciparum malaria in Fako Division.

Methods: Study procedures: Between July and September 2001, structured questionnaires responses were obtained from physicians and nurses who consult and prescribe antimalarials to patients in their respective communities in major health institutions in the province. Quinine sulphate identified as the first-line drug of choice during this phase was tested in vivo between May and August 2002. Study subjects between the ages 0.7 and 50 years who met the enrollment conditions were recruited after informed consent and treated under direct observation with standard doses of quinine sulphate (200 mg) tablets using the WHO 14-day test protocol (WHO, 2002). During follow-up, clinical, parasitological and hematologic parameters were assessed and evaluated. Thin and thick blood films were made on the same slide for parasite speciation and parasitemia evaluation, respectively.

Results: One hundred and seven responses were collected from caregivers. For uncomplicated malaria, the most frequently prescribed first line antimalarial drug was quinine sulphate tablets (56.1%; 60/107) followed by Artesunate (17.8%; 19/107) for second-line and quinine infusion (57.0%; 61/107) as third formulation antimalaria drug. Initially, 73 patients were enrolled into the in vivo survey and 69 (94.5%) completed follow-up. The 14-day adequate clinical and parasitological response was 58.0% (40/69) while 42.0% (29/69) of the infections tested were found to be resistant at the RI (10.0%) and RII (32.0%) levels with no case of RIII resistance detected. Clinically, the drug achieved a therapeutic efficacy (ACR) of 94.2% while four subjects (5.8%) did not respond to treatment. The prevalence of anaemia (PCV < 33%) was 27.4% at enrolment and decreased to 17.4% at day 14. Overall, the mean changes in PCV levels of subjects during follow-up were statistically different ($F = 60.29, P = 0.0001$).

Interpretation: The relatively high level of resistance (42.0%) observed in this study raises the possibility that quinine sulphate resistance genes may be present in the study community. There is therefore the need to closely monitor its resistance patterns so as to provide further data to policy makers.
Underdosage of artemisinin-derivative antimalarials in Kenya and Congo DR [MIM-MA-219240]

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Introduction: The use of artemisinin (AR), artemether (AM), arteether (AE), artesunate (AS) and dihydroartemisinin (DHA) for treating malaria has gained widespread use in almost all endemic areas, especially in Africa. Due to this surge, fake drugs can easily get to the market. Reports of artesunate counterfeits have recently been reported in part of Asia but not yet in Africa. In this work, we present some preliminary results for a group of artemisinin-derivative drugs and circulating in Kenya and Congo DR.

Methods: Oral formulations and IM ampoules containing either AM, AE, AS or DHA were bought in 2004 in East Africa from pharmacies and street vendors. All products were packaged and from this the following details were taken: product type, active pharmaceutical ingredient (API), dosage, preservative(s) (dry powders only), manufacturer, and manufacturing and expiry dates. All drugs were analysed before the expiry dates. The amounts of active ingredients were determined quantitatively using validated high performance liquid chromatographic (HPLC) methods with ultraviolet detection. Reference samples were obtained from accredited manufacturers or suppliers and all analyses were done according to pharmacopoeial requirements.

Results: Of the 21 drug samples analysed 43% (n = 9) did not comply with the pharmacopoeial limits and were either underdosed or in excess. 43% (3 of 7) of the substandard drugs was attributed to DHA alone. Arteether IM ampoules had the lowest drug content, only 77% of the claimed active drug was present. In addition, the presence of other peaks on the AE chromatogram were found which were not attributed to either AR, AM or DHA. All drugs contained at least some API. The overdosed drugs contained only slightly higher doses than the accepted limits. Contrary to the results in Asia, artesunate was the least counterfeited product (1 of 9). All of the substandard compounds were manufactured either in China or India, except for a DHA brand that had its address in Belgium. On further investigation, this address seemed to be fake. Macroscopic examination of some of the paediatric dry suspensions portrayed sticking of powder particles suggesting caking. This may have an influence on drug homogeneity, hence inappropriate dosaging.

Interpretation: The high proportion of underdosed drugs in this study suggests that nearly all artemisinin derivatives are at risk of promoting resistance development. There is urgent need for countries concerned to set up task forces on fake drugs at all levels.

Monitoring resistance to antimalarials used for intermittent preventive treatment: A comparison of resistance in symptomatic versus asymptomatic children [MIM-DC-231066]

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Introduction: Seasonal intermittent preventive treatment for malaria (S IPTc) can reduce malaria in seasonal transmission areas. However, it will soon be necessary to consider alternative drugs for IPTc as SP resistance is increasing. The acceptable level of SP resistance for IPTc is unclear. Since the IPTc target children are mostly asymptomatic and semi-immune, have lower parasite density than symptomatic children, the applicability of SP resistance seen in sick children, for IPTc needs to be examined.

Methods: The study was conducted in Navrongo, Ghana in 2003–2004. One hundred and sixty-four 19–45-month-old children who had asymptomatic parasitaemia were treated with SP and the parasitological clearance rate was assessed on post-treatment days 14 and 28. The day 14 parasite failure rate observed in the asymptomatic children was compared to that observed in 6 to 59-month old-febrile children (n = 116) determined using the standard WHO protocol as part of monitoring drug resistance in Ghana.

Results: The geometric mean parasite density on day zero was 2595/µl (range 120–48640/µl) in the asymptomatic children and 18,978/µl (range 2000–530,000)
in the symptomatic children, respectively. The day 14 parasitological failure rate was significantly lower in the asymptomatic children (5.5%, 95% CI 2.7, 9.8) compared to the symptomatic children (22.4%, 95% CI 15.2,31.1).

Interpretation: The estimates of drug resistance levels measured in children with clinical malaria may not be appropriate to decide on appropriate drug regimes for intermittent preventive treatment in children.

361A
Drug Policy change for effective case management: A synopsis of anti-malarial drug resistance and national drug policies in Southern Africa [MIM-NC-101654]
(1) World Health Organisation: Southern Africa Inter-Country Malaria Control Programme; (2) World Health Organisation: Malaria Unit, Regional Office for Africa; (3) National Malaria Control Centre, Zambia; (4) Department of Health, South Africa; (5) National Malaria Control Programme, Namibia; (6) National Malaria Control Programme, Angola; (7) National Malaria Control Programme, Zimbabwe; (8) National Malaria Control Programme, Malawi; (9) National Malaria Control Programme, Botswana

Introduction: Chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) have been the cornerstone for the treatment uncomplicated malaria in southern Africa. These two drugs formed the 1st and 2nd line treatment policies of the countries in the sub-region. However, the increase in P. falciparum resistance to these drugs has seen a shift towards other more effective antimalarials. Global fund support has helped support the accuralisation of drug policy change in the sub-region.

Methods: Objective is to describe the process of drug policy change in view of rising resistance to both CQ and SP, countries of the sub-region have embarked on process of drug policy change to Artemisinin-based Combination Therapy (ACTs). The change process has included establishment of evidence for the need to change, partnership building and consensus meetings. However, the process has been compounded with a number of challenges of which the cost of the new drugs, availability, and community based safety data for special groups’ especially pregnant women remain inadequate. Political commitment and the will by countries to provide better and effective treatment for malaria is very strong. Avenues to solicit for complimentary funding to support government budgets in the procurement of ACTs are being identified and the GFATM support is going a long way in ensuring countries overcome the challenge of cost in the short to medium term. Drug resistance to the commonly used drugs CQ and SP has seen countries move towards drugs containing artemisinin compounds. However, this movement has been associated with challenges in implementation such as cost of procurement of new drugs and the implementation of the change process.

Interpretation: The process of implementation of new policies has rather been too slow compounded mostly by unavailability of commodities.

362B
Malaria transmission intensity and the evolution of chloroquine resistant Plasmodium falciparum: Why have theoretical models generated conflicting results? [MIM-UD-103581]
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Introduction: The rate at which falciparum resistant malaria spreads in different transmission settings is still a controversial subject since inbreeding in malaria parasite populations was reported.
Methods: We have assessed the evolution of mutant Plasmodium falciparum parasites in six Ugandan populations with varying prevalence of chloroquine resistance (CQR), malaria transmission intensity, multiplicity of parasite clones and prevalence of CQ use. For each population, we have determined the wild and mutant allele frequency at codons 76 and 86 of the pfcrt and pfmdr1 genes, respectively.

Results: The highest frequency (median = 16.3%, range: 0.0–70.4%) of infections with two pure mutants (no wild genotype in either gene), adjusted for clone multiplicity, was observed at the extremes of malaria transmission intensity. The wild/mutant (W/M) allele ratio (an index for tracking the evolution of CQR) was less than one at all sites (median = 0.51, range: 0.09–0.98) for the pfcrt-76 gene, while it was greater than one at 2 of 6 sites (median = 0.75, range: 0.4–1.6) for the pfmdr1–86 gene, suggesting that the pfcrt-76 mutants were the predominant parasites at all sites. Furthermore, the pfmdr1–86 W/M allele ratio was consistently higher than that of the pfcrt-76.

Interpretation: The evolution of mutations linked to CQR in Plasmodium falciparum occurs faster at the extremes of the transmission spectrum. CQR starts with the pfcrt-76 gene mutations, followed later by the pfmdr1–86 gene mutations that modulate higher CQR.

363C
Size variations in a P. falciparum multidrug resistance protein (PfMRP) homologue and Coartem and artesunate + amodiaquine clinical response [MIM-SD-175726]
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Introduction: Multidrug resistance-associated protein (PfMRP)-like ABC transporters are known to be related to multidrug resistance in various organisms from Man to bacteria. Homologues of these types of proteins have recently been identified in P. falciparum. Size variation polymorphisms have been found in one of these homologues, herein referred to as pfmrp2. We intend to investigate the involvement of these size variations to parasite in vivo responses to Coartem and amodiaquine + artesunate (ASAQ).

Methods: The clinical study was conducted in Zanzibar from November 2002 to February 2003. 408 children with uncomplicated P. falciparum malaria on Zanzibar were enrolled and assigned either Coartem or ASAQ. Blood were collected on filter papers during the 42-day follow-up period for DNA extraction. Breakthrough infections were determined as recrudescences or reinfecions through pfmsp2 block 3 analyses. We analysed the presence of two insertions and one deletion of pfmrp2 in blood samples before (D0) and after the administration of the drugs (breakthrough infections), with nested PCR-based protocols as well as DNA sequencing.

Results: On Zanzibar the size variations in pfmrp2 were found to be highly polymorphic. For both the insertions and the deletion we could detect several sizes for each size variation, respectively by PCR. Since these variations all occur in minisatelites our findings of a wide range of sizes could be expected. Comparison of preliminary data of the frequency of each of the analysed polymorphisms among the D0 versus recrudescent infections shows notable prevalence variation, suggesting a selection event upon the administration of the drugs.

Interpretation: The analysed size variations are located in the open reading frame of pfmrp2 and may thus affect the transport capacities of the protein. This observation points for a possible involvement of PfMRP2 in the parasite responses to both Coartem and ASAQ.

In vitro sensitivity of Plasmodium falciparum susceptibility chloroquine, amodiaquine, quinine and artemisine in Bougoula (Mali) [MIM-sd-505818]
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Introduction: Falciparum malaria represents serious and an increasing world public health problem due to the acquired parasite’s resistance to the most available drugs. Although a large body of in vivo and molecular
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data is available for parasites collected in Mali, no in vitro results is available.

Methods: Following an intensive training and technology transfer session we used an isotopic microtest to assess the in vitro sensitivity of Plasmodium falciparum isolates collected in Sikasso (Mali) from October 2004 to January 2005 to chloroquine, amodiaquine, quinine and artemisinin.

Results: Overall 102 fresh isolates of P. falciparum were tested. Thirteen of these height were negative with the microscopic control. Eighty-nine were positive yielded a successful assay.

Interpretation: Final analysis and interpretation of these new in vitro susceptibility data is underway and will be presented at the meeting.

365B

Synergistic effects in vitro with chloroquine, of methanol extract of Bidens pilosa leaves on resistant Plasmodium falciparum isolate [MIM-BD-3144]


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Introduction: Artemisinine-based combination drugs, though highly efficacious are very costly and in short supply. Anti-histaminic drugs in synergy with anti-malarials have reversed resistance in parasites. Bidens pilosa a very safe and widely used plant in Africa and Asia for a multiplicity of inflammatory conditions was recently described to posses verapamil-like properties and so we undertook to investigate the effects of B. pilosa in combination with chloroquine in the growth of Plasmodium falciparum in culture.

Methods: Blood from consenting patients were collected in citrate buffer as anti-coagulant and used to set up an in vitro drug susceptibility assay essentially according to the WHO/MIM anti-malaria drug resistance Network protocol. Schizont inhibition was used to assess parasite growth and expressed as a percentage of the growth in control well and plotted against the chloroquine concentration. Dd2 parasite served as control parasites. Varying concentration of Bidpil extracts were used at three increasing concentrations and marched against verapamil.

Results: The association of Bidpil with chloroquine stimulated a significant drop in the IC50 of chloroquine on four out of nine isolates, the other five being sensitive parasites lines. When used in isolation Bidpil demonstrated a weak anti-malaria activity. The IC50 of chloroquine dropped from 36.4 to 508 ng/ml in be presence of 100 ng/ml of Bidpil extract, and to 0.3–5 ng/ml at an extract concentration of 10 ng/ml which curves re superimposed on those for verapamil.

The considerable left-shift characteristic of verapamil on resistant parasites was observed on other parasite isolates and on Dd2, a known chloroquine resistant cell line.

Interpretation: It was concluded that B. pilosa possesses a weak anti-malarial activity and its association with chloroquine synergistically limits parasite growth in vitro. Its further development may be helpful in new ways of using the age old chloroquine.

366C

Génotypage par MSP 1 et ses allèles K1, MAD 20 et RO 33 des souches de P. falciparum aprés traitement par les combinaisons d’antipaludiques au Senegal [MIM-HD-108720]


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Introduction: Le Sénégal a adopté la bithérapie comme politique de traitement des accès palustres simples à P. falciparum depuis juin 2003. Des éudes y ont montré des taux de RCPA à J28 de 97.5% pour l’association Artésumate + Amodiaquine (Artésumat®), 98% pour Artésumate + Méfloquine (Artéquinn®), 83.6% pour le Coartem® 4 doses 100% pour le Coartem® 6 doses et 98.8% pour Amodiaquine + Sulfadoxine–Pyriméthamine. L’objectif de ce travail était de connaître l’origine des quelques échecs observés.

Methods: Nous avons effectué un génotypage des souches de P. falciparum qui sont réapparus entre J21 et J28. Pour chaque souche, après extraction de l’ADN sur papier filtre, nous avons étudié le gène MSP 1 en faisant une double PCR: une première a amplifié la
totalité du gène MSP 1. Ensuite pour chaque amplicon, nous avons effectué trois PCR pour amplifier les allèles K1, MAD 20 et RO 33. Une migration sur gel a permis de comparer ces différents allèles pour les souches de J0 et celles du jour où l’échec thérapeutique ou parasitologique a été constaté.

**Results:** Pour Arsucam®, Artéquini® et Amodiaquine + SP tous les cas d’échecs apparus entre J21 et J28 sont des réinfestations donnant un taux de réponse clinique et parasitologique adéquate (RCPA) de 100% à J28 pour les trois associations. Concernant le Coartem 4 doses, 4 patients ont présenté des recrudescences. Le taux de RCPA est donc de 96.4%. La différence du taux de RCPA à J28 après génotypage entre ces quatre combinaisons n’était pas significative.

**Interpretation:** L’utilisation de MSP 1 et des ses allèles K1, MAD 20 et RO 33 à permis de faire le génotypage des souches de *P. falciparum* après traitement par quatre combinaisons d’antipaludiques.

**367A**

**Partial efficacy and poor tolerance of amodiaquine plus sulfadoxine–pyrimethamine in the treatment of uncomplicated *P. falciparum* malaria in Senegal**

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**Introduction:** Drug resistance is a major problem for malaria control. Because of increasing rates of chloroquine resistance, Senegal changed, in 2003, its first-line drug for the treatment of uncomplicated *Plasmodium falciparum* malaria from chloroquine to amodiaquine (AM) and sulfadoxine–pyrimethamine (SP) combination. The aim of this study was to assess the efficacy and the tolerance of this new first-line treatment in a Senegalese village with seasonal malaria transmission.

**Methods:** We used a modification of WHO standard protocol (2002) for measuring therapeutic efficacy and parasitological resistance: adequate clinical and parasitological response (ACPR), late parasitological failure (LPF), late clinical failure (LCF) and early treatment failure (ETF). All the patients, attending the Health Centre of the village, fulfilling inclusion criteria (≥2 years old, axillary temperature ≥37.5 °C, pure *P. falciparum* inclusions between 1000 and 100,000 parasites/µl of blood) were given recommended oral doses of AM and SP. With their informed consent, patients were followed parasitologically and clinically for 42 days. In vitro tests were performed and genotyping was used to distinguish recrudescence from reinfection.

**Results:** Among 100 confirmed cases of *P. falciparum* uncomplicated malaria attacks, 38 were included in the 42-day follow-up. Characteristics of these 38 patients were: mean age (95% CI) = 20.7 (16.3–25.1); sex ratio (M/F) = 1.11. Only nine thick films were missed (3% of the expected number for a complete parasitological follow-up (day 0, 1, 2, 3, 7, 14, 28, and 42) of all subjects). Efficacy. Late clinical failure occurred in 1/38 of the patients: on day 8, this 2-year-old boy had an axillary temperature of 37.8 °C and his parasitemia was positive. He was treated with quinine, the second-line drug for treatment failure. One patient presented a positive parasitemia on day 28 and two others on day 42. For these patients, tests to distinguish recrudescence from reinfection are pending. Tolerance: 12/38 (32%) patients never vomited, either before or after treatment. Among the other patients, 17 (65%) received a complementary antiemetic treatment. In fact, 15 patients were vomiting before SP + AM treatment and ten of them continued vomiting after treatment. Interestingly, 11 patients began to vomit only after SP + AM treatment. Finally, one patient presented scratching on the second day (day 1) of treatment.

**Interpretation:** Poor tolerance of SP + AM raises the problem of the acceptability of this treatment in the community. It is to be feared that this will result in delay in the treatment of malaria and will worsen the sanitary situation in malaria endemic countries.
368B
Sequence variations of *Plasmodium falciparum* cytochrome b in field isolates from a multicentric study [MIM-ME-142462]

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**Introduction:** Atovaquone-proguanil (Malarone), is used in some areas for treatment and prophylaxis of *Plasmodium falciparum*. Unfortunately, resistance of parasites is increasing. To better document polymorphism of cytochrome b, the main target of atovaquone, we conducted a molecular survey in Plasmodium endemic areas. The approach was based on full sequencing of the cytochrome b gene.

**Methods:** Health center surveys were conducted in Madagascar, Senegal, French Guiana and Cambodia. Standardized molecular procedures were used for DNA extraction and amplification. Oligonucleotide primers were designed using a reference sequence (PlasmoDB, AY282930) for full cytochrome b gene amplification. The amplicons were purified using a millipore system. The expected 1131 bp PCR product was visualized in 1.2% agarose gels. Automatic sequencing of the PCR products was performed in an Applied Biosystems ABI 3100 DNA sequencer. Sequence analysis of was done using the Seqscape software v.2.0. Single nucleotide polymorphisms (SNPs) were considered only if observed unambiguously on both strands.

**Results:** We successfully sequenced 192, 50, 179 and 150, isolates collected in 2000–2001 from Madagascar, Senegal, Cambodia and French Guiana, respectively. No mutations were observed in Madagascar and Senegal. In isolates from French Guiana and Cambodia, 12 coding SNPs were observed: four in French Guiana and eight in Cambodia. Interestingly, all 12 were site-specific. Except two coding SNPs, detected at 1.1% in Cambodia, the others were found at a low frequency. An interesting finding was the observation of one synonymous SNP occurring at a very high frequency in French Guiana (>90%). This SNP was not detected in other study-sites. The Y268N/S mutation (described as link to resistance) was not observed in any of the four sites. We are currently analysing a second subset of isolates collected in 2003–2004 after introduction of Malarone in French Guiana. These isolates have been monitored for in vitro drug susceptibility, including atovaquone.

**Interpretation:** So far, populations analysed in all four sites have a wild type codon 268. The cytochrome b gene polymorphism is another illustration of the difference in genotypes circulating in South America (French Guiana) compared to Africa and Asia.

369C
Assessment of artemisinine based combinations therapy (ACTs) efficacy for treatment of uncomplicated *P. falciparum* malaria in the Sudan [MIM-SE-218976]

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**Introduction:** After the spread of chloroquine resistance in Sudan, the previous first line of treatment, to levels exceeding the recommended level of 25%, a new antimalarial drug policy was introduced recently in 2004. The recommendation of artemisinin based combination therapy ACTs was strongly supported by the technical advisory committee in series of meetings held since 2002–2004. Six sentinel sites were selected for the ACTs trials which constituting different strata in the country, according to the transmission intensity.

**Methods:** We followed the WHO protocol 2003 of assessment and monitoring of antimalarial drugs efficacy for the selection of the study area(s), study design and the study population. Also in the treatment we used good quality drugs of artesunate + SP and artesether/humefantrine and the dose was given according to patients body weight by the medical team. The data was entered in WHO excel sheets for further analysis and clinical classification of response to treatment was done according to standard criteria WHO, 2003.
Results: A total of 4862 patients with fever or a history of fever were examined, of them 1518 were positive for *P. falciparum* malaria. A number of 379 patients who met all of the inclusion criteria were enrolled in the study for 28 days follow up following the WHO protocol, 2003. The results showed that, the first line (artesunate + SP) was found to be highly effective, 100% cure rate in three sites and 96% in one site in eastern Sudan. The second line artemether/lumefantrine was also found to be highly effective 100% cure rate in one site in seasonal transmission area in central Sudan and 97.2% in the other site in an area of intense transmission in southern Sudan.

Interpretation: No reported serious side effects accompanied the two lines of combined treatment and rapid parasite clearance was observed. We concluded that, the two new combined therapies were found to be highly effective, safe and well tolerated by patients with uncomplicated *P. falciparum* malaria.

370A

Pattern and progress of antimalarial drug resistance in relation to malaria endemicity in Sudan during the last 25 years [MIM-ME-47536]

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Introduction: Reduction of mortality from malaria depends on accurate early diagnosis and prompt effective drug treatment. During the last 50 years the development and spread of resistance to most front-line antimalarial drugs has given reason for grave concern, which is greatly worsened by lack effective vaccines. Resistance of *P. falciparum* to most antimalarials currently used has been documented in almost all countries where there is transmission, and Sudan is not an exception.

Methods: We surveyed and analysed data from more than 100 published and unpublished reports available on antimalarial drugs resistance in Sudan during the last 25 years including more than 20 reports from our own work in this field.

Results: Chloroquine resistance of falciparum malaria was initially shown in 1978 in Central Sudan, mesoendemic area, where it was found that 0.4 and 0.2% of the study patients were having R1 and R11 resistance, respectively. One year later chloroquine resistance was shown to be 3% and 5 years later it reached 25%. In Khartoum, hypendemic area, resistance to chloroquine was found to be 61.5% and it reached 75% in 2000. In Eastern Sudan, hypendemic area with unstable transmission, it was reported in the late eighties that 42% of the patients treated with chloroquine were resistant and few years later it reached 72.2% and by the year 2000 we reported 76.9% chloroquine treatment failure. In South of Sudan, an area of high endicity, adequate clinical response was observed up to 88.5%, recently. In Eastern Sudan 100% sensitivity to fansidar and quinine was reported in early nineties but the resistance progressed over the years to reach up 19.5%, 18.7% for the two drugs, respectively. Mefloquine was tested in Central and Eastern Sudan, and in both areas it showed 100% response. Recently, we used mefloquine for treatment of falciparum malaria during pregnancy with only 2.5% failure.

Interpretation: Regular mapping of antimalarial drug resistance is required and proper prescription of effective drug may be affected by level of malaria transmission and sociodemographic factors.

371B

Comparison efficacy, safety and tolerability of three treatment regimens of uncomplicated *P. falciparum* malaria in Mali: [artesunate + amodiaquine (3 jours) versus artesunate (3 days) + sulfadoxine–pyrimethamine (1 day) versus artesunate (5 days)] [MIM-BF-55250]


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Introduction: The future of *P. falciparum* malaria treatment is the use of combination therapies. To promote evidence based policy decisions and prepare the introduction of combination therapy for the treatment of malaria in Mali, we needed to know which combination of antimalarial will be most efficacious and best tolerate in local conditions. To address those questions, we have started an assessment of different combinations of antimalarials drug used in Mali. Here we describe an randomized open clinical trial we compare the
efficacy, safety and tolerability of those three treatments arms: artesunate + amodiaquine (3 days) versus artesunate (3 days) + sulfadoxine–pyrimethamine (1 day) versus artesunate (5 days).

Methods: Our objective was to test the hypothesis that three days artesininin combined with amodiaquine and sulfadoxine–pyrimethamine are at least as efficacious as 5 days artesininin in monotherapy. This study was conducted from December 2002 to October 2004 in Bougoula-hameau (5000 people) at Sikasso in southern Mali. The subjects included in this study were followed up for 28 days. The protocol received the approval of the Institutional Review Board of University of Bamako. Parasitological responses and clinical efficacy were assessed, the impact of each treatment arm on gametocyteemia were determined. Safety and tolerability were assessed through a questionnaire and measurement of hemoglobin, creatinemia, blood glucose and liver enzymes (ALT and AST), platelets counts, white and red blood cells counts were performed before and after treatment.

Results: Of a total of 753 patients included, the mean age was 4.4 years and overall 47% of the included patients were males. Our three treatment arms were comparable at inclusion with regard to median age, sex, prevalence of anemia and prevalence of gametocyteemia. At day 14 using the current WHO 2003 protocol, we found 0% (n = 250), 0.8% (n = 244) and 6.8% (n = 250) of cases of drug resistant malaria parasites in the AS/AQ, AS/SP and AS arms, respectively. Day 28 analysis shows that AS/SP is significantly more efficacious than AS/AQ which in turn is significantly better than AS monotherapy. However, molecular correction showed that AS/SP is significantly more efficacious than AS/AQ which in turn is significantly better than AS monotherapy. All treatment decreased the prevalence and incidence of abnormal values of markers were assessed before and after treatment.

Interpretation: No significant adverse event definitely attributable to any of the study drugs was found. Furthermore, each treatment regimen decreased the prevalence of abnormal values of markers of blood and kidney toxicity. Therefore, the combination regimens were comparable to AS monotherapy with regard to safety and tolerability.

372C
In vivo amodiaquine (AQ) resistance and molecular markers of chloroquine (CQ) resistance in P. falciparum isolates from Southwest Nigeria [MIM-OF-10274]
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Introduction: Amodiquine (AQ) a 4-aminoquinoline in combination with artesunate (AS) has been introduced as first line treatment for malaria in replacement for chloroquine (CQ) alone in Nigeria. Although the role of the AS in this combination is to prevent the development of AQ resistance, parasites may quickly develop resistance to AQ in this area of high CQ resistance. Therefore, there is a need to monitor the development and spread of AQ resistant parasites in Nigeria.

Methods: We investigated the association between molecular markers (pfcrT76 and pfmdr1Y86 alleles) of CQ resistance and in vivo AQ resistance in children in Southwest Nigeria as well as the clearance of parasites harboring these two alleles in patients treated with AQ. One hundred and one children (aged 6 months to 12 years) with acute uncomplicated P. falciparum infections were enrolled into the study, treated with standard dosage of AQ and follow-up for 28 days. Finger pricked blood samples from each child were blotted unto filter paper at enrollment and during follow-up. Parasite genomic DNA was extracted from the filter paper blood samples using chelex extraction. The nested PCR and RFLP approaches were used for parasite genotyping and identification of molecular markers of CQ resistance.

Results: The data show that 87 and 13% of patients were cured and failed treatment, respectively. In the pre treatment isolates obtained from all the children, pfcrT76 and pfmdr1Y86 were present in 62 and 28% of the isolates, respectively, while 16 and 28% of the isolates harbored the mixed allele of pfcr and pfmdr1 genes, respectively. Both pfcrT76 and pfmdr1Y86
alleles were present in 19% of all the isolates. In all the isolates that did not respond to treatment with standard doses of AQ, 92 and 86% had pfcrtT76 and pfmdr1Y86, respectively. Analysis of the post treatment isolates obtained from children whose infection did not respond to standard doses of AQ showed that all the isolates had mutant allele of pfcrtT76 and pfmdr1Y86 allele. Although infections in patients were polyclonal (as determined by msp-2 genotyping), the double mutants pfcrtT76 and pfmdr1Y86 alleles are strongly associated with in vivo AQ resistance ($p = 0.035$; OR = 4.38; 95% CI = 1.12–17.04). Treatment failure with the double pfcrtT76 and pfmdr1Y86 mutant as well as the ability of patients to clear these resistant parasites is strongly dependent on age, suggesting that host immunity plays a critical role in clearing AQ resistant *Plasmodium falciparum*.

**Interpretation:** Overall molecular markers of CQ resistance are reliable tools for surveillance of resistance to AS-AQ combination in areas of Africa where this new combination is being introduced.

### 373A

**Amodiaquine resistant *Plasmodium falciparum* malaria and gene polymorphisms [MIM-GF-133829]**

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**Introduction:** Resistance to the mainstay antimalarials is a major cause for the increased morbidity and mortality of *Plasmodium falciparum* malaria and a switch to artemisinin based combination therapy (ACT) is now becoming the new treatment policy. The choice of partner drug is most important for ACT to remain effective and prevent development of further resistance. Amodiaquine (AQ) has mainly been used in monotherapy in some areas in Africa and South-America and is an important partner drug to be evaluated.

**Methods:** In November 2004 we treated 81 children <5 years old with AQ alone, the second-line therapy in Kenya. We related the in vivo treatment outcome to the presence and possible selection of the known pfcrt 76T, pfmdr1 86Y, pfmrp1 191H, pfmrp 437S, pfcrt 152A and pfcrt 163R polymorphisms in recurrent (recrudescent and reinfecting) parasites as defined by msp-2 genotyping and to the blood concentration of the main metabolite desethylamodiaquine (DEAQ). In parallel with this work we have sequenced the pfcrt cDNA from 15 in vitro samples from Colombia and with different characterized AQ/DEAQ susceptibility. New SNPs identified will be analyzed in the Kenya in vivo material.

**Results:** During 21 days follow-up, 28 children had about equally recrudescences or reinfections, i.e. treatment failure rate (TFR) 37%. Neither genotyping of the polymorphisms before treatment nor DEAQ blood concentrations could predict the treatment outcome. Pfcrt 76T was however significantly selected for after treatment ($p = 0.02$, OR 9.6), especially among reinfections during the elimination phase of DEAQ. In the in vitro Colombian material new pfcrt SNPs were identified in highly AQ and/or DEAQ resistant samples. These mutations will be analyzed in the in vivo material from Kenya.

**Interpretation:** TFR after AQ monotherapy was 37%. Added artemisinine may eliminate recrudescences but would not prevent resistant re-infecting parasites to be selected by DEAQ. Pfcrt 76T may be associated with DEAQ resistance, but additional SNPs may be involved.

### 374B

**Transmission of multi-drug resistant *Plasmodium falciparum* after treatment with chloroquine and sulphadoxine–pyrimethamine in The Gambia [MIM-RH-22533]**


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**Introduction:** Malaria parasites carrying genes conferring resistance to antimalarials have a selective advantage leading to higher transmissibility from the treated host. Whilst the implementation of artemisinin-based combinations is being planned on a large-
scale in Africa, chloroquine (CQ) plus sulphadoxine/pyrimethamine (SP) is being employed as an interim combination in several countries. We studied the effect of adding SP to CQ on *Plasmodium falciparum* transmissibility and the selection of resistance markers.

**Methods:** In 2001 we conducted a randomised controlled trial of CQ versus SP versus CQ + SP in combination among Gambian children with uncomplicated *falciparum* malaria. A subset of patients, who were found to be carrying gametocytes 7 days after treatment, donated blood for direct membrane-feeding experiments. In the SP group, feeding also took place 10 and 14 days after treatment. Mosquitoes were dissected 7 days after feeding and oocysts counted. Malarial markers for CQ and SP resistance were examined in the pre and post-treatment blood samples and in oocysts after transmission to mosquitoes to investigate trends in selection and transmission of resistance-associated alleles of *pfcrt*, *pfmdr1*, *dhfr* and *dhps* under mono and combination therapy.

**Results:** The 26/70 (37%) feeds resulted in transmission to one or more mosquitoes. At day 7, adding SP to standard CQ treatment had no effect on the intensity of transmission to mosquitoes, as measured by the ratio of mean oocyst number. Treatment with SP alone resulted in a significantly reduced oocyst burden compared to CQ monotherapy (*P* < 0.001), even though gametocyte density in the feed donors was similar in both groups. Feeds carried out 14 days post-treatment with SP showed greater transmission success. The pre-treatment prevalence of SP-resistant dhfr triple mutant (codons 51, 59, and 108) was 82.5% in this selected group of feed donors, and 65/70 (93%) carried the chloroquine-resistant pfcrt-76T mutation. Genotyping data showed that oocyst genotype generally reflected that of the feed sample, and that most oocyst positive midguts resulting from one feed tended to carry the same genotype. The 3/4 positive feeds resulting from CQ + SP treated individuals allowed the transmission of some CQ and SP sensitive alleles to eight mosquito midguts. However, the most successful feed resulted in the detection of only SP-resistant dhfr alleles in all 11 PCR positive midguts, even though SP-sensitive genotypes had been present in the bloodmeal.

**Interpretation:** Adding SP to standard CQ treatment does not reduce the intensity of transmission to mosquitoes. Molecular markers indicate that multi-drug resistant parasites can be selected by CQ + SP treatment, are readily transmissible and are common in The Gambia.

375C

**Re-infection of* Plasmodium falciparum* recurs parasitaemias in patients selected for CQ efficacy trial in an area of low intensity transmission in Sudan [MIM-AH-138301]**

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**Introduction:** Chloroquine was the drug of choice in Sudan. Decisions regarding drug policy rely largely on the results of in vivo studies that assess clinical and parasitologic outcomes after therapy. Parasite genotyping using the PCR and molecular markers facilitated the differentiation of reinfection from recrudescence parasitaemias in malaria infections. MSP1, MSP2, and GLURP are the most polymorphic markers used, to genuinely identify whether reinfected parasites are resistant and the effects and selection pressure of CQ treatment under low transmission settings experienced in Sudan.

**Methods:** The study was carried out at Gedaref Teaching Hospital, eastern Sudan. Malaria transmission is seasonal, confined to 2–3 months following the offset of the rainy season. *Plasmodium falciparum* is responsible for >95% of clinical cases and the EIR is estimated to be around two to three infective bites/person/season. Patients from both sexes with different ages, clinical symptoms and microscopically confirmed malaria, were recruited. Standard questionnaire was used to record all essential information of the patients during all follow up days of the study (WHO, 2001). All samples was collected after oral consent of the patient. Slides were prepared, stained with Giemsa and parasitaemia per 1 ml of blood was calculated. A blood sample was spotted onto a filter paper for PCR analysis during all follow up days. DNA extraction was carried out using the chellex method. Poly morphic repetitive region, block 2 of MSP1 and block 3 of MSP2 was amplified using allele specific primers.

**Results:** We monitored clinically and parasitologically 33 patients selected for CQ efficacy trial in a
Hospital based study, conducted at the Gedaref Teaching Hospital, eastern Sudan. We used microscopy and the PCR to investigate the prevalence and diversity of *P. falciparum* parasitaemia in slides and filter paper blood samples series collected at days 0, 3, 7, 14, 21, and 28, from each patient followed up for 28 days (WHO, 2001). Allelic polymorphisms on GLURP, MSP-1 and MSP-2 genes loci were assayed for samples series with slide readable parasitaemias after day 14. From a number of 33 patients initially treated with CQ, six of them (18.2%) patients cleared their parasitaemias and clinical symptoms. Molecular analysis of the data approved that four patients were having diverse parasite clones during time point(s) of their follow up. Only two (6.1%), showed a newly different clone acquired after day 14, accompanied by clinical symptoms and followed by change of drug treatment and/drug regimen.

**Interpretation:** The use of polymorphic markers confirmed if patients with clinical recrudescence are genuinely resistant. This apparent recrudescence may therefore consist of parasites sensitive to drug. This also minimises the number of treatment failure based on uncontrolled in vivo tests, mistakenly classified and is useful in intervention studies as well as decisions regarding drug policy. The unsignificant underestimation of the true risk of treatment failure due to reduce the follow up period to 14 days, is important when studying drugs with short half-lives.

376A

**Linkage disequilibrium between two loci on chromosomes 7 and 5 of Plasmodium falciparum and in vivo chloroquine resistance in Southwest Nigeria [MIM-CH-60458]**

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**Introduction:** The mechanism of Plasmodium falciparum resistance to chloroquine (CQ) is still unclear and remains very controversial. However, CQ resistance in *P. falciparum* has been associated with polymorphisms in loci on chromosome 7 (pfcrt gene) and on chromosome 5 (pfmdr1 gene). This study was designed to determine the association between in vivo CQ resistance and *P. falciparum* polymorphisms in pfcrt and pfmdr1 genes.

**Methods:** One hundred and eleven children with falciparum malaria were enrolled into the study and treated with standard dosage of CQ and followed-up for 28 days. Blood samples were blotted onto filter paper at enrollment and at recrudescence of infections. The nested PCR and RFLP methods were used for detect of polymorphisms in pfcrt and pfmdr1 genes. Amplification of block 3 of msp-2 gene was used to discriminate between recrudescences and reinfections. Linkage disequilibrium analysis between the mutant pfcrtT76 and pfmdr1Y86 alleles in pre-treatment isolates of *P. falciparum* obtained from patients was estimated by linkage disequilibrium constants $D'$ and $r^2$, using only isolates with single allele detectable at both pfcrt and pfmdr1 alleles.

**Results:** Single alleles at both pfcrt and pfmdr1 loci were found only in 55 out of 111 *P. falciparum* isolates screened at enrollment. Of these 55 isolates, the double mutant pfcrtT76 and pfmdr1Y86 was found in 84% of isolates. Allelic frequency analysis showed that the frequencies of mutant pfcrtT76 and pfmdr1Y86 alleles were 0.636 and 0.718, respectively in the parasite’s population. In addition, these two mutant alleles were in Hardy–Weinberg equilibrium ($17.95; p<0.000$ for pfcrtT76 allele and 8.46; $p=0.0036$ for pfmdr1Y86 allele). Association and linkage disequilibrium analysis between paired single alleles on pfcrt and pfmdr1 loci showed that the mutant pfcrtT76 allele on chromosome 7 was in strong linkage disequilibrium with the mutant pfmdr1Y86 allele on chromosome 5 (31.44; $p=0.000$). The linkage disequilibrium constants, $D'=0.67$ (95% CI=0.537–0.753) and $r^2=0.28$ are greater than zero even at their lower extremity of the confidence interval.

**Interpretation:** This study suggests that both mutant pfcrtT76 and pfmdr1Y86 alleles on chromosome 7 and 5, respectively are involved in *P. falciparum* resistance to CQ in vivo and are in linkage disequilibrium likely maintained epistatically by CQ selective pressure.
Plasmodium falciparum resistance in Niger: Molecular markers scale up of the survey

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Introduction: The process to adapt the malaria treatment guidelines is based on data representative as much as possible of the national situation. The clinical surveys (28 days follow up) of therapeutic efficacy are essential, but because this method is resources consuming, it cannot be repeated in time and space. Molecular assays are relevant tools, (i) to distinguish true resistance from re-infestation during the survey, (ii) to scale up the surveillance of parasite resistance in large country such as Niger.

Methods: To support the national control program, the assays to detect pfcrt and dhfr gene mutations for chloroquine and sulphadoxine–pyrimethamin resistances by PCR-RFLP have been implemented. Resistance among clinical cases and asymptomatic carriers of parasite has been carried out. To extend the surveillance previously limited to the capital, a national network including primary health care centers and hospitals was built. Active collects of dried blood samples are collected from clinical malaria cases. These cases were either confirmed when microscopic diagnosis was disposable or presumptive and then screened by antigens before being tested. The results were analyzed according to the different transmission level areas.

Results: Samples were collected from several districts, principally from the West and South zones of the country. Biologically confirmed cases from hospitals allow quick testing and fed-back results. Although clinical febrile cases from primary health centers were more difficult to obtain, they are supposed to be less biased than hospitalized cases. Finally the prevalence of Pfcrt mutations seems to be more linked to the collection zone rather than to the clinical status of the patients. Urban areas appear to support a higher prevalence (about 50%) of Pfcrt muted strains than rural areas where a twice less prevalence is observed. This difference between urban and rural environments seems not to observed with the dhfr gene mutations when sulfadoxin–pyrimethamin is poorly used for mild malaria cases treatment.

Interpretation: Molecular tools make Niger able to survey the evolutions of resistances of (i) chloroquine after the switch of first-line treatment towards artemisin-combined therapeutic and (ii) pyrimethamin–sulfadoxin for intermittent treatment of pregnant women.

Assessment of the clinical impressions of Fansidar® on P. Falciparum malaria patients in Abakaliki Ebonyi State Nigeria

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Introduction: There are published data documenting Fansidar® treatment failures in many countries including Nigeria (WHO, 1997). At Nnobi, South East Nigeria Eneanya and Nwazelu et al. (2003) reported drug treatment failures in individuals treated with Fansidar®. This was earlier reported in Baissa, North Eastern Nigeria by Moltan et al. (1993). The main aim of the present investigation was to ascertain the cure rate of Fansidar® drug therapy against P. falciparum malaria in Abakaliki, Nigeria.

Methods: Regular materials and methods for staining thick and thin blood films for malaria diagnosis and identification of Malaria parasites as described by Ukaga et al. (2002) was adopted followed by the standard test procedure of WHO (1991) for clinical evaluations. Administration of Drug/Follow up Analysis. The fansidar drug was administered orally. Three tablets of Fansidar for one dose treatment as indicated by the drug manufacturers. The drug was only given when the patient tested malaria positive. Any malaria negative patient was excluded from the study.

Results: This study was designed to ascertain the effectiveness of fansidar® drug therapy in the principal target group of P. falciparum malaria patients in Abakaliki Ebonyi State Nigeria. Assessment was based on 14 day clinical and parasitological responses to the supervised therapy. Out of 132 patients studied, 61 (46.2%) were males and 71 (53.8%) were females stratified into different age groups. Ten to nineteen years had at Day 2 (D2), parasitological success (PS) of 30.3%
and parasitological failure (PF) of 18.2% and at Day 14 (D14) clinical success (CS) = 35.6%, clinical failure (CF) = 12.9%. 20–29 years.

379A
Pfcrt exon2 genotypes and Pfdhfr mutations in Dakar [MIM-RJ-164846]

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Introduction: Senegal is mainly an area of seasonal transmission of malaria. Level of drug resistance increased during the last 10 years and the national policy for the treatment of mild malaria moved from chloroquine to the amodiaquine–sulfadoxine–pyrimethamine association. The area of Dakar is a low seasonal area of transmission of malaria, with extensive exchange of population with the rural area. One out of three inhabitants of Senegal is leaving in this part of the country.

Methods: This phase of change requires an acute survey of the resistance of the field isolates. Blood samples were collected among patients attending dispensaries for malaria attack. Parasites were in vitro cultured according to the standard isotopic microtest and IC50 were calculated for drugs. The full sequences of Pfcrt exon2 and Pfdhfr were determined for these parasites.

Results: Mean IC50 of the strains collected in Dakar were 129 ± 109 nM for chloroquine, 38 ± 32 nM for monodesethylamodiaquine, 1.3 ± 65.5 nM for artemether and 3 ± 16.5 nM for lumefantrine. Among the stains collected in Dakar a high polymorphism was detected using MSA1 and MSA2. Multiple infections were frequent. For PfDHFR only three mutations were detected, mainly in the urban area of Dakar (C50R, N51I, and S108T). These mutations were associated with an increasing level of IC50 against pyrimethamine. For PfCRT, 190 strains were analysed. The main allele for exon2 was 70SVCVIETIFAKA81. Only 15% of the strains had a K76 allele. CVMDK, CVMEK, and VIDK were rarely detected. For the “sensitive alleles” a higher polymorphism was found, with mainly 78VIDK76, but also VMDK, VMEK, and VIDK. This polymorphism claim for a re-introduction of strains for different area of the country, associated with migrations of workers.

Interpretation: These data also argue for an invasion of very few resistant alleles in this area, rapidly selected by drug pressure. Amodiaquine and sulfadoxine–pyrimethamine can still be used in this area.

380B
Prevalence and risk factors for gametocyte carriage in Kenyan children with uncomplicated Malaria [MIM-EJ-4109]

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Introduction: Transmission of Plasmodium falciparum parasites from humans to mosquitoes requires the presence of infectious gametocytes in the human peripheral blood. Treatment with sulfadoxine–pyrimethamine (SP) is often associated with emergence of P. falciparum gametocytes.

Methods: We determined the prevalence and risk factors associated with P. falciparum gametocytaemia at presentation to hospital in Kenyan children with uncomplicated malaria. A total of 600 children <5 years with uncomplicated malaria were enrolled in a randomized trial of the efficacy of artesunate plus SP compared to SP alone at Siaya district hospital, western Kenya. At enrolment, malaria parasites, hemoglobin, and temperature were measured. These measurements were repeated on days 1–7, 14, and 28 after enrolment. All children were followed up for 28 days.

Results: A total of 86 (14%) children had gametocytes at enrolment. During follow up, 18 and 15% all children had gametocytes by days 7 and 14, respectively. Compared to those treated with a combination of 3 days of artesunate plus SP, the risk of developing gametocytaemia was significantly increased among those treated with SP on days 7 (OR = 9.32 [95% CI 4.73–18.37]) and on day 14 (OR = 18.2 [95% CI 7.64–43.5]) after treatment. The presence of gametocytes at enrolment was significantly associated with prolonged illness (OR 1.58 [95% CI 0.93–2.69]), severe anaemia (OR 2.85 [95% CI 1.49–5.44]) and low parasitaemia (OR 2.05 [95% CI 1.3.35]).
Interpretation: Adopting an artesunate-based combination therapy for uncomplicated malaria in Kenya will improve cure rates and reduce transmission of resistant parasites.

381C Increased prevalence of drug-sensitive *Plasmodium falciparum* in absence of drug pressure in the dry and transmission-free season of eastern Sudan [MIM-AK-36884]

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Introduction: Malaria in eastern Sudan lies at the fringes of endemicity, where transmission is low and seasonal. The main parasite, *Plasmodium falciparum*, survives the long dry and transmission-free season as asymptomatic sub-patent infections, and resurges following annual rains. Chloroquine resistance (CQR) first emerged in the mid 1980s and remained stable until the early 1990s. However, the CQR rate varied between years in association with the level of annual rain, and fluctuated significantly between seasons, increasing in the dry season but falling at the start of the next transmission season.

Methods: In the present study we extended the above work and carried out within-season analysis and close monitoring of drug sensitive and resistant parasites in Asar village in eastern Sudan throughout the dry season in the absence of drug pressure. A cohort of 30 *P. falciparum* infected patients was recruited in November 2001, treated with a dose of chloroquine (25 mg/kg), and then followed monthly until the end of December 2002. We used PCR to detect parasites that persisted at sub-patent levels in the dry season, and resurges following annual rains. Chloroquine resistance (CQR) first emerged in the mid 1980s and remained stable until the early 1990s. However, the CQR rate varied between years in association with the level of annual rain, and fluctuated significantly between seasons, increasing in the dry season but falling at the start of the next transmission season.

Results: A large proportion of the cohort maintained sub-patent asymptomatic *P. falciparum* infections throughout the dry season into the next transmission season. Mutant alleles of pfcr and pfmdr1 reached fixation following CQ treatment and remained high in the transmission season, a reflection of selection. However, at the start of the dry season, wild type alleles of both genes started to emerge and increased significantly in frequency as the season progressed (P = 0.03 for pfcr and P = 0.003 for pfmdr1 when ignoring mixed infections).

Interpretation: These data appear to reflect a compromised fitness of drug resistant parasite in the absence of drug pressure. This fitness cost is expected to slow the rate of spread of drug resistance in *P. falciparum* when drug usage ceases.

382A Molecular markers for sulfadoxine–pyrimethamine resistance in *Plasmodium falciparum* infections in children under 5 years at Kibaha District, Tanzania [MIM-WK-21527]

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Introduction: Sulfadoxine–pyrimethamine (SP), the current first line drug in Tanzania for treatment of malaria, may be compromised by rapid spread of resistance via evolved mutations in the parasite’s dhfr and dhps genes. The aim of the present study was to establish the baseline frequencies of pfdhfr and pfdhps mutant genotypes and their potential for predicting the in vivo efficacy of SP.

Methods: Ethical approval was given by the National Institute for Medical Research. Verbal informed consent was obtained from all parents or guardians. A total of 116 children were enrolled in February to March 2002, during establishment of SP efficacy. Infected blood samples were taken from children presenting with non-severe malaria at day 0, before and after treatment with SP, at days 3, 7 and 14. Blood samples were collected on Whatman filter paper, air dried and stored at room temperature. Genomic DNA was then extracted by the chelex method. Point mutation at positions 51, 59, 108 and 164 of dhfr gene and at 581, 540 and 437 of dhps gene were analysed by Nested Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism.
Results: Eighty-six percent (86%) of the subjects demonstrated an adequate clinical response by day 14. Sensitive parasites at dhfr locus was 8.2%, whereas those at the dhps was 73%. Frequency of triple mutant dhfr allele (Ile 51, Arg 59, Asn 108) was found to be 47%, double mutant dhps (Gly 437, Glu 540) 7.9%. No mutation was detected at codon 164 of the dhfr gene. The post treatment infections had higher rates of dhfr mutations at codon 108, 59 and 51 (Fisher’s exact test, \(p = 0.0007\)). The presence of triple dhfr mutant alleles did not correlate with clinical outcome (Fisher’s exact test, \(p = 0.5434\)).

Interpretation: Although SP is still efficacious at Mlan- dizzi, the higher rates of mutation on the dhfr do not spell a bright future for SP treatment. It is rational to think of an alternative drug, while retaining SP for chemoprophylaxis in pregnancy.

383B Rapid increase in resistance of Plasmodium falciparum to chloroquine–fansidar, the first line drug for treatment of uncomplicated malaria in Uganda [MIM-FK-92496]
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**Introduction:** Chloroquine (CQ) plus sulfadoxine–pyrimethamine (SP) recently replaced CQ monotherapy as the policy first-choice drug for uncomplicated parasite strains to either drug. In this report, we compare the clinical, parasitological and molecular outcomes from two studies. **Methods:** In one study, the efficacies of CQSP in one arm and amodiaquine (AQ) plus SP (AQSP), in the other arm, were evaluated using a 28-day follow-up WHO in vivo drug efficacy protocol. In the other investigation undertaken a year before, we assessed the efficacy of SP using a 14 day WHO follow-up procedure. **Results:** While adequate clinical response (ACR) for SP (14-day follow-up) was 92.7% in year 2002, the combination CQSP unexpectedly gave lower ACR of 80% 1 year later. Of the three regimens (SP, CQSP and AQSP) that we evaluated, AQSP gave the highest level of efficacy (ACR = 94.3%). There were no early treatment failures among patients treated with the AQSP combination. Nevertheless, AQSP therapy ultimately failed in 5.7% (14 day follow-up) and 11.5% (28 day follow-up, corrected by MSP2 genotyping) of the patients. On the other hand, for CQSP, the percent failure rates in 14- and 28-day follow-up protocols were, respectively, 20% (not corrected by genotyping) and 17% (corrected by MSP2 genotyping). We find that in one year, when CQSP was the first-line drug, the percent mutation rates at codons of amino acid residues putatively linked to SP resistance increased as follows: from 83.8 to 100% at codon 108, from 58.7 to 76% at codon 59 in the DHFR gene; yet, from 58.8 to 86% at codon 437 and from 33 to 43% at codon 540 in the DHPS gene. One year after introduction of the aminoquinoline–antifolate combination 'CQSP' as first line treatment in Uganda, there was a very rapid development of *P. falciparum* resistance to the regimen. **Interpretation:** The study strongly suggests that adding CQ to SP does not noticeably improve upon efficacy of CQ mono-therapy. Finally, our results corroborate the findings of others showing that AQSP is superior to CQSP.

384C High re-infection and treatment failures in children treated with amodiaquine for falciparum malaria in Muheza, Tanzania: A community-based study [MIM-ML-194879]
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**Introduction:** Plasmodium falciparum resistance to current first (sulfadoxine–pyrimethamine, SP) and second line (amodiaquine, AQ) antimalarials is a major public health problem in Tanzania. Monitoring efficacy of AQ was initiated in 2002 to complement drug failure surveillance in communities with high SP resistance. The objective of this study was to assess the efficacy
of AQ in two villages of Magoda and Mmpayu (with insecticide treated nets, ITNs) and Mgome (without ITNs) in Muheza, Tanzania.

Methods: The study was conducted in mobile clinic set up in May 2003. Sixty children (aged 6–59 months) who met the inclusion criteria were treated with AQ given over a 3-day period (10 mg/kg on day 0 and 1, and 5 mg/kg on day 2) under supervision. To ensure the drugs used were of required standards, the quality of AQ was evaluated by both quantity and dissolution tests. Parasitological and clinical follow-up were made on several occasions over a 28-day period. To differentiate recrudescence from re-infections, PCR amplification of the polymorphic regions of *P. falciparum* msp1 and msp2 genes was performed. Additionally, Pfcrt codon 72–76 polymorphisms were investigated by PCR and sequence-specific oligonucleotide probe (SSOP)-ELISA.

Results: In the 54 cases with complete follow-up, a significant difference in late treatment failure (LTF) rates was seen (60.7% in ITN versus 88.5% in non-ITN villages, *P* = 0.02), before PCR correction. However, after PCR correction, 23 cases (60.5%) were confirmed as re-infections; giving a true LTF rate of 35.3 and 39.1% in the above communities. Similar parasite clearance rates were seen in the first 14 days in both settings. However, a significantly higher proportion were parasite free on days 21 (*P* = 0.002) and by day 28 the rate was different but not significant (*P* = 0.079) in the ITN villages compared to the non-ITN village. The frequency of Pfcrt CVIET haplotype pre-treatment was 97.0%; remaining samples were CVMNK. The AQ tablets used passed quality assurance tests for quantity (104.5 ± 2.6%) and dissolution (86.9% in 30 min).

Interpretation: The similar parasite clearance seen in the first 14 days, in both settings, might be due to the added effect of amodiaquine; whereas prevention of re-infections by ITNs was apparent later on. The results indicate that AQ alone is no longer effective.

385A
Effect of amodiaquine, sulfadoxine/pyrimethamine, and amodiaquine plus artemisin in children with falciparum malaria at Ujiji, Kigoma, Tanzania [MIM-MR-47203]
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Introduction: Tanzania adopted sulfadoxine/pyrimethamine (SP) as first line antimalarial in 2001. In preparation for review of antimalarial drug policy, the National Malaria Control Programme planned studies to assess efficacy of SP, amodiaquine (AQ), and alternatives. In 2003, a study to assess the efficacy of SP, AQ, and AQ + artesunate (AQ + AS) combination therapy (CT) was conducted at Ujiji. Primary outcome was Day 28 treatment failure. Day 14 failure and haematological response were secondary outcomes.

Methods: Children aged 6–59 months with falciparum malaria (2000–200,000 rings/µl) meeting other inclusion criteria were randomized to one of the three treatment arms. Recruited children were 87 on SP, 88 on AQ, and 86 on CT. Treatment was given by body weight and under supervision. Single dose SP (1.25 mg/kg pyrimethamine), three divided doses over 3 days for AQ (10 mg/kg on day 0 and 1 and 5 mg/kg on day 2), and 3 days for the CT arm (AQ for 3 days and AS at 40 mg/kg daily for 3 days). Follow-up was made over a 28-day period. PCV was estimated at baseline and follow-up. Filter paper blood-spots were collected for PCR analysis of msp1 and msp2 to distinguish recrudescence from re-infection. All cases of treatment failure were treated with quinine.

Results: Results revealed that out of 645 children screened for malarial parasites 383 (59.4%) were positive. Day 28 efficacy data was available for 72 cases on SP, 75 on AQ, and 78 on CT where successful follow-up was made. Evaluation of treatment outcome revealed an overall high treatment failure with both
SP (88.9%) and AQ (61.3%), and considerable failure (39.7%) in the CT arm. Evaluation of data based on 14-day follow-up period showed adequate clinical and parasitological response (ACPR) of 42.1, 81.6 and 91.1% in the SP, AQ and AQ + AS treatment arms respectively. Initially, rapid clearance of parasites was seen in the CT arm; all cases with the exception of three were parasite free by day 2 of follow-up. Data from the three trial arms revealed early treatment failure (ETF) in the SP group only. Preliminary PCR data for the CT arm confirmed most of the parasites seen at day 14 and 28 were indeed due to re-infections. Within-day comparisons revealed significant differences in mean PCV at both day 14 and 28 of follow-up. The SP arm had minimal PCV gain whilst the other two arms had marked increases in mean PCV levels on both day 14 and day 28.

Interpretation: Results show clearly that SP, the current first line antimalarial drug, was not as effective as AQ and the CT. Day 14 cure rates with AQ and CT were good; the latter and other fixed dose ACTs merits further investigations.

386B
Etude de la relation entre la présence de la mutation Pfmdr-1 Y86 et la résistance in vivo de P. falciparum à l'Amodiaquine et au Burkina Faso [MIM-GL-186472]
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Introduction: L’amodiaquine (AQ) seul ou en combinaison avec les dérivés de l’artémisinine, constitue de nos jours une des meilleures alternatives face à la chloroquine-résistance (CQ-R). Cependant, bien que l’AQ soit plus efficace que la chloroquine (CQ), son activité diminue dans les zones de forte CQ-R. Nous avons réalisé une étude d’efficacité thérapeutique à l’AQ en relation avec la présence de la mutation Pfmdr-1 Y86 dans une zone de forte CQ-R au Burkina Faso.

Methods: L’étude s’est déroulé de Juillet à Décembre 2004, à Nanoro, au centre du Burkina Faso. L’AQ a été administré à la dose de 25 mg/Kg repartie en 3 jours. Le protocole de 28 jours de l’OMS a été utilisé pour le suivi des patients. La mutation Pfmdr1 Y86 a été détectée avant et après traitement, en utilisant la technique de Polymerase Chain Réaction (PCR) suivi d’une digestion enzymatique avec AflIII qui coupe uniquement le gène mutant en 2 fragments de 226 bp et 295 bp. Le test de Chi 2 (\( \chi^2 \)) de Pearson a été utilisé pour comparer les variables catégorielles avec un niveau de significativité de 95% (\( p < 0.05 \)).

Results: Ce sont au total, 217 enfants âgés de 6 mois à 15 ans qui ont été suivi au cours de l’étude. Le taux d’échec thérapeutique total a été de 12% (26/217), avec une forte proportion d’échecs tardifs (92%). La présence de la mutation Pfmdr1 Y86 a été observée chez 30% (65/217) de nos échantillons. Nous avons noté une forte (66%) proportion d’allèles purs parmi les mutants. Au total, seulement 10% (22/217) des échantillons étaient des infections mixtes. Nous n’avons pas trouvé de relation entre la présence de la mutation Pfmdr1 Y86 et l’échec thérapeutique à l’AQ (OR = 1.5 [95% CI: 0.6–3.9]; \( p = 0.3 \)). En effet, la plupart des échecs (61.5%) présentait le génotype sauvage à J0. Cependant, on notait une forte sélection de la mutation Pfmdr1 Y86 dans les échantillons post-traitement. En effet, on note une augmentation du taux de la mutation Pfmdr1 Y86 de 30% avant traitement à 70% après traitement, mais cette différence n’était pas statistiquement significative (\( p > 0.05 \)).

Interpretation: L’AQ pourrait être une bonne alternative face à la CQ-R, dans le traitement du paludisme simple au Burkina Faso. Cependant, la mutation Pfmdr1 Y86 ne serait pas un bon marqueur pour la surveillance épidémiologique de la résistance à ce médicament.
Analyse moléculaire de *Plasmodium falciparum* des infections palustres reinfestants par la Chloroquine, l’Amodiaquine et la sulfadoxine–pyriméthamine chez les enfants de moins de 5 ans à Kollé au Mali

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Introduction: Nous savons que la diversité antigénique de *Plasmodium falciparum* est le reflet d’un polymorphisme allélique important. Plusieurs études et techniques ont permis de mettre en évidence le polymorphisme allélique, que ce soit par RFLP ou par typage génétique d’isolats de zone d’endémie palustre par PCR ainsi que le séquençage de plusieurs allèles de certains gènes comme MSP-1.

Methods: Le but de ce travail était de définir les caractéristiques de la résistance parasitologique corrigée de *P. falciparum* à la Chloroquine (CQ), à l’Amodiaquine (AQ) et la sulfadoxine–pyriméthamine (SP) à Kollé (zone hyper-endémique). Pour atteindre cet objectif, nous avons réalisé d’août 2002 en février 2003 et de juillet 2003 en janvier 2004 une étude prospective chez les enfants paludéens de moins de 5 ans à Kollé. L’efficacité de ces trois antipaludiques a été évaluée par le test in vivo standard de 28 jours de l’OMS.

Results: Au total, 556 sujets ont été inclus. L’analyse révèle que les taux globaux de résistance parasitologique sont les suivants: En 2002 (CQ = 61.5%, AQ = 41.5% et SP = 14.5%); la différence entre les trois était significative (*p* = 0.001); En 2003 (CQ = 55.5%, AQ = 41.8% et SP = 16.4%); la différence entre les trois était significative (*p* = 0.001). En 2002–2003 (CQ = 58.3%, AQ = 41.7% et SP = 15.5%); la différence entre les trois était significative (*p* < 0.05).

Les taux de reinfestation observés en 2002 étaient respectivement de (CQ = 5%, AQ = 2% et SP = 0%); en 2003 (CQ = 10%, AQ = 3% et SP = 0%); et en 2002–2003 (CQ = 8%, AQ = 3% et SP = 0%). Ceci donne le taux réel de la résistance parasitologique corrigée pour la CQ, l’AQ et la SP en 2002 (56.5%, 39.5% et 14.5%) respectivement, en 2003 (45.6%, 38.8% et 16.4%) et en 2002–2003 (50.3%, 38.7% et 15.5%); la différence était très significative entre les trois groupes de traitement (*p* < 0.05).

Interpretation: Cette analyse montre que la chloroquine et l’amodiaquine ont une résistance parasitologique considérable à Kollé ce qui montre que ces molécules sont inefficaces à Kollé, quant à la sulfadoxine–pyriméthamine, elle est encore efficace mais présente un taux réel de résistance parasitologique remarquable à Kollé.

Selection for *Plasmodium falciparum* drug resistance after intermittent treatment of children with sulfadoxine–pyrimethamine

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Introduction: A study on intermittent preventive treatment in infants (IPTi) was performed in central Ghana to assess the effect of a periodical application of sulfadoxine–pyrimethamine (SUL/PYR). The aim of IPTi is to suppress parasitaemia and thereby clinical malaria episodes and to protect from reinfections for a prolonged period of time while simultaneously allowing the development of natural anti-parasite immunity. However, drugs with a long half-life inherently bear the risk of emerging drug resistance.

Methods: Blood samples from participants were monthly collected for a period of 2 years and parasite densities as well as first appearance of isolates were assessed. The detection of PYR and SUL resistance markers were performed using a mass-spectrometry based assay. To this end, a nested PCR was carried out covering the parts of the genes under question. Extant dNTPs were removed from yielded PCR products and subsequently a primer extension reaction
was initiated for each single nucleotide polymorphism (SNP) to achieve an elongated oligonucleotide corresponding to the respective gene variant under question. After purification, SNP detection could be performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Results: Three SNPs in the \textit{Plasmodium falciparum} dihydrofolate reductase gene (S108N, N51I, C59R) are commonly believed to be responsible for PYR resistance while two SNPs in the \textit{P. falciparum} dihydropteroate synthase gene (A437G, K540E) encode SUL resistance. Resistance to SUL/PYR is mediated by the occurrence of SNPs at all five codon positions. A high prevalence of SUL/PYR drug resistance markers could be assessed in parasites of three months old infants prior to the beginning of the IPTi study. This prevalence correlated to the prevalence pattern detected in asymptomatic adults living in the same area. In a subgroup of this study differences in the prevalence of drug resistance markers became apparent after the complete elimination of the drug (week 8), whereby the relative incidence of isolates with four mutations was two times higher in the verum group than in the placebo group (verum group \(2.6\;[CI\ 1.8–3.9]\), placebo group \(1.3\;[CI\ 0.8–2.1]\), \(p<0.05\)). Treatment, however, did not significantly affect the rate of \textit{P. falciparum} strains exhibiting less than four mutations (IR R07 \([CI\ 0.2–2.0]\), n.s.). Additional data on the longitudinal development of SUL/PYR drug resistance in comparison to clinical malaria episodes will be presented.

Interpretation: The parasitological rebound effect after single dose SUL/PYR treatment raises concern, particularly in areas with a preexisting high prevalence of resistance markers. IPT trials with other regimens should be initiated to provide worthy alternatives.

389B
Verapamil reversal of chloroquine resistance is enhanced in presence of PfCRT T76 by PfMDR1 N86Y mutation in \textit{P. falciparum} isolates from Uganda [MIM-KM-184808]

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Introduction: Reversal of \textit{P falciparum} chloroquine resistance (CQR) has a potential antimalarial clinical application. Compounds exemplified by verapamil (VP) reverse CQR. Mutations in the CQR transporter (PICRT) and multi-drug resistance 1 (PIMDR1) protein are linked to CQR yet their role in the reversal mechanism is unclear. We evaluated associations of mutations Lysine (K) to Threonine (T) at 76 in \textit{P falciparum} and asparagine (N) to tyrosine (Y) at position 86 in PIMDR1 with reversal of CQR by VP in field isolates.

Methods: Between April and May 2003 we obtained \textit{P. falciparum} isolates from uncomplicated malaria patients in Wakiso district. We determined the in vitro susceptibility of \textit{P falciparum} isolates to chloroquine (CQ) in absence and presence of VP (1 \(\mu\)M) by the WHO microtest. Reversal of CQR was assessed by percent reduction in 50% inhibitory concentrations (IC50 from sigmoid percent schizont maturation-CQ curves). DNA was extracted from dried filter paper blood spots by methanol fixation-heat method. Sequences flanking codons 76 and 86 in pfcr and pfmdr1 genes, respectively, were amplified by nested PCR. Alleles at 76 and 86 were detected by restriction fragment length polymorphism (RFLP) following agarose gel electrophoresis.

Results: The mean IC50 of 50 \textit{P. falciparum} isolates tested for in vitro susceptibility to CQ was 134.7 nM (95% confidence interval [CI] 104.4–164.9) indicative of CQR. The IC50(CQ)'s of all isolates were five-fold (95% CI 4.4–7.4) reduced compared to respective IC50's in presence of VP suggestive of reversal of CQR (range of percent reduction in IC50 44.4–99.9%). By PCR RFLP, the pfcr T76 mutation was present in all 50 isolates. In contrast, alleles at 86 in pfmdr1 varied; the wildtype (N86) was 16% (7/44) and mutant (Y86) 73% (32/44). With respect to PfMDR1 N86Y mutation, the percent reduction in IC50 (CQ) of mutant isolates was 82.0% (95% CI 77.0–87.0) compared with 93.3% (95% CI 83.0–103.6, \(p>0.05\)) in mixed and 63.1% (95% CI 52.7–73.5, \(p<0.01\)) in wildtype. Thus, reversal of CQR was similar between mutant and mixed isolates but greater than wildtype.

Interpretation: Our findings indicate that among \textit{P falciparum} isolates with the mutant (T76) allele in PICRT, further mutation of Asparagine to Tyrosine at 86 in PIMDR1 may enhance reversal of CQR by VP.
Prescription practice, anti-malaria drug trade and therapy seeking habits in Cameroon [MIM-BA-29275]

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Introduction: The emergence of drug resistance and the lack of new drugs has worsened the burden of disease and made control efforts difficult. Effective control strategies require the prior investigation of factors that drive drug resistance. Thus, an investigation was done to assess the level of understanding of the malaria problem by care givers, evaluate drug counterfeiting and examine the care-seeking habits of the Cameroonian people.

Methods: An investigation into knowledge, attitude and practice of taking antimalaria drugs was conducted with consenting adults in Yaounde, Dschang, Limbe and Nkambe. Another questionnaire for care givers, pharmacists, and drug street vendors was administered to assess knowledge of resistance, prescription and sales habits. The drug content of chloroquine and sulfadoxine/pyrimethamine were investigated by titration, thin layer chromatography and potentiometric assays. Blood samples were collected from patients for histology, microscopic speciation and for parasite DNA. Polymorphisms of the dhfr and pfcrt genes were determined by the PCR-RFLP. Data were analysed using the EpiInfo software.

Results: The most prescribed drugs by care givers, street vendors and pharmacists were quinine (45%), Fansidar® and chloroquine (35%). Analysis of 135 questionnaires revealed that chloroquine is known to be the least effective drug. Artemisinine-based drugs were sold more in the pharmacies than on the street. Pharmacists prescribed amodiaquine more than street vendors. Chloroquine and sulfadoxine/pyrimethamine drugs on the street were of similar quality – 4% for chloroquine and sulfadoxine; and 7.5% for pyrimethamine. Only street vendors reported knowing of resistance to artemisinine. Only 75% of those with a fever were positive for malaria. Of 400 individuals interviewed 75% had never listened to a health talk on malaria prevention or slept under bednets. Self-medication by tablets was the choice method for their most preferred drugs – chloroquine, Fansidar® and quinine. For caregivers, 80% did not complete treatment as opposed to 50% of the general population. Caregiver self-medication choice drugs were artemisinine-based combinations. About 33% of the investigated population took herbs. An inverse relation was found between the prevalence of mutation 108 N of dhfr in the sites and self-prescription of Fansidar®.

Interpretation: Chloroquine, quinine and Fansidar® are the most prescribed and sold drugs in Cameroon. Antimalaria drugs are of good quality. Where mutation 108 N is high, people less self-prescribe Fansidar®.

Drug resistance to sulphadoxine–pyrimethamine (SP) in Plasmodium falciparum malaria in Mlimba, Tanzania [MIM-EM-543567]

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Introduction: Sulphadoxine–pyrimethamine combination (SP) has been and is currently in use for treatment of uncomplicated Plasmodium falciparum malaria in many African countries. Tanzania has changed its policy to SP as an interim antimalarial drug for treatment of uncomplicated malaria since August 2001. This study was aimed at molecular investigation of drug resistance to sulphadoxine–pyrimethamine (SP) in P. falciparum malaria.

Methods: About 141 blood samples from children under 5 years of age collected on filter papers were
tested from malaria-endemic Mlimba, south eastern Tanzania. SP was given as a single dose equivalent to 25 mg/kg body weight based on sulphadoxine component. Parasite DNA was extracted by Chelex technique, DHFR and DHPS genes were analysed using PCR-RFLP techniques. These techniques made it possible to investigate the SP resistance-associated point mutations in the commonly reported codons 51, 59, 108 and 164 in the DHFR and codons 437, 540 and 581 in the DHPS domains.

Results: The results showed that 66.9% carried mutations at codon 108, 62.7% at codon 51 and 48.8% at codon 59 of DHFR domain. Fifty-six (43.7%) of samples carried mutations at codon 437, 39.2% at codon 540 and 0.8% at codon 581 on the DHPS domain. Proportions of mixed variants in the DHFR domain ranged from 0 to 21.5% and 0.8 to 6.3% in the DHPS domain. About 44 (36.4%) of isolates harboured triple mutant DHFR genotypes, whereas quintuple mutation was observed in 10 (8.3%) isolates. About 10 (8.3%) isolates possessed at least double DHFR and double DHPS mutants. This study found a high proportion of SP resistance-associated point mutations in Mlimba 2 years after deployment of SP as a first-line antimalarial drug in Tanzania. However, the adequate clinical response (89.4%) observed clinically reflects the role of semi-immunity component in the study population.

Interpretation: Monitoring drug resistance, be done simultaneously with immunological studies on malaria and the extensive use of antifolates other than SP for treatment of infections other than malaria. The therapeutic useful life of SP is questionable in Tanzania.
Methods: During 2001–2003, 66 subjects with positive smears of *P. falciparum* and treated with chloroquine were assessed to evaluate the association between the point of mutation Pfcrt (K76T) and clinical outcome. In vivo method was used according to WHO (1996) protocol to assess the sensitivity to chloroquine and PCR-RFLP was used to detect point of mutations.

Results: Results showed that 48 (72.7%) were sensitive to chloroquine and successfully treated, failure was observed in 18 (27.3%). R1, R11 and R111 levels of resistance were detected, while PCR-RFLP analysis showed the presence of mutant T76 allele in 42 (63.6%) isolates. The remaining 24 (36.4%) isolates carried the wild type (K76) allele and no mixed alleles were found.

Interpretation: The data showed that the allele of the Pfcrt gene with K76T is strongly associated with chloroquine resistance.

394A
Sentinel site surveillance of the in vivo therapeutic efficacy of artemether–lumefantrine and SP–artesunate in Zambian children aged 6–60 months [MIM-HM-39338]
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Introduction: Sentinel site surveillance of antimalarial drug regimens is one of the key activities earmarked for monitoring and evaluation. The studies are conducted annually in order to provide timely and reliable information on the status of the recommended regimens for malaria case management.

Methods: The design is a simple, one-arm, prospective evaluation of the clinical and parasitological response to directly observed treatment for uncomplicated malaria. The studies were conducted using the WHO standardised protocol for the assessment of therapeutic efficacy of antimalarial drugs in children aged 6–60 months in seven sentinel sites. Sesheke and Mansa which were for sulphadoxine/pyrimethamine (SP) and sulphadoxine/pyrimethamine–artesunate (SP–artesunate). Chongwe, Chipata, Choma, Mpongwe and Isoka, were for Coartem®. For all the drugs, a 28-day follow-up period was used and PCR genotyping for MSP1 and MSP2 was done in order to differentiate recrudescence from reinfections for parasites that appear after Day 14.

Results: Two hundred and eighty-seven children aged 12–60 months qualified for enrolment on Coartem® at five study sites. Twenty-eight-day ACPRs for all the Coartem® sites was found to be at 100% after PCR correction. Parasite and fever reduction was rapid in the first 48 h. Coartem® was found to have significant gametocyte reduction. One hundred and twenty-six children aged 6–59 months were enrolled on SP–artesunate at two sites and the 28-day ACPRs were found to be at 85.5% (Mansa) and 94.8% (Sesheke), respectively. One hundred and twenty-four children aged 6–59 months were enrolled on SP yielding 28-day ACPRs of 68.4% (Mansa) and 78.1% (Sesheke). All the patients with new infections were excluded from the final classification of therapeutic efficacy. All treatment failures were given oral quinine as stipulated in the malaria case management guidelines. Gametocyte clearance for SP was poor as compared to SP–artesunate.

Interpretation: The current therapeutic efficacy of Coartem® will provide a solution for multidrug resistance. The rates of reinfections however are an indication for coupling of effective treatment with integrated vector management.

395B
Assessment of pharmacopoeial quality of quinine tablets and artemisinin-based drugs available in DR Congo [MIM-OM-34336]
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Introduction: To investigate from local market the pharmacopoeial quality of competitive quinine tablets and artemisinin derivatives-based products representing 21 and 32 trade brands, respectively.

Methods: A blind assessment of quinine and artemisinin-based drugs in tablet form and dry syrup collected at Kinshasa with trade and manufacturers names
of the products veiled by blanking. We collected from June 2004 to March 2005, 100 samples of quinine tablets and 120 samples of tablets and syrups containing artemether, dihydroartemisinin or artesunate from local market. Drug quality was assessed by a simple colour reaction (using FAST RED salt for artemisinins) and semi-quantitative thin-layer chromatography at the University of Kinshasa, School of Pharmacy. The active ingredient content was assessed using UV spectrophotometric assay.

**Results:** According to TLC tests, 40 of 120 collected artemisinin-based drugs (33.33%) showed insufficient quality of active ingredient. The active ingredient content did not comply with authorized limits in 15 of 120 (12.5%). For quinine-based tablets, 85 of 100 collected samples complied with stated content, 15% of sampled drugs showed insufficient pharmacopoeial quality.

**Interpretation:** A significant variation in quality of tested antimalarial drugs demonstrated by TLC test and assay raises serious questions whether the less effectiveness of drugs claimed are due to substandard preparations. These drugs may lead to increasing therapeutic failure observed and contribute to the spread of drug-resistant malaria parasites.

396C The monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria infections at sentinel surveillance sites in Zimbabwe


**Introduction:** Chloroquine (CQ) was used for a long time on its own as treatment of choice for uncomplicated malaria in Zimbabwe until 2001. The first cases of CQ resistance were confirmed in 1984 and since then, various reports indicated the development of varying levels of CQ resistance in different parts of the country. Organised and systematic monitoring of *Plasmodium falciparum* parasite response to antimalarial drugs was established in 1999 to generate quality data that would guide decision making on the treatment of malaria by the Ministry of Health and Child Welfare.

**Methods:** The therapeutic efficacy of CQ alone and that of a free combination of chloroquine and sulphadoxine/pyrimethamine (CQ + SP) was monitored from 1999 to 2002 and 2001 to 2004, respectively, in all age groups that presented with uncomplicated monoinfections of *P. falciparum* at selected sentinel sites. Clinical and parasitological response to directly observed treatment for uncomplicated malaria was monitored using modified standard in vivo WHO (1996, 1999) protocols for assessment of monitoring antimalarial drug efficacy for the treatment of uncomplicated falciparum infections. Recruited participants were followed up and assessed on scheduled days for 14 days and their antimalarial drug response classified as Adequate Clinical Response, Early Treatment Failure or Late Treatment Failure.

**Results:** Studies conducted from 1999 to 2002 showed that treatment failure rates in *P. falciparum* infections treated with CQ alone ranged from 0 to 43.2%. These findings led to the recommendation in 2000 that there was need to change the first line antimalarial treatment for uncomplicated malaria in areas of high CQ treatment failure to a free combination of CQ + SP. Data from efficacy studies carried out from 2001 to 2004 indicated that adequate clinical response rates of CQ + SP against monoinfections of *P. falciparum* varied from 93 to 100%. These findings resulted in the recommendation in 2001 that a free combination of CQ + SP should be the first line antimalarial treatment policy for Zimbabwe.

**Interpretation:** CQ treatment failure in malaria infections is highly prevalent in most endemic areas of Zimbabwe. However, a free combination of CQ + SP is still effective against these infections in areas of high CQ resistance.
Molecular analysis and the spread of the knock-down resistance (kdr) gene in the *An. gambiae* and *An. funestus* complex populations from Kenya

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Introduction: Insecticide resistance affects the re-emergence of vector-borne disease and their control. The kdr allele is characterized by a single base pair substitution causing a change from leu to ser (East African) in codon 1014 sodium channel protein sequence. The use of pyrethrin indoor sprays and ITNs has been shown to contribute to development of resistance. The extent of kdr spread in the wild mosquito populations helps in designing sustainable ITN use, a top malaria management strategy in Kenya.

Methods: A total of 1860 adult mosquitoes were collected from Mbita, Mwea and the Kenya Coast by PSC/aspiration method. They were characterised to species level morphologically and further by a targeted ribosomal DNA-PCR. The *An. gambiae* s.s. was further characterised to the molecular forms by PCR-RFLP analysis. The *An. gambiae* s.s. was further characterised to the molecular forms by PCR-RFLP analysis. The kdr genotyping by PCR approach which comprised of two methods, a resistance assay (rkdr-PCR) and a susceptibility assay (kds-PCR). The kdr allele frequencies were calculated for each site and per species group and their conformity to HWE determined by X2 analysis. The Marascuilo multiple comparison procedure was used to test for the homogeneity of the observed kdr genotype frequencies across the three sites.

Results: The rkdr-PCR and kds-PCR were successfully adopted for the field screening of the *An. gambiae* complex and further optimised for screening of the *An. funestus* complex, achieving 98% success. The kdr alleles were in heterozygous form (RS) in the populations screened and no homozygotes (RR). The *An. gambiae* s.s. had the highest frequency (14%) followed by *An. arabiensis* (8%) and lastly *An. funestus* with 7%. The kdr frequencies were highest in Kenya Coast (12.4%), followed by Mbita (8%) and lastly by Mwea with a frequency of 5.75%. These findings conform to studies by Stump et al. (2004) where the kdr frequencies was 4-8% in Asembo, an ITN trial site in western Kenya. A X2 analysis (a = 0.05) of association between genotype and site revealed a strong association (X2 = 10.3, P = 0.0042) indicating that resistance was heterogeneous across the three sites. An observed resistance homogeneity analysis using the Marascuilo multiple comparison procedure revealed that the frequencies of Coast-Mwea and Coast-Mbita were significantly different (heterogenous) whereas Mwea-Mbita was homogenous. Conformance to HWE tests by chi-square analysis indicated that the populations sampled in the three sites were not in HWE and violated the equilibrium assumptions.

Interpretation: Presence of selection pressure attributable to the kdr frequencies was confirmed by the HWE conformity test. The use of rkdr-PCR and kds-PCR assays will facilitate screening of resistance for future resistance monitoring and management programmes.

In vitro activity of piperaquine on Kenyan *Plasmodium falciparum*

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Introduction: Artekin® (AK; piperaquine (PQ)/dihydroartemisinin combination), is a potential alternative to chloroquine and pyrimethamine/sulfadoxine for malaria treatment. However, data from China shows that resistance to PQ emerges rapidly, which could compromise AK’s therapeutic lifetime. Thus, there is need for monitoring of PQ chemo-sensitivity once AK comes to widespread use. We present baseline data on PQ chemo-sensitivity against *Plasmodium falciparum* laboratory reference and Kenyan field isolates.

Methods: In vitro culture of malaria parasites was carried out in RPMI 1640 medium (GIBCO) containing physiological concentrations of para-aminobenzoic
Acid (0.5 mg/ml) and folic acid (10 mg/ml), 10% (v/v) normal human serum, 25 mM bicarbonate and 25 mM HEPES buffer. Antimalarial activity was measured in the presence of varying concentrations of PQ using radio isotopic hypoxanthine incorporation, and results were expressed as the drug concentration required for 50% inhibition of parasite growth (IC50).

Results: We have analyzed the chemosensitivity profile of PQ against laboratory reference isolates, M24 (fully sensitive to all antimalarials including CQ), K39 (pyrimethamine resistant), V1S and W282. The later two isolates are multidrug resistant (MDR) isolates and are highly resistant to CQ. The in vitro activity of PQ against M24, K39, W282 and V1S are 6.40 ± 7.72, 10.89 ± 3.48, 14.48 ± 4.27 and 17.7 ± 17 ng/ml, respectively.

Interpretation: PQ is potent against CQ-sensitive and resistant isolates, but its activity is decreased against MDR isolates, indicating that PQ resistance could be selected quickly on the background of CQ-resistance.

We are currently testing Kenyan field isolates.

399C
Mutations in Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase in Senegal [MIM-DN-5966]
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Introduction: Senegal recently (2003) switched to sulfadoxine–pyrimethamine (SP) with amodiaquine as first line therapy for malaria in response to increasing chloroquine resistance. In anticipation of emerging resistance to SP as a result of this change in drug pressure, we set out to define the baseline prevalence of SP-associated mutations in the dhfr and dhps genes in Plasmodium falciparum using geographically diverse and longitudinally collected samples.
Methods: A total of 268 blood samples were analyzed from patients (5 years or older) with mild malaria after informed consent was obtained. Longitudinal samples were collected between 2000 and 2003 ($n$ = 145) in Pikine, a suburb of Dakar. Geographically diverse site sampling was carried out in 2003 (16 from Thies, 17 from Tambacounda) and 2004 (90 from Thies, Velingara, Kaoilack, and Richard Toll). DNA was extracted and a nested PCR strategy was used to amplify parasite dhfr and dhps loci for sequencing. To determine the relatedness of parasites strains containing triple mutations in dhfr (amino acids 51, 59 and 108), microsatellite sequences flanking this gene locus were analyzed.
Results: Mutation prevalence in dhfr codons 51, 59 and 108 was 65, 61 and 78%. The overall prevalence of the triple mutation that is associated with high-level pyrimethamine resistance is 55%. The mutation prevalence rate in dhps codons 436 and 437 was 21 and 40%, respectively. The dhfr triple mutation rate increased over time from 2000 to 2003 in Pikine from 42 to 56%. There is significant geographic variation in genotypic resistance, as samples from Pikine in 2003 had significantly higher mutation prevalence in the dhfr and dhps genes compared to samples from Tambacounda ($P < 0.02$). Microsatellite data will be presented to determine if the geographically distinct parasites containing the triple dhfr mutations in Senegal are secondary to a selective sweep across the region or have arisen independently. Interpretation in Summary, there is a high prevalence of SP genotypic resistance in Senegal (see Ndiaye et al., submitted for publication). Mutations in Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase in Senegal).
Interpretation: Other sites in Africa, with high (continued from previous section) prevalence of these mutations have demonstrated good outcome with SP–amodiaquine therapy (Dorsey et al., 2002. Lancet) O. Sarr will present an in vivo SP–amodiaquine drug efficacy trial undertaken in Pikine.

400A
Situation actuelle de l’efficacité des antipaludiques classiques (chloroquine, amodiaquine, sulfadoxine–pyriméthamine) chez l’enfant congolais vivant en milieu urbain et périurbain brazzavillois [MIM-MN-3728151]
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Introduction: Le Congo Brazzaville envisage de changer dans un futur très proche la politique de traitement du paludisme non compliqué encore basée sur la chloroquine. En attendant, nous nous sommes fixés comme objectif la connaissance du niveau actuel de l’efficacité de la chloroquine (CQ), de la sulfadoxine–yriméthamine (SP), de l’amodiaquine (AQ) et de l’association sulfadoxine–yriméthamine + amodiaquine (SP + AQ).

Methods: De mai 2003 à février 2005, des enfants de 0 à 5 ans atteints de paludisme non compliqué à Plasmodium falciparum ont été recrutés dans la zone sud de Brazzaville. Fifty-one ont été traités avec la CQ, 97 avec la SP, 61 avec l’AQ et 59 avec l’association SP + AQ à 57. Le protocole OMS de 28 jours a été appliqué.

Results: A J14, les taux d’échecs cliniques sont respectivement de 60.3% (IC 95%: 45.26–74.2) pour la CQ, 10.7% (IC 95%: 5.06–19.36) pour la SP, 13.8% (IC 95%: 6.1–25.4) pour l’AQ et 1.7% (IC 95%: 0.04–9.8) pour SP + AQ. Le suivi de 28 jours a donné les taux d’échecs cliniques suivants: 85.1% (IC 95%: 71.7–93.8) pour CQ, 35.4% (IC 95%: 25.0–47.0) pour SP, 38.2% (IC 95%: 25.4–52.3) pour AQ, et 26% (IC 95%: 10.4–33.0) pour SP + AQ. La SP, contrairement aux trois autres traitements, provoque une forte augmentation de porteurs de gamétocytes et de la charge gamétocytaire à partir du jour 7 de suivi.

Interpretation: Face à la chloroquine ayant perdu toute efficacité, la sulfadoxine–pyriméthamine et l’amodiaquine ne sont pas des alternatives crédibles si on considère les résultats d’un suivi de 28 jours. Toutefois, l’association de ces 2 médicaments, en raison du coût relativement bas, peut être dans une phase transitoire prescrite dans le traitement du paludisme non compliqué, en attendant les associations à base de dérivés d’artémisinine.
tion with a stop codon and two non synonymous mutations.

Interpretation: This difference of frequencies of mutations in AA and AS subjects strongly suggests that mutated PfTCTP strains have a greater capacity to infect SCT individuals and be thus maintained in the parasite gene pool.

402C
Choice and sources of antimalarials, self-treatment and malaria resistance [MIM-DN-210473]

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Introduction: Malaria is a major public health problem in Africa. This study was carried out to make a local analysis of the disease and to establish epidemiological and behavioural baselines and their implications for malaria control.

Methods: Design: observational cross-sectional study; Setting: community-based; Participants: two hundred and fifty-three participants of different socio-demographic status took part in the study. Three hundred and fifty participants were contacted and 253 volunteers were recruited for the study. Questionnaires were administered through a house-to-house survey.

Results: Antimalarials commonly cited were chloroquine (26.1%) and nivaquine (14.62%) and analgesics: panadol (22.92%) and paracetamol (12.25%) including native drugs (6.32%). Only 26.1% respondents knew the correct adult malarial dosage for chloroquine and/or nivaquine. One hundred and twenty-five (49.4%) participants said they get their antimalarials from the health center, 27 (10.67%) from the shop while 90 (35.5%) participants get theirs from other unauthorized sources, and 16 (6.3%) from the traditional doctor. Only 85 (33.6%) respondents had knowledge of malarial resistance compared to 168 (66.4) who did not. Of the 85 (33.6%) participants who had knowledge of malaria resistance, 52 (20.6%) ascribed malaria resistance to continuous fever for a long time during treatment, 15 (5.93%) to serious fever during treatment and 18 (7.12%) when chloroquine does not stop the malarial fever. Most (9.0%) of those who had the correct knowledge of malarial resistance were in the age group 31–35 bracket compared with other age groups (95% confidence interval, P > 0.05). There was no difference in correct knowledge of malarial resistance among the participants and different professions.

Interpretation: Malaria self-treatment is common but knowledge of malaria resistance is poor.

403A
The effect of artesunate-based combination therapy on haematologic recovery in Kenyan children with uncomplicated malaria [MIM-CO-46437]

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Introduction: Malaria associated anaemia is a major public health problem in children and women in malaria endemic areas of sub-Saharan Africa. Treatment for uncomplicated malaria aims to clear parasites, relieve symptoms and improve the haematologic recovery. The impact of antimalaria treatment efficacy on haematologic recovery has not systematically been evaluated.

Methods: To compare the effect on haematologic recovery of adding Artesunate to sulfadoxine–pyrimethamine (SP) therapy with SP alone, we enrolled 600 children presenting in 1999 to Siaya district hospital, western Kenya with uncomplicated malaria: they were randomly assigned to one of three treatment groups: (1) SP alone [SP], (2) SP plus one dose of artesunate [AS1], (3) SP plus three doses of artesunate [AS3]. We measured haemoglobin (Hb) at enrolment, 7, 14, 21 and 28 days after treatment. The primary end-point was mean Hb concentration at day 28. Secondary endpoints included Hb change from day 0, proportion still anaemic and the fractional fall in Hb.

Results: At enrolment the mean Hb in all treatment groups was 8.4 g/dl and 91% of the children were anaemic (Hb < 11 g/dl). This proportion reduced to 74% by the end of 28 days. By day 28 the mean Hb was 10.2, 9.8 and 10.2 g/dl for those in SP, AS1 and AS3 groups. Correspondingly, the proportion of anemic children by day 28, was 69%, 82 and 71%, respectively. Children were more likely to be anaemic during follow-up if they had anaemia at enrolment, prior treatment with chloroquine, recurrent parasitemia, early
therapy failure, age, weight or parasite density below the median. There was no significant difference in mean Hb or proportions of anemic children between those who received AS1 compared to those who received SP alone. Failure rates on day 28 were 46, 36 and 26% among those treated with SP alone, AS1 and AS3, respectively.

**Interpretation:** Addition of artesunate to SP therapy did not result into a marked improvement in haematologic recovery, when SP failure rate was unacceptably high.

**404B**

**Evaluation des traitements de première ligne en Côte d'Ivoire [MIM-aN-7704]**

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**Introduction:** Le premier cas de chloroquinorésistance a été suspecté en 1986 et confirmé en 1987 depuis elle s’est propagée et a atteint des proportions inquiétantes (60% voire plus). En 2003 le Programme de Lutte contre le Paludisme a préconisé l’Amodiaquine et la sulfadoxine–pyriméthamine comme traitements de première ligne en remplacement de la chloroquine. Le but de la présente étude est d’évaluer l’efficacité et la tolérance de ces deux molécules selon le nouveau protocole OMS (version 2002).

**Methods:** One hundred and twenty-three patients ayant une infestation mono-spécifique à *Plasmodium falciparum* avec une densité parasitaire supérieure ou égale à 2000 trophozoïtes par microlitre de sang ont été inclus. L’Amodiaquine à la posologie de 30 mg/kg repartis sur 3 jours consécutifs (10 mg/kg à J0, J1 et J2) ou à la sulfadoxine/pyriméthamine à la posologie 1cp/20 kg soit 1/2 comprimé/10 kg de poids corporel a été administrée. Des contrôles clinique et parasitologique ont été effectués à J1, J2, J3, J7 et J14.

**Results:** La prévalence du paludisme dans la population de l’étude était de 38, 39 avec 100% d’infestation à *Plasmodium falciparum*. On nota: 95.10% de réponses cliniques et parasitologiques adéquates dans le groupe Amodiaquine, contre 83.87% dans le groupe sulfadoxine/pyriméthamine. Un échec thérapeutique précoce de 4.9% pour l’amodiaquine contre 11.29% pour la sulfadoxine/pyriméthamine. Un échec parasitologique tardif de 3.23% pour la sulfadoxine/pyriméthamine.

**Interpretation:** Le taux d’échec à la sulfadoxine/pyriméthamine inspire des inquiétudes en raison du fait que cette molécule, la seule disponible, est indiquée dans le traitement préventif du paludisme de la femme enceinte.

**405C**

**Effect of pre-treatment chloroquine levels on parasitological response in children with acute uncomplicated malaria in Ibadan, Nigeria [MIM-SO-91430]**

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**Introduction:** The emergence and spread of parasites resistant to antimalarial drug especially chloroquine has become a major barrier to malaria control. One major factor influencing spread of chloroquine resistance is drug pressure. Thus, this study was designed to determine the chloroquine (CQ) level in patients with uncomplicated falciparum malaria before treatment and at recrudescence in order to investigate the effect of pretreatment CQ level on clinical and parasitological response of patients.

**Methods:** Of the sixty-one patients (age 0.5 < 13 years, 51% male, 49% female) that were recruited into the study, 56 patients completed the study and are presented. Each patient was treated with a standard dose of CQ (25 mg/kg body weight) and followed up for a period of 28 days. Filter paper blood samples as well as thick blood films for drug analysis and microscopy were collected on day 0 before treatment and day 1, 2, 3, 4, 5, 6, 7, 14, and 28 and at recrudescence. Chloroquine levels were determined using HPLC techniques.

**Results:** Seventy-five percent of the patients had chloroquine in their blood samples before treatment (mean concentration = 884.6 ± 826.0 ng/ml). Infection
in 24 patients (42.85%) adequately responded to treatment (ACR) while infection in 14 patients (25%) exhibited early treatment failure (ETF). Infection in 18 patients (32.14%) exhibited late treatment failure (LTF). The chloroquine levels on day zero were 753.8 ± 747, 682.0 ± 689 and 970 ± 606 ng/ml in patients with ACR, ETF and LTF, respectively. No significant (P > 0.05) difference in chloroquine levels in the three groups on day zero was observed. The mean blood levels of CQ on day 3 and 7 in the CQ sensitive group were found to be higher than that in the CQ resistant groups. However, these differences were found to be not significant. On the day of recrudescence of infection, chloroquine concentration was 826.8 ± 480 ng/ml indicating no significant difference from day 0 chloroquine level. Moreover, this CQ level at recrudescence was significantly higher than the minimum chloroquine therapeutics level in whole blood.

Interpretation: High level of CQ prior to treatment appears not to have a positive influence on patient response to treatment with CQ and may be a contributing factor for selection of CQ resistant parasites.

406A

In vitro evaluation of current susceptibility of patient isolates of Plasmodium falciparum to standard antimalarial drugs [MIM-AT3-400554]

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Introduction: The need to monitor and document the drug sensitivity pattern of Plasmodium falciparum using accurate and appropriate techniques cannot be overemphasized in order to control malaria in endemic countries. One of the techniques is the in vitro drug susceptibility testing of P. falciparum, which is useful for epidemiological purpose in detecting and monitoring drug resistant infections.

Methods: The in vitro susceptibility profile of 154 patient isolates of P. falciparum was evaluated using a modification of the standard WHO schizont inhibition assay. The patients were aged between 0.4 and 12 years and enrolled for drug efficacy study between years 2002 and 2004 in the two study sites; Adeoyo Maternity Hospital (AMH) and the Institute for Advanced Medical Research and Training (IMRAT) both in Ibadan South-west Nigeria. The ability of verapamil (VER) to potentiate the intrinsic antimalarial activities of chloroquine (CQ), amodiaquine (AQ), and quinine (QN) in resistant parasites was used as a marker for detecting drug resistant P. falciparum in vitro.

Results: The in vitro sensitivity of the parasites to CQ between 2002 and 2004 in isolates from patients at IMRAT and AMH sites ranged from 52.4 to 43.6% and 69 to 41.4%, respectively. Similarly, in vitro sensitivity of the parasites to AQ between 2002 and 2004 in isolates from patients at IMRAT and AMH sites ranged from 81.8 to 76.9% and 78.9 to 75%, respectively. In addition, in vitro sensitivity of the parasites to Quinine in isolates from patient at IMRAT and AMH sites ranged from 30 to 31% and 68 to 64% in 2003–2004, respectively. The in vitro sensitivity profiles of patient isolates of P. falciparum to CQ and AQ in both study sites are similar (P = 0.24 and 0.68). However, the sensitivity profile to QN in both sites differed significantly (P = 0.0005). Fifteen percent and 14% of the isolates were cross resistance to CQ and AQ in AMH and IMRAT, respectively. Also, 17 and 19% of the isolates were cross resistance to AQ and QN in AMH and IMRAT, respectively. Similarly, 31 and 42% of the isolates were cross resistance to CQ and QN in AMH and IMRAT, respectively.

Interpretation: Difference in drug pressure in both centers and sensitivity pattern of the infecting parasites may be responsible for the difference in QN sensitivity in the two centers.

407B

In vivo chloroquine resistance and prevalence of the pfcrt codon 76 mutation in Plasmodium falciparum isolates from the republic of Congo [MIM-MP-105168]

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Introduction: Chloroquine (CQ) resistance in Plasmodium falciparum has been particularly associated with mutations in the pfcr gene. The present study was carried out in the malaria hyperendemic town of Brazzaville (Republic of Congo, Central Africa) where CQ is still recommended and used as a first-line drug for P. falciparum malaria. We assessed the efficacy of CQ in vivo, and the association between pfcr mutation at codon 76 and response to CQ treatment in children with uncomplicated malaria.

Methods: We recruited 137 children aged 6–60 months presented criteria of assessment efficacy of CQ at the Térinkyo Hospital in 2003. They were treated with CQ and followed during 28 days. Due to the high number of early treatment failure among the 50 first patients, the 87 next patients were not treated with CQ and blood samples from children were collected for determining the prevalence of K76T pfcr mutation in P. falciparum isolates. Parasites DNA was extracted from blood samples collected before treatment and during the follow-up. Analysis of point mutation in codon 76 in pfcr gene was done by nested mutation specific restriction enzyme digestion PCR reactions. P. falciparum isolates were also genotyped for the polymorphic MSP-1 and MSP-2 loci.

Results: Among 50 patients aged 6–60 months followed, high-grade resistance to CQ was observed (failure rate on day 28 of 95.7%). Notably the pfcr K76T mutation was present in 100% of pre- and post-treatment isolates. Two pre-treatment isolates were from patients who recovered after CQ therapy suggesting the contribution of other factors to clinical outcomes. The pfcr mutation was examined in a further 87 isolates from uncomplicated patients not treated with CQ. Eighty-five where (98%) showed T76 mutation. P. falciparum isolates were also genotyped for the polymorphic MSP-1 and MSP-2 loci.

Interpretation: The strong association between the mutation at point 76 pfcr gene and in vivo evaluation of efficacy of CQ for the treatment of uncomplicated malaria suggest that pfcr T76 can be applied for predicting chloroquine resistance in Brazzaville.

Efficacité et tolérance de la sulfaméthoxypirazine/pyrénéméthamine/artésunate versus artéméther/luméfantrine dans le traitement du paludisme simple [MIM-LP-7056]


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Introduction: Le paludisme constitue un grave problème de santé publique en Côte d’Ivoire et les taux de résistance du parasite aux monothérapies, notamment à la chloroquine et à l’association sulfadoxine–pyrénéméthamine sont devenues de véritables préoccupations. L’OMS recommande ainsi désormais l’utilisation de combinaisons thérapeutiques. Pour autant, celles-ci doivent être efficaces, bien tolérées et de coûts abordables.

Methods: Cette étude est un essai ouvert, comparatif, randomisé à 2 bras, sans insu sur le traitement, testé pour évaluer l’équivalence de deux traitements, sulfaméthoxypirazine/pyrénéméthamine/artésunate (SMP/AS) versus artéméther/luméfantrine (ART/LF). Un échantillon de 150 patients âgés d’au moins 2 ans et remplissant les critères d’inclusion ont bénéficié d’un suivi clinique et parasitologique aux jours 0, 1, 2, 3, 7, 14, 21, 28 et d’un bilan hématochimique à 30 et à J7. Des confettis sur papier Whatman pour les marqueurs moléculaires ont été faits à J0, J28 et entre J7 et J12 en cas de repositivation de la parasitémie.

Results: Les résultats suivants suivants ont été obtenus: Tous les patients étaient apyrétiques après 48 heures de traitement et 99.5% des patients présentaient une goutte épaisse négative à J1 dans les deux groupes thérapeutiques. De même, à J28 tous les patients avaient une goutte épaisse négative dans les deux bras de traitement. Entre J2 et J28, aucun patient n’a eu une goutte épaisse reposi-

Interprétation: L’arsenal thérapeutique du paludisme, relativement réduit, dispose ainsi d’une autre alternative, la SMP/AS dont l’efficacité avait été déjà démontrée par d’autres auteurs.

409A
Low prevalence of pfCRT mutant Plasmodium falciparum in Madagascar [MIM-MR-32450]
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Introduction: Chloroquine has been used in Madagascar for the last six decades, and is still used for home case management of “fever” (presumptive treatment of malaria).

Methods: As part of the national network activities (called RER) dealing with the surveillance of Plasmodium falciparum chloroquine-resistance in Madagascar, PCR/RFLP is performed to detect pfcr K76T mutation.

Results: We detected the PfCRT K76T mutation in 51 clinical isolates and 132 isolates from pregnant women, using a PCR/RFLP method. This detected six (3.3%) isolates containing the K76T mutation, which is critical for chloroquine resistance. We analyzed a DNA segment of the pfcr gene, spanning codons 72–76 in these mutant parasite-containing samples. The K76T mutation was found in the five good quality sequences. Two haplotypes (CVID[T] and CVIE[gaa]T) were observed. The wild type haplotype is CVI[NK]. This study provides the first evidence of the occurrence of mutant pfcr in Madagascar. The low prevalence of mutant pfcr in Madagascar contrasts with the situation in the Comoros Archipelago, where >75% of P. falciparum isolates harbor the PICRT haplotype CVIETF. Home case management of fever in children under five – based on the use of prepackaged freely distributed for free at the primary health centers, is part of the ongoing strategy to fight against malaria in Madagascar. Such practice will increase the drug pressure related to chloroquine, and the dissemination of chloroquine-resistant parasites will affect this strategy.

Interpretation: Thus, the genotyping of CQ-resistance marker remains a useful tool now that we are far from saturation in the prevalence of pfcr mutant P. falciparum.

410B
Introgression and spread of sulphadoxine/pyrimethamine resistance determinants in West Africa [MIM-CR-9614]
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Introduction: The evolution of drug resistance in Plasmodium falciparum is the most important limitation of malaria treatment in Africa today, yet the number of origins and the dynamics of emergence are poorly understood.

Methods: To examine the evolutionary origins of SP resistance determinants in the West African region we have screened samples of P. falciparum collected in Cameroon, Senegal, Ghana and Burkina Faso for resistance mutations in the dhfr gene. To investigate their ancestral origins we have taken the approach of analysing microsatellite markers flanking the dhfr. These indicate whether the resistance alleles identified have a related or independent ancestry.

Results: The results of these investigations are part of a wider project to map the present day distribution of SP
Resistance lineages across the African continent. We shall describe the resistance alleles found at dhfr in these four countries and examine their relationship to each other and to previously described lineages identified in the southern and east African regions (Roper et al., 2003. Lancet).

**Interpretation:** The results indicate a pivotal role for migration and introgression in the establishment of SP resistance in African *P. falciparum*.

### 411C

**Geographical differences in antimalarial efficacy of aminoquinoline–antifolate combinations across Uganda are not explained by parasite polymorphisms [MIM-PR-161700]**

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**Introduction:** We compared efficacies against uncomplicated malaria of chloroquine (CQ) + sulfadoxine–pyrimethamine (SP) and amodiaquine (AQ) + SP at six sites in Uganda and showed marked differences between sites, with decreasing risks of failure with increasing transmission intensity. Differences could be explained by variations in antimalarial immunity or parasite populations. To study the contribution of parasites, we assessed the impacts of mutations that confer resistance to CQ and SP on treatment outcomes.

**Methods:** Patients were treated with CQ + SP (1158) or AQ + SP (1180) and followed for 28 days; outcomes were adjusted by genotyping based on MSP-2. Seven mutations (pfcrt 76T; dhfr 51I, 59R, 108N, and 164L; dhps 437G and 540E) were characterized in 99% of pretreatment samples using nested PCR and restriction endonuclease digestion, and prevalences were compared across the six sites. Differences in risks of treatment failure were compared using a Cox proportional hazards model. This analysis was then restricted to patients with samples containing six of the tested mutations (all but dhfr 164L) to examine whether relative differences in risks of treatment failure across the six sites could be explained by differences in prevalences of parasite polymorphisms.

**Results:** Sites were ranked based on transmission intensity: low (one site, unstable malaria, EIR < 10), medium (three, stable, EIR < 10), and high (two, stable, EIR > 500). Ninety-nine percent of samples contained the pfcrt 76T, dhfr 51I, and dhfr 108N mutations. All but 1 were wild-type at dhfr 164. The prevalences of the dhfr 59R, dhps 437G and dhps 540E mutations were 82, 89 and 89%, respectively. The prevalence of samples with six mutations was 62% at the low, 58–77% at the medium, and 85–90% at the high transmission sites. Using the low site as the baseline, the relative hazard of treatment failure with CQ + SP was 0.45–0.46 at the medium and 0.20–0.39 at the high transmission sites. Restricting the analysis to patients infected with parasites containing all six mutations had little effect on the relative hazards of treatment failure (0.35–0.49 at the medium and 0.14–0.28 at the high transmission sites). Similar results were seen with the AQ + SP treatment group. Among patients infected with parasites containing six mutations the risk of treatment failure with CQ + SP was 79% at the low, 49–52% at the medium, and 22–36% at the high transmission sites. With AQ + SP these risks were 37% at the low, 13–17% at the medium, and 8–19% at the high transmission sites.

**Interpretation:** Decreasing treatment failure with increasing transmission was not explained by key parasite polymorphisms, as their highest prevalence was at sites with the lowest failure risks. Thus, differences in efficacy were primarily influenced by immunity.

### 412A

**Effects of treatment of falciparum malaria with chloroquine, amodiaquine or sulfadoxine–pyrimethamine on gametocytogenesis and infectivity to mosquitoes [MIM-IS-3848]**

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Introduction: This study aimed to examine the effects of treatment with chloroquine (CQ), amodiaquine (AQ) and sulfadoxine–pyrimethamine (SP) on the spread of *Plasmodium falciparum* infection and resistance in South Cameroon.

Methods: One thousand eighty-one individuals living in the district of Yaoundé or Mengang in South Cameroon, harbouring at least 1000 *Plasmodium falciparum* trophozoites/µl of blood were treated with CQ (n = 604) or AQ (n = 326) or SP (n = 151) and followed-up for 14 days in surveys of antimalarial treatment efficacy.

Results: The prevalence of resistant infections was 58.9, 20.1 and 13.8% in those treated with CQ, AQ and SP, respectively. The risk of post-treatment gametocyte increase were 3.5, 2.1 and 3.2 times higher for treated individuals with CQ-resistant (C.I. 95% [2.1–5.8]), AQ-resistant (C.I. 95% [1.0–4.5]) and SP-resistant (C.I. 95% [1.0–10.2]) parasites, respectively, than for those with CQ-sensitive ones. For individuals infected with drug-sensitive parasites, the risk were 1.7 and 4.5 times higher when treated with AQ (C.I. 95% [1.0–4.5]) and SP-resistant (C.I. 95% [2.5–8.0]), respectively than when treated with CQ. Post-treatment gametocyte infectiousness of mosquitoes increase with gametocyte density (p < 0.0001) or when harbouring resistant parasite (p = 0.02), or decrease with asexual parasite density (p = 0.008) at the day of experimental infection.

Interpretation: This study underlines the preferential risk of dissemination of resistant parasites if the CQ was continued used or if it is replaced by AQ or SP in this area.

413B
Assessment of antimalarial drug resistance in Senegal [MIM-OS-34879]
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Introduction: We report in vivo outcomes and pfcr genotype data for 2002 and 2003 when chloroquine (CQ) was first line therapy for uncomplicated malaria in Senegal. The high prevalence of CQ failure resulted in the change in treatment to SP–amodiaquine (SP/AQ) in 2004. We present 28 day in vivo data for this new regimen from Pikine, a hypoendemic area near Dakar. We also evaluated the prevalence of gametocytes after treatment with these two different regimens.

Methods: One hundred and seventy-four patients (mean age 19 years and mean parasitemia 20,058 parasites/µl) diagnosed microscopically with mild malaria were recruited and treated with CQ in 2002 and 2003; and 80 patients (mean age 18 and mean parasitemia 21,550 parasites/µl) were similarly diagnosed, followed, and treated with SP/AQ in 2004. Drug efficacy studies were carried out using the WHO 28 day in vivo protocol. Parasite DNA isolated from blood samples collected from these patients was analyzed for mutations in pfcr gene by PCR-RFLP and clonality of infection was determined by msp1 genotyping. The gametocyte count determined by microscopy, was also recorded.

Results: The 2002 and 2003 studies showed that the T76 polymorphism in pfcr was associated with treatment failure among children under 10 years of age (P = 0.02), but not among those 10 years or older (P = 0.2). An association between parasite density and treatment failure was also observed among the under age 10 group (P = 0.006). The DELI-microtest demonstrated that 23% of 2002 in vitro cultured isolates were CQ-resistant and early treatment failure (ETF) was observed in 16% of CQ-treated patients. In contrast, no in vivo failure was observed in patients treated with the new SP/AQ combination therapy. The peak gametocytemia was observed on day 7 in 4% (8/174) of CQ-treated patients, and on day 28 for 30% (25/80) SP/AQ-treated patients (P < 0.0001).

Interpretation: In vivo resistance is prevalent with chloroquine therapy. However, in vivo failure is not seen with SP/AQ treatment suggesting this combination is clinically efficacious despite the presence of molecular resistance (see abstract by Ndiaye et al.).
Evaluation of quantitative nucleic acid sequence based amplification assay to predict Fansidar treatment outcome of uncomplicated falciparum malaria [MIM-HS-155092]

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Introduction: In view of widespread emerging drug resistance, laboratory techniques are becoming more important. Availability of a fast, sensitive, reliable and quantitative method to detect parasite survival during and after drug treatment would help clinicians to monitor and – if needed – adjust treatment regimen. Quantitative nucleic acid sequence based amplification (QT-NASBA) technology is an isothermal molecular diagnostic test that allows for the quantification of Plasmodium species in clinical samples.

Methods: A quantitative nucleic acid sequence-based amplification (QT-NASBA) assay was employed to predict retrospectively the outcome of sulfadoxine–pyrimethamine (SP, fansidar) treatment of uncomplicated malaria in children aged <6 years in an endemic region in Kenya. Success of treatment was assessed according to WHO 2003 guidelines. Blood samples were collected at initial diagnosis and during follow-up. Mutation-specific nested PCR methods to analyse DHFR (Arg-59) and DHPS (Glu-540) mutations that are associated with SP drug resistance were applied. Parasite genotyping on msp1 and 2 and GLURP was performed in order to distinguish between re-infection and recrudescence.

Results: Eighty-six patients were recruited of which 66 were available for followed up. Nine children were classified as early treatment failure (ETF), 13 cases were classified as late clinical failure (LCF), 32 as late parasitological failure (LPF) and only 12 children had an adequate clinical and parasitological response (ACPR). DHFR and DHPS mutations conferring SP resistance were abundant in the Plasmodium population. Blood samples obtained 7 days after treatment were used to predict retrospectively the outcome of SP treatment. QT-NASBA was able to give a correct prediction of treatment outcome in 85.7% of the cases. Positive predictive value (PPV) of QT NASBA case was 95% (95% confidence interval = 88.3–100) and negative predictive value (NPV) was 63% (95% C.I. = 39.5–86.5). In contrast, microscopy correctly predicted outcome in only 37.5% of the cases. PPV of microscopy was 100% (95% C.I. = 73.9–100) and the NPV was 25.5% (95% C.I. = 13.0–38.0).

Interpretation: The analysis of a day 7 blood sample with QT-NASBA allows for the prediction of late clinical or parasitological treatment failure in the majority of the cases analysed in the present study.

Mutation of Plasmodium falciparum dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) genes from different cities in Senegal [MIM-PS-377664]

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Introduction: Blood samples were collected on filter paper from Plasmodium falciparum malaria positive patients; during the 2004 malaria transmission season, in three different places. Twenty-seven were collected: 11 from Dakar, 6 from Thies and 10 Velingara. Using PCR and direct sequencing we defined prevalence of sulfadoxine–pyrimethamine resistance associated mutations.

Methods: Blood samples were collected on filter paper from Plasmodium falciparum malaria positive patients; during the 2004 malaria transmission season, in three different places. Twenty-seven were collected: 11 from Dakar, 6 from Thies and 10 Velingara. Using PCR and direct sequencing we defined prevalence of sulfadoxine–pyrimethamine resistance associated mutations.

Results: For the dhfr gene, 10 of the 11 samples from Dakar had a triple mutation 51-Ile, 59-Cys and 108-Arg; one was wildtype. Samples from Thies showed 16.7% at 51-Ile, 33.3% at 59-Cys and 33.3% at 108-Arg. For those from...
VELINGARA, 40% had mutation at 51-Asn/Ile, 40% at 59-Cys/Arg, and 50% at 108-Ser/Asn. For the dhps gene, 6 of the 11 samples (54.5%) from Dakar, had 437-Ala/Gly mutation and 45.5% were wild type. For those from Thies 16.7% 437-Ala/Gly and 73.3% were wild type. In Velingara, 30% had 436-Ser/Ala and 40% had 437-Ala/Gly mutation. For the association of the two genes, 10/27 of the samples (37.0%) had three mutations in the dhfr gene and one mutation of the dhps gene. The prevalence of four mutations was 54.5% in Dakar, 16.7% in Thies and 30.0% in Velingara.

Interpretation: This study shows a high prevalence of SP resistance associated mutations in P. falciparum in Senegal, especially in the capital where there is a higher drug pressure. This might compromise the effectiveness of this drug for the treatment of malaria.

416B Effects of a malaria home treatment intervention on chloroquine resistance development in rural Burkina Faso [MIM-FS-182826]
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Introduction: The majority of African malaria cases are treated at the household level. Malaria home treatment is considered a promising intervention in such communities. It is unknown, if home treatment interventions have an effect on the development of resistance against first-line drugs.

Methods: A cluster-randomized controlled study took place in 13 villages of Nouna Health District in northwestern Burkina Faso. The mothers of preschool children from the six intervention villages were trained on correct malaria home treatment with chloroquine and the intervention was integrated into the governmental health services. All children aged 6–59 months were examined in a baseline survey in 2002 and again at the end of the intervention in 2004. Children diagnosed with malaria during these surveys were treated with chloroquine and followed up for 14 days according to the WHO protocol.

Results: One hundred and fourteen and 181 children with falciparum malaria (fever + >5000 par/µl) were enrolled at baseline and follow-up, respectively. In children from intervention and control villages respectively, adequate clinical and parasitological response (ACPR) was 66 and 67% at baseline and 52 and 44% at follow-up. There was a significant effect of time on the outcome (OR 0.4, 95% CI 0.2–0.7), but not of the intervention (OR 1.4, 95% CI 0.8–2.5).

Interpretation: Resistance against chloroquine is now rapidly increasing in rural Burkina Faso, but malaria home treatment interventions do not appear to accelerate resistance development.

417C Controlling malaria in the context of chemoresistance: Status in Senegal, challenges and prospects [MIM-OG-6765]
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Introduction: The different malaria control strategies based on the use of chloroquine have reached their limits due to the increase of the level of Plasmodium falciparum resistance; clinical failure rates higher than 25% were noted during these last years in several sentinel sites in Senegal. Combinations therapies and Intermittent Preventive treatment are the new options. We carried out studies to give evidence to the decision makers.

Methods: Comparative studies using the PCR corrected cure rate on Day 28 have been carried out in 2003–2004. Eight hundred patients with acute uncomplicated malaria received in randomisation clinical trial artether-lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine. Non ACT were also evaluated and compared with the cotreatment amodiaquine plus sulfadoxine-pyrimethamine and sulfalene-pyrimethamine plus amodiaquine. RDTs studies were carried out with 200 patients receiving in a randomisation trial Core Malaria which HRP2 test and OPTIMAL detecting PLDH. In vivo study with SP and DHPS and DHFR genetic markers were evaluated due to the increase of SP resistance.

Results: Clinical trials with ACT showed high efficacy cure rate ranging from 98 to 100%. No seri-
ous adverse events and no abnormal laboratory values have been noted. The efficacy cure rate of amodiaquine plus sulfadoxine–pyrimethamine at Day 28 was 98%, this combination is adopted for the interim phase from June 2003 to July 2005. The efficacy cure rate at Day 28 with the 216 patients who received sulfalene–pyrimethamine plus amodiaquine was 100%. RDTs studies carried out with 200 patients noted a sensitivity and specificity higher than 90% with HRP2 test and 100% with OPTIMAL test based on the principle of the secretion of the PLDH enzyme. Results of studies undertaken on 600 pregnant women showed that the strategy is effective and well tolerated (absence of serious clinical side effects on the mother and the new born; morbidity is not increased compared to the data before the use of the IPT). In vivo study with SP show 13% of failure rates. Thirty-seven percent of samples analysed had four mutations in DHFR and DHPS.

Interpretation: Two consensus meetings have lead to policy changes. Some aspects constitute bottlenecks for a good deployment of these options: The still high cost of the ACTs, their availability, Home based management with ACTs, Prequalifications and Packaging aspects. The increase of SP resistance may lead to look at alternative option as CT which is proposed for our research program.

418A
A randomized trial of chloroquine, amodiaquine and sulfadoxine–pyrimethamine in children under 5 years with uncomplicated malaria in Kolle, MALI [MIM-MT-16878]

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Introduction: Increasing chloroquine resistance has initiated the reassessment of the efficacy of potential alternative treatments against Plasmodium falciparum malaria in Mali, West Africa.

Methods: In a prospective, randomized open trial, we compared the clinical and parasitological efficacy of chloroquine, sulfadoxine–pyrimethamine and amodiaquine, three cheap and widely available antimalarials in our country. The period of inclusion extended from August 2002 at January 2003 for the first year and from July 2003 to January 2004 for the second year of study and took place in Kolle, a village near Bamako, southern Mali. All children aged 6–59 months and admitted with uncomplicated malaria were included and randomly assigned to one of the three treatment arms. We used the WHO standard protocols of 28 days, but for the analysis of parasitological and clinical resistance we used 14 days analysis also.

Results: Overall, 455 children were included with 100, 56 and 57 for the first year and 119, 59 and 64 for the second year in the chloroquine, amodiaquine and sulfadoxine–pyrimethamine arms, respectively. The three groups were comparable with regard to mean age, sex, fever, hemoglobin levels and mean parasitemia at inclusion in each year. Analysis for clinical and parasitological resistance showed that 50.55, 11.11, and 7.14% for the first year and 44.95, 30.36, and 8.06% for the second year had, respectively chloroquine, amodiaquine and sulfadoxine–pyrimethamine resistance at day 14 analysis. While at the day 28 these rates were, respectively, 85.71, 42.59, and 10.07% for the first year and 81.65, 51.79, and 16.13% for the second year for the chloroquine, the amodiaquine and the sulfadoxine–pyrimethamine. During the study we met one case of early therapeutic failure with the SP. For the patients treated with CQ and AQ, the rate of early treatment failure were, respectively, 5.43 and 1.85% for the first year and 6.4 and 5.36% the second year while the rates of late clinical failures at 14 day analysis were 7.69 and 0% for the first year and 3.7 and 1.82% for the second year. Amodiaquine was the most effective on the fever.

Interpretation: The implications of these results on the evolving process for changing antimalarial treatment policy in Mali are discussed.
In vitro susceptibility of *Plasmodium falciparum* to monodesethylamodiaquine, dihydroartemisinin and quinine in an area of high chloroquine resistance in Rwanda [MIM-HT-263384]


(1) Institut de Recherche en Sciences de la Santé, Centre Muraz, Bobo Dioulasso, Burkina Faso; (2) National Malaria Control Program, Kigali, Rwanda; (3) Laboratoire National de Santé Publique, Kigali, Rwanda; (4) Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium; (5) University of Antwerp, Belgium

Introduction: In 2002, a study comparing the efficacy of amodiaquine (AQ) alone or combined with artesunate (AS) was carried out in Rwanda. In two sites, a higher resistance to AQ alone was found. These results were worrying though it was difficult to estimate whether this was a chance finding or a true increase in resistance. Therefore, *Plasmodium falciparum* in vitro susceptibility to chloroquine, monodesethylamodiaquine, the active metabolite of AQ, quinine and dihydroartemisinin was investigated in 2003.

Methods: The isotopic microtest described by Desjardins was used to assess the proliferation of parasites in the presence of drugs. The results of cellular proliferation were expressed as the counts per minute and analysed to determine the 50% inhibitory concentration (IC50) values. The threshold IC50 for in vitro resistance was estimated to be >100 nM for chloroquine, >60 for monodesethylamodiaquine and >800 nM for quinine. The threshold for dihydroartemisinin is still undetermined. Correlation of the IC50 values for different drugs was calculated using Spearman rank-order correlation test. The activity of monodesethylamodiaquine, quinine and dihydroartemisinin against chloroquine-resistant and -sensitive strains was compared.

Results: Dihydroartemisinin was the most potent (GM IC50 = 2.6 nM, 95% CI: 2.2–3.2) among the drugs tested. Resistance to chloroquine was 45% (33/74) and, that to monodesethylamodiaquine 7% (5/74), confirming previous observations that amodiaquine might still be effective where CQ resistance is high. However, this is in contrast with the results of the in vivo study carried out about a year earlier and raising the question on the relationship between the in vitro threshold and the in vivo outcome. All the tested isolates were susceptible to quinine. However, almost 30% of them had a high IC50, some of them close to our defined threshold of 800 nM. The mean IC50 of monodesethylamodiaquine, quinine and dihydroartemisinin was significantly higher for chloroquine-resistant than for chloroquine-sensitive strains (P < 0.05). This might indicate that CQ resistance parasites have acquired a common ability to tolerate several antimalarial drugs, even if they can be classified as sensitive according to threshold used for in vitro tests. The IC50 of each drug was significantly and positively correlated to that of the other three drugs (P < 0.005) and this correlation was higher between CQ and monodesethylamodiaquine (r = 0.8).

Interpretation: The in vitro CQ resistance is linked to that of the other drugs tested. Most worrying is the correlation between the IC50 of dihydroartemisinin and the other drugs, more particularly with CQ, suggesting an increased tolerance to all these drugs.

Molecular surveillance of mutations on dhfr and dhps genes in *Plasmodium falciparum* infection in Ethiopia [MIM-TW-82683]

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Introduction: It has been reported that point mutations in the gene for DHFR and DHPS of the *Plasmodium falciparum* isolates are associated with sulfadoxine and pyrimethamine (S/P) treatment failure, respectively. Knowing the prevalence of point mutations in DHFR and DHPS genes can be used as a surveillance tool to measure the level of S/P resistance. This study was designed to assess the prevalence of point mutations in the two genes of *P. falciparum* isolates collected from Jimma region, Ethiopia.

Methods: The genetic profile of the *P. falciparum* isolates with respect to DHFR and DHPS genes can be used as a surveillance tool to measure the level of S/P resistance. This study was designed to assess the prevalence of point mutations in the two genes of *P. falciparum* isolates collected from Jimma region, Ethiopia.
tions at codons 16, 51, 59, 108, and 164 as well as DHPS mutations at codons 436, 437, 540, 581, and 613 have been analyzed using nested PCR and DNA sequencing techniques. The prevalence of single, double and multiple mutations in the two genes has been calculated.

Results: The sequence profile displays that all isolates (100%) had double mutations at N51I and S108N. 67 (54.03%) of the isolates had a triple mutation in the DHFR gene (N51I, C59R, S108N). With respect to the DHPS gene, all isolates had mutations, at two codons (A437G and K540E) and two isolates (1.61%) were additionally mutated at codon 581 (A581G). Codons 16 and 164 of the DHFR gene, and codons 436 and 613 of the DHPS gene were of the wild type in all isolates. The finding reflects higher mutation rates in the two genes indicating the occurrence of higher level of resistance against SP treatment in Ethiopia, like the previous in vivo studies suggested. The other antifolate drug, chlorproguanil-dapsone (CPG-DDS) is dependent on the presence of a point mutation at position 164 of the DHFR gene. In this study, as previously reported in other African countries, there is no point mutation at this position in any isolates.

Interpretation: To replace S/P, it is possible to use a cheaper and readily available drug like CPG-DDS, since the current treatment recommendation, artemisinin combination therapy, is not affordable in Ethiopia.

421A Multi-site trial of combination antimalarial therapy: Evaluation of efficacy, safety and tolerability in Uganda [MIM-AY-255780]


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Introduction: Drug resistance in Plasmodium falciparum poses a major threat to malaria control. Combination antimalarial therapy including artemisinins has been advocated recently to improve efficacy and limit the spread of resistance, but artemisinins are expensive and relatively untested in highly endemic areas. We compared artemisinin-based and other combination therapies in four districts in Uganda with varying transmission intensity.

Methods: The studies were randomised single blind trials with 28 day follow up. Study sites, selected for geographic diversity, were: Jinja (medium high endemicity, entomological inoculation rate [EIR]=7), Arua (very high endemicity, EIR = 393), Tororo (very high endemicity, EIR=591) and Apac (very high endemicity, EIR=1564). We enrolled 2160 patients aged 6 months or greater with uncomplicated falciparum malaria. Patients were randomized to receive chloroquine (CQ) plus sulfadoxine-pyrimethamine (SP); amodiaquine (AQ) plus SP; or AQ plus artesunate (AS). Primary endpoints were the 28-day risks of parasitological failure either unadjusted or adjusted by genotyping to distinguish recrudescence from new infections.

Results: Two thousand and eighty-one patients completed follow-up, of which 1749 (84%) were under the age of 5 years. The risk of recrudescence after treatment with CQ + SP was high, ranging from 22 to 46% at the four sites. This risk was significantly lower (P < 0.01) after AQ + SP or AQ + AS (7-18% and 4-12%, respectively). Compared to AQ + SP, AQ + AS was associated with a lower risk of recrudescence but a higher risk of new infection. Defining treatment failure as any need for retreatment for malaria over 28 days, the overall risk of failure was similar at two sites and significantly higher for AQ + AS at the two highest transmission sites (risk differences = 15 and 16%, P < 0.003). Median duration to failure was shorter with recrudescences compared to new infections (26 days versus 27 days, p = 0.03), although the difference was marginal, and over 75% of both recrudescences and new infections occurred after 20 days of follow-up. No differences between recrudescences and new infections were found with respect to the proportion of patients who were symptomatic, the risk of complicated malaria, parasitite density, or changes in hemoglobin. Gametocytes during follow-up were more common with recrudescences (51% versus 43%, p = 0.02). Serious adverse events were uncommon with all regimens.

Interpretation: AQ + AS was the most efficacious regimen for preventing recrudescence, while AQ + SP was the best regimen for preventing re-treatment. Both regimens were markedly superior to CQ + SP. The high
endemicity of malaria in Africa may impact on the efficacy of ACT’s.

**422B**

Amodiaquine, sulfadoxine–pyrimethamine, and combination therapy for uncomplicated falciparum malaria: A randomized controlled trial from Burkina Faso [MIM-IZ-273312]


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**Introduction:** In Burkina Faso, increasing resistance to chloroquine requires the testing of alternative antimalarial therapies. Artemisinin combination therapies are now widely advocated, but we were interested in evaluating cheaper, more readily available drugs. We therefore compared the efficacies against uncomplicated falciparum malaria of amodiaquine (AQ), sulfadoxine–pyrimethamine (SP), and AQ + SP in Bobo-Dioulasso, a city in western Burkina Faso that is holoendemic for falciparum malaria.

**Methods:** We enrolled 944 patients, aged over 6 months, who presented with uncomplicated falciparum malaria at three clinics in Bobo-Dioulasso. Patients were randomized to AQ (317 patients), SP (304), and AQ + SP (323), all administered via directly observed therapy. Patients were followed for 28 days and outcomes assessed based on 2003 WHO criteria. Primary outcomes were day 28 treatment response, both unadjusted and adjusted by genotyping (based on MSP-2) to distinguish new infections from recrudesences. Risks of treatment failure were estimated using Kaplan–Meier survival analysis techniques. Secondary outcomes included fever clearance, parasite clearance, change in hemoglobin, gametocytes during follow-up, and treatment safety and tolerability.

**Results:** Of the 944 patients enrolled 829 (87.8%) were assigned 28-day efficacy outcomes and included in the per-protocol analysis. Fifty-three percent of patients were under 5 years of age (range 0.5–52). For all regimens, early treatment failures were uncommon (<2%). Considering all treatment failures, the most efficacious regimen was AQ + SP (failures in 4.2%), followed by SP (9.1%) and AQ (17.9%; p < 0.02 for all pairwise comparisons). Considering only clinical failures, relative efficacies were similar (AQ + SP: failures in 2.1%; SP: 6.5%; AQ: 13.2%; p < 0.02 for all pairwise comparisons). Considering only recrudescences, the risk of failure was lower with AQ + SP (2.1%) compared to SP (6.1%, p = 0.02) and AQ (8.1%, p = 0.001). Risks of new infection were lower with AQ + SP (2.1%) and SP (2.4%) compared to AQ (9.1%, p < 0.001 for both comparisons). The proportion of patients with persistent parasitemia on day 3 was greatest for SP and lowest for AQ + SP. Fever resolution was most rapid with AQ + SP. Gametocytes were seen during follow-up most commonly in SP-treated patients. The incidence of adverse events was similar across the three treatment groups except for pruritis, which was more common with AQ. No serious adverse events were seen.

**Interpretation:** All regimens were quite efficacious, but the combination of AQ + SP was superior. AQ + SP appears to offer a highly efficacious, inexpensive, available, and safe regimen for the treatment of uncomplicated falciparum malaria in Burkina Faso.

**18: Severe malaria in children**

**Posters 423–455**

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday
423C Interactions of elevated nitric oxide with platelet parameters in Nigerian children with asymptomatic and symptomatic Plasmodium falciparum malaria [MIM-IA-164511]

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Introduction: Elevated nitric oxide (NO) level and platelet dysfunction are known as separate pathogenic elements in falciparum malaria. The interactions of these factors in modulating malaria episode and informing appropriate therapeutic interventions remain poorly understood. This study has determined the association between plasma NO levels, platelet aggregation and secreted aggregation modulating enzymes: apyrase and 5′-nucleotidase in asymptomatic and symptomatic Plasmodium falciparum malaria.

Methods: A total of 113 Nigerian children aged 2–12 years with asymptomatic falciparum malaria (AM) \( (n = 31; \text{parasitaemia} = 300–1050 \text{parasites}/\mu\text{L}) \) and those with symptoms stratified into acute uncomplicated malaria (UM) \( (n = 53; \text{parasitaemia} = 2350–31500 \text{parasites}/\mu\text{L}) \) and severe malaria (SM) \( (n = 29; \text{parasitaemia} = 70,150–115,400 \text{parasites}/\mu\text{L}) \) were consecutively enrolled into the study. They were submitted to plasma NO determination by spectrophotometric method and platelet function tests: platelet aggregation by platelet count ratio technique, hypersensitivity reaction by in vitro exposure to agonists and enzyme assays based on Pi released per min/mg protein. Apparently healthy children with zero parasitaemia \( (n = 25) \) were also analyzed as control.

Results: A significant \( (P < 0.05) \) increase in plasma NO level was observed in AM children \( (21.6 + 1.4 \text{nmol/L}) \), children with UM \( (22.4 + 1.8 \text{nmol/L}) \) and those with SM \( (41.5 + 3.8 \text{nmol/L}) \) compared to control \( (16.4 + 1.3 \text{nmol/L}) \). Compared to control \( (0.89 + 0.02) \), a non-significant \( (P > 0.05) \) reduction in platelet count ratio \( (0.86 + 0.03) \) but significant \( (P < 0.05) \) increases in the activities of apyrase \( (14.1 + 1.3 \text{U/mgprotein}) \) and 5-nucleotidase \( (6.7 + 0.2 \text{U/mgprotein}) \) with both enzymes showing significant positive correlation \( (r = 0.86–0.93; P < 0.05) \) with NO level were observed in the AM children. Children with UM also elicited significantly \( (P < 0.05) \) reduced platelet count ratio \( (0.78 + 0.07) \) and mild hypersensitivity reaction \( (58.7 \text{versus} 51.6\%) \) to ATP and collagen compared to the control. Elevated apyrase \( (13.2 + 0.7 \text{U/mgprotein}) \) and 5-nucleotidase \( (6.1 + 0.6 \text{U/mgprotein}) \) activity with the latter showing positive correlation \( (r = 0.75; P < 0.05) \) with NO were also found. In SM, platelet aggregation, a non-significant alteration in apyrase \( (11.2 + 2.1 \text{U/mg protein}) \) but significant decrease in 5-nucleotidase activity \( (5.0 + 0.8 \text{U/mgprotein}) \), which correlated negatively \( (r = –0.67; P < 0.05) \) with NO level and were found compared to the control.

Interpretation: Elevated nitric oxide in children with falciparum malaria functions to inhibit platelet aggregation in asymptomatic and uncomplicated malaria and acts in synergy with altered platelet parameters to exacerbate the pathogenesis of severe malaria.

424A Antigenic variation in severe Plasmodium falciparum malaria: A comparison between severe malarial anemia and cerebral malaria [MIM-TA-7406]

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Introduction: The variant surface antigens (VSA) of infected erythrocytes are important pathogenic marker,
and a set of variants (VSASM) was found to be associated with severe malaria (SM). However, severe malarial anemia (SMA) and cerebral malaria (CM) are clinically quite diverse. Only limited numbers of studies were carried out in the area of antigenic variation in severe malaria (SM) using wild field strains, and verify between the individual complications of SM, such as CM and SMA.

Methods: This study was carried out in Gedarif Teaching Hospital (GTH). Gedarif town is located in eastern Sudan, where the malaria transmission is described as seasonal, moderate, and highly unstable. Parasites/plasma were obtained from patients with different types of SM and uncomplicated malaria (UM). Parasites were cultured and the mature forms were enriched using the magnetic cell sorter. Flow cytometry technique (FACS) was used to study the parasite VSA expression and host immune response in SM (including, SMA and CM).

Results: In general, individuals who recognized a broader range of isolates had higher level of VSA Abs against the recognized isolates, (CC, 0.727, P < 0.001). Of our main observations are; unexpectedly we found that, at the time of malaria diagnosis, plasma from patients with CM recognized a significantly larger number of isolates than did the plasma from patients with SMA (P < 0.001). While parasites obtained from patients with SMA or from children were better recognized than isolates obtained from patients with UM or from adults, P < 0.001 and 0.021, respectively.

Interpretation: Taken together, the VSA response determinants suggest a greater role for VSA immune incompetence in pathogenesis of SMA than in that of CM.

425B
Safety of a modified gelatin colloid (Gelofusine) for correction of volume deficits in severe malaria [MIM-SA-81528]

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Introduction: Recent evidence shows presence of hypovolaemia in severe malaria. In a formal RCT we showed volume expansion with albumin was associated with a significantly lower mortality in children with severe malaria acidosis, especially those with coma. As albumin is costly and scarce in Africa, we now aim to examine the safety and determine dose (efficacy) for the correction of hypovolaemia of a cheaper colloid, Gelofusine in children with severe falciparum malaria complicated by acidosis.

Methods: Children >3 months old with malaria and severity feature (impaired consciousness and/or deep breathing) plus metabolic acidosis (base deficit >8 mmol/l) included. Eligible children were randomised to receive Gelofusine (~10.50 L−1) or 4.5% human albumin solution (~120 L−1). Children received 20–40 ml/kg of resuscitation fluid over first hour, volumes given dependent upon degree of acidosis and shock. Other management were identical. Efficacy was determined by the volume of Gelofusine or albumin required to correct features of shock. Safety was defined by whether Gelofusine resulted in “harm” (>20% mortality in the non-coma group or >40% mortality in the coma group) compared to albumin or the development of an adverse event.

Results: In a mid-trial review in February 2005: 55 children had been recruited, 28 received albumin, 27 received Gelofusine. There was no difference between the two colloids in the volumes required to correct the clinical features of shock. There were two deaths in the Gelofusine group (2/27, 7%) and no deaths in the albumin group (0/28). At admission approximately 50% of the children entering the trial were in deep coma, Blantyre Coma Scale <2 (cerebral malaria) – a group we had hypothesised would benefit most from the use of colloids rather than crystalloids for volume resuscitation. There were no major adverse events (pulmonary oedema, raised intracranial pressure, allergic reaction or significant coagulopathy) and no evidence of neurological sequelae in survivors at discharge or at follow up 1 month later.

Interpretation: Gelofusine appears to be as safe as human albumin solution in children with severe malarial acidosis and was at least as effective in correcting clinical features of shock. Its efficacy, in terms of mortality, should now be tested in a larger randomised controlled trial.
Association of adhesion molecules and the clinical severity of malaria in south-west Nigeria [MIM-OA-127381]

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Introduction: Falciparum malaria remains a major public health hazard in sub-Saharan African children. While the factors that determine the variations in clinical outcome of a malaria infection have not been completely defined, both parasite and host factors, as well as the complex molecular interactions between them are implicated. The cytoadherence of \( P. falciparum \)-infected erythrocytes is an important step in the pathogenesis of severe malaria and adhesion molecules have been associated with the process.

Methods: We have studied the association between adhesion molecules at five different loci (ICAM-1 exons 2, 4 and 6, E-selectin exon 2, PECAM exon 3) and the severity of disease in childhood malaria in Ibadan, south-west Nigeria. Clinical information and blood samples were collected from 222 children (median age of 34.5 months) presenting with different clinical manifestations of malaria; asymptomatic malaria (AsyM), acute uncomplicated malaria (UM) and severe malaria (SM). Genotyping of five genetic loci was done by PCR-RFLPs using the appropriate restriction digests for the different loci.

Results: All the loci were at Hardy–Weinberg equilibrium (HWE). The E-selectin locus had very low heterozygosity (~0.06) in contrast to the other loci (0.23–0.44). Controlling for covariates (age and parasite density), shows that the E-selectin locus is associated with both uncomplicated malaria and severe malaria (SM). Genotyping of five genetic loci was done by PCR-RFLPs using the appropriate restriction digests for the different loci.

Interpretation: Findings show the polymorphism at the exon 6 of the ICAM-1 locus is associated with severe malaria. It is concluded that adhesion molecules genotypes especially in the ICAM-1 exon 6 locus are a risk factor in the severity of malaria in children.

Acute clinical \( P. falciparum \) malaria causes shifts in B cell populations in children from malaria endemic region of western Kenya [MIM-AA-148080]

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Introduction: Acute clinical malaria has been linked to increases in antibodies, loss of specific immune responses, but it is unknown whether there are changes in lymphocyte subsets around an episode of acute clinical malaria. Hence the need to determine whether an episode of acute clinical malaria results in perturbations of the frequency of lymphocyte subsets.

Methods: Using flow cytometric techniques, we characterized the surface phenotype of T and B-cell surface markers CD3, CD4, CD8, CD19, CD27, CD10, CD23, CD38 and IgD in the peripheral blood of 15 children aged 2–5 years during episodes of acute clinical malaria, post-recovery and of six healthy age-matched controls who were parasitaemic but asymptomatic.

Results: We did not find significantly altered B cell expression of memory or naïve B cells. However, there was significant increase in the frequencies of CD38+ B cells during acute clinical malaria, CD10+, CD23+ B cells during post-recovery suggesting that there are shifts in the B cell that result from \( P. falciparum \) infection. Of note is the significant increase in B cells expressing CD10 the cell surface marker of Burkitt’s lymphoma cells in these children.

Interpretation: These results demonstrate that acute episodes of \( P. falciparum \) malaria cause alterations in B cell phenotype and differentiation suggesting that malaria is not just increasing B cell numbers but also shifting the B cell pool.
A correlation between levels of antimalarial specific IgE and eosinophils may confer protection in children with severe malaria [MIM-TA-23061]

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Introduction: Eosinophils and monocytes are potent bearers of CD23 (FcεRII), the low affinity receptor for antimalarial IgE. The binding of these IgE to CD23 may activate an antiplasmodial effect or induce the production of TNF-α by monocytes.

Methods: We measured plasma levels of antimalarial IgE and TNF-α by ELISA in Cameroonian children with different disease categories: severe anemia, cerebral malaria, other forms of severity, uncomplicated malaria and normal control children. Levels obtained together with eosinophil and monocyte counts were compared between the five study groups during active infection (at onset of treatment) and during convalescence (seven days post-treatment).

Results: Plasma levels of antimalarial IgE were elevated in children with severe malaria, with an increase observed at day 7 after treatment. There was a positive correlation between the levels of these specific antibodies and age of the children (r = 0.162, P = 0.0225). Plasma levels of TNF-α were higher in all malaria groups during active infection, with a significant decrease observed on day seven (P = 0.047). We found no significant correlation between the levels of TNF-α and those of antimalarial specific IgE (r = 0.123, P = 0.0839); but TNF-α levels strongly correlated with poor outcome (r = 0.214, P = 0.0058). Monocytes counts did not show any specific change during both active and convalescent phases of malaria while eosinophil counts showed lower levels during active infection in children with malaria (P = 0.0135) except those with severe anemia. A control on day 7 showed an increase although only significant in children with uncomplicated malaria (P = 0.0493).

Interpretation: Thus, specific IgE may not essentially induce TNF-α production in falciparum malaria; they may activate rather an ADCI/ADCC process by eosinophils against malaria parasite.

An evaluation of the WHO haemoglobin colour scale among young children in the Kintampo District of Ghana [MIM-KA-25200]

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Introduction: Anaemia is a common complication of malaria. Early diagnosis and treatment of anaemia is important for the prevention of severe complications. In areas were laboratory facilities are unavailable, diagnosis of anaemia is by clinical signs or Tallqvist Colour Scale method. The WHO haemoglobin colour scale has been found to be more effective than the two methods mentioned above. There has been the need to evaluate it in Ghana for possible use in areas where laboratory facilities are unavailable.

Methods: We determined the frequency of deviations of haemoglobin colour scale (Copack GmbH, Germany) readings from that of the automated haemocue (Leo Diagnostics, Sweden) among 408 children in the Kintampo District. Sensitivity, specificity and predictive values were also determined.

Results: The mean age of children who participated in the study was 5.0 years. The mean hemoglobin level determined by the haemocue and haemoglobin colour scale were 10.4 and 10.9 g/dL, respectively. About 63 and 88% of HCS readings deviated from the Haemocue readings within ±1 and ±2 g/dL, respectively. Sensitivity and specificity values were 50 and 93%, respectively. Positive and negative predictive values were 93 and 52%, respectively.

Interpretation: In malaria endemic areas where laboratory facilities are unavailable, the WHO haemoglobin colour scale may be used in the determination of anaemia as a screening method.
430A
Impaired Ab responses to critical targets of *P. falciparum* merozoite antigens in patients with cerebral malaria: Are they markers of fatal outcome? [MIM-MB-3486]

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Introduction: Cerebral malaria is a major complication of *P. falciparum* infection, resulting from a complex cascade of events, possibly including insufficient qualitative and/or quantitative Ab responses against key targets of anti-parasite immunity such as the merozoite, the erythrocyte invading form of the parasite.

Methods: We investigated Ab profiles of responses against a set of several recombinant merozoite surface proteins (MSP) in 122 hospitalized urban patients from Dakar (Senegal) with confirmed cerebral malaria (CM), recruited during two successive transmission seasons: young individuals (*n* = 45, 2–13 years) and 77 adults (*n* = 77, 14–70 years), of which 24% deceased [FCM] (*n* = 29). We analyzed IgG responses against several merozoite-associated Ags by ELISA, using crude merozoite extracts and MSP recombinant proteins: MSP1p19, MSP3, MSP4 constructs and MSP5 Ags. Functionality of anti-merozoite IgG response was measured by a polynuclear cell mediated phagocytosis assay.

Results: We found high prevalence of Ab responses to merozoite Ags and every recombinant MSP Ag tested (>80%). Levels of IgG responses against all Ags were elevated, comparable to the levels of antibodies found in immune individuals from endemic areas. Antibody responses were age-unrelated (except for MSP3 and contrary to immune individuals) and showed a significant degree of colinearity (*P* < 0.01, Rho 0.4–0.9) between each other. IgG responses against the crude merozoite Ags and MSP constructs were significantly correlated with phagocytic indexes [PI] (*P* < 0.01, Rho #0.5). Importantly, a significantly lower IgG response was found in fatal cases compared to surviving individuals, it was selectively detectable with PI, and IgG to MSP1p19 and to MSP4 (*P* < 0.01).

Interpretation: These results point out the role of IgG responses to merozoite-associated Ags in the protection against fatal outcome in CM. The PI assay allows a discrimination between CM an FCM, contrary to ELISA measures, calling for further investigations.

431B
Effect of home environment on the cognitive outcome in childhood cerebral malaria [MIM-PB-130764]

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Introduction: Cerebral malaria (CM) is a neurological complication of malaria and it is associated with cognitive sequelae. Socio-economic indicators like low nutritional resources, low parental education and occupation level have been shown to worsen this sequelae. However, the quality of the home environment is a better indicator of cognitive functioning in at risk children than socio-economic factors. We therefore looked at the effect of the home environment on cognitive functioning after childhood CM.

Methods: Forty-six children aged 5–12 years admitted with CM at Mulago Hospital, Uganda had cognitive assessment using the Kaufmann Assessment Battery for Children (K-ABC), Tactual Performance Test (TPT) and the Test of Variables of Attention (TOVA) before discharge and 3 months later. The quality of the home environment was assessed before discharge using the Middle Childhood Home Observation for Measurement of the Environment (HOME). Fifty-four children treated for uncomplicated malaria (UM) and sixty healthy controls (HC) received the same assessments.

Results: All three groups had similar anthropometric measurements and home environment status. Children with CM had significant deficits on most K-ABC subtests including the Mental Processing Composite (*p* = 0.001), TPT’s bimanual task (*p* = 0.002) and on TOVA’s *D*’ score for attention capacity (*p* = 0.001).
at discharge compared to the UM and HC children. Children with CM still had deficits in mental processing ($p = 0.001$), bimanual task performance ($p = 0.002$) and attention capacity ($p = 0.001$) at 3 months. Children with CM from a superior home environment (HOME score of 33–47, $n = 21$) performed significantly better than those from a poorer home environment (HOME score of 0–32, $n = 24$) on most K-ABC subtests and on TPT and TONA measures at discharge and at 3 months. Similar effects of home environment on test performance were also observed in children with UM and healthy control children. Although children with CM generally did worse on cognitive testing at 3 months than healthy control children, children with CM from a good home environment did significantly better at 3 months than healthy control children from a poorer home environment on most of the tests.

Interpretation: The home environment plays a significant role in the cognitive functioning of children and this is more important for those recovering from CM. Home environment enrichment may lessen the burden of CM on cognitive development in children.

432C Plasma levels of tumour necrosis factor-alpha (TNF-$\alpha$) and interleukin-10 (IL-10) in children with severe and uncomplicated malaria [MIM-RB-719888]

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Introduction: In malaria endemic areas, children constitute the most vulnerable group to malaria. Pro- and anti-inflammatory cytokines have been implicated in both protection and pathogenesis of malaria. To gain insight into the putative roles and mechanism of action of cytokines in malaria immunity and pathogenesis, levels of TNF-$\alpha$ and IL-10 were assayed in children with severe and uncomplicated malaria and compared with levels in healthy controls of same provenance.

Methods: A total of 117 children (1–14 years) with malaria from five hospitals in Fako Division, South West, Cameroon and 45 afebrile school children were recruited into the study between May and December 2004, after informed consent from parents/guardians and from the Provincial Delegation of National Education. A questionnaire was completed by the physician and patients were categorised into cerebral malaria (CM, $n = 25$), severe malarial anaemia (SMA, $n = 46$) and uncomplicated malaria (UM, $n = 46$). Malaria parasitaemia was determined by microscopic examination of stained thick blood films. Plasma TNF-$\alpha$ and IL-10 levels were determined by ELISA.

Results: Cytokine levels were significantly elevated in malaria patients compared to controls. Mean TNF-$\alpha$ levels were significantly higher in severe malaria (CM and SMA) than in UM and controls ($P < 0.04$), CM = 31 pg/ml, SMA = 30 pg/ml, UM = 22 pg/ml and controls = 9 pg/ml. Mean IL-10 levels were significantly lower ($P < 0.03$) in SMA (266 pg/ml) compared to CM (493 pg/ml) and UM (403 pg/ml). There was a positive correlation between TNF-$\alpha$ and IL-10 levels ($P < 0.001$). The IL-10/TNF-$\alpha$ ratio was significantly lower ($P = 0.001$) in SMA than in CM and UM. Log parasite density (PD) correlated positively with TNF-$\alpha$ ($P = 0.002$) and IL-10 ($P < 0.001$) levels. There was a negative correlation ($P < 0.03$) between TNF-$\alpha$ and haemoglobin concentration among patient categories, though this was not significant. A negative nonsignificant correlation between IL-10 levels and age was observed. TNF-$\alpha$ levels also correlated negatively with age, but this was not significant.

Interpretation: These results suggest an imbalance in TNF-$\alpha$ and IL-10 expression in severe malarial patients. IL-10/TNF-$\alpha$ ratio may serve as a marker for SMA but not for CM.
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433A
Relationship between cerebral malaria and epilepsy: A case-control study in Libreville, Gabon [MIM-NE-275819]
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Introduction: Cerebral malaria is one of the most serious complications of Plasmodium falciparum infection. It is a potential cause of epilepsy in tropical areas but little information are available to quantify it. The purpose of this study was to evaluate the relationship between CM and epilepsy in young subjects in Gabon.

Methods: A case-control study was carried out on a sample of Gabonese subjects aged 6 months to 25 years and hospitalized between 1990 and 2004 within three hospitals of Libreville. In this study CM was defined according to the WHO clinical and biological criteria. Epilepsy was defined according to the epidemiological definition (ILAE) and confirmed by a neurologist.

Results: Five hundred and ninety-two subjects (296 epilepsy cases and 296 controls) were included. Thirty-six (26 epilepsy cases and 10 controls) had a CM antecedent. The odds ratio to develop an epilepsy with a CM antecedent was 3.4 [CI 95%: 1.6–7.4] p < 0.001. But CM (coma alone) did not represent a statistically significant risk factor compared to CM (convulsions-coma associated) OR = 3.9 [CI 95%: 1.7–8.9] p = 0.001. Other risk factors were identified: epilepsy family antecedent OR = 6.0 [CI 95%: 2.4–14.1] p = 0.0001 and febrile convulsions OR = 9.2 [CI 95%: 4.0–21.1] p = 0.0001. Sickle cell disease represented a protective factor OR = 0.3 [CI 95%: 0.1–0.7] p < 0.001.

Interpretation: This study show that epilepsy-related CM is an important problem but under-recognized. It emphasizes the needs for studies to better appreciate the role of convulsions during CM and sickle cell disease.

434B
Correlates of red blood cell deformability (RCD) with adverse outcome in severe falciparum malaria: The effect of sequestered parasitized red cells [MIM-AE-70550]
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Introduction: Reduced (r) RCD correlates with poor outcome and severe anaemia in falciparum malaria. Alpha + thalassaemia causes rRCD and mild anaemia in health, but protects against severe anaemia and fatality in falciparum malaria. In severe falciparum malaria and non-malarial severe sepsis, lactic acidosis indicates a fatal outcome. We hypothesize that sequestered parasitized red cells modify the relationship between rRCD and lactate levels, but that RCD predicts anaemia regardless of underlying disease.

Methods: We studied three groups: (1) 134 children admitted to the Paediatric High Dependency Unit (HDU) with severe malaria, (2) 134 healthy controls from a community cross sectional survey and (3) 43 children admitted to the HDU with non-malarial sepsis. Blood was tested for haemoglobin (Hb), mean cell volume, malaria thick and thin film, RCD (using a laser assisted optical rotational cell analyzer) and thalassaemia and sickle status (by multiplex PCR). Sequestered parasite biomass is estimated using plasma histidine rich protein-II level (Cellabs, Australia). Regression analysis is with lactate levels and Hb as outcome measures. The possible modifying effect of sequestration on the relationship of RCD and plasma lactate levels is assessed.

Results: Results confirm that reduced RCD (measured by elongation index [EI]) correlates with blood lactate concentration in group 1 [severe malaria] (r = 0.45, p < 0.0001), and not group 3 [severe sepsis] (r = 0.0, p = 0.977). Reduced RCD was correlated with the severity of anaemia in both group 1 (r = 0.53,
In healthy children with α-thalassaemia (from group 2), RCD was reduced (mean EI = 0.17) compared to healthy controls without α-thalassaemia (mean EI = 0.21, p = 0.0001). The relationship of RCD: lactate and anaemia in children with α-thalassaemia in groups 1 (severe malaria) and 3 (severe sepsis); the effect modification of sequestered parasite biomass on the above relationships; and the correlation of the degree of sequestration, RCD and lactate are also examined.

**Interpretation:** This comparative study suggests that rRCD may play a role in anaemia through splenic clearance of rigid cells irrespective of underlying disease, but that sequestered parasitized red cells may be a prerequisite for the effect of rRCD on lactate.

**Red cell aging and red cell surface molecules in Tanzanian children with malarial anemia [MIM-MG-5805]**

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**Introduction:** The loss of uninfected red blood cells contributes to malarial anemia, and may be related to changes in levels of key surface molecules, including decreased levels of complement regulatory proteins (CR1 and CD55), increased surface IgG, and increased exposure of phosphatidylserine. Because levels of these surface molecules also vary as part of the normal cell aging process, we investigated whether RBC aging could be influencing the membrane changes that have been associated with malarial anemia.

**Methods:** Blood samples used in the study were obtained from children participating in the Mother Offspring Malaria Study Project, in Muheza, Tanzania. RBCs were separated on Percoll density gradients to obtain fractions of different densities/ages, then labeled with monoclonal antibodies to measure surface CR1, CD55, CD59 (BD, PharMingen, USA) and IgG (Molecular Probes, USA), and with Annexin V-FITC (Sigma-Aldrich) to detect surface-exposed phosphatidylserine. Levels of RBC surface molecules measured by flow cytometry were compared between children with uncomplicated malaria, Hb > 10 g/dl (n = 43) and children who had malaria complicated with anemia, Hb < 10 g/dl (n = 36). Differences with P < 0.05 were considered statistically significant.

**Results:** Separation of RBC by Percoll produced four distinct fractions: RBCs prior to separation were designated as FR0, whereas the RBC subpopulations were designated as follows: FR1 = very young; FR2 = young; FR3 = intermediate; and FR4 = old. Compared to other children, children with anemia displayed significant increases of cells in FR1 (P = 0.0115) fraction and significant decreases in FR3 (P = 0.0061) and FR4 (P = 0.0301) fractions. By Wilcoxon Signed Rank test, in all RBC subpopulations anemia was associated with a decrease in the proportion of cells expressing CR1 (FR0: P = 0.0284; FR1: P = 0.0382; FR2: P = 0.0015; FR3: P = 0.0236 and FR4: P = 0.6573), CD55 (FR0: P = 0.0506; FR1: P = 0.0015; FR2: P = 0.004; FR3: P = 0.0043 and FR4: P = 0.0962, and with significantly low proportion of cells showing surface IgG (FR0: P = 0.0117; FR1: P = 0.0032; FR2: P = 0.003; FR3: P = 0.0042 and FR4: P = 0.0079). Surface levels of CD59 and phosphatidylserine did not differ between children with and without anemia, when either unfractionated RBC samples or individual RBC fractions were compared.

**Interpretation:** Children with malarial anemia have RBCs with decreased levels of CR1, CD55, IgG and shortened half-life. These changes were in all RBC populations regardless of age suggesting that RBC aging is unrelated to the changes in surface molecules.

**Study of Plasmodium falciparum genetic diversity in severe malaria cases in Cotonou, Benin [MIM-EI-210043]**

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Introduction: This study was designed to investigate the genetic diversity of *Plasmodium falciparum* in children with severe malaria infections.

**Methods:** One hundred and fifty-one children with severe malaria were enrolled into the study. Finger pricked blood sample were blotted onto 3 MM Whatmann filter paper. Parasite genomic DNA was extracted from the filter paper blood sample using the methanol with heat extraction method. Polymorphic region of the different allelic families of MSP1, MSP2 and GLURP were amplified from the parasite genomic DNA by nested PCR technique.

**Results:** Analysis of samples obtained at enrolment showed that the frequency of K1 allelic family of MSP1 was significantly higher \((p < 0.001)\) than MAD20 and RO33 of the same gene. In the case of MSP2, the frequency of the FC27 allele was also significantly higher \((p = 0.032)\) than the ICI/3D7 allele. GLURP was observed to be more polymorphic than both MSP1 and MSP2. An equal distribution was observed in MSP2 and GLURP genotypes and are significantly higher \((p > 0.001)\) than MSP1 in all the isolates obtained from the children with severe malaria. There was no association \((p > 0.05)\) between age of patients or parasites density and family specific alleles of MSP1, MSP2 or GLURP.

**Interpretation:** Data obtained suggest that K1 and FC27 allelic families of MSP-1 and MSP-2 genes may be involved antigenic diversity in severe malaria patients and this may have some immunological implications for the future development of an antimalarial vaccine.

437B
The influence of malaria on calcium and zinc levels in the blood serum, of children aged from 0 to 14, in the city of Douala in Cameroon [MIM-MJ-19118]

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Research Associates in the Chore Team of Researchers on Malaria Attached to the Faculty of Sciences at the University of Douala

Introduction: In order to study the influence of malaria on calcium and zinc levels in the blood serum, an assessment was conducted, in the city of Douala, on a sample of 160 children (aged from 0 to 14 years), whom children attended the Medico-Social Centre at Cité SIC-Bassa, Douala, during a 2 months survey period from January to February 2004.

**Methods:** These children were identified in two categories of 80 individuals each as follows: The group of children tested negative for malaria or, control sample. The group of malaria positive children, (presenting clinical symptoms), amongst which 27 have undergone a longitudinal survey. their blood were sampled and analysed.

**Results:** The results obtained subsequently reveal that about 62% of control the whole sample shows a calcium level lower to the normal (81–104 mg/l). The calcium level is significantly low \((p < 0.05)\) to the malaria positive (90.69 ± 20.62 mg/l) as compared to the control subjects (98.20 ± 24.64 mg/l). In the more, an increase of calcium’s rate is observed during the convalescence (98.88 ± 14.33 mg/l) as compared to the beginning of the illness (92.12 ± 20.77), but in a non significant way \((p = 0.09)\). To malaria positive children with, the calcium level increases with age until 5 years, whereas it decreases with a moderate and/or high level of parasitaemia.

**Interpretation:** As far as zinc is concerned, about 31% of individuals on the whole sample show a blood zinc level lower to the normal 7.60–15.30 μmol/l and 9.8–16.80 μmol/l The mean level of zinc increases during the convalescence (18.63 ± 6.82 μmol/l)

438C
Epidemiological mapping of Malaria in Muheza District: All causes and malaria specific mortality by verbal autopsy [MIM-MK-17626]

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Introduction: Many deaths in developing countries occur outside health care settings; hence health facility based data underestimate the true picture. Verbal autopsies (VA) are used to determine the underlying cause of death, and the probable diagnosis helps to estimate reasonably cause specific mortality.

**Methods:** Verbal autopsy survey involved eight villages (four villages from each stratum in low and highlands) and was conducted following a rapid census, which was done to identify households that had lost one or more member within a period of two years from the
date of census. Trained research assistants administered VA questionnaires to parents/close relatives. Two physicians reviewed each report independently and a third opinion was sought if there was discordant report between the two.

Results: In 9872 surveyed populations, 134 deaths were recorded of which 71.6% (96/134) were from lowland villages representing high malaria transmission. Majority (74.4%) of the reported deaths occurred at home whilst 25.6% occurred in health facility settings. Overall, severe malaria was the leading cause of death accounting for 34.3% deaths. Infants were the most affected group accounting for 43.5%. Pulmonary tuberculosis ranked second (8.2%) and was exclusively in age group 15 years and above. Probable cause of death could not be determined in 13.4% of deaths.

Interpretation: Majority of deaths still occur at home of which the immediate cause is usually unknown but VA technique could be utilized to bridge the gap by complementing the establishment of probable cause of deaths that occur outside health care settings.

439A
Severe falciparum malaria and working memory: Neuropsychological and electrophysiological findings [MIM-MK-423252]
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Introduction: Neurocognitive assessment of children exposed to severe malaria in resource poor settings is difficult due to lack of age- and culture-specific assessment tools. In this study, we used neuropsychological tests that have been found to be sensitive to brain function and compared the results with those obtained using cognitive Event Related Potentials (ERP). ERP’s are EEG changes that are time-locked to sensory or cognitive events and reflect the neurophysiological processing of these events.

Methods: Thirty-three children (mean = 6.6, S.D. = 0.86) previously hospitalized with severe falciparum malaria were selected from the hospital database and randomly matched with 34 (mean = 6.94, S.D. = 1.13) community controls. Neuropsychological tests used included self ordered pointing test (working memory), SCORE (sustained attention) and vigilance (selective and sustained attention). Each child further had an auditory ERP in which a series of auditory stimuli consisting of standard tones (p = 0.76), target tones (p = 0.12) and distracter novel sounds (p = 0.12) were presented randomly. ERPs were recorded from 15 scalp locations with amplitude and latency of the p300 from the distracter assessed and those of P100 and N250.

Results: No Statistical significant differences were found between falciparum malaria patients and unexposed children with respect to gender, age and education. Neuropsychological results: test results showed that children exposed to falciparum malaria performed poorer than unexposed children in the SOPT (p < 0.05) task even when schooling and home environment were included into the model. They also committed more errors (p < 0.05) than the unexposed in the vigilance task. ERP results: there were no significant differences in the novelty P300 between exposed and unexposed children. The amplitude of the P100 was significantly lower (p < 0.05) in the exposed children in the frontal brain locations. The P100 amplitude and SOPT were highly correlated (p < 0.01) in the unexposed children but not in the exposed children.

Interpretation: The P100 amplitude is hypothesized to be a neural correlate of working memory and fluid intelligence. The strong correlation between P100 amplitude and SOPT suggests that exposure to falciparum malaria results in impaired working memory.

440B
Levels of IgE and anti-glycosylphosphatidylinositol (GPI) IgG antibodies in children with uncomplicated and severe malaria [MIM-AK-9147]
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Introduction: IgE has been shown to have a pathogenic role during malaria infection with elevated levels most pronounced in severe disease. Anti-glycosylphosphatidylinositol (GPI) IgG antibodies may prevent symptomatic malaria by blocking the induction of pathologic host inflammatory responses to parasite-derived toxins, glycosylphosphatidylinositol (GPI). We investigated the role of Plasmodium falciparum specific IgE and anti-GPI IgG antibodies in the pathogenesis of severe malaria.

Methods: A prospective case-controlled hospital-based study was carried out in Fako Division, Cameroon involving patients with cerebral malaria (CM), severe malaria anaemia (SMA), uncomplicated malaria (UM) cases and healthy controls (HC). We recruited 150 children (1–14 years): CM (n = 17); SMA (n = 44); UM (n = 45) and HC (n = 44). Malaria parasitaemia was determined by microscopic examination of thick blood films. Total IgE, P. falciparum IgE and anti-GPI IgG antibody levels were measured by indirect ELISA in plasma samples.

Results: We observed a significant positive correlation between P. falciparum IgE (p = 0.023) and anti-GPI IgG (p < 0.001) antibody levels with age. Total IgE antibody levels were similar among the patient categories and controls while P. falciparum – specific IgE was significantly different among the groups (p = 0.012). Mean anti-GPI IgG levels were higher in the HC and UM groups than in the CM and SMA groups although this difference was not significant. However, when patients were grouped into groups of severe (CM and SMA) and mild disease (UM), both P. falciparum IgE and anti-GPI IgG levels were significantly lower (p = 0.015) in the severe disease group when compared with the others. P. falciparum IgE and anti-GPI IgG levels were similar in the mild disease and control groups. There was a positive though insignificant correlation in total IgE and P. falciparum IgE with anti-GPI IgG levels as well as between all three antibody levels with parasite density. A significant positive correlation was observed in P. falciparum IgE (p = 0.001) and anti-GPI IgG (p = 0.031) with haemoglobin levels.

Interpretation: Malaria-specific IgE and anti-GPI IgG antibodies may contribute to protection against severe disease in malaria endemic areas.

Spatial profile of activated caspase-3 in experimental cerebral malaria [MIM-PL-153254]

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Introduction: Cerebral malaria (CM), the most severe complication of Plasmodium falciparum malaria, is still associated with a high death rate. Survivors may suffer from neurological sequelae. The underlying pathomechanisms are yet not fully understood. However, there is increasing evidence that apoptotic mechanisms are involved in the pathogenesis of long term deficits.

Methods: C57BL/6 mice were infected with Plasmodium berghei blood stages. Clinical severity of the disease was assessed by SHIRPA primary screen, a battery of 40 standardized tests for evaluating neuromuscular, spinocerebellar, sensory, neuropsychiatric and autonomic functions in mice. The extent of apoptotic cell death was evaluated by detection of cleaved caspase-3 with a monoclonal antibody. Brain homogenates and cryostat sections of mice with CM, infected animals without cerebral involvement and non infected control animals were analyzed. Cryostat sections were counterstained with hematoxylin to assess nuclear morphopathology. Nonparametric statistical methods were used to check for significance.

Results: Western blot analysis of brain extracts for cleaved caspase-3 showed an induction of immunoreactivity in cerebrum and cerebellum of animals with CM. No immunoreactivity could be detected in infected animals without CM or non infected control animals. Densitometric analysis revealed significantly higher reactivity in cerebellum than in cerebrum. Furthermore, animals having a worse clinical score showed higher immunoreactivity. Immunohistochemistry revealed cleaved caspase-3 positive cells, showing nuclear morphology consistent with apoptotic cell death, throughout different brain regions. Immunopositive parenchymal cells were frequently clustered around vessels showing immunopositive sequestered leukocytes and endothelial cells. Groups of immunopositive neurons were predominantly found in
brainstem and cerebellum. The data in the current study provide direct evidence for caspase-3 activation in experimental CM. Interestingly, cerebellum and brainstem showed increased immunoreactivity for activated caspase-3 compared to cerebrum.

**Interpretation:** These results indicate a putative role of apoptotic cell death in the pathogenesis of delayed cerebellar ataxia, the most common manifestation of post malarial neurological syndrome.

**442A**

**Pentoxifylline as adjunct therapy in children with cerebral malaria [MIM-BL-72864]**


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**Introduction:** Pentoxifylline (PTX) affects many processes involved in the pathogenesis of severe malaria, including production of the cytokines, cytoadherence and red blood cell deformability. Small studies of PTX as an adjunct therapy in children with cerebral malaria showed a strong beneficial effect of the drug, whereas trials in adults have shown conflicting results. We performed a pilot trial to assess pharmacokinetics, safety and efficacy of PTX in African children with cerebral malaria, with the aim to determine the dosage for efficacy evaluation in large multi-centre trials.

**Methods:** Ten children admitted at the high dependency unit of the Kilifi District Hospital in Kenya with cerebral malaria (BLANTyre coma score of 2 or less) received quinine plus a continuous infusion of 10 mg/kg/24 h PTX for 72 h. Five children were recruited as controls and received normal saline instead of PTX. Plasma samples were taken for drug levels of PTX (measured by high performance liquid chromatography) and TNF-Blantyre Coma Score, parasitaemia, hematology and vital signs were assessed 4-hourly. Neurological status was assessed at follow-up visits at 1 and 3 months after admission.

**Results:** One child (20%; 95% CI: 0.5–72%) in the control group died, compared to four children (40%; 95% CI: 12–74%) in the PTX group (p=0.60). PTX infusion was stopped early in two subjects because of severe adverse events possibly related to the study drug (hypotension and gastrointestinal bleeding with vomiting). Laboratory parameters and clinical data (coma resolution time, neurological sequelae, fever clearance time, duration of hospitalisation) were comparable between groups. Between 8 and 72 h after admission, TNF levels were lower in children receiving PTX compared to placebo (43 versus 116 pg/mL at 8 h). The maximum concentration of PTX was 176 + 83 ng/ml achieved at 12 + 8 h after the start of the infusion.

**Interpretation:** PTX led to a reduction in TNF levels. Mortality was unexpectedly higher in the PTX group. However, the study was not designed as an efficacy study and the low sample size does not permit definitive conclusions.

**443B**

**Vbeta profile of circulating T lymphocytes in African children suffering from cerebral or uncomplicated malaria [MIM-SL-127488]**


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**Introduction:** Pathogenesis of cerebral malaria (CM), one of the major malaria complications, is still poorly understood. Rodent models have shown a critical role for T cells in the inflammatory processes associated with CM and highlighted expansion of specific Vbeta, suggesting implication of superantigens. The massive T cell activation in human malaria remains poorly documented.

**Methods:** By using a whole blood flow cytometry kit, we studied the peripheral Vbeta T cell repertoire of Ghanaian children with CM, uncomplicated malaria (UM) and asymptomatic control children (AC) to look
for abnormal specific Vbeta stimulation, which would be more pronounced in CM. Among them, 9 UM and 16 CM were followed at day 3 and 7 after the treatment.

Results: The Vbeta repertoire of AC children was found not different from that of Caucasian populations. At admission, the repertoire of CM and UM patients were similar, and there was no major distortion compared to the AC group, apart from a gradual significant increase of the Vbeta 21.3 subset frequency, from AC to UM and UM to CM, attributed to the CD4 subset. Analysis of the repertoire on day 3 and 7 after treatment showed limited fluctuations except for a progressive decrease of the V beta 21.3 subset and increase of the Vbeta 20 subset, pointing to different kinetics of activation of distinct T cell subsets during malaria.

Interpretation: This study suggest that cerebral malaria cannot be viewed as a T cell mediated shock syndrome driven by a dominant superantigenic activity. However, preferential expansion of Vbeta 20 and Vbeta 21.3 T cell subset might play an important role.

Identification of Plasmodium falciparum variant surface antigens expressed by parasites causing malaria in non-immune individuals [MIM-PM-16758]

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Introduction: The variant surface antigen (VSA), Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1) mediates antigenic variation and cytoadherence and are encoded by ~60 var genes per genome. Parasites causing malaria in young children who have not yet acquired immunity express semi-conserved VSA associated with severe disease syndromes (VSASM). We investigated PF11_0008 (var5), which was highly transcribed in an isolate from a non-immune human host experimentally infected with NF54 parasites.

Methods: VAR5 domains DBL1a, DBL2g, DBL3d and CIDR2h were expressed in the Baculovirus expression system and the recombinant proteins used to raise specific antibodies in rabbits. To investigate for VAR5 surface expression, the specific antibodies were used to stain live P. falciparum-infected erythrocytes and analyzed by flow cytometry. The parasites tested included isolates from malaria naive human hosts experimentally infected with NF54, NF54, an NF54 line selected to express VAR2CSA, 3D7, and a 3D7 line selected to express VSASM. Recombinant VAR5 domains were used in ELISA to measure antibody levels in individuals from a malaria endemic area.

Results: VAR5 was expressed on the surface of NF54 isolated from naive human hosts and reacted with VAR5 antibodies raised against DBL1a. This NF54 line did not react with antibodies against VAR2CSA (implicated in pregnancy associated malaria) or VAR4 (expressed by 3D7-VSASM and implicated in severe disease). The VAR5 antibodies did not react with the surface of any of the other parasites tested. However, 3D7-VSASM and NF54-VAR2CSA parasites reacted strongly with VAR4 and VAR2CSA antibodies, respectively. ELISA results showed that 80–100% of 2–4 years old children living in an area of intense malaria transmission in Tanzania had acquired antibodies against recombinant DBL1a, DBL2g and CIDR2h VAR5 domains.

Interpretation: The results indicate that VAR5-like PIEMP1s are expressed by parasites infecting individuals who have not developed immunity, implying that VAR5 expressing parasites obtain high growth rates and may be involved in the pathogenesis of severe malaria.

Malaria Parasitaemia and helminthic infections in asymptomatic school-pupils from Fako Division, South Western Cameroon [MIM-EM-244080]


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**Introduction:** Malaria and helminth infections constitute serious public health and socio-economic problems especially in children from sub-Saharan Africa. Although school-pupils in endemic areas are known to be asymptotically parasitaemic all year round, little is known about the frequency and density of such infections in their locality. We investigated the prevalence of Plasmodium spp. and helminth co infections in 4–12 year old pupils attending mission and public schools in Buea area.

**Methods:** A total of 219 subjects were recruited and information relating to height, weight and body temperature was recorded. Blood samples were analysed for PCV, red and white cell counts, glucose levels and parasites detected by microscopy. Early-morning stool samples were examined for the presence of ova by Kato Katz technique.

**Results:** Overall malaria parasite rate was 66.7% (146/219); 69.3% (61/88) for public schools compared to 63.4% (85/131) for mission schools ($p > 0.05$). A higher ($p = 0.0001$) prevalence (85.9%; 73/85) was recorded in Muea located at 300 m compared to schools in Buea town (700–1096 m) (56.5%; 73/134). 22.8% of the pupils were febrile and 12.8% (28/219) were also parasitaemic but parasites rates in febrile (56%; 28/50) and afebrile (69.8%; 118/169) cases were similar ($p = 0.69$). The geometric mean parasite density was 569. The mean parasite density was significantly different ($p < 0.05$) amongst the three age groups (0–5, 5.1–9, >9 years) but their parasite rates were similar ($p > 0.05$). Ascaris was the most prevalent helminth spp (62%; 32/51) and 28.2% (51/181) of subjects harboured Ascaris and/or Trichuris spp. 23.7% (36/152) of the pupils harboured both malaria and helminthic co-infections.

**Interpretation:** Children from the study area particularly those resident at a lower altitude frequently carry malaria parasites asymptomatically with some co-infected with helminths.

446B

**Plasmodium falciparum** microsatellite polymorphisms are associated with clinical outcomes in malaria infections in Ugandan infants [MIM-GM-23616]

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**Introduction:** Plasmodium falciparum infections result in different clinical manifestations in African children. The *P falciparum* genome is rich in microsatellites especially (TA)n and (TAA)n. Microsatellites are highly polymorphic and might be ideal markers for *P falciparum* genetic diversity and virulence. We employed two microsatellite markers (TA17 and C3M85) to test the hypothesis that the carriage of specific parasite genotypes is associated with the different clinical manifestations of malaria.

**Methods:** Children with severe and complicated malaria (cases) and age-matched children with mild uncomplicated malaria (controls) were enrolled during a case-control study of severe malaria in Apac District in Northern Uganda. The study population were children 6–60 months old attending Apac Hospital. DNA was extracted from blood samples collected from 92 cases and 98 controls. Polymerase chain reaction (PCR)-based genotyping of TA17 and C3M85 was carried out and fixed bin analysis was used to define the allele classes for the microsatellites.

**Results:** Alleles ranging from 174–390 bp and 222–537 for TA17 and C3M85, respectively, were identified by size differences using an ID Image analysis software (Kodak Digital TM Science, version 3.0). We observed that 53 and 44% of the isolates for TA17 and C3M85, respectively, were identified by size differences using an ID Image analysis software (Kodak Digital TM Science, version 3.0). We observed that 53 and 44% of the isolates for TA17 and C3M85, respectively, were not associated with any of the clinical outcomes. Five allele classes of 50 bp each, save for the last class, were designated for C3M85 and TA17. In general there was a decrease in the risk of manifesting severe malaria with the carriage of parasites with increasing TA17 allele size as indicated by the Chi-squared test for trend. In contrast, there was an increase in the risk of manifesting severe malaria with the carriage of parasites with increasing C3M85 allele size. Specifically, carriage of alleles of TA17 between 300 and 390 bp (TA17 allele class D) and alleles of C3M85 between 251 and 300 bp (C3M85 allele class B) was associated with mild malaria whereas carriage of parasites with C3M85 microsatellite class E (351–400 bp) was associated with severe malaria. Multiple clone infections as indicated by the number of bands were associated with a reduction in the risk of developing severe malaria.

**Interpretation:** *P falciparum* associated with severe or uncomplicated malaria might be genetically distinct. Polymorphic TA17 and C3M85 microsatellite repeats
might be associated with virulence factors; the underlying mechanisms remain to be elucidated.

447C

Genetic diversity of *P. falciparum* in Bolifamba on the slope of Mount Cameroon: Influence of MSP1 allelic variants on symptomatic malaria and anaemia [MIM-AN-258552]

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Introduction: *Plasmodium falciparum* shows high genetic diversity and in endemic areas infected individuals harbour several parasite genotypes. Several studies have shown discordant associations of parasite genotypes with clinical disease and anaemia in children. As part of an ongoing study on genetic polymorphisms of *P. falciparum* and immune responses in individuals in a rural community of Mt Cameroon, this study aims to identify *P. falciparum* MSP1 allelic variants and its association with clinical disease.

Methods: Blood was analysed for 174 children aged 1–15 years. Children were grouped according to clinical status and anaemia. Symptomatic status was defined as parasitaemia and axillary temperature >37.5°C and asymptomatic status as parasitaemia and temperature <37.5°C. Anaemia status was defined as PCV of <31%. Fifty-five percent of children were anaemic and 50% had temperature >37.5°C. Parasite DNA was analysed with allelic specific primers which identify the k1, MAD20 and R033 variants of MSP1 gene.

Results: Of the *P. falciparum* infections in Bolifamba 22% was of MAD20, 8.5% R033, 15.3% K1, 5.1% MAD20 + R033, 6.8% MAD20 + K1, 28.8% RO33 + K1 and 6% all three alleles. There was a significant difference in the prevalence of MSP1 allelic variants between age groups and the presence or absence of anaemia (p < 0.001). RO33 + K1 mixed infections were highly associated with the age groups 1–3 and with anaemia (p < 0.001). There was also a significant difference in the prevalence of MSP1 allelic variants with temperature >37.5°C and <37.5°C (p=0.01). Fever was highly associated with the presence of R03 + K1 allelic variants. There was no significant difference in the presence of allelic variants with age and mean parasite density.

Interpretation: Children in Bolifamba are highly exposed to all three allelic variants early in life and this may subsequently determine the level of acquired immunity.

448A

Characteristics of severe anaemia and its association with malaria in under-five children in Ebonyi State, Nigeria [MIM-JO-57552]

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Introduction: Malaria is the most important single cause of anaemia in Nigeria and a frequent cause of hospital admission. Blood transfusions are frequently given to treat severe paediatric anaemia. Severe anaemia in children depends on age and parasitaemia and older children have a lower risk of severe anaemia. We sought to elucidate factors associated with or influencing severe anaemia. This study will help improve case management and identify priority research areas.

Methods: Malaria is the most important single cause of anaemia in Nigeria and a frequent cause of hospital admission. Blood transfusions are frequently given to treat severe paediatric anaemia. Severe anaemia in children depends on age and parasitaemia and older children have a lower risk of severe anaemia. We sought to elucidate factors associated with or influencing severe anaemia. This study will help improve case management and identify priority research areas.

Results: One hundred and ninety-three children (73.9%) met our inclusion criteria, M = 108, F = 85 (M:F 1.3:1). 55 (28.5%) had Hb < 5 g/dL, 87 (45.0%) 5–9 g/dL and 9 (4.7%) >11 g/dL. The mean Hb < 5 g/dL was 3.78 ± 0.66. More males than females had severe anaemia (30 versus 25, ratio 1.2:1). Mean age of those with Hb < 5 g/dL (20 ± 10.77 months) was lower than those ≥ 5 g/dL (23.9 ± 14.8 months) though not statistically significant (p=0.1). Survey identified statistically significant associations between severe anaemia and weight and fever. Mean weight of those with Hb < 5 g/dL (9.9 ± 2.3 kg) was statistically lower than those with Hb ≥ 5 (11.5 ± 3.7 kg) (p=0.01). Relative to children with Hb ≥ 5 g/dL, those with Hb < 5 g/dL were
more febrile (OR, 5.05; 95% CI, 2.52–10.13). Those with Hb <5 g/dL were more hyperparasitemic (33/55; 60.0%), than those ≥ 5 g/dL (79/138; 57.2%), but not statistically significant (p = 0.5). Severely anaemic children by age showed a fairly uniformity in sampling over age groups 5–24 months. Eighty-five (44.0%) received blood transfusion, but 55 (28.5%) had Hb < 5 g/dL, 64/85 (75.3%) were in infants <25 months with equal sex spread. Few >36 months were transfused. Transfusion peaked with rainy season, as with severe anaemia more boys were transfused (45/85; 52.9%).

Interpretation: Severe malaria anaemia and blood transfusion appear to be a seasonal event and occurred more in younger, smaller, more febrile and male children and is a valuable index that reflects the intensity of malaria in the state.

449B
Distinct haematological characteristics of malarial anaemia in infants and young children presenting at a rural district hospital in western Kenya [MIM-JO-61809]

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Introduction: Severe malaria anaemia (SMA) due to Plasmodium falciparum is a major public health problem in young children in endemic areas. The pathogenesis of malaria-induced anaemia is multi-factorial and not fully understood. To address the complex aetiology of malarial anaemia (MA), a hospital-based prospective longitudinal study was designed to examine the haematological characteristics associated with malaria in children residing in a holoendemic area of malaria transmission in western Kenya.

Methods: As part of our ongoing activities in western Kenya, we have currently enrolled 483 children aged 3 months to 3 years presenting at the Siaya District Hospital with varying degrees of MA. Children were divided into five groups based on the presence of P. falciparum parasitaemia and haemoglobin (Hb) levels: SMA (n = 149; Hb 6.0 g/dL + parasitemia); moderate MA (ModMA; n = 134; Hb = 6.1–7.9 g/dL + parasitemia); mild MA (MlMA; n = 125; Hb = 8.0-10.9 g/dL + parasitemia); uncomplicated malaria (UM) (n = 28; Hb 11.0 g/dL + parasitemia); and healthy controls (HC) (n = 47 Hb 11.0 g/dL without parasitemia). Complete blood counts, reticulocyte indices, and parasite density were determined on all participants prior to anti-malarial treatment and/or supportive therapy.

Results: Children with SMA had thrombocytopenia, lymphocytosis, monocytosis, and an elevated absolute reticulocyte number (ARN) relative to the other malaria-infected groups. Except for the UM group, malaria infection resulted in lower mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values relative to the HC group. Hb concentrations were inversely correlated with white blood cells (WBC; r = −0.210, p = 0.001), lymphocytes (r = −0.219, p = 0.001), and monocytes (r = −0.333, p = 0.001), but positively associated with platelets (r = 0.333, p = 0.001). There was no association between Hb levels and parasitaemia. The ARN was positively correlated with lymphocyte (r = 0.132, p = 0.004) and monocyte (r = 0.214, p = 0.001) numbers, but inversely associated with granulocyte levels (r = −0.167, p = 0.001) and parasitemia (r = −0.132, p = 0.006). There was no association between age and Hb levels or age and the ARN. Presentation with fever was significantly associated with parasitemia (p = 0.004).

Interpretation: SMA is defined by a distinct haematological profile of thrombocytopenia, lymphocytosis, monocytosis, and a reticulocytosis that does not appear proportional to the degree of anaemia, suggesting suppression of erythropoiesis in children with SMA.
Suppression of circulating RANTES is associated with enhanced pathogenesis of malarial anemia in Kenyan children [MIM-TW-166110]


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Introduction: Effective innate immunity to Plasmodium falciparum malaria requires a rapid but controlled proinflammatory cytokine response. Chemokines, such as macrophage inflammatory protein (MIP)-1, MIP-1, and regulated on activation, normal T-cell expressed and secreted (RANTES), are emerging as important inflammatory mediators of infectious diseases and haematopoiesis. As such, the relationship between malarial anaemia (MA) and circulating levels of MIP-1, MIP-1, and RANTES was investigated.

Methods: As part of our activities in western Kenya investigating the pathogenesis of severe malarial anaemia (SMA) in children less than 3 years of age, circulating levels of MIP-1, MIP-1, and RANTES were measured. SMA was categorized according to malaria disease severity: healthy asymptomatic aparasitaemic (HC), uncomplicated malaria (UM), mild malarial anaemia (MlMA), moderate malarial anaemia (ModMA), and SMA.

Results: Circulating RANTES concentrations at admission were significantly lower in children with SMA (P < 0.01) versus the other categories, while MIP-1 was significantly lower in the SMA group compared to ModMA (P = 0.05) and MlMA (P < 0.05). In contrast, MIP-1 levels at admission were non-significantly elevated in the MIMA and SMA groups (P = 0.07), and significantly higher in the ModMA group (P < 0.05), relative to the HC and UM groups. Plasma RANTES levels were positively correlated with haemoglobin (Hb) concentrations (r = 0.31, P < 0.01), and the numbers of RBC (r = 0.41, P < 0.001), platelets (r = 0.45, P < 0.001), and reticulocytes (r = 0.23, P < 0.05). Both MIP-1 and MIP-1 were inversely correlated with the number of monocytes (r = −0.25, P < 0.05 and r = −0.31, P < 0.01, respectively), and MIP-1 was positively associated with granulocyte numbers (r = 0.29, P < 0.05). Circulating MIP-1 and MIP-1 levels were not associated with Hb concentrations.

Interpretation: Children with falciparum malaria have dysregulation of chemokine production in which suppression of RANTES is associated with enhanced pathogenesis of malarial anaemia, suggesting a role for RANTES in suppression of erythropoiesis.

Photoparoxysmal response among children in a malaria endemic area [MIM-GO-333270]

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Introduction: Photoparoxysmal response (PPR) is thought to be a marker of epilepsy, yet it is reported to be less frequent in Africa where epilepsy is reported to be more common. Recently we have reported that exposure to malaria is associated with the development of epilepsy (Carter et al., 2004. Epilepsia 45 (8), 978–981). We assessed whether the frequency of photosensitivity is increased in children exposed to severe malaria was increased.

Methods: We studied three groups of children aged 6–9 years: 152 exposed to cerebral malaria (CM), 156 exposed to malaria + complicated seizures (M/S) and 176 children chosen from the community unexposed to either condition. A detailed history of epilepsy was obtained from the parents. Each child had a 16-channel EEG using 21 scalp electrodes with standard 10–20-
system electrode placement, recording for 20 min and activation procedures, with hyperventilation for 3 min and intermittent photostimulation.

**Results:** There were three children with photic stimulation abnormalities (2 (1.3%) M/S; 1 (0.54%) unexposed) and five children with abnormalities on hyperventilation (2 (1.3%) CM; 1 (0.64%) M/S; 2 (1.1%) unexposed). All the children seen with photostimulation abnormalities were females. The photic abnormalities were seen at flash frequencies of 20–24 Hz. These EEG abnormalities were associated with a history of seizures (Fischer’s exact test $p = 0.014$).

**Interpretation:** These results suggest that children previously exposed to severe malaria do not have increased PPR response relative to children unexposed to these complications of malaria.

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**452B**

**Heterogeneity of severe paediatric malaria in six sites across Africa: A prospective, hospital-based cohort study [MIM-MP-305592]**


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**Introduction:** The severe malaria in African children (SMAC) clinical network has been established to conduct mortality-based trials. To inform trial design we have quantified and described children admitted with severe falciparum malaria across the variety of African epidemiological settings represented by the SMAC sites. Here we compare case presentations and fatality rates.

**Methods:** The six sites were Banjul, The Gambia; Blantyre, Malawi; Kilifi, Kenya; Kumasi, Ghana, and Lambarene and Libreville, Gabon. Standardized clinical and laboratory data were collected on admission to hospital from all parasitaemic children (2–180 months), for whom consent was given, and outcomes were recorded. Syndromes of severe malaria were defined in these parasitaemic children as follows: respiratory distress (presence of deep breathing), severe malarial anaemia (PCV <15%) and cerebral malaria (Blantyre coma score <3). To encompass complete malaria transmission periods in each site, data from 1 January to 31 December 2003 were analysed. In this period, 7205 patients were enrolled and complete data were available for 7129 (98.9%).

**Results:** The annual variation in enrolled patients followed site-specific transmission patterns, e.g. two clear peaks in Kilifi and no seasonal variation in Lambarene. The incidence of each severe syndrome ($p < 0.001$ for each) and the associated mortality varied across sites ($p < 0.005$ for each). The proportion of patients with at least one severe syndrome varied from 21% in Lambarene to over 45% in Banjul and Kumasi ($p < 0.001$). Mortality associated strongly with the presence of severe syndromes; the presence of one or more severe syndromes at admission increased the risk of fatality by 3% in Banjul to 20% in Blantyre. In all sites, over 50% of fatalities occurred within 2 days of admission. Severe malarial anaemia was the most common severe syndrome in all sites and as a single syndrome, carried a fatality risk of 0–7%. Cerebral malaria and respiratory distress were less common but, as single syndromes, carried fatality risks of 5–25%. The presence of more than one severe syndrome greatly increased the risk of mortality; in general, for patients presenting with two severe syndromes the risk doubled and increased further if all three severe syndromes were present [but there were inter-site variations which will be presented].

**Interpretation:** The severity of malaria at admission varied between sites and appeared crucial in determining outcome. Inter-site variations in patterns of severe disease may be due to differences in malarial epidemiology, admission criteria and health care systems.

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**453C**

**A model of the relationships between malaria transmission, severe morbidity and mortality [MIM-AR-110376]**

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Introduction: The transmission intensity of *Plasmodium falciparum* has multifarious and sometimes counter-intuitive effects on age specific rates of severe morbidity and mortality in endemic areas. This has led to conflicting speculations about the likely impact of malaria control interventions. We propose a quantitative framework to reconcile various observations relating morbidity and mortality rates to malaria transmission.

Methods: We consider two sub-categories of severe malaria episodes. These comprise (i) episodes with extremely high parasite densities in hosts with little previous exposure; and (ii) uncomplicated malaria episodes accompanied by age-dependent co-morbidity or other risk factors enhancing susceptibility. In addition to direct malaria mortality, this model considers that clinical episodes accompanied by co-morbidity contribute to subsequent indirect mortality risk, after the parasites have been cleared. We fit this model to summaries of field data from endemic areas of Africa.

Results: This model can simultaneously account for most of the observed age- and exposure-specific patterns of paediatric severe malaria and malaria-associated mortality in children. The predicted pattern of incidence of severe episodes in children under 9 years by transmission intensity followed that of published data. Severe episodes arising through co-morbidity contributed a greater proportion of the total in high transmission settings. We were also able to reproduce patterns of direct malaria mortality in children under 5 years by transmission intensity, and to predict all-cause infant mortality rates in settings with different transmission intensities.

Interpretation: This model can make predictions of the long-term impact of potential malaria interventions. Predictions for children will be more reliable than those for older people because there are few studies of severe malaria morbidity and mortality in adults.

454A

IgG reactivities with high-molecular-weight human brain proteins correlate with circulating TNFa concentration in cerebral malaria [MIM-GV-48492]

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Introduction: To investigate whether antibody-mediated self-reactivities to brain are involved in CM pathogenesis, we analysed reactivities of circulating IgG from uninfected and *P. falciparum* infected Gabonese children with a human brain proteins extract. Furthermore, as Th1 type pro-inflammatory cytokines such as interferon γ (IFNg) and tumor necrosis factor a (TNFa) play an important role in CM, we analysed whether they regulate the self-reactive antibody response to malaria.

Methods: One hundred and forty-eight patients from Gabon were included in the study. They are under 5 years old and divided into four groups including endemic controls (U; n = 25). *P. falciparum* infected individuals were divided into three groups: uncomplicated malaria (UM; n = 66); severe non-cerebral malaria (SNCM; n = 36), and CM (n = 21). The plasmatic concentrations of IFNg, TNFa and IL-10 as well as IgGs were determined using Sandwich type ELISA. The comparison of anti-brain reactivity levels between the groups was done using a specialised combined approach based on quantitative immunoblot (PANAMA-Blot) and multivariate analyses. For quantitative comparisons between groups, either Mann–Whitney (between two groups) or Kruskal–Wallis tests (>2 groups) were used.
Results: No significant difference was observed in total levels of IgG between UI, UM, SNCM and CM groups. The IFNγ concentrations were not significantly different between the UM, SNCM and CM groups. The plasma concentrations of TNFα were significantly higher in SNCM and CM than in UM group (p = 0.001 and 0.03, respectively). IL-10 levels were significantly lower in the UI group than in the UM (p = 0.0005) and severe malaria groups (p = 0.0001), whereas there was no significant difference between the UM, SNCM and CM groups. The repertoire of brain antigens recognized by plasmatic IgGs was more diverse in infected patients than in UI. The anti-brain reactivities were significantly higher in CM group than in SNCM and UM groups (p = 0.007 and 0.006, respectively). In addition, these reactivities were correlated with age (R = +0.4, p = 0.001) and plasma IgG levels (R = +0.21, p = 0.02). Pertinently, 90% of CM patients, compared to 50% of SNCM and 44% of UI and 39% of UM, reacted with a high molecular weight band that contains at least three proteins. The reactivity with this band was correlated with the concentration of TNFα only in the CM group (R = +0.76, p = 0.006), while no correlation was found between anti-brain reactivity and IFNγ or IL-10 levels.

Interpretation: These results highlight the existence of an antibody-mediated self-reactive response to brain associated with pathogenesis in Plasmodium falciparum infected patients.

455B
Persistent Epstein–Barr viral reactivation associated with severity of Plasmodium falciparum malaria in Gabonese children [MIM-CY-166551]
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Introduction: EBV and Plasmodium falciparum have overlapping distributions and are thought to have causal interactions, particularly concerning the etiology of Burkitt’s lymphoma. However, the association between malaria-induced EBV-reactivation and the severity of P. falciparum malaria has not been investigated.

Methods: To address this question, we quantified and monitored by means of DNA-based real-time PCR the profile of EBV genome loads in the peripheral blood of Gabonese children with either mild or severe P. falciparum malaria before and at least 6 months after anti-malarial treatment.

Results: EBV DNA levels were similarly high prior to treatment in both groups of children, declining significantly in the mild but persisting over time in the severe malaria group. EBV DNA loads and the proportion with EBV DNA were consistently higher in the severe malaria group, significantly so when they were healthy and parasite-free (67% versus 39%, p = 0.013). Moreover, EBV DNA was detected in a higher proportion of under-five year olds. Children with the highest EBV DNA loads had shorter delays to their first P. falciparum reinfections and suffered more malaria attacks. Pre-treatment plasma IL-12 levels were lower but post-treatment IL-12p40 and TNFα levels higher in those with EBV DNA.

Interpretation: EBV reactivation during acute P. falciparum malaria persists at higher frequency in children with severe malaria, and is associated both with enhanced susceptibility to P. falciparum malaria and with altered pro-inflammatory cytokine activity.

19: Health systems research

Posters 456–479

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

456C
The role of the Kenyan retail sector in provision of antimalarial services [MIM-AA-190928]
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Introduction: Across much of Africa, the informal retail sector plays an important role in fever management of young children. The characteristics and structure of the retail market, however, are rarely described.

Methods: We did a national survey of antimalarial (AM) drugs in circulation, their costs and registration
status and followed this with an audit of 880 randomly sampled retail outlets in four districts of Kenya in 2002. Outlets were categorized into pharmacies, large shops and small shops and retailers interviewed using a standardized, pre-tested questionnaire to capture information on brands of AM drugs, pharmacological groups and retail prices.

**Results:** Although many brands of sulfapyrimethamine (SP, 65 brands, 52% registered with the PHARMACY and Posons Board (PPB), amodiaquine (AQ, 33 brands, 52% registered), chloroquine (CQ, 67 brands, 66% registered), and artemisinin products (ART, 12 brands, all registered) were in circulation nationally, far fewer product ranges were in circulation in the districts (45, 25, 21 and 9 brands of SP, AQ, CQ and ART, respectively). SP, AQ, and CQ all cost less than one USD per adult and paediatric treatment course, whilst the cost range for the same for ART products was 4.6–7.4 USD. There was no consistent pattern in the mark-up prices of these drugs between the factory gate prices and prices at the periphery. The smallest mark-up was on SP suspensions at 14% and the highest on CQ suspensions at four and half times the price of the cheapest national source. AQ tablets in retail pharmacies were priced almost three times the cheapest national price (344%), while SP tablets and AQ suspensions were priced twice the national price (100 and 101%, respectively).

**Interpretation:** The data presented demonstrate a largely unregulated, fragmented sector that demands improved regulation for better engagement with RBM activities at the national level.

457A

**The impact of malaria and intestinal helminth co-infections on anaemia in children living in a malaria-endemic setting of mount cameroon**

[MIM-CC:175959]

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**Introduction:** In tropical countries, malaria anaemia has been described as one of the most widespread health problems with important public health, social and economic consequences. Pre-existing intestinal helminth infections, which have been described as the most prevalent parasitic infections may aggravate the severity of malaria anaemia. The study was aimed at investigating the impact of malaria and helminth co-infections on anaemia in children living in Boli/Famba, a rural mount Cameroon setting.

**Methods:** Four hundred and twenty-five children of both sexes aged 9 months to 14 years were enrolled in a cross-sectional study. Venous blood samples were collected for the assessment of malaria parasitaemia (MP), haemoglobin concentration, packed cell volume (PCV) and red blood cell count (RBCC). The prevalence and intensity of Ascaris lumbricoides, Trichuris trichiura, and hookworm infections were assessed by the Kato–Katz technique.

**Results:** The prevalence of anaemia (PCV < 31%) was 30.8% (131/425); 87, 10.7 and 2.3% being mild (PCV from 21 to 30%), moderate (PCV from 15 to 20%) and severe (PCV < 15), respectively. Of the anaemic cases, 45.8% were associated exclusively with malaria parasitaemia, 10.7% exclusively associated with helminth infections and 24.4% associated with malaria and helminth co-infections. About 19% of the anaemic cases did not harbour any of the infections. Males and children <5 years old had the highest prevalence of anaemia. However, the difference was not significant. There was no significant difference in the mean levels of PCV and red cell indices in Plasmodium infected subjects with and without intestinal helminth infection. The same pattern was observed among the sexes and age groups. Overall, the geometric mean parasite density was higher in anaemic subjects ($F = 6.59, P = 0.011$). A negative correlation was observed between low PCV (anaemia) and the intensity of the various infections (parasite density for malaria and egg count for helminth infection) ($P < 0.05$). A negative association was also observed between anaemia and age ($r = 0.0341, P < 0.001$).

**Interpretation:** Malaria, hookworm infection and fever were the strongest predictors of anaemia among the subjects. Integrating helminth and malaria intervention programmes could serve as an effective strategy in reducing anaemia in the study population.
458B
Déterminants socio-économiques et perceptions du risque de paludisme au sein de ménages résidant dans une agroforesterie du sud Cameroun [MIM-BD-34040]

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Introduction: L’objectif général de la recherche est d’étudier comment est géré le paludisme par les populations qui vivent en milieu forestier et en zone de transmission continue afin de savoir si le paludisme est perçu comme une fatalité ou comme un problème d’environnement et de santé auquel on peut remédier. Il s’agit d’une recherche financée par le Programme PAL+ du ministère français de la recherche.

Methods: Nous présentons dans cette communication les premiers résultats d’une enquête réalisée par questionnaire en février 2003 dans une plantation agro-forestière du Sud Cameroun (Hevecam) auprès de la totalité de la population de 5 des 16 villages de la plantation. Cette enquête était destinée à décrire respectivement les caractéristiques socio-économiques des ménages (966 ménages enquêtés) et les perceptions, connaissances et comportements en matière de paludisme des chefs de ménages et de leur conjoint éventuel (1478 personnes interrogées).

Results: Les premiers résultats montrent que s’agissant des perceptions du risque de paludisme, 62% des personnes interrogées ont une bonne perception de cette maladie (elles en apprécient sa gravité et son caractère mortel, pensent que le paludisme n’est pas une fatalité et qu’il est possible d’agir efficacement contre cette maladie). La bonne perception est liée à une bonne connaissance, elle augmente avec l’âge des personnes interrogées, et avec leur niveau d’instruction (55% pour le niveau primaire contre 77% pour le niveau secondaire) et de revenus. En ce qui concerne les comportements de soins et de prévention des travailleurs, 43% des personnes interrogées disent avoir dormi sous moustiquaire la nuit qui précède l’enquête. Si les moyens de protection utilisés sont majoritairement la moustiquaire, 23% des personnes interrogees reconnaissent avoir une action destinée à assainir l’environnement et citent ensuite une diversité de mesures (propreté corporelle 10%, utilisation d’insecticides 5% etc …). La lutte anti vectorielle est plus importante chez les ménages avec un bon niveau socio économique. On notera aussi que la prévention est plus élevée pour les enfants et chez les femmes chefs de ménage.

Interpretation: Ces résultats confirment que les conditions socio-économiques et culturelles déterminent la perception du risque et les pratiques des populations. L’impact de l’âge sur les perceptions du paludisme, ou du sexe sur les comportements préventifs sont à noter.

459C
Vaccines and malaria: Micro-analytic approach on published outputs in the past 40 years [MIM-SD-3936]

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Introduction: Evaluations of publication outputs in international databases (like MEDLINE) are standard approaches to assess direct output from scientific activity. This study was done to review publications on malaria vaccines, assess publication patterns, characterize the changes on the type of vaccine, and study other bibliometric issues in malaria vaccines. This method may broadly equate with advances in knowledge on malaria vaccines.

Methods: A bibliometric study was conducted to assess the current status of vaccine development. The analysis primarily employed on MEDLINE database from 1963 to 2004. The analysis of all the articles was organized according to medical subject heading. The qualitative indicators (publication type – clinical trial phases; type of vaccine, country of research,) and quantitative indicators (the number of publications, number of journals carrying highest number of articles) were obtained.

Results: A total of 2682 articles, published on 504 journals for the period 1964–2004 were evaluated. The number of articles increased from eight in the year period 1965–70; 590 in 1981–1990 to 1360 in 1991–2000. The journal with highest output on malaria vaccine articles include Infect. Immunol.; Am. J. Trop.
Med. Hyg.; J. Immunol.; Mol. Biochem. Parasitol.; Sci. Nat. On critical analyses, 1811 (68%) of articles were original articles. Eight hundred and forty-eight articles of them were animal experiments (including comparative study). Twenty-three articles were phase I trials; 54 articles were phase III trials (including RCT) and one was a validation study. The vaccine discussed in articles showed, in 1964 malaria immunization against mice, owe monkeys; in 1970’s antibodies against sporozites, recombinant Pfs 25, SPf66 vaccines and in 2003 blocking vaccine of vivax. Language categorization showed 2480 articles in English followed by 84 in French. The country of publication had risen from 3 in 1970’s to 17 in 1990 with United States contributing 1080 articles followed by England (758) and The Netherlands (228). Malaria endemic countries like South Africa had far less publication in MEDLINE database.

Interpretation: The wide spread dispersion on vaccine trials (decade-wise, continent wise) shows vast development. Ways to capture vaccine evaluation reports, not appearing in peer reviewed journals and in local languages of endemic countries needs to be developed.

460A Socio-economics differentials in health seeking for the treatment of malaria in Nigeria: An agenda for health care reform [MIM-OE-153765]
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Introduction: Malaria is the number one public health problem in Nigeria. The disease incidence and prevalence have been rising, partly due to the breakdown of existing malaria control structures due to resource constraints and the emergence of drug resistant malaria. As a result of resource constraint, socio-economic differences exist in health seeking for treatment of malaria in Nigeria. It is important to address equity in malaria treatment in line with achieving the millennium development goals.

Methods: The study areas are four malaria holoendemic communities in Enugu State, Southeast Nigeria. Focus group discussions (FGDs) were held with two different groups of mothers and fathers in each town. Pre-tested questionnaires were used to collect data from 370 households from each community using simple random sampling. Socio-economic status (SES) index was used to examine whether there were systematic differences in health-seeking variables across the SES groups. Principal components analysis was used to generate the SES index. The SES index was used to divide households into poorest, very poor, poor and the least poor.

Results: Malaria was a major burden to adults and children living in the study areas. However, self-diagnosis was the major procedure used to determine that someone had malaria. There were varying levels of inequity in treatment seeking for adult malaria, with the poorest SES group most commonly consuming services of low level providers and least poor SES consuming services of high level providers. All SES were likely to incur similar level of costs to treat malaria implying that the poorest are spending a greater proportion of their income on the disease. The community members suggested that improving equity of malaria treatment should be through the overall improvement of accessibility and affordability of services in public health facilities, to be supplemented by community-based health workers where there is dire paucity of such facilities. Subsidies, free treatment and communal donations for the poor where other strategies that that stake-holders suggested that could be used to improve the equity in treatment of malaria.

Interpretation: The finding identifies inequity among the SES groups in seeking for malaria treatment in Nigeria and possible societal strategies for overcoming them.

461B Health worker performance and case management of underfives at health care facilities in Mkuranga district, Tanzania [MIM-JE-298672]
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Introduction: Effective treatment of malaria episodes is a fundamental pillar of the malaria control strategy. Studies have shown inadequate clinical assessment by health workers at primary health care facilities. This could lead to misdiagnosis, inappropriate treatment and drug resistance. Improved performance of health workers is thus an essential factor for better malaria control. We assessed the quality of malaria case management in underfives at primary health facilities in a rural district of Tanzania.

Methods: To assess the health worker performance, we performed passive observations of patient-health worker consultations at health facilities using standardised checklists. Prescriptions and diagnoses were recorded and exit interviews with the guardians of the children where performed. From all the children in the consultations observed, blood was sampled for analysis of blood drug levels of chloroquine (CQ) and sulfadoxine/pyrimethamine (SP) and malaria parasite density.

Results: We observed 117 consultations in eight different health care facilities (HCF). The preliminary results show poor clinical examination of the children. Fifty-two percent had clinically significant parasitaemia, but of these 10% did not receive antimalarial treatment. However, 64% of those without parasitaemia got antimalarial treatment. The blood drug analysis showed that 13 and 2% of the children had detectable levels SP and CQ, respectively. There was a decrease in self-treatment of antimalarials compared to previous studies. We have also developed a scoring system to be able to assess the quality of the care at the HCFs. Data analysis is still in progress and the final results will be presented at the MIM congress.

Interpretation: Management of the febrile children at HCFs was poor and a potential risk for appropriate treatment of the children. However, our findings raised questions about what good quality of care and what good health worker performance is.

462C

An economic analysis of the retail market for fever/malaria treatment in rural Tanzania: Implications for implementing combination therapy

[CIM-CG-25145]

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Introduction: In many African settings over 50% of fever/malaria treatments are provided through commercial retailers, but treatment quality is often poor. With the widespread adoption of artemisinin-based combination therapy (ACT), shops selling drugs may be seen as both a threat to effective ACT implementation, and as a means to expand coverage of appropriate treatment.

Methods: The potential role of the retail sector in ACT delivery was assessed through an analysis of the current market for fever/malaria treatment in rural Tanzania, which plans to adopt ACT in 2006. Data were collected between 2000 and 2002 in the demographic surveillance areas of three rural districts: Ulanga, Kilombero and Rufiji. Data collection encompassed a census of all private drug outlets (n = 684), semi-structured interviews with 18 purposively selected outlets, a structured survey of 334 randomly selected outlets, and a retail audit of antimalarial sales in 126 randomly selected outlets. Data on treatment seeking behaviour were collected through a household survey (n = 1250 households).

Results: The main providers of fever/malaria treatment were public and mission facilities, drug shops and general shops/stalls. Retailers were an important source, accounting for 65% of provider visits, and 38% of antimalarial sales. This reflected their relative accessibility: there was one health facility for every 5198 people, but one shop stocking drugs for every 248, and average shop opening hours were double that in facilities. Moreover, shops provided an important antimalarial source when facilities were out of stock. There was close competition between facilities and drug shops, which all stocked antimalarials, attracted custom through expertise, and were seen as sources of “complete treatment”. By contrast general stores were
perceived to provide “first aid” only, and the proportion stocking antimalarials had fallen from 29% in 2000 to 14% in 2001. Geographical segmentation and high concentration in the antimalarial market led to weak price competition, with total markups over international reference prices frequently greater than 500%. Over 90% of drug stores stocked prescription-only antimalarials illegally, a practice widely known to health care staff and communities, and to some degree tacitly accepted by regulatory authorities.

Interpretation: Retail sector ACT delivery has the potential to increase coverage but may also pose risks in terms of unsupervised treatment, drug pressure, and diversion of funds from other needs. Key features of a potential retail sector strategy are discussed.

463A
Community referral links home management of malaria to the facility-based health system in Western Uganda [MIM-KK-291920]
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Introduction: Home Based Management of fever (HBM) was introduced as a national policy in Uganda to increase access to prompt presumptive treatment of malaria. Pre-packed chloroquine/fansidar combination is distributed to all febrile children <5. Persisting fever or danger signs are referred to the health centre. We assessed the overall referral rate, causes of referral, referral completion rate and reasons for non-compliance with referral advice under the HBM strategy.

Methods: The study was conducted in one sub-county of Kasese District, West Uganda where under-5 population was approximately 3600. Six health centres and one NGO hospital serve the area. Malaria is hyper-endemic and under-5 mortality rate estimated at 170/1000. In late dry and early rainy season all 40 Drug Distributors (DDs) and children referred were included and followed for a total of 528 weeks of observation. DDs were visited fortnightly and referred children visited in homes. Primary caretakers were interviewed on symptoms, DD actions, referral timing and completion. Coping behaviour and reasons for non-compliance were explored. Referred children were traced in the outpatient registries in seven health facilities.

Results: Referral completion rate was 93% for ‘urgent referrals’ versus 84% for ‘non-urgent referrals’ ($p = 0.31$). Lack of money (5/10) and improvement (4/10) were main reasons for non-completion. Instead, five were taken to a drug shop. ‘Urgent referrals’ were more likely to access referral care <24 h (69%) compared to ‘non-urgent referrals’ (39%) ($p = 0.016$). Lack of money and waiting for malaria drugs to finish were main reasons for delay. Mothers complained mainly of fever (93%), general illness symptoms (85%) and various ARI symptoms (30–66%). DDs mainly referred persisting fevers (32%), convulsions (15%) and vomiting (11%). 32% of caretakers complained of fast breathing. Of these, 50% were ‘urgently referred’.

Interpretation: Thirty percent complained of HBM integrates with the health system and links with health services. Given the potential hazard for children with pneumonia, local terms for ‘fast breathing’ deserves exploration and village volunteers may need to be empowered to manage pneumonia.

464B
Factors affecting adoption or use of sulphadoxine–pyrimethamine for home management of malaria in children less than five years of age, Ndola, Zambia [MIM-SK-311766]
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Introduction: Due to resistance to chloroquine, SP was being introduced as interim drug of choice before Coartem. The general objective of study was to determine factors affecting use of SP for home management of malaria in children under the age of five years. Specific objectives were: to conduct a KAP on SP for treating malaria in children; to identify motivators or processes to facilitate change to accelerate use of SP;
Methods: Household of 246 respondents to collect Information on Knowledge of malaria, treatment-seeking behaviour, Sources of drugs, knowledge and use of SP in children, availability of SP, perception of severe malaria in children, sources of information on malaria and perceived credibility of each source, barriers and willingness to accept SP. In-depth interviews were conducted with traditional healers, traditional birth attendants, mothers, heads of households, and opinion leaders on perceptions of SP. 

Results: Nineteen percent of respondents were male, 81% female, 75% were married, 75% were literate. Over 20% had completed either primary or senior secondary. Over 80% were either heads or spouses to the head of household. Knowledge of SP was 98%, and 15% for Coartem. Thirty-one percent still had confidence in chloroquine, 30% stocked it for emergencies, 15% stocked SP. Seventy-six percent stocked to SP after chloroquine failure. 18% ever used SP in children though 75% indicated it is appropriate to use SP. Forty-seven percent were strongly willing, 38% somewhat willing and 12% unwilling to give children SP. Forty-six percent had never given SP to children. Twelve percent sourced it from a drug store, 44% from health facility. Twenty-eight percent reported their children reacted to SP. Eighty-two percent had heard of bad side effects of SP. Side effects included headache (30%), rash (6%), worsening condition (75%), joint pains (10%), body swelling (4%). Twenty-five percent needed approval from spouse before giving SP to the child. Eighteen percent knew dosage for the 2–11 months old, 9% for the 1–2 year olds and 34% for the 3–5 year olds. Decision of dose was based on weight (14%), age (73%), perceived severity of malaria (16%), duration of infection (10%), other considerations (11%). Forty percent did not know why SP has been introduced. Five percent saw IEC on SP in health facilities. 

Interpretation: Study showed there are misconceptions regarding SP that need addressing at household level. Caregivers fear SP, continued using chloroquine in spite of information that it is ineffective. Fears of SP stem from rumours and myths.

Published research on malaria: Critical review

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Introduction: Analysis of publication outputs on Malaria represents relatively convenient measure of Research productivity. This will obtain indicators of knowledge advancements in Malaria science. The quantitative indicators include number of publications, journals carrying highest number of articles in Malaria, etc; qualitative indicators include country of publication, language of publication and such journal publication profile.

Methods: A MEDLINE bibliographic search was conducted on Malaria for the period 1963–2004 through SPIRS. The search using FoxPro in a free text format on MALARIA. A total of 31,767 articles published during the above period were evaluated.

Results: Thirty-one thousand seven hundred and sixty-seven articles were published in 2266 journals. The number of articles arose from three in 1963 to 1050 articles in 1990. The highest number of publications was seen in the year 2002 numbering to 1753. The journals contributing for publications of MALARIA were from 89 countries in 36 languages. The highest number of articles were from Trans-R-Soc-Trop Med-Hyg (1698) followed by Am-J-Trop Med-Hyg (1656), Lancet (907), the non-english Journal Mem-Inst-Oswaldo-Cruz (161) comes in 40th position. By language categorization, English was the main language used (26,769, 84% articles) and the next language was French (7%) and Germany (6%), Serbian, etc. Based on publication type, 26,549 were original journal articles, clinical trials (1295), commented authors letter (1254), case reports were (341). The medical search heading (MeSH) showed a high proportion of articles in malaria prevention and control followed by Therapeutics. The subject content of the research relates mainly to Biography, Evaluation studies, and case-reports. A big proportion of articles are published in journals of developed countries than developing countries ($P < 0.05$).

Interpretation: The result emphasizes that world wide research on malaria is growing. Publishing in develop-
ing countries journals will facilitate easier to countries, which bears the burden of malaria.

466A
Perceptions and other factors affecting choice and use of different approaches for the diagnosis of malaria in Mkuranga, Tanzania [MIM-MM-302839]
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Introduction: Malaria control programs in Africa depend heavily on early diagnosis and effective treatment of cases. The evolution of rapid immunochromatographic diagnostic tests (RDTs) has drawn attention to inadequacies in current diagnostic practices. Aside from technical limits of sensitivity and specificity, few studies have examined how these tests might be interpreted by health workers and patients accustomed to microscopic or clinical diagnosis alone.

Methods: We collected qualitative data in four focus group discussions with community members and 46 in-depth interviews with stakeholders in diagnostic services provision in Mkuranga District, Tanzania. Stakeholders were 20 malaria patients and 26 health service providers including clinicians, laboratorians, drug store vendors, and local health officials. Data collectors led participants in discussions about diagnostic testing in general, then demonstrated a RDT for malaria (Paracheck). Participants were then asked to comment on how they perceived this new technology might be used. Data collectors produced detailed field notes. Content analysis was used to identify common themes and contrast findings from different types of participants.

Results: Among health workers, the most frequently mentioned reason for requesting laboratory diagnosis for malaria was to confirm diagnosis, especially where clinical signs indicated a severe form of the disease. Long queues at the laboratory, clinical signs of uncomplicated disease, prior use of antimalarial drugs and lack of reagents were reasons for not obtaining a blood test. Malaria patients and focus group participants valued microscopic diagnosis but also recognized that health workers were expert at identifying malaria based on clinical symptoms alone. Both health workers and consumers identified advantages to the RDT. Consumers in particular, valued the fact that they could see the RDT result for themselves and did not have to rely on the word of a laboratory technician. Both malaria patients and focus group participants said they would have confidence that they did not have malaria if the RDT was negative. Health workers, on the other hand, universally agreed that they would continue to treat patients on the basis of clinical symptoms regardless of a negative result from microscopy or RDT.

Interpretation: RDTs may be an acceptable approach for improving malaria diagnosis in this setting. For the test to be effective, however, health worker knowledge and perceptions should be addressed.

467B
Community knowledge, attitude and practices towards national malaria treatment policy change in Tanzania [MIM-JM-540735]
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Introduction: The alarming chloroquine (CQ) resistant falciparum malaria, led the government of Tanzania in 2001 to revise its treatment policy by adopting sulfadoxine/pyrimethamine (S/P) in treatment of uncomplicated malaria. When there is policy change; questions arise on how the community will cope and how health providers will administer the drug. Therefore, we conducted an exploratory study to establish knowledge, attitude and practices of caretakers and health providers on the new treatment policy.

Methods: The study was conducted in twenty randomly selected communities of Muhuru district, north-eastern Tanzania between June and October 2002. The study population composed of 453 randomly selected community health care providers of which 35 were health personnel 20 from government and 15 from private dispensaries, 18 owners/sellers from drug medical stores and 13 from fixed/kiosks sellers. Structured face-to-face interviews was used to collect information from caretakers and health care providers while focus group discussions were adopted for key informants. Inter-
views were held in the home of the caretakers and drug sellers or health facility while focus group discussions occurred at primary schools.

**Results:** All health personnel 35 (100%), 11 (90.3%) drug sellers and 358 (79.0%) caretakers were aware of malaria treatment policy change. Reasons for policy change included: ineffectiveness of CQ, CQ causing skin irritation, and government officials personal invested interests in companies producing S/P. Other reasons included inappropriate use of CQ for committing suicide and inducing abortion. Above half (52.2%) of caretakers used S/P as a first-line drug for treatment of last clinical malaria attack while 34.2 and 1.9% used AQ and CQ respectively. Of 447, 126 (28.2%) caretakers were not willing to use S/P due to associated adverse effects; the outstanding ones were: increasing body temperature (ina pandisha joto) 53 (42.1%), causing skin lesions (kubabua ngozi) 44 (34.9%) and inducing general body weakness (ina vunjavunja mwili) 29 (23.0%). Other effects associated with unpopular use of S/P included ineffectiveness, unavailability in informal outlets such as kiosks and high cost. Fifty-nine (14.7%) out of 225 caretakers who used S/P reported to have given out a repeated dose at interval of 7 days, with a proportion of 5/18 (27.8%) drug sellers from medical stores advised their clients to take S/P twice at an interval of one week.

**Interpretation:** The community is aware of malaria treatment policy change and S/P is used on malaria attack despite being perceived to cause adverse effects and ineffective.

**468C**

**Quality of malaria slide reading in hospitals of north east Tanzania [MIM-RM-16072]**

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**Introduction:** Microscopy for malaria parasites is the most common investigation undertaken in district hospitals in Africa, but studies of its accuracy in routine practice suggest variable standards. In 2000, Reyburn et al. demonstrated at 10 hospitals, low values of sensitivity (75%) and specificity (59%) of blood slide results that may result in clinicians either making unsafe decisions or ignoring the results. We evaluated reasons for low accuracy of malaria slide results in nine of the 10 hospitals.

**Methods:** Eight district hospitals and one regional hospital at varying malaria transmissions were selected. Laboratory staff are technicians and assistants (3 and 2 years training, respectively), supported by attendants who have no formal training. Total out patients, malaria diagnoses and the number of slides examined and proportion positive in 2002 were recorded. We interviewed staff regularly reading malaria slides and clinicians. The primary question was their opinion on constraints to their work. We inspected the laboratory space and noted any clerical errors in results recording. We used 10 thick blood films with known results to assess the readers’ performance and 20 randomly selected slides from each hospital for quality of preparation.

**Results:** Consistent with our previous work we found the proportion of children <5 years diagnosed with malaria and the slide positivity rate increased with increasing transmission. All slide readers identified (n = 39) participated and the mean duration of experience in slide reading was 16 years (range 3–27 years). Although physical space and equipment were generally satisfactory, examination of hospital slides suggested poor preparation. Performance of readers on test slides was low (correct results mean 67% range 50–83%) and did not correlate with training or experience. Most readers said excessive workload was a constraint to good results and lack of ongoing professional training was also a problem. Twenty-two clinical staff participated in the study and overall they trusted laboratory slide results, though they said these became unreliable when workload was high. Ninety-five percent (21/22) of clinicians stated that even an expert slide reader could give a negative result in a large proportion of ‘true’ malaria cases due to biological reasons, e.g. sequestration. Clinicians rarely visited laboratories or provided clinical information on the slide request form; laboratory staff were divided on the benefits of these visits or provision of information.

**Interpretation:** Our data show low levels of accuracy in slide reading attributed to a high workload and lack of training. However, the contribution of the accurate slide reading to the high level of malaria over-diagnosis is unclear and needs further investigation.
Building a Geo-Medical Information System (GeoMedInfo) for severe malaria anemia research in western Kenya [MIM-SM-97776]


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Introduction: Investigators from the University of Pittsburgh and the Kenya Medical Research Institute (KEMRI) initiated studies of severe malarial anemia (SMA) in Siaya District Hospital (SDH), western Kenya. To strengthen demographic surveillance and gain a better understanding of the etiologic basis of SMA, a Geographic Information System (GIS) is under development that is being integrated with an existing Patient Management Information System (PMIS).

Methods: This project component involves linking the PMIS data with the GIS data. The GIS software for the project is ArcView 9 or ArcGIS. The procedure involves comprehensive mapping of Siaya District. Spatial information is then incorporated in the PMIS with information based on location, distance from the road, and distance to the nearest health facility as well as regional socioeconomic profiles. Important geographical features are geo-referenced using the Global Positioning System (GPS). Once fully established, the district GIS database will be linked with PMIS with spatial analytical functionalities.

Results: The system, GeoMedInfo, is interactive and allows the project staff to maintain and update the database in real-time. The spatial context for the study of disease dynamics and the provision of service infrastructure is ongoing. Comprehensive mapping for the drainage and road network for Siaya District has been completed. LANDSAT satellite imagery of Siaya dating back to 1980 has been acquired and is undergoing interpretation and layering out onto the ArcGIS platform. Distances between individual houses and relevant environmental factors are being obtained using the NEAR command IN Arc/Info. The GPS data collection has been incorporated as part of the regular information acquisition procedures by the project staff.

Interpretation: A GeoMedInfo system can be used to incorporate relevant geospatial data into a complex database that will aid in defining the multifactorial nature of malarial anemia, and allows for improved management, control, and treatment of malarial anemia.

Malaria notification and deaths over 10 years in Agincourt, South Africa: From 1992 to 2001 [MIM-JM-160512]

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Introduction: Malaria is a life threatening disease in many tropical countries. In South Africa, it is endemic in parts of three provinces, i.e. Limpopo, KwaZulu-Natal, and Mpumalanga. Climatic change, migration, drug resistance and resistance to pyrethroids have been implicated in its transmission and mortality. Temperature and rainfall have contributed malaria epidemics associated with malaria mortality. Evidence suggests that climatic variability has direct effect on epidemiology of malaria.

Methods: This descriptive study of deaths from malaria was conducted in the Agincourt Demographic Surveillance Site (DSS) located 500 km north-east of Johannesburg in Limpopo province of South Africa. This study was done for the period 1992–2001. Climatic and mortality data for this study was analyzed, and the Agincourt Health Demographic Surveillance Site malaria mortality was compared with that of the national malaria mortality.

Results: For the 1992–2000 period a total number of 51 (0.08%) people died from malaria in Agincourt. Fifty-one deaths from malaria in Agincourt represent 4% of the 1331 nationally reported malaria deaths for the same period. From the people who died from malaria, 22% of them were migrants from Mozambique.

Interpretation: Deaths due to malaria tended to increase whenever there was a significant increase in temperature during the period 1995 and 2000. This data
suggests that malaria morbidity and mortality could greatly be influenced by climatic changes, and migration.

471C
Cost and effects of underfive malaria case management using community’s women resources in rural Tanzania [MIM-PM-115830]

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Introduction: A costing exercise was conducted to estimate the cost of the intervention aimed at bridging the gap between health facilities and mothers/guardians of underfives, with the aim of recommending scaling up the intervention, and evaluate its sustainability. Women in respective villages were used to manage underfive malaria cases using SP and paracetamol drugs.

Methods: Health workers trained in Good Malaria Practice were used to train women from Women Groups in intervention villages in Good Malaria Self Practice. Women were given a “treatment kit” for managing and referring children. In the villages, women sensitised mothers/guardians of underfives on causes and treatment of malaria. Women were supervised and retrained twice monthly by the same trainers for one year. A negotiated amount was paid monthly as a compensation for their time, which was given during the monthly meeting of all women, supervisors and researchers. Costs related to malaria and other episodes were recorded for each child attended. Researchers and supervisors recorded all the financial and economic costs related to the intervention.

Results: About 2690 underfives’ malaria episodes were attended in the 12 months. Out of these about 140 were referred to health facilities. The average “cured days” was estimated to be 2 days per episode. The preliminary economic incremental analysis shows that, on average, an episode costs between Tshs 2530 (USD 2.10) and Tshs 5400 (USD 4.50). Further analysis is being conducted on the data.

Interpretation: Cost monitoring of an interventions is important exploring the best ways of allocating resources to interventions that have a great value for money in reducing the burden of diseases.

472A
Vouchers for ITNs: Lessons from a pilot scheme in Tanzania [MIM-JM-4914]

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Introduction: In October 2004, the Tanzanian Ministry of Health launched the Tanzanian National Voucher Scheme (TNVS) for targeting subsidized insecticide-treated nets (ITNs) to pregnant women in Tanzania. To assess the impact of the scheme, and to inform the national roll-out, UNICEF-Tanzania commissioned a ‘pilot’ voucher scheme in two districts (Kilosa and Kibaha) from June 2003 to July 2004. This paper will present findings from a voucher tracking study and household survey undertaken in the two districts.

Methods: The voucher tracking study aimed to successfully ‘track’ the outcome of 100 issued vouchers in each district. We randomly selected 150 voucher numbers from project records listing all vouchers issued to health facilities. Field workers visited each facility to obtain additional information about the recipient of the selected voucher. They then attempted to locate and interview the identified recipients. The household survey aimed to estimate coverage of ITNs in the target group and estimate coverage of the voucher scheme among those women who were eligible during its operation. We adopted a cluster survey design with the final sample size comprising 11 clusters of 25 households in Kilosa and 15 clusters of 25 households in Kibaha.

Results: A total of 102 and 99 interviews were conducted with voucher recipients in Kilosa and Kibaha respectively. Nearly all of those interviewed in both districts had received a voucher and over three-quarters had used it to purchase a net. Almost all recipients confirmed that they still had the net and this was verified by the interviewer in most cases. Of those who did not buy a net, most said it was because they could not afford it. Interviewers were unable to track 35 (28%) vouchers in Kilosa and 73 (60%) vouchers in Kibaha either because there was no record of their use at the health facility or the woman or village identified on the voucher
was not known. A total of 153 ‘eligible’ pregnancies (i.e. exposed to the voucher scheme) were sampled in the household survey. The results confirmed that the majority of voucher recipients used it to buy a net. However, we found that coverage was low among pregnant women. Only 22% of eligible pregnancies in Kilosa and 50% in Kibaha received a voucher from an MCH. However, in both districts use of nets and treated nets during pregnancy was higher than among the general population.

Interpretation: The study reveals important lessons for the TNVS. In particular, the low level of receipt of vouchers by eligible women indicates the need for a focus on health facility level implementation issues. This and other findings will be discussed.

473B
Creating an enabling environment for insecticide treated nets scaling up: The Tanzanian experience

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Introduction: Malaria is the largest cause of health service attendances, hospital admissions and child deaths in Tanzania and a major impediment to social and economic development in the country. Tanzania committed itself at the Summit of African Heads of State in Abuja in April 2000 to protect 60% of its population at high risk of malaria by 2005. The country is therefore determined to ensure that sustainable malaria control using insecticide treated nets is carried out at national scale.

Methods: We have put together evidence to show that Tanzania has been involved for two decades in the research process for developing insecticide treated nets as a malaria control tool, from testing insecticides and net types, to assessing their efficacy and effectiveness, and exploring new ways of distribution. Since 2000, the emphasis changed from a project approach to that of a concerted multi-stakeholder action for taking insecticide treated nets to national scale (NATNET). This means creating conditions that make insecticide treated nets accessible and affordable to all those at risk for malaria in the country.

Results: This paper describes Tanzania’s experience in (1) creating an enabling environment for insecticide treated nets scale-up, (2) promoting the development of a commercial sector for insecticide treated nets, and (3) targeting pregnant women with nearly free insecticide treated nets through a national voucher scheme. As a result, nearly 2 million insecticide treated nets and 2.5 million re-treatment kits were distributed in 2004. Here we summarise the extensive Tanzanian experience with ITNs, as well as the long and complex process leading to a national scale-up of ITNs. We also attempt to review some of the issues that are crucial for this process, with the hope that this experience might prove useful for other countries.

Interpretation: Current evidence shows that, National upscaling of insecticide treated nets is possible when the programme is well designed, coordinated and supported, and the Abuja target of protecting 60% of those at risk is feasible even for a large endemic country like Tanzania.

474C
The economic and financial costs of introducing artemisinin-based combination therapy: Evidence from district-wide implementation in rural Tanzania

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Introduction: Malaria endemic countries are being urged to adopt artemisinin-based combination therapies (ACTs), which are efficacious and believed to inhibit resistance. Evidence on the costs of ACT implementation is essential to inform the process of policy change. However, to date costings have relied on simple models and estimates. We have collected data on
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the drug and non-drug costs of ACT implementation in Rufiji District in southern Tanzania.

Methods: As part of the Interdisciplinary Monitoring Project for Antimalarial Combination Therapy, we documented financial and economic costs related to implementing ACT (SP + artesunate) in formal facilities between late 2002 and mid 2005. Drug costs were estimated from drug dispensing data from sentinel health facilities, valued using International Reference Prices. Non-drug costs included all activities implemented in support of ACT introduction, comprising consultation with health officials, incremental costs of procurement and drug delivery, repackaging costs, developing treatment guidelines and training manuals, training of district health personnel, media communication and a sensitization campaign.

Results: Data will be presented on financial costs to indicate budgetary requirements, and economic costs to indicate the overall value of resources used and potential burden to the health system. Non-drug costs will be presented by both activity and line item. The value of drugs dispensed will be calculated as both the total drug cost and the incremental drug cost of ACT compared with SP monotherapy in adjacent districts. The Rufiji District ACT programme costs will be scaled up to estimate the national costs of implementing ACT in Tanzania, using information on population size, health facility numbers, staffing levels, and geographical location. Sensitivity analysis will be used to assess how national costs vary with changes in the combination used and the implementation strategies employed.

Interpretation: This study provides reliable estimates of the costs of ACT implementation, which is vital for both donors and national policy makers faced with the choice of a suitable delivery model and time frame for implementation.

475A
Nutritional status and malaria infections in children in malaria endemic area of Kenyan coast [MIM-AN-251750]
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Introduction: The interaction between nutritional status and malaria disease is complex and often controversial. Repeated malaria infections have been associated with subsequent malnutrition. On the other hand, malnutrition is associated with either susceptibility or resistance to infection with malaria infections.

Methods: In this study, we have investigated the relationship between nutritional status, anti-malaria antibody levels and P. falciparum malaria in a cohort of children less than 8 years old living on the coast of Kenya. The study involved anthropometric measurements and immunological assays at cross-section surveys and longitudinal follow-up for malaria episodes.

Results: We have found that malaria was associated with malnutrition in an age-dependent fashion. Malaria was associated with subsequent underweight (incidence rate ratio [IRR], 1.965; 95% CI: 1.10, 2.20; p = 0.01) in children under the age of 2 years, but this effect was not there in older children. Also, we observed that iron deficiency was associated with reduced risk of clinical malaria in the cohort. Children who were iron deficient had a lower incidence of malaria episodes as compared to those who were iron replete (the IRR, 0.70; 95% CI, 0.51–0.99; p < 0.05). Also, the iron deficient children had lower malaria-specific IgG, IgG2, IgG4 and IgE immunoglobulin levels compared to the iron replete children.

Interpretation: We conclude that malaria has a greatest effect on nutritional outcome in the youngest children and that iron deficiency is associated with a reduced risk in clinical malaria outcome.

476B
Vers une approche géo-anthropologique de prévention du paludisme: le point sur une expérimentation en cours à Hévécam et à Kribi (Cameroun) [MIM-AR-13430]
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Introduction: Le paludisme fait partie des maladies tropicales qui doivent leur développement et leur pérennité aussi bien aux conditions physiques du milieu qu’aux comportements des groupes humains, vis à vis de l’espace qu’ils occupent ou qu’ils pratiquent. Les
ravages qu’il cause sont en partie liés à la façon dont les hommes gèrent leur environnement immédiat et plus précisément ceux des éléments qui conditionnent la vie et la prolifération de l’agent vecteur: humidité, couverture végétale, déchets.

Methods: La méthode de prévention que nous proposons d’exposer prend appui sur deux pendants du fléau, à savoir les milieux naturels et les comportements humains. L’expérimentation présentée a été conduite dans le cadre de deux projets réalisés en continuum et financés par le Ministère français de la Recherche (PAL+ et ATC Environnement Santé) et dans une collaboration Sud/nord. Les enquêtes de terrain ont été réalisées en 2002 et 2005 à partir d’entretiens semi-directifs auprès des populations vivant dans 2 sites au Sud Cameroun (une agro-industrie HEVECAM et la ville de Kébi et ses environs). Les statistiques collectées lors de ces enquêtes ont fait l’objet de traitements cartographiques.


Interpretation: L’approche interdisciplinaire, associant géographes et anthropologues et permettant la confrontation des données recueillies sur les mêmes sites fait la richesse de notre expérimentation. Cette méthode convient parfaitement à la nature du paludisme.

477C
Are there spatial differences in maternal responses to childhood malaria in south east Nigeria?

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Introduction: Childhood fevers due to malaria remains a major cause of morbidity and mortality among under-five children in Nigeria and this has been attributed to ignorance and poor service delivery. The degree of vulnerability perceived by mothers will affect their perception of the severity and threat of their child’s fever and the patterns of health care use. This study was undertaken to compare maternal responses to childhood fever in urban and rural areas of Enugu, Nigeria.

Methods: Data was collected with interviewer-administered questionnaires from 400 randomly selected mothers aged 15–49 years who lived in both urban and rural areas for at least one year and have at least one child less than 5 years old.

Results: Malaria was mentioned as the commonest cause of childhood fevers (84% urban, 100% rural; \( p < 0.05 \)). Rural mothers are more likely to recognize danger signs and symptoms that warrant medical attention than urban mothers (95.2% versus 72%; \( p < 0.05 \)). Rural mothers use more of informal (patent medicine dealers, village health workers and traditional healers) than formal health services (hospitals, clinics and health centers). Home management of the fever is more with urban than rural mothers (10.5 versus 4.6%; \( p < 0.05 \)). The time before first action in urban (1.05 \( \pm \) 1.67 days) was significantly shorter than in rural areas (2.32 \( \pm \) 0.82 days; \( p < 0.05 \)). The time before second action was taken was however not significantly different between urban and rural communities (2.81 versus 4.5; \( P > 0.05 \)). Chloroquine (44.5% rural versus 48.2% urban; \( p < 0.05 \)), SP (2.8% rural versus 3.4% urban; \( p < 0.05 \)) and paracetamol (87% rural versus 66.5% urban; \( p < 0.05 \)) were more commonly used in rural communities but not significantly so.
urban; \(p < 0.05\) are the main drugs given at home and the drugs were mainly bought from a patent medicine dealers. The total cost of treatment, including transport and drugs was 325.5 Naira, (2.5 dollars) in urban and 220 Naira (1.69 dollars) in rural areas.

**Interpretation:** Urban and rural mothers are aware that malaria is the major cause of childhood fevers, but differences exist in their responses to malaria which may be important for National malaria control programs.

478A

**Anti malaria drug information in patent medicine dealers in south east Nigeria** [MIM-BO-76272]

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**Introduction:** Early effective case management is a cornerstone of malaria control in the tropics. This depends on effective management, reliable supply, and rational use of antimalarials. Patent medicine dealers (PMD) are frequently patronized in Nigeria for malaria treatment and should provide correct information on drug use to customers, in such a way that the customers understand and can follow the advice. This study therefore looked at the interaction between PMD and malarial clients.

**Methods:** The study was carried out using three data collection methods: structured interviews with patent medicine dealers (PMD), structured interviews with exiting customers with fever, and observations of the interaction between the PMD and malarial clients.

**Results:** A majority of PMDs (94%) used chloroquine and sulphadoxine–pyrimethamine of various brands to treat presumptive malaria and in incorrect doses. Most clients (83%) made an independent decision to buy antimalarial drugs, without having a prescription or a recommendation from a health personnel (42%). Anti malaria drugs were dispensed on the demand of clients or depending on their ability to pay. About 76% of clients received information on drug use from the PMD and only 56% of clients could remember the provided information. About 29% of customers received an incorrect written drug label, 48% received no written drug label and 44% received a mix of various drugs in the same bag. PMDs advised patients to use chloroquine injection in 32% of cases, citing reasons such as faster action.

**Interpretation:** Antimalaria drugs are used irrationally. Both PMDs’ and clients’ knowledge of anti malaria drug use should be improved, although different approaches should be taken to improve knowledge for each group.

479B

**Geographic and socioeconomic differences in rural households’ perceptions and prioritization of malaria and other tropical diseases in Nigeria** [MIM-MU-265903]

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**Introduction:** Disease prioritization is important in health care policy because it guides priority setting and strategic planning by policy makers as no health system can afford to pay for every service it wishes to provide especially in developing countries like Nigeria. However, there is no consensus on the best methods to carry out priority setting and how rural households perceive and prioritize malaria and other tropical endemic diseases is not known in Nigeria.

**Methods:** A household survey using questionnaires to a female household primary care giver, or the household
head in her absence was conducted. The Primary Health Care house numbering system was used as frame for the sampling of 100 households in each of the 16 communities to give a two-stage sample of 1600 households. A socio-economic status (SES) index was used for analysis.

**Results:** Malaria is perceived and prioritized highly by these communities. This is followed closely by typhoid fever, HIV/AIDS and malnutrition. The least poor ranked malaria and tuberculosis as most serious more than the poorest while the reverse is the case with typhoid fever and malnutrition. A few thought the diseases are not serious for any group of people. Marked differences exist across the LGAs.

**Interpretation:** Malaria is perceived and prioritized highly by these communities Community ranking of diseases and the perception of their seriousness can be adapted to compliment the burden of disease and cost effectiveness approaches used for priority setting.

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### 20: Epidemic forecasting, warning, detection and response

**Poster 480–490**

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

#### 480C

Maps of the Sri Lanka malaria situation preceding the tsunami and key aspects to be considered in the emergency phase and beyond [MIM-OB-252000]

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**Introduction:** Following the tsunami, a detailed overview of the area specific transmission levels is essential in assessing the risk of malaria in Sri Lanka. Recent information on vector insecticide resistance, parasite drug resistance, and insights into the national policy for malaria diagnosis and treatment are important in assisting national and international agencies in their control efforts.

**Methods:** Monthly records over the period January 1995 to October 2004 of confirmed malaria cases were used to perform an analysis of malaria distribution at district spatial resolution. Also, a focused review of published reports and routinely collected information was performed.

**Results:** Although relocated people may be more exposed to mosquito bites, and their capacity to handle disease will be affected, the environmental changes caused by the tsunami are unlikely to enhance breeding of the principal vector. The incidence of malaria was one case per thousand population in the 10 months leading up to the disaster, in the districts with the highest transmission. Despite some losses, the Sri Lanka public health system is capable of dealing with the possible threat of a malaria outbreak after the tsunami. The influx of foreign medical assistance, drugs and insecticides may interfere with malaria surveillance and the long term malaria control strategy of Sri Lanka, if not in accordance with government policy.

**Interpretation:** As the environmental changes caused by the tsunami are unlikely to enhance breeding of the principal vector, and the present parasite reservoir is low, the likelihood of a malaria outbreak is low. However, close monitoring is necessary.

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### 481A

The importance of model formulation in developing a pre-control malaria risk map for Botswana [MIM-MC-28566]

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**Introduction:** Spatial methods are increasingly being applied to map distributions of vector borne diseases. The Mapping Malaria Risk in Africa project, working towards a malaria risk atlas to guide rational and targeted control, has collated historical prevalence data from malarious countries and several risk maps have been produced. In this paper we explore different environmental explanatory variables and statistical approaches, and the sensitivity of resulting malaria risk maps to model formulation.
Methods: Of the 1063 age-specific malaria prevalence rates collected for Botswana, 125 were available from the 1961/62 national survey for the 1–14 year age group. The 116 unique locations were fairly well distributed across the country. Non-spatial uni-variate and multi-variate logistic regression analysis was carried out against a range of potential explanatory co-variates generated from environmental data available for the African continent. Automated step-wise as well as manual co-variate selection procedures were applied. Spatial statistical analysis against the four most plausible co-variates was carried out through Bayesian estimation implemented, via Markov chain Monte Carlo methods. Risk maps were generated using the model results.

Results: Most of the 64 potential predictors were strongly associated with malaria prevalence in uni-variate logistic regression analysis. Good overall model fit was achieved in multi-variate analysis. When using automated step-wise selection procedures the model outcome was mainly affected by how many and which co-variates were included in the starting list. The more co-variates remained in the model, the better the overall fit tended to be. However, the resulting risk maps did not represent actual malaria distribution well when compared to expert knowledge and an interpolation of the data obtained through ordinary kriging. Manual selection of a few plausible co-variates produced much more realistic and parsimonious models, though the statistical fit tended to be weaker. Half the prevalence rates were below 15% so that all the risk maps were considerably more accurate in low risk than in high risk areas, though the residuals on the logit scale were quite evenly distributed. Applying Bayesian estimation methods to one simple plausible model containing four variables only (maximum monthly rainfall, summer vapour pressure, winter mean temperature and elevation), increased the range of predicted prevalence values and improved the risk map.

Interpretation: The best predicted risk maps were obtained using only a few plausible predictors, even while much residual variance remained unexplained. Bayesian estimation improved the results, but co-variate selection determined the plausibility of the model.

Solar ultraviolet radiation UV-B and malaria in Mali [MIM-MI-83160]

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Introduction: Depletion of the ozone layer allows the flux of solar radiation. Our main concern is the detrimental effect of short-wave radiations such ultraviolet (UV) on human health. UV-B can activate a latent disease or accelerate the clinical progression of the disease. Impact of Solar UV-B radiation should be assessed. No study was performed to seek relation between malaria and UV-B. Study goals were to examine monthly variation of solar UV-B and compare its intensity with malaria prevalence in Bamako.

Methods: The study was carried out in the Hill of Badalabougou (12.5 North and 8.35 South) in Bamako capital city of Mali between September 2000 and October 2001. A spectrometer (PMA2101) with a special filter for UV-B mounted on a sun tracker device was used. A 15 min interval was inserted between 2 measures from 8:15 h the morning to 5:30 h in the afternoon. The data obtained in minimal erythema doses/h. Sky was observed during each measure of UV-B for the presence of clouds. Malaria prevalence was obtained by estimating the number of malaria cases between October 2000 and September 2001 at Hospital Mère-Enfant “Luxembourg”. Thick smear was obtained from each febrile patient.

Results: We observed high values of UV-B in September and March right one month with a large peak of morbidity in October and a small peak in April. Radiations were correlated with rainfall and the temperature with a significant degree (Pearson correlation $r = 0.631$; $p = 0.028$ and Pearson correlation $r = 0.681$
and \( p = 0.015 \), respectively. We did not find a relation statistically significant between the intensity of UV-B radiation and malaria (Pearson correlation = \(-0.529\) and \( p = 0.077 \)) probably due to a weak sampling of our patients. The clouds were more frequent during the wintering and could attenuate the strong intensities of UV-B radiations observed between September and October.

**Interpretation:** There is a month lag between the peaks of malaria morbidity and intensity of UV-B in Bamako. In human host, residual parasitemia may be the target of UV-B to induce disease in period of the year when malaria transmission does not occur.

**Variations in entomological indices and incidence of malaria in the highlands of East Africa in relation to climatic factors [MIM-MK-251736]**

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**Introduction:** Malaria epidemics in the East African highlands are a recurring problem. When local weather conditions become favourable for transmission and the density of Anopheles vectors increases rapidly, there is a high risk of malaria outbreaks, often associated with high mortality. However, our understanding of the interactions between climatic factors, entomological variables and malaria incidence has not yet reached a stage where it can be used for effective epidemic early warning.

**Methods:** Locality-specific meteorological, entomological and malaria morbidity data are being collected within the Highland Malaria Project (HIMAL) at two sentinel sites in Kenya (Sengera and Kilibwoni in Gucha and Rukungiri districts, respectively). Entomological data are being collected since January 2003 by using weekly pyrethrum spray sheet collection method, in 12 houses at each site. Vector species composition and densities, seasonality, blood meal analysis and infection rates among mosquitoes have been determined.

**Results:** Preliminary results show that Anopheles gambiae s.s. was the main vector species in Gucha and Rukungiri districts, especially during the rainy periods. However, An. funestus predominated in the drier, intervening periods. The density of An. arabiensis was generally much lower, except in Kilibwoni, where there was a sudden increase in the density in April 2003, following a dramatic increase in temperature one month earlier while rainfall was minimal. There was a lot of variation in vector density from month to month. However, even in the months when transmission was relatively high, vector density remained low and sometimes no vectors could be found at all. Bloodmeal analysis showed that in all the sites, the three vector species fed predominantly on humans and in Rukungiri, they fed exclusively on humans. Animal bloodmeals have been detected in An. gambiae s.s., An. arabiensis and An. funestus. Human biting rates seem to mirror the vector density in relation to seasonal and temporal variation. Only one An. gambiae s.s. from North Nandi was found to be sporozoite positive out of 15 tested from that site. New entomological samples, meteorological data and malaria morbidity data are currently being analysed.

**Interpretation:** All the data collected will form the basis for modelling epidemic risk and the implications of the results for the on-going transmission studies to develop epidemic prediction systems are discussed.

**Towards empirical description of malaria seasonality in southern Africa: The example of Zimbabwe [MIM-MM-67416]**

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**Introduction:** Quantitative description and mapping of malaria seasonality is important for timely spatial targeting of interventions and for modelling malaria risk. There is a need for seasonality models that predict quantitative variation in transmission between months.
Methods: We use Zimbabwe as an example for developing an empirical map of malaria seasonality. We describe the relationship between seasonality in malaria and environmental covariates for the period 1988–1999, by fitting a spatial-temporal regression model within a Bayesian framework to provide smoothed maps of the seasonal trend. We adapt a seasonality concentration index used previously for rainfall to quantify malaria cases load during the peak malaria transmission season.

Results: Mean monthly average temperature range from 28 to 32°C and mean monthly maximum temperature from 24–28°C and high rainfall are suitable predictors of seasonal transmission. Extremely high temperatures and mean monthly minimum temperatures limit the season of transmission. The intensity of seasonal transmission was highest in the northwestern part of the country from February to May with the peak in April and lowest in the whole country from July to December. The northwestern lowlands had the highest concentration of cases (>25%) followed by some districts in the north central and eastern part with a moderate concentration of cases (20–25%) and the central highlands and southeastern part of the country had the lowest concentration of cases (<20%). This pattern was closely associated to the geographic variation in the seasonality of climatic covariates particularly rainfall and temperature.

Interpretation: Our modelling approach quantifies the geographical variation in seasonal trend and the concentration of cases during the peak transmission season and therefore has potential application in malaria control.

485B
Seasonal changes in haematological parameters in children 0–5 years old residing in a rural malaria endemic area of Mount Cameroon [MIM-I -54804]
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Introduction: In malaria endemic areas, morbidity and mortality from Plasmodium falciparum mainly affects children and severe anaemia is a frequent life threatening complication. Seasonal epidemics of malaria occur in areas where transmission intensity varies according to rainfall. We investigated haematological indices, clinical and parasitologic parameters in 120 children <5 years and the extent to which these parameters are influenced by high and low malaria transmission periods.

Methods: This study was carried out in Bolifamba in September and October 2002 (high transmission period; rainy season) and in November and December 2002, and January 2003 (low transmission months; dry season). Parasites were detected on thick blood films. Hb concentration was measured using Drabkin’s solution. ABO blood group was determined using agglutination tests. PCV was determined by the micro-haematocrit centrifugation method. Blood cells were counted using the improved Neubauer counting chamber. Fever was defined as temperature ≥37.5°C. Spleen rate was determined by palpation of the spleen and graded according to the classification of Hackett.

Results: The prevalence of malaria, fever occurring with parasitaemia, and anaemia occurring with parasitaemia was 65.8, 58.2, and 60.8%, respectively in the rainy months compared with 46.7, 41.4, and 57.1% in the dry months (P=0.05, 0.04, and 0.03, respectively). Children <2 years had a higher prevalence of malaria, fever, anaemia and splenomegaly compared with those older, but these differences were not significant (P>0.05). No significant difference was seen in geometric mean parasite density between seasons (1673.8 versus 1532.3 parasites/μl; P=0.32). Malaria prevalence and parasite density were highest in group O+ (51.1% and 6822.3 parasites/μl, respectively) and lowest in group AB+ children (2.2% and 153.5 parasites/μl, respectively). Mean PCV, Hb, RBC, counts, MCH and MCHC indices were significantly higher in the dry compared with the rainy season.

Interpretation: Our findings show that malaria infection is a primary determinant factor of fever, anaemia and splenomegaly in these children. Anaemia severity increased with a decrease in haematological parameters during the high transmission period.
Abstracts / Acta Tropica 95S (2005) S1–S506

486C
Characteristics of *P. falciparum* infections in children in a suburb of Ibadan, Nigeria

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Introduction: Infections with *Plasmodium falciparum* can present with a wide spectrum of clinical features but it is poorly understood why some infections are symptomless or resolve without complications while others are fatal. We determine through a cross-sectional survey, the characteristics of *P. falciparum* infections in children living in a suburb of Ibadan, Nigeria. This study is important for future malaria interventions in this area.

Methods: This study was carried out in Igbanda, a rural community in Ibadan, southwest Nigeria where malaria is holoendemic. Four hundred and twelve children (6–96 months old) were screened for *P. falciparum* infection. Those who had positive blood films were classified into two groups: Asymptomatic malaria (AM) group had asexual forms of *P. falciparum* in blood smear, axillary temperature <37.5 °C and no history of febrile illness in the preceding 2 weeks. Uncomplicated malaria (UM) group had a history of fever in the 24 h preceding presentation or pyrexia at presentation (>37.5 °C) and asexual forms of *P. falciparum*.

Clinical, parasitological and haematological data were recorded for each child.

Results: The prevalence of *P. falciparum* infection in this community was 82.5% (340/412). Asymptomatic infection was found in 64.1% (264/412) of the children while 72 (17.5%) children had uncomplicated malaria. Geometric mean parasite density was slightly higher in the UM group (2326 per μl) compared with that of ASM (964) but this was not statistically significant. No significant difference was also found in the mean haematocrit level in both group (32.2 and 30.5% for ASM and UM, respectively). No significant correlation was found between parasitaemia and age or haemoglobin level in both groups.

Interpretation: The consequence of the high prevalence of asymptomatic infection on malaria morbidity remains to be investigated in subsequent studies as asymptomatic infection have been shown to play a role both immunity and pathology.

487A
Use of GIS in planning monitoring and evaluation of Malaria Control Activities in Swaziland

S. Kunene, S. Chambers, Z. Zulu, Z. Dhlamini

Ministry of Health, Swaziland

Introduction: Malaria is a major public health problem in Swaziland with a population of 30% (300,000) at risk of malaria infections in the country. Malaria transmission is unstable hence the high risk of epidemics. The malaria programme has a comprehensive malaria information system for use in decision making. A GPS project was introduced in 2004 with the objective of mapping all households in selected localities where vector control (IRHS and ITNs) interventions are implemented.

Methods: The GIS uses the Healthmapper program to manage data from different control interventions collected using simplifies forms and databases. These interventions include a weekly surveillance system which collects data on malaria cases, deaths, number of referrals, and number of chloroquine treatment failures and status of drugs at the facility; distribution of ITNs to children under 5 years and pregnant women from health facilities; indoor residual house spraying of structures; and spatial distribution of a number of indicators.

Results: The GIS displays data on distribution of malaria cases, deaths and other indicators collected through the weekly surveillance system by health facility. Weekly trends for the indicators over a period of time for each facility are monitored. The spatial distribution of confirmed malaria cases is compared with the clinical malaria cases to determine existence of under and over-reporting of malaria cases in the weekly system. The spatial distribution of mosquito nets by health
facility and Inkhundla level is monitored monthly. The spatial distribution of mosquito nets per household, households sprayed during the 2004/2005 malaria season, number of people by age group per household, households with a rural health motivator and/or a traditional healer, number and type of toilets per household and other indicators per household can be shown on a map for each area of interest. Analysis on the indicators collected and their relationship to the distribution of malaria cases at the health facilities can be made. The data on the spraying coverage is currently only available by locality in a database and cannot be mapped for each locality due to lack of digital maps of localities.

**Interpretation:** GIS is being successfully used to monitor trends and spatial distribution of a number of indicators by health facility and household. Useful information can be collected using currently existing spraying structures with minimal additional cost.

488B

**Responding to malaria epidemics in Botswana**

T. Phindela, E. Mase

Ministry of Health, Botswana

**Introduction:** Malaria is a major public health problem in Botswana with between 59,000 and 102,000 cases of unconfirmed malaria occurring each year. Malaria transmission with a high risk of epidemics. The burden of malaria is greatest in the northern part of the country. Malaria epidemics occurred in Botswana in 1988, 1993, 1996 and 1997. The worst malaria epidemic in 1996 and 1997 resulted in more than 8000 malaria cases and more than 140 deaths being reported in both years.

**Methods:** After the outbreak in 1996/7 a malaria epidemics and preparedness sub-committee was put together. Staff in malarious districts were trained in malaria epidemic preparedness and response. Efficient drug supply and distribution system was put in place. Seven epidemic containers were acquired and placed in six strategic points in the malarious areas and the seventh back-up container maintained at the national level. Each container contains equipment for responding to malaria epidemics including tents, linen, mattresses, camp beds, drip stands and a generator. In addition, a malaria contingency fund is available at the national level. Surveillance system was strengthened. Partnership with the military and national disaster office was strengthened.

**Results:** Since 1997 no major malaria epidemic was reported. During the malaria season the national epidemic preparedness and response committee meets every month. Data is received from all districts with number of unconfirmed and confirmed malaria cases and malaria deaths. The data is monitored every week. When district notification reaches/exceeds 600 unconfirmed cases/week, extra manpower is deployed to the district. Tents are erected where there is need and volunteers including the defence force are deployed in hard to reach areas. A bi-weekly newsletter to inform community about epidemic is produced. When district notification reaches/exceeds 800 unconfirmed cases/week mobile teams consisting of a nurse and support staff are deployed. When district notification reaches/exceeds 3000 unconfirmed cases/week a district disaster is declared. Tents from epidemic container are erected at affected areas.

**Interpretation:** A combination of improved drug supply and distribution system and epidemic detection system has led to control of malaria epidemics in Botswana. A number of outbreaks have been responded to before becoming epidemics.

489C

**A stochastic simulation model of Plasmodium falciparum epidemiology**


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**Introduction:** We report the development of an integrated mathematical model for predicting the epidemiological and economic effects of malaria interventions. The model includes components relating the entomological inoculation rate (EIR) for Plasmodium falciparum malaria and the force of infection, a model of infectiousness to mosquitoes, models for parasite tolerance and thus the incidence of acute episodes of clinical malaria.
Methods: We have constructed a stochastic simulation incorporating effects of (i) host age on exposure to mosquito bites, (ii) naturally acquired pre-erythrocytic immunity, and (iii) the success probability of inoculations as a function of EIR; (iv) acquired immunity on parasite densities (v) recent parasitaemia on parasite tolerance. The model is fitted to multiple field malariological datasets by maximum likelihood, using a simulated annealing algorithm.

Results: The model reproduces reasonably well the parasitological patterns seen in malariological surveys in endemic areas and can account for non-monotonic relationships between the age of the host and the parasite prevalence and incidence of disease. As seen in field studies it predicts a non-monotonic relationship between lifetime incidence of clinical malaria and transmission intensity.

Interpretation: We provide a parsimonious explanation for faster acquisition of immunity in older people. No intrinsic age dependence is assumed in the infection outcome. The model provides a basis for predicting impacts of a range of interventions against malaria.

490A
Prevalence of malaria parasite in pregnant women in Ihiala local Government area of Anambra State, Nigeria [MIM-YS-173808]

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Introduction: The work was carried out to determine the incidence of malaria parasitemia in Ihiala Local and also to suggest possible management of the disease in the area. This was because within the years the incidence of malaria among pregnant women in the area has been a serious public health problem in the Local Government area. The work is perspective study of 10 years from 1994 to 2003.

Methods: During the period 1994–2003, 2241 pregnant women aged 15–44 years attending hospitals in the locality presenting with clinical features suspected to be malaria infections, were examined. The data for the study were obtained from the records of all malaria parasite request made between January 1994 to December 2003. The results were documented at the parasitology unit register of the hospitals. The laboratory unit employs 3% Giemsa solution in pH 7.2 distilled water for staining fixed thin and thick blood film smears. The slides are viewed using X100 (oil immersion) objective. The method of determining results is the use of the simple ‘plus methods’ of enumerating malaria parasite density according to WHO (1985) which ranged from + to ++++. Negative results were designated −ve (o).

Results: From the total of 2241 pregnant women examined, 1472 (65.7%) were infected with malaria parasite while 769 (34.3%) were unaffected. The most dominant species were Plasmodium falciparum (76.4%) followed by Plasmodium malariae (23.6%). The monthly distribution of the malaria parasite showed that August had the highest occurrence in both hospitals and the lowest occurrence from December to February. The yearly distribution of the malaria parasitemia was highest in 2000 with a total of 177 positive cases and lowest incidence occurred in 1997 with a total of 106 positive cases. In age distribution, 25–29 years had the highest incidence with 559 positive cases and 290 negative cases. Lowest number of cases occurred in age group between 40 and 44 with 35 positive cases and 29 negative cases.

Interpretation: Generally, there was no significant difference between (p > 0.05) the prevalent rate of malaria in pregnant women within the months as well as within the years. The studies also showed that malaria is on the high side in the L.G.A. and travelers going to Ihiala should be advice to take anti-malaria therapy.

21. Vaccine trials

Posters 491–495

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: posters: Wednesday; C-posters: posters: Thursday
TRAP-based vectored vaccines are more immunogenic and protective than CSP-based vectors: Correlation of efficacy with long-lived memory T cells

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Introduction: The choice of antigen for inclusion in subunit vaccines for malaria is complicated by the huge number of available candidates. Direct comparisons of different antigens in the same trial have been very rare. Here we compare the immunogenicity and efficacy against sporozoite challenge of vectored vaccines expressing either the thrombospondin-related adhesion protein or the circumsporozoite protein using vectors and regimes designed to induce protective T cells against the liver-stage parasite.

Methods: Full length TRAP and CS from P. falciparum were expressed in plasmid DNA, modified virus Ankara (MV A) or a fowlpox strain FP9, and manufactured to clinical grade. Healthy UK volunteers were immunized with either DNA-MVA or FP9-MVA regimes. Two doses of the first vaccine and one of the second were administered at monthly intervals. Volunteers were challenged about 2 weeks after the last challenge with five bites of A. stephensi mosquitoes laden with P. falciparum 3D7 strain sporozoites. Efficacy was measured by 12 hourly blood films and real-time PCR performed in real-time. T cell immunogenicity was measured both by ex vivo and cultured ELISPOT assays to measure induced effector and central memory T cells.

Results: In a series of small-scale phases I/IIa trials we demonstrated previously that heterologous prime-boost immunization regimes with DNA-MVA and FP9-MVA vaccine vectors expressing the TRAP insert could induce substantial protection, reducing liver-stage parasites by up to 92%. Here in a trial with 16 vaccinated and challenged volunteers, eight receiving each antigen, we find that DNA-MVA expressing TRAP is more immunogenic than DNA-MVA expressing CS and only the former is protective (P < 0.05). Also, FP9-MVA expressing CS was only weakly immunogenic even with very high dose regimes and challenge studies showed no protection. This contrasts with the very protective FP9-MVA TRAP regime. Analysis of gamma-interferon ELISPOT responses showed a marked correlation with protection (P < 0.01) of vaccine-induced cultured ELISPOT responses to TRAP, a measure of long lived central memory-type T cells. This immune response was found to be more durable post-vaccination than the more frequently measured ex vivo ELISPOT response, which measures circulating effector T cells. Re-challenge of selected protected volunteers receiving the FP9-MVA TRAP vaccine has shown durable protection extending to 20 months after the last vaccination.

Interpretation: TRAP is more immunogenic and protective than the CS antigen and induces strong cell-mediated immunity against the liver-stage of falciparum malaria. The immunological correlation identified suggests that the protection induced may be durable.

The design of a phase 2 malaria vaccine trial based on a cohort study [MIM-MF-125074]

(1) National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA; (2) Emory University, Atlanta, GA, USA; (3) Malaria Research and Training Center, University of Bamako, Bamako, Mali

Introduction: With multiple vaccines targeting blood stage antigens of malaria under development, we need efficient methods for phase 2 trials aimed at demonstrating a biological impact of the vaccine. There are two important factors: (1) picking endpoints that will predict final efficacy in reducing death and severe morbidity; (2) trial designs that minimize the number of volunteers. Using data from an endemic population in Mali, we studied several possible clinical, parasitologic or combination endpoints.

Methods: We assume that the differences in endpoints between 4 years old control subjects and 4 years old...
vaccinated subjects are similar to observed differences between non-vaccinated 4 and 8 years old from a longitudinal study in Doneguebougou, Mali. Children had weekly visits with monthly blood smears, with additional unscheduled visits when malaria symptoms were present. Children with malaria symptoms had smears read immediately and if positive, were treated. Sample size calculations were done by: (1) only using data from children close to the target ages and using the associated means and variances in standard sample size formulas, or (2) fitting a mathematical model of the age related response, then using the predicted response at the target ages.

Results: During the 1999 (and 2000) malaria transmission seasons, 184 (193) children aged 3 months to 20 years were followed for at least 140 days, of which 23 (18) were 3–5 years old and 25 (39) were 7–9 years old at enrollment. 67 children were followed in both seasons. Both maximum and average parasitemia over all scheduled visits during a season produced large sample size estimates, while all but treatment follow-up (ABTFU) visits gave smaller ones. For the ABTFU visits, maximum parasitemia gave smaller sample sizes than average parasitemia. Parasite densities above 1000 or above 10,000, with or without fever, performed similarly to maximum parasitemia, although there was considerable variability in the estimates. For example, by method (1), for a 100% efficacious vaccine, using maximum parasitemia over ABTFU visits resulted in an estimated sample size per vaccination group of 61 for the 1999 season (80% CI 18–524, by bootstrap) versus 27 for the 2000 season (80% CI 14–1650) for 2000. A vaccine that was efficacious in only half the subjects required a greater than four-fold increase in sample sizes.

Interpretation: Assuming that an effective vaccine will convert the malaria profile of a young child into the profile of an 8 years old, phase 2 trials in Mali require group sizes of a few hundred to detect a significant effect with 50% of the vaccinees responding.

493A Malaria infection diagnosed by PCR as a means of evaluating pre-erythrocytic candidate malaria vaccines [MIM-BI-278784]

(1) Medical Research Council Laboratories, The Gambia; (2) London School of Hygiene and Tropical Medicine, UK; (3) Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, University of Oxford, UK

Introduction: The ability to test candidate pre-erythrocytic malaria vaccines, using a well-established sporozoite challenge model, in a field setting with group sizes of tens rather than hundreds of volunteers would greatly facilitate identification of the most promising vaccine candidates. We assessed the suitability and acceptability of this method in a field trial in semi-immune volunteers exposed to natural infection during the high malaria transmission season.

Methods: Prior to the high malaria transmission season, 102 volunteers in three groups received either the pre-erythrocytic candidate malaria vaccine regime FP9 ME-TRAP/MVA ME-TRAP or rabies vaccine as control. All volunteers received antimalarial drugs primaquine and Lapdap plus Artesunate as radical cure for gametocytes and to clear asexual stages of the parasite. In addition, one group received one dose of Fansidar and acted as negative control. Follow up, which commenced 7 days after final vaccination, was by daily finger pricks to obtain 0.5 ml of blood for PCR analysis and duplicate blood films for a 28-day period. DNA extracted from blood samples were analyzed by quantitative real-time PCR for the presence of parasite 18S RNA genes.

Results: Vaccines were well tolerated and no SAE’s observed. Eighty-five percent of volunteers (87/102) received three doses of vaccines and were followed up. During the 28-day follow-up period an average of 70% of volunteers gave daily blood samples. Kaplan-Meier curves obtained using varying PCR parasite densities (20, 100 and 1000 ppml) were similar to results obtained from earlier trials in non-immunes.
494B
Safety and immunogenicity of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP in semi-immune adult males [MIM-OK-87312]
(1) Kenya Medical Research Institute, Centre for Geographic Medicine Research Coast, Kilifi, Kenya; (2) Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK; (3) Wellcome Trust Laboratories, Kilifi, Kenya

Introduction: Vaccines inducing strong T lymphocyte responses against a pre-erythrocytic malaria antigen, thrombospondin-related adhesion protein (TRAP) and multiple T and B cell epitopes (ME), can protect against malaria in sporozoite challenge of non-immune volunteers.

Methods: Sixty adult males were immunised with different regimes using attenuated fowl pox strain 9 (FP9) and modified vaccinia virus Ankara (MVA), both recombinant for ME-TRAP. Heterologous prime-boost regimes, single vector immunisations and alternating vectors for three sequential immunisations were compared. Antigen specific interferon gamma producing T cells were counted by ELISpot.

Results: Prime-boost combinations of attenuated fowl pox strain 9 (FP9) followed by modified vaccinia virus Ankara (MVA), both recombinant for ME-TRAP, induced stronger immune responses compared to single vector immunisations. Three sequential immunisations were required for significant responses to immunisation; hence single vaccination regimes are immunogenic in endemic areas. Furthermore, novel vaccination regimes produce more long-lasting T cell responses.

Interpretation: The very intensive blood sampling required in this method was acceptable in this setting. This is the first demonstration of the use of malaria parasitaemia detected by PCR as the primary endpoint in a field trial.

495C
Implementing phase I trials of malaria vaccine candidates in Mali, West Africa [MIM-DC-135470]
(1) Malaria Research and Training Center, University of Bamako, Bamako, Mali; (2) Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, USA; (3) Malaria Vaccine Development Branch, National Institutes of Health, Rockville, MD, USA

Introduction: Safe and efficacious malaria vaccines require that phases 1–3 clinical trials be conducted in malaria-endemic countries. Such trials must comply with international standards to ensure high quality and worldwide acceptance of the data generated, thus enabling eventual licensure of these vaccines. As part of an international network the Malaria Research and Training Center in Mali started capacity building in 1998, and since 2003, three malaria vaccine trials have been implemented.

Methods: Three double blind randomized controlled phase 1 vaccine trials were conducted since 2003. The first and third trial aimed to assess safety and immunogenicity of MSP142 and AMA1 vaccines co-developed by the Walter Reed Army Institute of Research and GlaxoSmithKline Biologicals in adults living in Bandiagara. The second trial evaluated the National Institutes of Health’s candidate vaccine, AMA1-C1, in adults in Donégou, Mali. All three trials were implemented in a framework that involved international partnerships. We applied ICH/GCP compliant procedures for individual written informed consent, data management and quality control, cold chain maintenance, sample preservation and transport, monitoring and reporting of adverse events.

Results: Overall, 154 volunteers were enrolled in the three trials and followed for at least 12 months. Lost to follow up rates were less than 5%. Operation issues encountered at the first trial were resolved and served to build experience for subsequent trials. The experience acquired enabled the design of more functional study site facilities in Donégou, establishment of a more confident relation with international partners and implementation of more efficient operational procedures. The challenges encountered in conduct-
ing malaria vaccine trials according to international standards in these settings, including weaknesses and strengths of the MRCT will be discussed.

**Interpretation:** Conducting malaria vaccine trials according to international standards in developing countries is challenging. It paves the only road that will fasten availability of malaria vaccines. Lessons learned in Mali may benefit other vaccine trial sites.

24: Socio-economic realities of malaria

**Posters 496–519**

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

496A  
Cost-effectiveness evaluation of intermittent preventive treatment (IPT) for malaria and anaemia in Mozambican children [MIM-PA-06072]


(1) Manhiça Health Research Centre, Manhiça, Mozambique; (2) Centre for International Health, Hospital Clinic (Universidad de Barcelona), Barcelona, Spain

**Introduction:** Malaria and anaemia represent a considerable economic burden for developing countries. In a context of limited resources control measures need to be cost-effective to be used for endemic countries. Implementation of malaria control interventions through and existing health infrastructure such as the Expanded Program of Immunization (EPI) is likely to be highly cost-effective, making the Incremental Cost-Effectiveness Ratio (ICER) relatively low for each extra.

**Methods:** A total of 1581 Mozambican children younger than 5 years who presented to hospital with malaria and/or anaemia were enrolled into this study. The costs of a malaria and/or anaemia episode were calculated from two perspectives: the household and the health system. The costs at the household level, direct and indirect, were calculated on the basis of data collected through standardized questionnaires during interviews of mothers/guardians of ill children who attended the Manhiça Health Centre. The costs for the health system were calculated, using data from this centre, taking into account all costs derived from the visit to the health centre (outpatients visits and inpatients admissions) of children with malaria and/or anaemia.

**Results:** The main efficacy results on which the effectiveness evaluation will be estimated, are still confidential and will be presented at the conference as well. The following results will be presented at the time of the oral exposition: the cost per episode of malaria and/or anaemia in children, the DALYs saved by IPT, and the cost-effectiveness ratio with its 90% confidence interval.

**Interpretation:** Will be presented during the oral exposition.

497B  
Malaria control among children of purdah and non-purdah mothers in Zamfara state of Nigeria [MIM-DA-20188]

D. Akinbode, M. Agboolade, B. Owodunni, R. Livingstone, M. Falade, O. Abiodun

(1) Department of Zoology, University of Ibadan, Ibadan; (2) Department of Biological Sciences, Olabisi Onabanjo University, P.M.B 2002, Ago-Iwoye; (3) Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan

**Introduction:** Children are mostly affected by malaria and women exhibit better treatment seeking behaviours for children, than men. Women are more frequently found with sick children in hospitals, than men. Movement of women in purdah is restricted, reducing participation in essential activities. To control malaria, information about treatment seeking behaviours, prevalences and responses of children to malaria treatment, among purdah and non purdah mothers, in a muslim dominated area of Nigeria is important.

**Methods:** Children included in this study were aged 1–11 years, and resident in Zamfara State of Nigeria. Questionnaires were administered to 579 mothers of patients, who were clinically diagnosed for malaria in order to obtain information about places commonly visited by patients, for malaria treatment. Out of these, thick and thin blood films were prepared from 235 patients, stained with Giemsa stain
and examined for malaria parasites on days 0, 3 and 7. These were examined for malaria parasites. The children were treated with chloroquine on day 0, 2 and 3. Other drugs like sulphadoxine-pyrimethamine and halophantrine were also administered on days 3 or 7, depending on the response of the child to the initial treatment.

**Results:** Out of 235 patients examined for malaria parasites on day 0, 202 (85.95%) were positive. Negative cases, 26 (11.0%), were from children of mothers in purdah, while 7 (3.0%) were from children of non-purdah mothers. Children belonging to mothers in purdah on day 0, who had malaria parasites, were 156 (66.38%), giving 77.2% of the total number with malaria. Forty-six (19.57%) belonged to non-purdah mothers, giving 22.8% of the total, of those with malaria parasites. On day 3, eighty-four out of all the patients reported back, but a follow up in their homes made it possible to locate others. Those who did not report back were found negative for malaria parasites, while 35 (17.3%), who were all among those who reported back, were still positive. On day 7, only 11 patients reported back but after a follow up, it was discovered that 3 (0.74%) out of the total, were positive. Places commonly visited by patients, for malaria treatment were chemists (57.7%), herbalists (21.86) and hospitals (20.3). Respondents treated malaria for different number of days, which spread from 1 to 6 days.

The number of children who had malaria among mothers in purdah was significantly higher than the number of children from non-purdah mothers.

**Interpretation:** Higher prevalence of malaria infection was identified among children of purdah mothers. Knowledge of treatment regimen was lacking. Education of women in Purdah about malaria treatment is recommended, to alleviate children’s sufferings.

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**498C**

**Malaria and resource loss in the Nigerian agricultural sector: Implications for the millennium development goals [MIM-OA-107614]**

**O. Alaba**

Department of Economics, University of Ibadan, Ibadan, Nigeria

**Introduction:** Malaria slows economic growth in Africa by about 1.3% per year; compounded over 53 years, this means that GDP is about a third lower than it might have been in African countries where malaria is endemic. Because of the close connection between malaria and poverty, addressing malaria may be a vital factor in achieving the Millennium Development Goals (MDGs) for Africa most populous country, Nigeria.

**Methods:** Data for the study was collected using multi-stage-sampling techniques. Three health zones from Oyo State namely Ibadan, Ogbomoso, and Oyo were used as the base strata. Using structure questionnaires and 4 weeks recall period, responses from 784 households were collated and used for the analysis, out of which 53% had a bout of malaria ($n=416$). The Cost of illness (COI) method was used to measure the indirect implications of malaria attack on the agricultural households. The indirect COI in monetary terms was calculated by multiplying the net days lost to malaria by the households’ average daily earnings. The total implication for economic resource loss from the agricultural sector of Oyo State is calculated based on the 1991 census figure.

**Results:** Our results reveal that the estimated indirect costs of illness which calculated by monetizing the productive time loss by not only the malaria patients but also the caregivers per agricultural household is about N22,266.60 per bout. Given that the agricultural household size obtained in our survey is 4.1 and the average bouts per household is as high as 16.4 per year per agricultural household. Also, given that the rural population figure estimated from the 1991 census is 3.1 million in Oyo State. Translating this to aggregate resource loss to malaria in the agricultural sector of Oyo State, our estimate reveals that the total annual resource loss to malaria incidences in the State from this sector is N1.09 trillion (USD 8.1 billion).

**Interpretation:** We identify that with the substantial resources being lost to malaria, deepening poverty within the household system which trickles down to worsening national poverty may reduce the chances of Nigeria meeting the MDGs on schedule.
499A
Socioeconomic factors in drug resistant malaria in Ibadan-linking molecular epidemiology with social analysis [MIM-CA-24531]
(1) Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Nigeria; (2) Malaria Research laboratories, Institute for Advanced Medical research and Training, College of Medicine, University of Ibadan, Ibadan Nigeria; (3) Department of Geography, University of Ibadan, Nigeria; (4) Africa Regional Centre for Information Science, University of Ibadan, Nigeria

Introduction: Resistance to antimalarials is deeply rooted in the socio cultural behavior of the people. Drug resistance a biosocial phenomena and malaria “a social disease” would require interdisciplinary efforts to proffer a lasting solution to drug resistant malaria. this study focuses on cq resistant markers (pfmdr1 and pfcrt) from a socio-epidemiological point of view by linking molecular epidemiology with social analysis in a population of asymptomatic and symptomatic Nigerians.

Methods: Questionnaires were administered to 130 volunteers recruited. Most of them had not taken antimalarial drugs in the last few days before the study. Any who had clinical symptoms were treated with chloroquine at recommended dosage. Treatments were based on microscopic examination for malaria parasites in a 14-day follow up using Giemsa stained thin and thick blood films. Parasite dna was extracted from finger prick blood samples blotted onto filter paper by methanol fixation and heat extraction. A nested mutation-specific PCR was used for the pfmdr1 and pfcrt genes. Following amplification of the fragments obtained, polymorphisms in the pfcrt and pfmdr1 were assessed by an RFLP digest with restriction enzymes APO1 and AFI III.

Results: All 130 questionnaires were recovered; 40 (30.8%) questionnaires were administered to adults and 90 (69.2%) to school children. Generally, all respondents were low-income earners below $219, therefore making grouping into social class difficult. However, using the monthly income per the number of household as the poverty index, 18 (45.0%) were high-income earners and 22 (55.0%) low-income earners. On day 0, 8 (20%) of the adult respondents had parasitaemia. Some of the adult respondents self-medicated 13 (32.5%), and their mode of prescription was mostly by hearsay, usually for over the counter, cheap one-dose antimalarials. Many of the low income earners 19 (47.5%) combined different western drugs or with herbs if the first treatment failed. All the high-income earners were 100% cured by day 7, unlike the low-income earners that were parasitaemic until day 14. The PCR analysis of Pfmdr1 N86Y showed that there was amplification of this marker in seven samples, mostly low income earners, while 30 (23.1%) randomly selected parasite negative samples yielded no amplification. There were two samples with a mixed infection of mutant and wild type N/Y, three were pure clones of the mutant type.

Interpretation: This study showed that the poor are more economically vulnerable to malaria attack due and may have difficulties in clearing chloroquine resistant malaria parasites. Low income earners have Pfmdr1 N86K alleles.

500B
Do health care providers and consumers agree on the meaning of good quality malaria treatment? [MIM-LC-197856]
L. Conteh, V. Wiseman, W. Stevens, K. Hanson, B. Mcelvoy
(1) Gates Malaria Partnership, LSHTM; (2) Health Economics & Financing Programme, LSHTM; (3) MRC, The Gambia; (4) University College, Cork

Introduction: Understanding notions and subsequently trying to measure quality has historically been difficult and inconsistent in demand analysis due to the uncertain and complex nature of the health care market. This research explores whether households’ notions of quality mirror those of health care providers. More specifically we identify whether ‘good quality malaria treatment’ as depicted by a range health care providers is recognised and valued by clients? What quality attributes are most prized by clients? Are these the same ones identified by health care providers?

Methods: Quantitative and qualitative approaches were used to explore the role of quality in the demand for
malaria treatment in The Gambia. In depth interviews were conducted with 40 health care providers and 40 health care consumers to explore their notions of good quality health care. In addition, 1760 households responded to a pre-coded questionnaire and stated their preferences for visiting different providers when suffering from malaria.

**Results:** A diverse range of both technical and perceived notions of quality were highlighted. Preliminary analysis suggests that, the quality attributes deemed important to households were similar to the ones given by providers. However, across the different providers, different quality attributes were rated more highly. For example households referred to drug availability as being important when choosing to visit hospitals, pharmacies, shops and kiosks (29, 24 and 19% of responses, respectively), whereas traditional and spiritual powers accounted for 61% of reasons to visit a marabout. Proximity accounted for 24% of the reasons why people chose to visit a shop or kiosk, but less than 10% for all other providers. The reasons why people chose not to visit particular providers was also revealing. For example of the small group of households that did not visit hospitals, 44% stated it was due to lack of money. Lack of trust accounted for 46% of the reasons households did not frequent marabouts. Initial interpretation of the in-depth interviews with providers showed that they recognised and responded to a range of consumers preferences this data is currently being analysed and will be presented at the conference in November.

**Interpretation:** In terms of policy, this research highlights the danger of only using conventional measures of quality, such as drug availability in trying to analyse barriers to accessing and delivering effective malaria treatment. In addition to the more conventional quality attributes, attention must be given to a number of less tangible dimensions of quality that are important to both providers and users such as trust, that to date have rarely featured in demand analyses.

**501C**

Spatial assessment of malaria hazard, vulnerability and risk related to urban agricultural land use in Dar es Salaam, Tanzania [MIM-SD-199375]


(1) City Medical Office of Health, Dar es Salaam City Council, Dar es Salaam, Tanzania, (2) Institute of Physical Geography, University of Freiburg, Freiburg, Germany, (3) Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland, (4) Department of Geography, University of South Carolina, Columbia, SC, USA; (5) School of Biological and Biomedical Science, University of Durham, Durham, UK; (6) Institute of Anthropology, University of Basel, Basel, Switzerland; (7) Ifakara Health Research and Development Centre, Ifakara, Tanzania

**Introduction:** Urban agriculture is a common livelihood strategy for residents of cities in developing countries. Malaria transmission in Dar es Salaam is a significant problem and assumed to be closely linked to agricultural activities, where farmers and mosquitoes share common water resources. Although urban agriculture often provides breeding sites for malaria vectors, it is not clear if this increases actual malaria transmission in the surrounding areas.

**Methods:** Malaria risk is considered as a function of hazard and vulnerability, i.e. malaria hazard created by urban agriculture and vulnerability of citizens to this hazard. In the first phase of the study, malaria risk resulting from urban agriculture will be assessed on a large scale with quantitative methods. In the second phase, based on the findings, specific areas for further investigation will be selected and qualitative methods applied. In the first phase, a comprehensive land use mapping exercise focussing on agriculture will be conducted over a study area of 55 km² of urban Dar es Salaam.

**Results:** The result will be a ‘hazard map’. In addition, ‘vulnerability maps’ will be created. They will be based on parasitological data and comprehensive household characteristics which potentially impact people’s vulnerability to malaria. GIS overlays of the hazard and vulnerability maps as well as spatial analysis will lead to a ‘risk map’. This risk map can be used as a basis for
addressing the prevailing antagonistic position between regulations restricting urban agriculture and policies which promote it.

**Interpretation:** This risk map can be used as a basis for addressing the prevailing antagonistic position between regulations restricting urban agriculture and policies which promote it.

**502A**
Socioeconomic differentials in perceptions of accessibility and utilization of malaria treatment and approaches for improving treatment of the disease in southeast Nigeria [MIM-BE-253836]

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Health Policy Research Unit, College of Medicine, University of Nigeria Teaching Hospital, Enugu, Nigeria

**Introduction:** There is paucity of knowledge about how socio-economic status (SES) differentials in perceptions of ease of accessibility of different healthcare providers determine health seeking behaviour and utilization of malaria treatment services. Such evidence is needed to develop strategies for equitable treatment of malaria in Nigeria.

**Methods:** Structural questionnaires were used to collect information from 1480 primary care givers from four communities on their socioeconomic status, accessibility, and utilization of different providers. Principal Component Analysis was used to create a SES index that was used to examine inequities in perceptions and health seeking.

**Results:** Patent medicine dealers were the most perceived and actually nearest providers to the people. The next nearest providers to the people were private hospital/clinics in two communities while it was traditional healers in one. There were inequities in perception of accessibility and use of different providers which were tilted against the poorest socio-economic status (SES).

**Interpretation:** Inequities exist in how different SES groups perceive ease of accessibility and utilization of different providers for malaria treatment. Strategies for remediating this are suggested.

**503B**
Impact des déterminants socio-économiques sur la morbidité palustre et les recours aux soins en zone forestière camerounaise (provinces de l’Est et du Sud) [MIM-FF-282014]

F. Félicien
IFORD & IRS/UCAC Yaoundé, Cameroun

**Introduction:** La persistance de l’endémie palustre au Cameroun et plus spécifiquement dans la zone forestière en dépit des efforts thérapeutiques et prophylactiques déployés pour l’éradiquer nous a conduit au questionnement suivant: qu’est ce qui explique le fait que malgré les mesures prises les indicateurs de morbidité et de mortalité palustre ne s’améliorent pas ? Et comment les populations se comportent-elles face à cette maladie ?

**Methods:** Nous avons exploré par questionnaires les perceptions, connaissances et comportements des populations face à la maladie et les types de recours adoptés pour les soins curatifs lors d’un épisode palustre. Les enquêtes ont été réalisées à Hévécam au Sud Cameroun auprès de 1477 adultes (dans le cadre d’un projet Pal+) et à l’Est auprès de 562 femmes Baka et Bantou (dans le cadre d’un projet réalisé par AFGRID pour le CRS). Les données ont été exploitées grâce aux méthodes statistiques d’analyses descriptives bivariées et explicatives.

**Results:** L’analyse des données, dans les deux régions étudiées, met en évidence que la prise en compte de la réalité socio-économique est nécessaire pour la compréhension de la prévalence de la morbidité et du comportement (préventif et curatif) des populations forestières face à la maladie. Ainsi on note qu’au Sud et à l’Est, parmi les populations enquêtées, 1 personne sur 2 dit avoir dormi sous la moustiquaire la nuit précédente mais que les utilisateurs sont essentiellement des hommes adultes qui cherchent surtout à éviter les nuisances des moustiques (bruits et piqûres); le niveau d’instruction du chef de ménage et le niveau de vie du ménage influencent fortement les comportements de prévention antivectoriel (moustiquaire, insecticide etc …). De même, les travailleurs qui bénéficient d’aide financière pour les soins ont plus recours aux soins modernes qu’à l’automédication traditionnelle que les autres. On relève que les femmes sont plus attentives à la prévention au sein des ménages et en par...
ticulier à celle des enfants mais que le chef de ménage reste décisionnaire pour les soins de santé. De manière globale, il y a une sous-utilisation des moyens de prévention et une forte automédication "moderne" qui peut favoriser la résistance aux antipaludiques utilisés. 

**Interpretation:** Un effort devrait être fait en matière d’information, d’éducation et de sensibilisation des populations vis-à-vis du paludisme. Il doit en priorité viser les chefs de ménage qui prennent les décisions au sein du ménage.

**504C**

**Home management of urban malaria in Abidjan (Côte d'Ivoire) – Experience, meaning and practice related to palu** [MIM-SG-27965]  
S. Granado, M. Weiss, G. Cissé, M. Tanner, B. Obrist  
(1) Swiss Tropical Institute, Basel, Switzerland; (2) Centre Suisse de Recherches Scientifiques (CSRS) en Côte d’Ivoire, Abidjan, Côte d’Ivoire

**Introduction:** Up to now, most studies on malaria and its local interpretation have focused on rural areas, although the population living in urban areas is steadily increasing. Thus, there is still little known about home management of urban malaria. But we expect the heterogeneous and dynamic urban environment to influence the experiences, meanings and practices related to the illness. Our study therefore focuses on palu (French paludisme, i.e. malaria), the local term for malaria in the city of Abidjan.

**Methods:** Our study combines a classic ethnographic approach with an EMIC approach (Cultural Epidemiology). Furthermore, we chose a comparative approach looking at a slum and a better-off area. The EMIC interview was developed to investigate the distribution of experience, meaning and practice related to palu in a sample of 80 recently affected adults in each area. The ethnographic part examines the dynamics and the context in which palu is occurring.

**Results:** Although health providers and patients apparently use the same term, namely palu, the interpretations of the local population differ from the biomedical view. Members of the local population appropriated the term palu into the local disease system and health-related practices so that its notion was transformed into something distinct from the biomedically defined malaria. According to the local view, palu is a common illness in Abidjan. Its symptoms are very vague but could still largely correspond to signs of clinical malaria. In contrast the parasite carrying mosquito is only one of many causes in this harsh urban environment. Palu is rather used to express the daily risks of urban life for which people have little means to cope. In this context palu becomes an embodied metaphor for urban vulnerability. While it is beyond their means to change these causes, people can at least treat the resulting illness by taking local remedies and pharmaceutical pills. So palu becomes a strategy to do something about the urban risks. The consequential demand for medicines is covered and further encouraged by a great diversity of health providers all offering something to fight palu.

**Interpretation:** Public health implications of our findings are over- or mistreatment of fever episodes which can only be tackled by access to more effective diagnosis and high quality treatment in combination with a better control of the flow of pharmaceuticals.

**505A**

**Evaluating the Tanzania National Voucher Scheme** [MIM-KH-23484]  
K. Hanson, R. Nathan, T. Marchant, H. Mponda, C. Jones, S. Armstrong  
(1) London School of Hygiene and Tropical Medicine, London, UK; (2) Ifakara Health Research and Development Centre, Tanzania

**Introduction:** The National ITN strategy in Tanzania involves a two-pronged strategy of creating an enabling environment for promoting private manufacture and distribution of ITNs, and at the same time providing targeted subsidies to pregnant women through the Tanzania National Voucher Scheme. The voucher scheme began distributing vouchers in October 2004. A monitoring and evaluation strategy for the voucher scheme has been defined collaboratively by researchers and the National Malaria Control Programme.

**Methods:** Twenty-four districts, roughly one-fifth of the entire country, have been randomly selected as “sentinel districts” for M&E activities, stratified by implementation stage. In these districts there will be multiple rounds of household, health facility and retail level data collection. The first full round of data collection is ongoing. Data will be collected on ITN coverage...
of target groups, uptake and use of the voucher scheme, barriers to scheme uptake, status of implementation at the facility level, and availability of nets in local shops.

Results: Findings from the first round of TNVS household and facility surveys will be presented.

Interpretation: Results will be interpreted in the context of the phased roll-out of the TNVS. Implications for the design and implementation of ITN distribution schemes will be drawn.

506B

Human behaviour and socioeconomic factors as determinants of malaria vulnerability in urban Dar es Salaam, Tanzania [MIM-KK-32232]

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Introduction: Malaria transmission through human-mosquito contact is influenced by human behavioral and socio-economic characteristics as well as geographic location. Successful and sustainable implementation of control strategies requires understanding of mitigating factors that reduce malaria risk.

Methods: Detailed questionnaire, parasitological and entomological surveys will be conducted throughout a 55 km² study area in urban Dar es Salaam Tanzania. These will be overlapped with estimates of effective coverage with available prevention measures.

Results: We will identify the major human behaviors and socio-economic factors associated with vulnerability to malaria infection in urban Dar es Salaam. We also aim to determine whether community-based mosquito abatement reducez malaria risk in the most vulnerable groups and compare this approach with social marketing of insecticide treated nets.

Interpretation: This study will allow the effectiveness of of alternative vector control strategies to be evaluated in an operational urban setting in sub-Saharan Africa.

507C

The role of traditional healers in the treatment of malaria related illnesses among children in Tanga, Tanzania [MIM-MK-289728]

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Introduction: Since the Alma Ata declaration, the role of traditional healers in health care has been acknowledged and there have been efforts to incorporate them into the official health care systems. However, this process has not been fully realized because of lack of in-depth understanding of the context in which they operate. We attempted to contribute to this by carrying out an exploratory study on the role they play in the treatment of malaria related illnesses among children in Tanga, Tanzania.

Methods: We used individual interviews and participant observations to obtain information from a total of 58 traditional healers over a period of 18 months. Our objectives were to explore their ideas and knowledge about malaria related illnesses, modes of payment for their health care services, the kinds of training they received and their perceptions and the nature of relationship they have with modern health care providers.

Results: More than half of the traditional healers interviewed recognize the key symptoms of convulsions but attributed the cause to supernatural sources, i.e. sorcery and punishment for breach of moral codes. A few knew of the link between ‘malaria’ and convulsions but they also hold onto the personalistic aetiology idea. The treatment for convulsions involves elaborate symbolic healing (rituals) as well as use of herbal medication. Most traditional healers do not seem to treat ‘normal fever’. They however understand the link between ‘normal’ malaria and mosquitoes but they attribute its cause to other factors as well. They acknowledge that ‘nor-
mal malaria’ is better treated at the hospitals after blood test. Most traditional healers understood the symptoms of ‘anaemia’ among children. They acknowledge their limitations in managing it and preferred to refer patients to doctors. Payment for traditional health care services may not necessarily be cheaper but is more attractive because the family is given the discretion to wait and assess the progress of the patient before making payment.

Interpretation: Findings show that most traditional healers are willing to refer cases of ‘anaemia’ and fever but not convulsions. We suggest a dialogue with them so as to understand their worldview and to share with them why referrals are important in all cases.

508A
Role of traditional health practitioners in management of severe malaria among children below five years in Kilosa and Handeni Districts, Tanzania [MIM-EM-401280]

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Introduction: Prompt identification of malaria and adequate treatment are essential for preventing irreparable complications and most malaria deaths can be avoided. It is common for children to be brought to health facilities late or not at all, and the traditional healers are blamed as main cause. We studied the role of traditional healers in the management of severe malaria among children under five years in Kilosa and Handeni Districts, in Tanzania.

Methods: Community cross-sectional study was conducted in malaria holo-endemic Kilosa and Handeni Districts involving four villages selected purposively on the basis of statistics on number of traditional health practitioners involved in the management of severe malaria in children. In the study area, there is ongoing WHO study to evaluate the efficacy of Rectocap on severe malaria. A total of 41 traditional health practitioners and 84 caretakers were identified purposively at the community level. In-depth interviews were used to gather information from caretakers and healers. Sixteen Focus Group Discussions (FGDs) involving traditional health practitioners, caretakers and community leaders were carried out.

Results: Home management of fever involving sponging or washing by hot water at the household level, was widely practiced by caretakers. In traditional healers’ management, five stages through which a child suffering from severe malaria in the course of healing were identified at the practitioners’ compound, including reception, bathing/sponging, diagnosis, treatment and prevention. There was normally a pause after treatment, whereby a child was referred for modern care for malaria treatment and consequently, the child had to be brought back for preventive rituals. There was a tallying finding that, traditional health practitioners’ and mothers were not linking severe malaria, the local illness term degedeged to biomedically-defined malaria. The healing process was therefore organized in stages and failure to abide to the procedure could lead to relapse of degedege, which was believed to be caused by evil spirits. Majority of mothers (75%) considered degedege among children to be caused by evil spirits. It is worth noting that referrals to health facility increased by 75% with introduction of Rectocap Project whereby the project staff facilitated the process after traditional medical care with the provision of suppository.

Interpretation: Traditional healing not necessarily an impediment in seeking modern care. Fostering joint training to manage malaria between healers and health workers periodically including operations research to identify modalities of collaboration is essential.

509B
Influence of socio-economic factors on utilization of mosquito nets in Zambia [MIM-FM-272304]

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Introduction: It is acknowledged that the value of an effective intervention for saving human life can be compromised if socio-economic factors are ignored. This paper connects socio-cultural factors to low utilization of insecticide treated net (ITN) and highlights close linkage between malaria, education, poverty and equity.

Methods: This study is a desk review of a Roll Back Malaria Baseline Survey, 2001 and the Zambia Demo-
graphic Health Survey (Z DHS) for 2001–2002. Data includes ownership of mosquito nets, net utilization, i.e. “having slept under the mosquito net the previous night before the interview” and socio-economic issues. A classification of poor (quintile 1) to richest (quintile 5) modified World Bank Classification was used to indicate socio-economic status of respondents.

Results: Overall, 14% in poorest (quintile) slept under an untreated net versus 47% in the richest quintile. Seven percent pregnant women in poor quintile slept under an ITN compared to 14% in the rich quintile. Also, 9% pregnant women with no education slept under an ITN compared with 30% who had secondary school education. Over 60% of respondents slept outside a house during a funeral, with no difference between poor and rich and 20% slept outside during a church activity. Less than 7% slept outside during wedding ceremony, hunting, mining or political campaigns. This study shows low ITN utilization, which calls for increased advocacy and awareness.

Interpretation: Inequalities in ownership and use of ITNs vary according to education levels and socio-economics which underpin the need to promote disease interventions within a socio-economic framework of poverty, education and disease elaborated in the MDGs.

510C
Les impacts psychosociologiques et économiques du paludisme chez les femmes ayant des enfants en dessous de 5 ans [MIM-RM-460306]

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Introduction: Le but de cette étude est d’étudier les facteurs psychosociologiques et économiques du paludisme et de mettre en évidence les impacts sociaux et économiques. Les femmes et les enfants de moins de 5 ans ont été inclus dans l’étude. Le but est de comprendre la manière dont le paludisme affecte les femmes et leurs enfants et de mesurer les conséquences économiques. L’étude a été réalisée dans la région de Lambarene, au Gabon.

Methods: Des entretiens en profondeur ont été menés avec des femmes de différentes catégories socio-économiques afin d’évaluer les impacts du paludisme sur leur vie quotidienne et leurs économies. Des enquêtes ont également été effectuées auprès des enfants de moins de 5 ans.

Results: Les résultats ont montré que le paludisme a des impacts significatifs sur la vie économique des femmes et des enfants. Les femmes ont rapporté des problèmes de santé financière liés à la maladie et des difficultés à couvrir les coûts des médicaments. Les enfants ont été affectés par l’absentéisme scolaire et la malnutrition. Les impacts économiques ont été plus prononcés dans les catégories socio-économiques les plus défavorisées.

Interpretation: Ces résultats soulignent l’importance de la lutte contre le paludisme afin de prévenir les impacts sociaux et économiques sur les femmes et leurs enfants. Des stratégies de soutien socio-économique peuvent être mises en place pour réduire ces impacts négatifs.

511A
Malaria: metaphor or disease? A study of malaria diagnosis among women in moshi district, kilimanjaro region, tanzania [MIM-RM-20895]

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Introduction: The introduction of more expensive anti-malarials is driving a greater awareness of the problems of over diagnosis of malaria. While technical aspects of diagnosis have been explored, little is known about using a diagnostic label of malaria to achieve social ends. This is particularly important in women who care for or suffer from the greatest burden of disease. We thus conducted a qualitative study among women to explore their attitudes to malaria, and their experiences.

Methods: We conducted 20 in-depth interviews among women exiting from a medical consultation or caring for a sick relative in a Regional hospital and focus
group discussions among women recruited from those waiting for antenatal or gynecological consultations. Both were located in an area of low-medium malaria endemicity. Data were transcribed verbatim and analyzed by thematic analysis.

Results: Women reported a dual conception of malaria. On one hand the biomedical model of malaria was recognized. But this was complemented by a personalized view of ‘malaria’. In this view women reported highly variable symptoms, which only they could accurately identify. Thus, women spoke of ‘my malaria’ as being ‘usually slide-negative’ or ‘not accompanied by fever’, etc. In this model, malaria was removed from the biomedical sphere. Women reporting such attitudes were reluctant to accept denial of malaria as a diagnosis and were willing to seek alternative opinions. Women described using malaria for social ends; to avoid social or work duties, to avoid the embarrassment associated with painful menstruation, early signs of pregnancy, or conceal stigmatizing illness including gynecological problems, or to avoid unwanted sex with their spouses. Women who did not report using malaria in this way acknowledged that such behavior was common. However, women felt somewhat embarrassed by these admissions, but there was a recognition that such behavior was in some respects ‘wrong’ but was part of a necessary range of life skills; malaria was chosen for such a role due to its lack of associated stigma and non-specific symptoms.

Interpretation: Our findings suggest that in malaria-endemic areas of Africa women are active players in the sphere of malaria diagnosis, effective responses need to include the attitudes, feelings and social strategies of women in relation to malaria diagnosis.

512B
Malaria treatment seeking and the influence of trust: Experiences from NE Tanzania [MIM-AM-101348]
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Introduction: Treatment seeking for malaria has been widely studied but its full complexity is still not understood. The factors commonly identified as influencing this behaviour include patient attitude and beliefs, cost and perception of quality of services. Yet most studies focusing on malaria have primarily considered patients’ understanding of the causes of malaria as the key influence over treatment seeking and, therefore, have led to recommendations about the need to improve user education.

Methods: A grounded analysis on care seeking with concentration on trust was conducted. Data was collected from villages and households using methods of Rapid Rural Appraisal (RRA), Focus Group Discussions (FGDS) and in-depth interviews with cases of malaria. Households were selected by rotating a pencil, using the public health facility as a starting point, and picking households in its direction of line. The selection criteria for the cases included episode of high or low fever or other symptoms of malaria in the past two weeks, as the most important. Other factors considered included socio-economic status, sex, age and distance from the health facility.

Results: Regardless of the village or household socio-economic level, there is always a considerable delay in initiating treatment, highly influenced by perceptions of symptoms. Even after the decision to seek care is made, there is always considerable delay, due to influences of cost of care and gender dynamics. Choice of providers was highly influenced by trust, both personal and institutional. Institutional trust was based on institutional motives, indicators of good quality of care, competence and training. Personal trust reflected knowledge of training and personal relationships. The type of antimalarial drugs dispensed was sometimes used to judge quality of care, which indirectly influenced trust of a provider. Fees for care undermined trust of all providers as they were associated with profit motives or even corruption.

Interpretation: This study highlights the influence of patient’s interactions with the health systems and its influence on malaria health seeking behaviour. It also suggests that changing drug treatment can have unintended effects on health seeking behaviour.
Effects of users’ educational status on the effectiveness of mosquito bednets in bama, borno state of nigeria and mora far north province of cameroon [MIM-AN-2966597]

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Introduction: Roll back malaria treated bednets distribution in bama and mora led to the adoption of the “net culture” for malaria prevention in these areas. Field trips were done between june and october 2004 to catch anopheles for entomological surveys in nigeria and cameroon. Remarkable observations were made on the effectiveness of malaria prevention and control using mosquito nets at these sites. They need to be properly addressed to improve the efficiency of bednets among users of low educational level.

Methods: A two steps random sampling technique was used to select 50 users from 30 and 25 houses with treated nets and 100 users from 45 and 35 houses with untreated nets in bama and mora, respectively. An unstructured interview was done with 300 bednet users from 120 houses to assess their ability to efficiently use bednets. Inspections were done by 7 a.m. and 5 p.m. to sort out treated from untreated net users, rbm skills learners from others and appraise the state of nets after before use. Surveys of 10 min each were made in selected houses for anopheles collection. Anopheles densities in the two categories of houses were evaluated and variations in rooms adjacent to those with treated nets determined. Data analysis was with epi info and spss 11.0.

Results: The distribution of treated nets incited 90% purchase of untreated ones; 75% of users did not differentiate hospital from market nets. Considering prices rather than their preventive qualities that 90% understood well, 60% bought any net from the market. Treated nets hung, even not in use, reduced mosquito density in human dwellings. No mosquito caught (1700) in sampled rooms in bama and mora was from rooms with treated nets or those adjacent to them. However, rooms (5%) sprayed the previous night still had 10–20 mosquitoes. The residual effect of itn led to 90% mosquito reduction in adjacent rooms without net; this effect decreased as the distance to the treated netted room increased. Rooms located near exit doors or breeding pools, had lower mosquito reduction of 35% and 40%, respectively. Houses (50%) with untreated nets had 15–20 fed anopheles within the nets; they had entered the eve or at night, and preyed on baits. Itn users (75%) attended rbm demonstration seminars, 85% did not master it; no untreated net user was taught any skill, thus 90% had fed mosquitoes in their net every morning. No user had the skills required for successful use of bednets; 60% started the nights’ rest outside and went in after 11 p.m. when it is cooler.

Interpretation: Rbm teams may teach untreated net users how, where and when to use it for effective prevention. Mora and bama are arid zones where many humans sleep outdoors; adapting the use of treated or untreated nets to their environment will achieve prevention.

Influence of education and knowledge about malaria on perceptions and practices to its control in southeast Nigeria [MIM-UO-5410]

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Introduction: Many methods for reducing malaria burden depend on knowledge about the disease and its control. Education is one of the major determinants of health behaviour and increases people’s awareness of disease etiology and control. Widespread epidemiologic evidence shows that social structures that undermine individual educational attainment can influence health outcomes, above and beyond well known individual socio-economic risk. The study examined how education and knowledge that people have about malaria influence their actual practices in treating and preventing the disease.
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Methods: The study was undertaken in four malaria holo-endemic villages in southeastern Nigeria to investigate whether the people’s level of education and what they know about malaria affect how they seek treatment and prevention for the disease. Pre-tested interviewer-administered questionnaires were used to collect data from randomly selected householders. In each selected household, one woman (primary care giver) or in her absence, a representing head of household was interviewed. Information was sought from the respondents on their knowledge of the causes and symptoms of malaria. They were also asked who within the household malaria was more serious to, different methods of treating and preventing malaria, the malaria preventive tools the households had, especially untreated and insecticide-treated mosquito nets.

Results: There was low to moderate levels of knowledge about malaria and its control. However, level of formal education statistically significantly explained the perception that mosquitoes transmit malaria, and the correct identification of the symptoms of malaria. In addition, educated people and people who thought mosquitoes caused malaria were more likely to own mosquito nets.

Interpretation: The findings indicate that improved education (even just a few years of primary education) can have a positive impact on malaria burden. Hence, medium/long-term improvement of overall literacy rates and short-term health education campaigns will have a positive impact on malaria control.

515B

Scaling-up of intermittent preventive treatment of malaria in pregnancy: Health providers’ knowledge, attitudes and practices in urban/rural Nigeria

[MIM-ES-303456]

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Introduction: Malaria poses a substantial risk to the pregnant mother and child. As a result, Nigeria has adopted intermittent preventive treatment (IPT) as one of the malaria control tools. There are plans to scale up the intervention but the key issue is determining whether healthcare providers have the required knowledge and their responsiveness to the use of IPT for malaria control in pregnancy. We investigated the knowledge, attitudes and practices of healthcare providers in Nigerian communities.

Methods: Pre-tested structured questionnaires exploring the knowledge, attitudes and practices (KAP) were administered to healthcare providers in two urban (n=85) and two rural (n=67) local government areas (LGAs) in Enugu State, Nigeria. The two urban LGAs namely Enugu North and Enugu South were purposively selected to include Enugu (the State capital), and the two randomly selected rural LGAs were Ezeagu and Udi. The questionnaires were administered under strict supervision by trained field workers. The healthcare providers were heads (or appropriate representatives) of all private and public health facilities (hospitals, clinics, medical centres, maternity homes, health centres/posts.) in each study area.

Results: There was a significant difference (59.87, d.f. = 6, p < 0.0001) in the distribution of health facilities in the study communities manned by the respondents. In the urban, 63.58% of the respondents were male medical doctors and in the rural, most (49.25%) were female community health workers. Although there was no significant difference (3.625, d.f. = 1, p = 0.0569) in the respondents’ knowledge of IPT, 56.72% in the rural communities knew of IPT compared to 41.18% in the urban. A significant proportion (7.152, d.f. = 1, p = 0.0075) of the respondents in the rural (73.68%) knew of the federal government policy on IPT when compared to the urban (42.86%). Most of the respondents in the study communities know that IPT is useful and effective, and will be acceptable to consumers. There was a significant difference (40.41, d.f. = 9, p < 0.0001) in the knowledge of the drugs used for IPT in both communities. In the urban, chloroquine, CQ (51.43%) and sulfadoxine/pyrimethamine, SP (57.14%) are used for IPT but CQ (65.79%) and pyrimethamine (52.63%) are used in the rural. However, only 22.86 and 5.26% of respondents in the urban and rural communities, respectively, know that the drugs are administered twice or three times from 16 weeks of gestation.
**Abstracts / Acta Tropica 95S (2005) S1–S506**

**516C**

Malaria treatment-seeking practices in a highland area of Kenya [MIM-PS-385679]

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**Introduction:** Epidemics in highland areas of Kenya cause severe morbidity and mortality, but treatment-seeking behavior for malaria in these areas has not been characterized. Patterns of treatment-seeking may affect clinical outcomes in malaria. In the present study, treatment-seeking practices for malaria were assessed in the highland area of Kipsamoite, Kenya.

**Methods:** A questionnaire on malaria treatment-seeking practices was validated and administered to 117 randomly selected households, representing 20% of all households in the highland area of Kipsamoite, Kenya.

**Results:** Episodes of malaria, as defined by a local term specific to malaria, were reported in 100 adults and 66 children under the age of 12 in these households. The most frequent initial sources of treatment for malaria were assessed in the highland area of Kipsamoite, Kenya.

**Methods:** A questionnaire on malaria treatment-seeking practices was validated and administered to 117 randomly selected households, representing 20% of all households in the highland area of Kipsamoite, Kenya.

**Results:** Episodes of malaria, as defined by a local term specific to malaria, were reported in 100 adults and 66 children under the age of 12 in these households. The most frequent initial sources of treatment for malaria were a health center (66.0 and 66.7%), local shops (19.0 and 30.3%), and traditional healers or herbalists (9.0 and 1.5%). Adults and children who initially visited a health center for malaria treatment were significantly more likely to recover from their illness and require no further treatment than those who initially went to a local shop for medication (recovery rate for health centers versus local shop in adults, 84.9% versus 36.8%, p < 0.0001, and in children, 79.6% versus 40.0%, p = 0.002). This was largely attributable to the provision of anti-malarial medication at health centers but not local shops. Among individuals who could remember the medication they were given, 100% of adults and children seen at health centers received anti-malarial medication, but only 29.4% of adults and 5.8% of children seen at local shops received anti-malarial medication.

**Interpretation:** A significant proportion of this highland population seeks care for malaria at local shops. Education of local shopkeepers in the symptoms and treatment of malaria may lead to improvement in the initial care of malaria in highland areas.

**517A**

Reducing morbidity of under-five children by empowering community members [MIM-ET-0]

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**Introduction:** In order to create a sustained reduction in infant and child mortality through increased access to community-based health care and improved quality of health services in the project area, the Plan child survival project supported the MOH community outreach policy to empower communities to improve local health resources and increase access to health services. This project was located in three Health Districts of the western part of Cameroon’s heavily forested Eastern Province.

**Methods:** The project targeted 38,009 children under five, 8447 infants 0–11 months and 48,568 women of reproductive age. It strengthened the capacity of community resource persons. Women’s groups were supported to set up community based ITN distribution centres. BCC activities raised awareness about health issues and promote health-seeking behaviors. Health facilities were provided supplies and equipment and re-training of staff. Outreach services to affiliated communities were supported. The Knowledge, Practices and Coverage survey was used to assess the capacity of randomly selected mothers of children 0–23 months to prevent, promote and take care of sick children at home at the beginning and end of the project.

**Results:** Household possession of a bed net increased from 6 to 44%. While the proportion of children aged 0–23 months who slept under an ITN increased from
0 to 33.6%. Three out of every five pregnant women are prescribed chloroquine for malaria prevention during pregnancy. Early breastfeeding initiation increased from 10% in 2000 to 16.4% in 2004. The proportion of mothers who declared they exclusively breastfeed increased from 29% at base line to 57%. The proportion of children assessed who were under weight decreased from 19% in 2000 to 16.4% in 2004. Only 24.7% of infants were fully vaccinated at their first birthday in 2000, and 54.9% in 2004. The proportion of pregnant mothers who received at least the second dose of tetanus toxoid before the birth of their youngest child increased from 47 to 55%. Only three out of every five mothers know at least two danger signs that make them seek out-of-home care for the sick child. Even though 56.2% of mothers declare that they give same or increased amounts of fluid to child with diarrhoea, only 18.2% give ORS/HMF. Only 23.4% of the children with diarrhoea are given more fluids and food. Maternal hand washing behaviour remains generally low but increased from 2.6% in 2000 to 20.6% in 2004.

Interpretation: Significant improvement in treatment and prevention indicators for malaria can be achieved within the context of an integrated community-based child health program.

518B
Equity of coverage of interventions to reduce child mortality: Untreated nets, insecticide treated nets and immunisation [MIM-JW-224880]
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Introduction: Insecticide treated nets (ITNs) and childhood vaccinations are two of our most powerful interventions for reducing child mortality. The poor and vulnerable should not be excluded. We tested two hypotheses (1) public health programmes and projects have achieved greater equity of coverage of ITNs than local commercial markets have in untreated nets, and (2) coverage achieved by local commercial markets in untreated nets is less equitable than that of childhood vaccination through EPI.

Methods: We analysed coverage of four interventions using Demographic and Health Survey (DHS) and Multiple Indicator Cluster survey (MICs) data from 26 countries (28 datasets), which were any net, ever treated net (ITN), never treated net, and EPI vaccination. As these surveys were conducted before 2003 we assumed that almost all of the ‘ever treated’ nets were delivered through public health programmes or projects; and ‘never treated’ nets were from local commercial markets. We used the concentration index to compare the equity of coverage of these interventions across socio-economic quintiles. Comparisons of coverage and equity between interventions were made using the paired t-test.

Results: Coverage of both never treated nets and EPI was significantly more equitable than coverage of ever treated nets across the 26 countries (p < 0.001 and p < 0.001). Across countries of East and Southern Africa, coverage of EPI was more equitable than that of never treated nets (p = 0.003), conversely in countries of West, Central and Sahelian Africa coverage of never treated nets was more equitable than that of EPI (p = 0.04).

Interpretation: Commercial markets have achieved greater equity of net coverage than public health programmes have with ITNs. There are marked regional variations in the equity of coverage achieved by EPI, which has implications for delivering ITNs through EPI.

519C
Household expenditure on mosquito control in The Gambia and Tanzania: An analysis of patterns of expenditure and determinants of demand [MIM-VW-263010]
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Introduction: While increased efforts are being made to promote the large-scale use of ITNs as a strategy for malaria control, many households continue to invest in a wide variety of mosquito protection products/activities that absorb a significant amount of their income. Gaining a better understanding of expenditure can help inform the effective take-up of nets as well as highlight ways in which limited family resources could be more effectively used to promote better health.

Methods: One thousand seven hundred households from both Farafenni (The Gambia) and Tanga (Tanzania) were interviewed about their expenditure on malaria prevention over the past 2 weeks. Interviews were staggered over a 12 month period. Comparisons in fortnightly household expenditure are made across several forms of prevention including bed nets, treating and repairing bed nets, aerosols, coils, indoor spraying, smoke and other prevention strategies such as drinking herbs and cleaning outside environment. Expenditure is also compared between wet and dry seasons. This is followed by an analysis of the principal determinants of each household’s expenditure on each form of protection against mosquitoes.

Results: The Gambia: over a 2 week period households spent an average of 23.11D on mosquito protection. Overall expenditure on nets, including treatment and repair, constituted only 10% of total fortnightly expenditure on prevention. Considerably more is spent on other types of prevention. An average of 8.40D is spent on coils; 4.20D on indoor sprays; 3.09D on smoke and 3.06D on aerosols. In total, these four measures make up 81% of total fortnightly expenditure on prevention. Expenditure on coils is 54% higher in the wet season compared to the rest of the year, 122% higher for smoke, and 140% higher for ‘other prevention’. Wealthier households spent significantly more on mosquito prevention as did those aged 20–29 years and those in ‘skilled’ occupations. The same analysis is underway for Tanzania and results will be available for the MIM conference.

Interpretation: Households need to be informed about the potential net savings in morbidity and productivity that can be achieved by using ITNs. Barriers to investing in ITNs such as their relative high upfront cost also need to be addressed.

26. Pregnancy-associated malaria

Pregnancy-associated malaria

Methods: One thousand seven hundred households from both Farafenni (The Gambia) and Tanga (Tanzania) were interviewed about their expenditure on malaria prevention over the past 2 weeks. Interviews were staggered over a 12 month period. Comparisons in fortnightly household expenditure are made across several forms of prevention including bed nets, treating and repairing bed nets, aerosols, coils, indoor spraying, smoke and other prevention strategies such as drinking herbs and cleaning outside environment. Expenditure is also compared between wet and dry seasons. This is followed by an analysis of the principal determinants of each household’s expenditure on each form of protection against mosquitoes.

Results: The Gambia: over a 2 week period households spent an average of 23.11D on mosquito protection. Overall expenditure on nets, including treatment and repair, constituted only 10% of total fortnightly expenditure on prevention. Considerably more is spent on other types of prevention. An average of 8.40D is spent on coils; 4.20D on indoor sprays; 3.09D on smoke and 3.06D on aerosols. In total, these four measures make up 81% of total fortnightly expenditure on prevention. Expenditure on coils is 54% higher in the wet season compared to the rest of the year, 122% higher for smoke, and 140% higher for ‘other prevention’. Wealthier households spent significantly more on mosquito prevention as did those aged 20–29 years and those in ‘skilled’ occupations. The same analysis is underway for Tanzania and results will be available for the MIM conference.

Interpretation: Households need to be informed about the potential net savings in morbidity and productivity that can be achieved by using ITNs. Barriers to investing in ITNs such as their relative high upfront cost also need to be addressed.

26. Pregnancy-associated malaria

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

520A

Pharmacokinetics of quinine and its metabolites in pregnant Sudanese women infected with Plasmodium falciparum [MIM-IA-9191]

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Introduction: Quinine is the principal alkaloid of cinchona bark. It is an important drug for the treatment of severe or multidrug-resistant Plasmodium falciparum malaria. Quinine is eliminated mainly by metabolism and only 18% of absorbed drug is excreted unchanged in the urine. The pharmacokinetic parameters of quinine are reported to be significantly altered during malaria and this alteration is in proportion to the severity of the infection. In Africa each year some 24 million women become pregnant in malaria endemic areas. Pregnant women in comparison to non-pregnant women are more susceptible to malaria and develop clinical attack of malaria and serious complications. There are no pharmacokinetic data from healthy pregnant women, but patients with falciparum malaria in the third trimester of pregnancy showed significant pharmacokinetic differences when compared with other adults in the acute phase of malaria. There is a lack of detailed pharmacokinetic studies of this drug in Sudan therefore there is urgent need for the optimisation of the use of this drug through pharmacokinetic studies. The aim of this study is to investigate the pharmacokinetics of quinine and its metabolites in pregnant Sudanese women with acute Plasmodium falciparum malaria.
Methods: In a randomised study, nine pregnant and eight non-pregnant Sudanese women in their second and third trimester infected with Plasmodium falciparum received a single dose of quinine hydrochloride (10 mg/kg body weight) given as intravenous infusion during 2 h. Blood and urine samples were collected before quinine administration and up to 72 h thereafter. Plasma and urine samples were analysed for quinine and its metabolites, 3-hydroxyquinine (3-OHQ), (10R)-10,11-dihydroxyquinine ((10R)-DOHQ) and (10S)-10,11-dihydroxyquinine ((10S)-DOHQ) using high performance liquid chromatography.

Results: During phase II, the mean AUC of quinine decreased significantly by 30% in pregnant women \((P < 0.001)\) and by 48% in non-pregnant women \((P < 0.01)\). In non-pregnant women there was a significant decrease in mean AUC of 3-hydroxyquinine by 20% \((P < 0.01)\) and \(C_{\text{max}}\) of quinine, \(t_{\text{max}}\), \(t_{1/2}\) of 3-hydroxyquinine in both groups. No significant change in the pharmacokinetic parameters of (10R)-10,11-dihydroxyquinine and (10S)-10,11-dihydroxyquinine was observed. No significant variation in the pharmacokinetic parameters of quinine and its metabolites were observed between pregnant women and controls neither during phase I nor II.

Interpretation: The results of this study show that no need for quinine dose adjustment when treating pregnant women infected with *P. falciparum*.

521B
Prevalence and risk factors for *Plasmodium falciparum* malaria in pregnant women of eastern Sudan

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Introduction: Pregnant women are more susceptible to malaria, which is associated with serious adverse effects on pregnancy. The presentation of malaria during pregnancy varies according to the level of transmission in the area. Our study aimed to demonstrate the prevalence and risk factors for malaria (age, parity and gestational age) among pregnant women of eastern Sudan, which is characterized by unstable malaria transmission.

Methods: The prevalence and possible risk factors for *Plasmodium falciparum* malaria were investigated in 744 pregnant Sudanese women attending the antenatal clinic of New Halfa Teaching Hospital, eastern Sudan, during October 2003 to April 2004.

Results: A total 102 (13.7%) had *P. falciparum* malaria, 18 (17.6%) of these were severe cases (jaundice and severe anaemia). Univariate and multivariate analysis showed that, age and parity were not associated with malaria. Women who attended the antenatal clinic in the third trimester were at highest risk for malaria \((\text{OR} = 1.58, 95\% \text{ CI} = 1.02–2.4; P < 0.05)\). Women with malaria had significantly lower mean haemoglobin \((9.4 \text{ g/dl}, 95\% \text{ CI} 9.1–9.7 \text{ versus } 10.7, \text{ CI} 10.6–10.8, P < 0.05)\).

Interpretation: The results suggest that *P. falciparum* malaria is common in pregnant women attending antenatal care and that anaemia is an important complication. Preventive measures (chemoprophylaxis and insecticide-treated bednets) may be beneficial in this area for all women irrespective of age or parity.

522C
Effectiveness of quinine monotherapy for the treatment of *Plasmodium falciparum* infection in pregnant women in Lambarene, Gabon

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Introduction: To date there is a limited choice of approved treatments for chloroquine resistant *Plasmodium falciparum* malaria in pregnancy in most African countries. In these countries quinine remains the first line therapy for pregnant women. The aim of
this clinical trial was to assess the actual usefulness of quinine monotherapy when prescribed under routine health care facilities in sub-Saharan Africa.

**Methods:** Pregnant women participating in a longitudinal immuno-epidemiological survey in Lambaréne, Gabon, and presenting with *Plasmodium falciparum* parasitemia at monthly blood smear examinations were offered treatment with oral 7-day quinine monotherapy according to national health guidelines. Pregnant women were offered 7-day oral quinine sulphate 10 mg/kg thrice daily. Clinical examinations and laboratory tests were performed on admission, on days 28 and 56, to assess the effectiveness of this standard regimen.

**Results:** A total of 50 pregnant women were included in this study. During the first month of follow-up 20 women presented with peripheral falciparum parasitaemia and 30 had negative thick blood smears. The majority of patients included in this study presented with asymptomatic *P. falciparum* parasitaemia. The effectiveness of seven days quinine monotherapy was therefore 60% (95% CI: 46–72) in our study population by day 28.

**Interpretation:** We conclude that a 7-day course of quinine has a poor effectiveness and that alternative treatment regimens for malaria in pregnant women should be assessed.

523A

La diminution érythrocytaire du récepteur au complément de type 1 (CR1) au cours de la grossesse est accentuée par l’infection à *Plasmodium falciparum*. Notre objectif était de comparer l’expression de CR1 au cours de la grossesse et lors d’une infection à *Plasmodium* chez la femme enceinte.

**Methods:** Quatre vingt femmes enceintes ont été incluses dans l’étude. 71 femmes dépistées négatives pour *Plasmodium* par goutte épaisse et frottis, et 9 femmes positives pour *Plasmodium falciparum*. Vingt quatre femmes se présentaient au premier trimestre de grossesse, 14 au second, 19 au troisième et 14 étaient analysées au moment de l’accouchement. Les femmes enceintes porteurs de *Plasmodium* ont été incluses au moment de l’accouchement. La quantification de l’expression érythrocytaire du CR1 a été réalisée par cytométrie (Cohen et al, 1987) à partir de sang périphérique.

**Results:** Les résultats montrent que le taux de CR1 diminue au cours de la grossesse pour être au plus bas au moment de l’accouchement. Ainsi, en moyenne, au premier trimestre de grossesse, le taux est de 1365 ± 425 récepteurs par hématie, il est de 1089 ± 390 pour le second trimestre, de 898 ± 324 et 730 ± 208 respectivement pour le troisième trimestre et au moment de l’accouchement. Aucune corrélation n’a été observée avec l’âge de la femme. Seules 9 femmes présentaient un nombre de grossesses supérieur à 2. Aucune corrélation n’a donc été observée avec le nombre de grossesses. Chez les femmes dont la parasitèmie variait de 11 à 2250 parasites pour 100 leucocytes, le taux moyen de CR1 était de 386 ± 83. Une différence significative est observée entre les femmes accouchantes porteurs de *Plasmodium falciparum* et les femmes accouchantes négatives. Aucune corrélation n’a été observée avec l’intensité de la parasitèmie.

**Interpretation:** Le CR1 érythrocytaire est donc régulé au cours de la grossesse mais aussi par l’infection à *Plasmodium*. L’implication du CR1 érythrocytaire dans la physiopathologie du paludisme et la grossesse reste à déterminer.
Malariometric indices, malaria parasitisation and systemic cytokine bias in pregnant women from South Western Cameroon [MIM-TA-222295]

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Introduction: Diminished pre-pregnancy immunity to Plasmodium predisposes expectant mothers to malaria in endemic areas. Although numerous studies have investigated the interaction between pregnancy and the disease, information on the effect of malariometric indices especially on systemic immunity in this high-risk group is limited. A study was therefore undertaken to determine the effect of age, gravidity/parity and gestation on malaria parasitisation and functional Tβ cell heterogeneity in pregnancy.

Methods: A cohort of 175 women attending antenatal clinics in South Western Cameroon was recruited from March to September 2002. Malaria parasitaemia was determined by the microscopic examination of Giemsa-stained thick blood smears of the peripheral blood and plasma cytokine levels by sandwich enzyme-linked immunosorbent assay (ELISA). Questionnaires were administered to establish age, gravidity/parity and last menstrual period (LMP) and gestational age (GA) calculated from the LMP.

Results: It was observed that 25.7% (45/175) had microscopic malaria parasitaemia (MP), with a decreased risk observed with increasing GA (P = 0.049) and gravidity (P = 0.061). Mean interleukin-4 (IL-4) levels were significantly higher than interferon gamma (IFN) (P = 0.0004) and this balance was not altered by parasitisation as well as gravidity and parity status. IFN levels were, however, higher in older women compared to their younger counterparts (P = 0.04) and in the second compared to first trimester of gestation (P = 0.04). IL-4 levels were impaired in parasitised individuals to levels similar to IFN (P = 0.118).

Interpretation: The ability to control MP, in gestation, is age, gravidity and parity dependent, with a possible role for GA. Systemic cytokine profile is also biased towards Th2 responses and unperturbed by indices and malaria parasitisation in this endemic area.

Malaria in pregnancy and use of insecticide treated bed-nets among primigravids attending antenatal clinic in private hospitals in Anambra state [MIM-DA-258478]

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Introduction: Pregnant women in malaria endemic communities are at risk of malaria infection. The principal effect of malaria during pregnancy is anaemia. The presence of parasites in placenta of mothers result to impairment of fetal nutrition leading to low birth weight in babies born to such mothers.

Methods: Thick blood films were prepared using blood from placenta of 50 primigravids after delivery. This was stained with giemsa stain and examined for malaria parasites. Also and already pre-tested structured questionnaire was administered to these women. This is to determine their level of knowledge on use of ITNs for prevention of malaria.

Results: Out of the 50 placenta of primigravids examined for malaria parasites, 66% were positive for Plasmodium spp. There was a significant difference (p = 0.05) in the mean birth weight of babies born to mothers positive for malaria (2.7 kg) and babies born to negative mothers (3.8 kg). Also 12 out of the 50 women (24%) interviewed had knowledge about insecticide treated bed-net but none of primigravids have ever seen or used ITNs.

Interpretation: There is need for vigorous effort and emphasis on prevention of malaria especially during pregnancy through distribution of nets to pregnant women and creation of better venues for information dissemination.

Infected erythrocyte surface exposure of DBL-domains of the PfEMP1 VAR2CSA [MIM-LB-382162]

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Introduction: CSA adhering parasites express a unique PIEMPI VAR2CSA on the surface of the infected erythrocyte (IE). Women acquire antibodies against VAR2CSA as a function of parity and having these VAR2CSA antibodies is associated with protection from PAM. Due to the large molecular weight of VAR2CSA it is not possible to determine the protein structure and predict surface exposed domains. Here we look for surface exposure of VAR2CSA on a CSA adhering parasite using antibodies raised in mice and rabbits.

Methods: We cloned and expressed all 6 DBL domains in an E. coli expression system and in the eukaryotic baculovirus expression system. The recombinant proteins were quality tested by SDS page, western blot analysis and used to immunize mice and rabbits using different adjuvants. The animal sera were tested for domain specificity in ELISA. To examine the specificity of the antibodies against VAR2CSA expressed on the surface of IE, NF54CSA IE were stained with the sera using a flowcytometry-based assay. The NF54CSA parasite line is a NF54 line selected for the ability to bind to CSA and further selected with rabbit anti VAR2CSA antisera as described in Salanti et al. (2004).

Results: Immune sera from animals immunized with the recombinant proteins expressed in E. coli or insect cells showed clear domain-specificity when tested in ELISA. When sera from mice immunized with E. coli produced proteins were used for staining of VAR2CSA on the surface of IE, none of the sera showed any recognition of the IE. Mice immunized with the proteins deriving from the same gene-segments but expressed in insect cells did recognize the VAR2CSA on the surface of the IE. With the immune sera from mice immunized with E. coli produced proteins were used for staining of VAR2CSA on the surface of the IE. With the immune sera from mice immunized with proteins expressed in insect cells it was possible to stain for DBL1-X, DBL2-X, DBL3-X, DBL5-e and DBL6-e but not DBL4-e. An N-terminal sequencing of the DBL4-e protein identified the leader sequence suggesting that the protein used for immunizations was recombinant DBL4-e. Furthermore the antisera reacted with a protein of the size of VAR2CSA in western blot. When comparing the adjuvants there was no clear pattern as to which was the most efficient but it seems like domains three and five were more immunogenic than the other domains since functionally anti-sera were achieved using all four adjuvants. In the rabbits the highest surface reactivity was seen with sera after about eight immunizations.

Interpretation: We report for the first time that five out of six DBL domains of VAR2CSA are surface exposed on the IE in such a way that antibodies can bind to them. The importance of using eukaryotic cells for recombinant protein expression is also emphasized.

527B Incidence of placental malaria among women presenting with abortions, still births and premature deliveries at University Teaching Hospital, Lusaka: A preliminary report [MIM-MC-218362]


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Introduction: Malaria is endemic in all the nine provinces of Zambia. It is among the most pressing health problems in Zambia with children and pregnant women being at higher risk. Malaria in pregnancy could lead to maternal mortality, anaemia, abortion/miscarriage, stillbirth, and low birth weight. The latter is the main factor in infant mortality. This is due to the malaria parasite’s ability to render infected erythrocytes adhesive and sequester in the intervillous space of the infected placenta thereby reducing placenta integrity. Further, the placenta becomes a protected site for parasite sequestration and growth without exhibiting symptoms. Studies show that Presumptive Treatment of malaria in pregnancy reduces morbidity and mortality, decreases anaemia in pregnancy and improves birth weights (Verhoeff et al., 1978; Okeyeh et al., 1996). The impact of malaria on adverse outcomes of pregnancies at different periods during pregnancy has not been well documented. The
Intermittent Presumptive Treatment (IPT) programme is designed to prevent the adverse outcomes of pregnancy by presumptively treating pregnant women with three (3) doses of sulphadoxine–pyrimethamine (SP) administered after the sixteenth (16th) week. Some studies have shown that most adverse outcomes of pregnancy occur much earlier than the 16th week. It is, however, not known how many adverse outcomes are prevented by IPT.

Methods: The aim was to study the pattern of specific outcomes of malaria infection at different gestation periods and assess the effectiveness of the Intermittent Presumptive Treatment of malaria in pregnancy. The study was designed to investigate the prevalence of malaria in primigravidae and multigravidae women of Lusaka delivering at the University Teaching Hospital (UTH). It was also designed to investigate the pattern of adverse outcomes of pregnancy in women attended to at UTH. Ninety-nine pregnant women were screened for malaria by peripheral blood, placental blood and placental tissue histopathology investigation. Blood smears were prepared from peripheral and placental blood while histopathology processing and staining was done on placenta biopsies.

Results: Prevalence of malaria in pregnant women admitted to UTH with adverse pregnancy outcomes was 5% by peripheral blood examination, 10% by placental blood examination and 13% by histopathological placenta examination. The prevalence of malaria in primigravidae women was found to be 15.6% while that of multigravidae was 7.4%. Furthermore, it was observed that adverse outcomes of pregnancy were low birth weight (4%), premature delivery (6%), and abortions/miscarriages (90%). Sixty-nine percent of all the adverse outcomes of pregnancy occurred during or before the 20th week of gestation. As also recently reported in literature (Rogerson et al., 2003) the study further showed that monocyte influx on the placenta was an indication of placental malaria infection. Monocyte cell counts of more than 15 per 500 neutrophils correlated well with placental malaria infection.

Interpretation: Conclusion: Prevalence of malaria among adverse pregnancy outcomes in Lusaka was found to be 10%. Primigravidae are twice at risk of malaria than multigravidae. The most common adverse outcome of pregnancy was found to be abortion/miscarriage. The study showed that 90% of the abortions/miscarriages took place before the 20th week of gestation. The current protocol of Intermittent Presumptive Treatment of malaria in pregnancy may need review and modification to enable adequate coverage and prevention of these malaria related adverse pregnancy outcomes.

Acknowledgements

This work is part of a Pilot study on the “Impact of HIV infection on host immune response to Plasmodium falciparum malaria” and was supported in part by MIM/WHO/TDR Project proposal development grant A10631.

528C

Prevalence of asymptomatic malaria parasitaemia in pregnant women in Zambia: Preliminary report from a pilot study [MIM-JC-38702]


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Introduction: Despite numerous interventions, the last two decades have seen an upsurge in malaria disease burden in Zambia and other sub-Saharan African countries. Morbidity and mortality are especially worse among pregnant women and children under the age of 5 years. Current studies ascribe this malaria disease burden upsurge, in part, to a negative impact of HIV co-infection. However, details of this impact are not well understood and need further elucidation. In this pilot study, we aimed to document the prevalence of asymptomatic malaria parasitaemia and anaemia among pregnant women in Lusaka, the capital city of Zambia, with the view of designing investigations to elucidate the impact of HIV infection on acquisition and maintenance of protective immunity to P. falciparum.
Methods: Peripheral blood was collected from 1140 pregnant women attending antenatal care (ANC) at University Teaching Hospital (UTH) and two Urban ANC centres from February to September 2003. Of these, 977 were analysed. Thick and thin blood smears were checked for malaria parasitaemia, as were results of haemoglobin levels on full blood count. We also assessed anaemia, fever, parity, gravidity, gestation age and checked whether the women were on any of the available ANC interventions including intermittent presumptive treatment of malaria (IPT), use of insecticide treated Mosquito nets (ITNs) and iron and folic acid supplements.

Results: A total of 42 women (4.3%) of the 977 analysed had microscopic malaria parasitaemia; 52.6% of these were either primigravidae or in their second pregnancy, and 47.3% were multigravidae. Parasitaemia density ranged from 80.0 to 384,000.0 parasites/mL with the primigravidae and second gravidae together having a mean parasite density of 24,352 mL$^{-1}$ and multigravidae 3984 mL$^{-1}$. The average haemoglobin of the 977-screened women was 10.76 g/dL. By definition only 9.8% of all the screened women had normal haemoglobin levels (HB > 12.5 g/dL), 4.1% had severe anaemia (HB < 8 g/dL), and the majority (86.1%) had mild to moderate anaemia (HB 3 8 < 12.5 g/dL). Parasitaemia density correlated well with anaemia severity and low rates of pregnancy. However, there was no correlation between access to ANC interventions and microscopic malaria parasitaemia nor anaemia.

Interpretation: Taken together these results suggest that, despite the available ANC interventions, prevalence of anaemia and malaria in pregnancy is unacceptably high in Zambia. Primigravidae and second gravidae women are the most susceptible to malaria infections. Further studies are urgently needed to help design an effective regimen of ANC malaria and anaemia prophylaxis.

Acknowledgements
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**529A**

Sulphadoxine–pyrimethamine (SP) efficiency in pregnant women in the context of chloroquine (CQ) chemoprophylaxis failure (Burkina Faso) [MIM-SC-392]

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Introduction: CQ has been the first line treatment for malaria attack and for chemoprophylaxis drug in pregnancy in Burkina Faso, but the progressive increase in resistance to CQ will require the country to change malaria treatment policy. SP will probably be chosen to replace CQ for prevention in pregnancy. Our study aimed to evaluate resistance to SP in pregnant women in order to help orientate the policy change.

Methods: Efficacy trials were undertook: (1) To evaluate the therapeutic efficacy of SP in primigravidae and secundigravidae in Ouagadougou; (2) To evaluate the efficacy of SP in terms of effects on birth weight, anaemia, peripheral and placental parasitemia in primigravidae and secundigravidae.

Results: A 28 day therapeutic efficacy trial was carried out and 74 subjects presenting with uncomplicated malaria attack were included. Sixty-two of them completed the follow up. Their median age was 21 years and mean axillary temperature at inclusion was 38.1°C. Primigravidae and second gravidae women are the most susceptible to malaria infections. Further studies are urgently needed to help design an effective regimen of ANC malaria and anaemia prophylaxis.

Acknowledgements
This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Multilateral Initiative for Malaria in Africa (MIM) under a Project Proposal Development Grant WHO/MIM/TDR A10631.
Interpretation: SP remains efficient enough to be used in malaria prevention during pregnancy.

530B
Systematical analysis of var gene transcription during the erythrocytic development in phenotypically distinct 3D7 Plasmodium falciparum parasites [MIM-MD-323782]

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Introduction: The var multigene family encodes PfEMP1, which are expressed on the surface of infected erythrocytes and bind to various host receptors. PfEMP1 plays a key role in malaria pathogenesis, and to understand var gene switching it is important to determine the timing of transcription and the number of var genes transcribed. We developed a method to measure transcriptional levels of all 3D7 var genes simultaneously, and here we apply it to parasite lines selected to express different phenotypes.

Methods: We analysed the var transcription during the intra-erythrocytic life cycle in two laboratory parasites, NF54 (unselected) and NF54VAR2CSA (selected for high homogeneous expression of VAR2CSA using antibody-coated Dynabeads). It has previously been determined that 3D7 and NF54 are isogenic with respect to var gene repertoire. Parasites were synchronised and samples for RNA extraction were harvested every 2 h throughout the whole 48 h cycle. Total RNA from each parasite sample was extracted and used for subsequent cDNA synthesis. Quantitative real time RT-PCR was performed using gene-specific primers for each of the full-length var genes.

Results: To compare transcription levels at different time intervals, all var gene measurements were related to a housekeeping gene and quantified as the Ct value of gene specific reaction minus Ct value of the housekeeping gene (DCt value). Taking into account the known bias of the primers and using standard curves, it is possible to calculate the absolute amount of each var transcript. Using the DDct method we calculated the fold changes in var gene transcription between different stages in the life cycle and in the isogenic parasite isolates. We show that some var genes are constitutively expressed regardless of adhesion phenotype. Some previous findings showed that a broad range of var genes are transcribed by ring-stage parasites and a single var transcript, claimed to be responsible for the PfEMP1-mediated binding phenotype is present in mature trophozoites. Our results seem to be more in line with other studies suggesting that multiple var genes also are transcribed by late trophozoite stage parasites indicating that association of binding phenotype with the detection of a specific var transcript should be made with caution.

Interpretation: We have found that several var genes are transcribed at high levels regardless of adhesion phenotype, and that the transcription pattern of these genes differs from the phenotype-related var gene.

531C
Do Plasmodium falciparum parasites from the placenta and the peripheral blood belong to the same population? [MIM-PD-40908]

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Introduction: Pregnancy-associated malaria is characterized by selection and multiplication in the placenta of a distinct Plasmodium falciparum population expressing particular variant surface antigens (VSA) that binds chondroitin sulfate A (CSA), causing complications for both mothers and newborn babies. Due to placenta sequestration of mature parasites, the analysis of parasites populations in peripheral blood may give a partial picture of a Plasmodium falciparum infection.

Methods: Msp-1 and msp-2 genes were quantified by fragment-analysis in 39 matched placental/peripheral blood samples from Senegalese women. A fluorescent PCR analyzed block 2 of msp-2 and msp-1. Amplification products were processed in a Genetic analyzer and analyzed with Genescan software. For each genotype, size and area under the curve (proportional to PCR products quantity) allow precise quantification. The binding of 10 fresh paired peripheral/placental isolates to human placenta chondroitin
proteoglycans (CSPGs) was explored. Peripheral blood red cells were incubated for 18–20 h for rings to form trophozoites. The suspension was allowed to bind CSPGs spots. All matched pairs (peripheral and placental) were tested on the same plate in duplicate.

**Results:** All samples but one had a polyclonal infection. The multiplicity of infection was similar in the two compartments. Qualitative analysis demonstrated only a partial overlap of the parasite populations in the two compartments, as previously reported. Conversely, quantitative analysis demonstrated that divergent alleles represented only minor parasite populations, and that identical genotypes represented 80 to >95% of the overall parasite populations in the two compartments. The mean level of adhesion on CSPGs (p = 0.69) was similar in peripheral and placental parasites. The binding to CSPGs of peripheral and placental parasites from the same women was strongly correlated (r = 0.66; p = 0.04).

**Interpretation:** Genotype and cytoadherence data demonstrate peripheral and placental blood parasite populations are more similar than previously thought, and suggest that peripheral ring stage parasites are mostly the progeny of mature forms sequestered in placenta.

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**532A**

**Monocytes increase TH1 cytokines in Plasmodium falciparum infected human term placenta [MIM-NF-213520]**

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**Introduction:** Pregnant women are at increased risk for malaria and *Plasmodium falciparum* accumulates in the placenta. The immune mechanisms of placental malaria are largely unknown. Placental inflammatory responses, via IFN-γ and TNF-α secretion are involved in functional damage of villies and troubles of fetal-maternal exchanges. IFN-γ and TNF-α control parasite growth and need to be produced during pregnancy. The monocyte cytokine IL-12, enhanced during normal pregnancy, is a major IFN-γ precursor.

**Methods:** At delivery 17 *P. falciparum* infected and 12 non-infected women were recruited in the maternity of Guédiawaye, near Dakar, Senegal. For each woman, term placenta and 10 ml heparinized venous blood sample were collected immediately after delivery. The impact of placenta parasite development on cytokine production by monocytes (CD14+), lymphocytes (CD4+ and CD8+) in intervillous and peripheral blood mononuclear cells was investigated and compared. The proportion of CD4 and CD8 cells secreting IFN-γ or TNF-α were similar in both compartments after stimulation by MPA/iono or IE. The proportion of monocytes secreting IL12 or TNF-α in response to IE were higher in peripheral than in intervillous blood (both p = 0.03). In *P. falciparum*-infected placentas, the proportions of placental CD4+ and CD8+ cells secreting IFN-γ and TNF-α after stimulation by MPA/iono were increased (CD4+: p = 0.08 and 0.02; CD8+: p = 0.01 and 0.02, respectively). We observed a dichotomy in monocytes responses in response to LPS. In infected placentas, the proportions of peripheral and placental monocytes secreting IL12 were increased (significant for placental p = 0.043), while those secreting TNF-α were lower (significant for peripheral p = 0.02). *P. falciparum* via hemozoin may down-regulate TNF-α responses and allow IL12 responses. Interpreted: IL12 is maintained and is an important factor to induce protective IFN-γ response by CD4+, CD8+. IL12 and IFN-γ may synergistically allow protective response in placenta malaria.

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Malaria in pregnancy; Promoting and scaling up the implementation of the strategy in Southern Africa [MIM-WD-81120]

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Introduction: Malaria in pregnancy remains a major cause of morbidity and mortality in southern Africa. Interventions directed towards malaria in pregnancy vary depending on the intensity of malaria transmission. Those countries with stable malaria transmission embrace all the three components of MIP, viz; good case management, use of IPT and ITNs. However, in those countries with unstable transmission case management and personal protection remain the main intervention areas.

Methods: Aim of paper is to highlight the adoption, implementation and scaling up of malaria in pregnancy in countries of Southern Africa. Eight of the countries in southern Africa are implementing MIP strategy. The other countries are only looking at effective case management and personal protection. The process of adoption of MIP in most of the countries involved partnership participation in the development of the policy strategy and followed by consensus meeting to agree on the way forward for MIP. Sensitization of MIP strategy to the health workers and involvement of RH for the implementation of MIP has strengthened integration between malaria and RH units. It has also supported the first line health worker in managing pregnant women in a holistic manner. Major challenges of MIP include inadequate personnel and commodities at ANC units. The concept of integrated information gathering is also a big challenge. Scaled up implementation for MIP is possible as shown by early adopter countries. Impact on country wide implementation on maternal and peri-natal morbidity and mortality remains. Other challenges include SP stock-outs, the unavailability of nets at ANC and the capturing of MIP data correctly within the HMIS.

Results: As data collection for MIP in most countries is rather lacking a deliberate process of developing indicators that can be collected via the HMIS was initiated. In addition, the antenatal card was modified to reflect the delivery of IPT as DOT, nutritional supplements and ITN use. For the early adopter countries, Tanzania, Malawi and Zambia, coverage of both IPT and ITNs is at scale and coverage indicators are constantly improving. The involvement of RH in the implementation of MIP has strengthened integration between malaria and RH units. It has also supported the first line health worker in managing pregnant women in a holistic manner. Major challenges of MIP include inadequate personnel and commodities at ANC units. The concept of integrated information gathering is also a big challenge. Scaled up implementation for MIP is possible as shown by early adopter countries. Impact on country wide implementation on maternal and peri-natal morbidity and mortality remains. Other challenges include SP stock-outs, the unavailability of nets at ANC and the capturing of MIP data correctly within the HMIS.

Interpretation: Finally the increased resistance of *P. falciparum* to SP implies the intervention may be short-lived and urgent need for alternative drugs is critical.

The human choriocarcinoma cell line BeWo as an in vitro model for adhesion of *Plasmodium falciparum* infected red blood cells to syncytiotrophoblast [MIM-TS-19588]


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Introduction: Pregnancy-associated malaria is characterised by sequestration of infected erythrocytes (IE) in the placenta. These parasites express variant surface antigens (VSAPAM) that are recognised in a sex- and parity-dependent manner and mediate binding to chondroitin sulphate A (CSA). Selection for VSAPAM expression in vitro and adhesion assays with such parasites are central for molecular characterisation of VSAPAM. We have used the human choriocarcinoma cell line BeWo for these purposes.

Methods: Three *P. falciparum* lines, 3D7, FCR3 and Hb3 were panned × 3 on BeWo cells. The selected and unselected lines were tested for expression of VSAPAM by labelling purified IE with plasma from
a panel of malaria-exposed men and multiparous women. Parasites from pregnant Ghanaian women and control parasites were radio-labelled with 3H-hypoxanthin and incubated either with monolayers of BeWo cells or immobilised CSA. After washing, parasite adhesion was quantified by scintillation counting.

Results: After three rounds of panning on BeWo cells FCR3 and HB3 expressed VSA that were recognised exclusively by plasma IgG from malaria-exposed multiparous women but not by malaria-exposed men, even though these men had IgG recognising the unselected control parasite. The selected 3D7 parasites appeared to express a mixture of VSAPAM and the original VSA type as the women in the test panel showed a marked increase in recognition relative to the unselected 3D7 while the men’s recognition decreased. All three parasite lines have previously been panned repeatedly on CSA-coated plastic. Only in the case of FCR3 did this result in expression of VSAPAM. Unselected 3D7, FCR3 and HB3 did not bind BeWo cells or CSA. The BeWo-selected sublines all adhered to BeWo cells, whereas only selected FCR3 and HB3 adhered to CSA. Field isolates obtained from pregnant Ghanaian women that expressed VSAPAM on their surface as judged by a sex-specific and parity-dependent recognition profile were also assayed. All field isolates that expressed VSAPAM adhered to BeWo cells as well as to CSA. In most cases adhesion to BeWo cells was much stronger than adhesion to CSA.

Interpretation: In conclusion, BeWo cells can effectively replace primary syncytiotrophoblast when selecting for and measuring adhesion of VSAPAM-expressing parasites. Furthermore, BeWo cells are much easier to culture than primary syncytiotrophoblast.

535A
The prevalence of placental malaria in Owerri, Imo State, South-Eastern Nigeria I [MIM-OI-101246]


Introduction: Malaria in pregnancy jeopardizes the outcome of pregnancy, affecting both the mother and the foetus. The prevalence of placental malaria amongst women who attended and completed the routine ante-natal clinical visits in the Federal Medical Centre (F.M.C), Owerri and St. David’s Hospital, Owerri was studied between March to December 2004.

Methods: The study was carried out with signed consent of the participants. A total of 480 pregnant women who had no signs and symptoms of malaria were involved in this study. Placental blood (5 ml) was collected from incisions made on the cleared maternal surface (basal plate) of expelled placenta within an hour post-delivery. Thick and thin blood films stained with Giemsa were used for the detection of the malaria parasites. Parasitaemia was expressed as the number of parasite per microscope field. The hemoglobin Hb level was assessed using the cyanomethaemoglobin method.

Results: Despite the routine weekly prophylactic malaria drugs given at each ante-natal visit a total of 135 (28.1%) of these women had malaria parasite in their placenta. The study population was made up of 205 (42.7%) primigravidae women, 140 (29.26%), secundigravidae women and 135 (28.1%) multigravidae women. Placental malaria was detected in 50.4% of the primigravidae with an overall percentage infection rate of 14.2%. The picture amongst the secundigravidae and multigravidae were 32.6 and 17.0% infection rate, respectively within the groups and 9.2 and 4.8% infection rates, respectively in overall infected women. Assessing the relationship between age and the prevalence of placental malaria, the age groups 20–25 years were the most infected with an infection rate of 57.0%. A statistical significant difference was not observed between parasitaemia and anaemia; between anaemia and age of the women. It was however observed that anaemia was more prevalent amongst the younger mother’s in the 20–25 age groups. Anaemia was also more prevalent in the primigravidae through a statistically significant difference between anaemia and gravidity was not observed.

Interpretation: Findings from these studies indicate a very strong need for a better cost effective malaria control strategy amongst susceptible pregnant women.
The effect of pregnancy and Plasmodium falciparum transmission intensity on IgG subclass responses to variant surface antigens [MIM-RM-213971]

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Introduction: Plasmodium falciparum parasites causing pregnancy-associated malaria (PAM) express particular variant surface antigens (VSAPAM) that differ from non-PAM type VSA expressed by parasites in non-pregnant individuals. Previous studies demonstrate the importance of VSAPAM-specific IgG in protection against adverse pregnancy outcomes, but few data are available on VSA-specific IgG subclass responses in relation to level of transmission and none in pregnancy or specific for VSAPAM.

Methods: We used 283 plasma samples from pregnant and non-pregnant women in two cohort studies in Cameroon. None had malaria during the sample collection although some had asymptomatic infections. We used purified late-stage infected erythrocytes (IE) from two sub-lines of the long-term in vitro-adapted P. falciparum FCR3 line: one that express VSAPAM and another that does not. We used flow-cytometry to measure plasma levels of IgG and IgG subclass antibodies specific for both types of VSA.

Results: We found that VSAPAM-specific responses depended on pregnancy status, parity, gestational age and parasite transmission intensity, whereas only the last influenced levels of IgG specific for non-PAM type VSA. For both types of VSA, responses were dominated by the cytophilic IgG1 followed by IgG3. Interestingly, Levels of VSAPAM-specific IgG1 increased with increasing pregnancy, while levels of the corresponding IgG3 tended to decrease with increasing gestational age in sample from low endemcity (Yaounde). Such a decrease was not seen in samples from Eto (high level of malaria transmission). Levels of non-PAM type VSA antibodies were not significantly affected by pregnancy.

Interpretation: Our results suggest that the rate of acquisition of cytophilic antibodies depends on transmission intensity. This is the first study showing the dominance of cytophilic IgG1 and IgG3 subclasses in acquired VSAPAM-specific immunity.

Paludisme et Grossesse: Apoptose, activation et distribution des sous populations lymphocytaires T dans les compartiments sanguins et placentaires [MIM-nm-126718]

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Introduction: les femmes enceintes sont une population à haut risque pour le paludisme. La séquestration du parasite au niveau du placenta entraîne une augmentation de la morbidité et de la mortalité néonatale et un retard pondéral. La grossesse est associée à une immunosuppression de type TH 1 plus importante. Cette immunosuppression cellulaire observée durant la grossesse serait-elle associée à un phénomène du genre d’apoptose ou globalement à une anergie des cellules dans le cas de l’infection palustre?

Methods: Notre étude est réalisée sur 25 femmes enceintes présentant une infection placentaire à Plasmodium falciparum et 25 autres ne présentant d’infection. Le recrutement s’est effectué dans le centre de santé Roi Baudoin situé à Guédiawaye, zone périurbaine et hypoendémique, avec une moyenne de 6500 accouchements/an. Les taux d’anticorps déterminés par ELISA et le marquage lymphocytaire par des anticorps monoclonaux ont été utilisés pour comparer nos deux groupes. Les test non paramétriques de Wilcoxon et de Mann Withney ont été utilisés pour comparer les résultats.

Results: Nous avons observé que la présence du parasite est associée à une augmentation du taux d’apoptose (Apo 2.7: P=0.001), des marqueurs d’activation (CD69 et HLADR), du taux des cellules NK (CD16), des cellules en prolifération (CD71), des cellules...
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mémories (CD45RO+) du sang placentaire. Le parasite induirait une anergie des lymphocytes du sang placentaire, car ceux-ci ne pouvant plus répondre, par une activation, à des stimuli antigéniques spécifiques comme l’extrait 0703 et même à un stimulus non spécifique que la PPD.

**Interpretation:** Ces constatations pourraient contribuer à l’explication de la séquestration de *P. falciparum* dans le placenta et de surcroît, de la grande susceptibilité de la femme enceinte au paludisme.

**538A**

**Epidemiology of pregnancy-associated malaria in the Ugandan highlands [MIM-SC-75243]**

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**Introduction:** Pregnancy is a time of increased malaria risk for mother and foetus, and is well characterised under high transmission. Less is known about malaria during pregnancy in areas of low transmission. Hospital records show foetal death to be inversely related to transmission intensity, and stillbirths to increase during epidemics. Yet to better characterise the epidemiology of malaria and impact on foetal outcome amongst pregnant women with little pre-pregnancy immunity requires prospective study.

**Methods:** Intervention trial in the epidemic-prone highlands of Kabale district, SW Uganda to compare protective efficacy of ITNs and IPT (singly and in combination). Women attending antenatal clinic before 29 weeks gestation are consented and individually randomised to one of three arms: IPT + ITN, IPT alone or ITN alone. All women receive at least one preventive measure. Women are followed during pregnancy and at birth to assess impact on maternal haemoglobin at week 36, parasitaemia and birthweight. Data is also recorded on abortions, stillbirths, maternal and perinatal deaths. Mothers are visited at home and interviewed by a midwife using a modified verbal autopsy method. Independent clinical review ascertains probable cause of foetal or infant death.

**Results:** Over 2000 women of all parities were recruited by end February 2005 (mean age: 26 years). The homes of study women are situated between 1219 and 2000 m altitude. No epidemics have occurred during the study period to date. A total of 1037 live births and 14 stillbirths have been recorded: a stillbirth rate of 13/1000 births. The mean birthweight was 3152 g, with 7% babies born with low birthweight. Maternal anaemia at 36 weeks gestation (Hb < 100 g/L) was observed in 29% of women. A total of 25 abortions were recorded in the same period, equivalent to 1% of pregnancies and 2% of births. Nineteen verbal autopsies have been completed. The majority of women had a symptom history of infectious illness prior to abortion (12/19, 63%). Malaria/anaemia was judged to be a primary cause of abortion in almost half the cases (8/19, 40%). The malaria-related abortions occurred during periods of peak transmission following seasonal rains. Five occurred before week 16 and the first dose of IPT. Only two women slept under an ITN. Recruitment and follow-up are ongoing. By November 2005 substantially more birth outcomes will be available and a fuller analysis will be possible. Birth outcomes will be presented in relation to altitude and other risk factors.

**Interpretation:** In an area of low and unstable transmission, almost half of abortions were attributable to malaria. During malaria epidemics this figure will be higher. Measures to prevent infection and clinical attacks of malaria should be given early in pregnancy.

**539B**

**Grossesse et paludisme: Prise en charge des femmes enceintes lors des consultations prénatales (CPN) dans une plantation du Sud Cameroun [MIM-VN-59997]**

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**Introduction:** Les femmes enceintes constituent avec les enfants de moins de 5 ans les populations les plus...
vulnérables face au paludisme et les CPN constituent un moment privilégié pour leur délivrer une information préventive. L’objectif de notre étude était d’étudier, à partir d’une enquête rétrospective, les connaissances sur le paludisme des femmes ayant suivies une consultation prénatale et d’évaluer leurs satisfactions et leurs attentes vis-à-vis des services de santé.

Methods: Au cours du 1er semestre 2003 nous avons réalisé une étude auprès des professionnels de santé et de 196 femmes ayant accouchées depuis moins de six mois dans une plantation du Sud Cameroun (Hévécam). Les données ont été recueillies auprès des femmes par questionnaires et complétées par l’analyse de leur carnet de santé. L’étude s’est appuyée également sur des entretiens auprès des femmes et des professionnels de santé et les femmes et une observation des relations de soins à l’hôpital et dans les dispensaires. Cette étude a été réalisée dans le cadre du projet PAL+ “Traiter et/ou Prévenir. Mères, enfants et soignants face au paludisme”.

Results: L’examen des carnets de santé montre que 53% des femmes ont bénéficié de 3 CPN, que 2/3 d’entre-elles ont utilisé une prophylaxie antipaludique adaptée et que 40% ont eu un accès palustre durant la grossesse. Nous avons pu également évaluer les pratiques professionnelles à travers le récit de l’expérience de femmes du processus de la CPN. 47% des femmes disent avoir reçu des informations sur les traitements prescrits et en ont effectivement une bonne connaissance. Néanmoins, on relève une insatisfaction vis-à-vis des professionnels de santé et des femmes et une observation des relations de soins à l’hôpital et dans les dispensaires.

Interpretation: Pour une meilleure compréhension des comportements des femmes enceintes les messages informatifs qui leur sont destinés et qui constituent des supports de prévention doivent tenir compte des phénomènes de ré-appropriation et de ré-interprétation.

**VAR2CSA expressed during Plasmodium falciparum pregnancy associated malaria is partially resistant to proteolytic cleavage by trypsin [MIM-MN-3161100]**

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Introduction: Immunity to malaria is gradually acquired during childhood in endemic areas. Regardless, first-time pregnant women become susceptible to pregnancy-associated malaria (PAM). PAM is caused by parasites expressing unique variant surface antigens (VSAPAM) that enable the accumulation of infected erythrocytes (IE) binding to chondroitin sulphate A (CSA) in the placenta. Protective VSAPAM-specific antibodies that block cytoadhesion in the placenta are acquired as a function of parity.

Methods: Antibodies against domains of the VSAPAM antigen VAR2CSA were raised in rabbits and used for surface labelling of IE. Parasites were selected for expression of VAR2CSA on BeWo cells. Binding to CSA was performed using BeWo cells in a static assay and IE binding was inhibited with soluble CSA. Trypsin sensitivity was evaluated by incubating IE in RPMI with 1 mg/ml trypsin for variable lengths of time.

Results: We have shown that placental isolates and parasites selected for adhesion to CSA express high levels of var2csa. Furthermore VAR2CSA is expressed on the surface of the IE and recognised by protective antibodies developed during PAM. Here we present data showing that VAR2CSA expressed by some isolates is resistant to proteolytic cleavage by trypsin. The protein remains on the surface but appears to be partially digested altering the CSA binding phenotype and antibody recognition of the IE. VAR2CSA in other isolates is sensitive to trypsin, which completely removes the protein from the surface of the IE.

Interpretation: Our findings extend previous data on the trypsin resistance of VSAPAM-type antigens. The data furthermore shows that not all VAR2CSA domains...
appear to generate antibodies in rabbits that recognise the surface of the IE.

541A
Les problèmes de communication entre soignants et femmes enceintes primipares lors de la prise en charge du paludisme dans un hôpital du Sud Cameroun [MIM-OO-338895]

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Results: Nous avons constaté que le personnel des consultations prénatales communique peu avec les femmes enceintes et en particulier les primipares: - les échanges sont laconiques et organisés sur le mode instructions/ordres, ce qui induit un rapport hiérarchisé de type subordonné/supérieur, - les éléments inscrits sur le carnet de santé lui sont rarement expliqués. Il est intéressant de mentionner que dans un premier temps, il lui est prescrit une série d’examens sans explication sur l’importance de ceux-ci et la nécessité de les subir. Lorsque ces examens sont faits, une ordonnance est prescrite. La parturiente, qui ignore de quoi elle souffre, est sommée d’acheter les médicaments et de les consommer. L’absence de communication nous amène à nous intéresser aux multiples implications de cette attitude qui ont une incidence sur la prévention et l’observance. Ces actes mécaniques du personnel de santé n’informent pas la primipare sur le danger que constitue le paludisme, et encore moins sur les moyens de le prévenir. Sans explication il est difficile pour le profane d’établir une corrélation entre le paludisme et ses complications dans la mesure où ses symptômes (fièvre, maux de tête . . .) sont similaires à ceux d’autres pathologies.

Interpretation: Ce travail pose la question plus générale de l’observance des traitements chez les primipares et leurs façons de s’approprier l’initiative de la prévention et de la demande des soins.

542B
Community based study of intermittent preventive therapy with sulfadoxine–pyrimethamine and chloroquine in preventing malaria during pregnancy in Mali [MIM-AO-180090]

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Introduction: After the first efficacy trial of intermittent preventive therapy using sulfadoxine–pyrimethamine, in Mali (Kayentao et al., 2005), the Malian National Malaria Control Program recommended in collaboration with WHO to implement this new strategy in a large scale to reduce malaria burden during pregnancy during the transition period of the implementation, we conducted a community based study in a rural village of Bancoumana, Mali.

Methods: The study was carried out in Bancoumana, a rural village of 8000 inhabitants located at 60km north from Bamako in the Savana transmission region. The minimum age during the enrolment was 15 years old from July 2003 to February 2005. Pregnant women with all parities were included. At enrolment we measured haemoglobin level, peripheral parasitemia, and determined socio-demographic characteristics such us age, marital status, parity, height, gestational age. At delivery, peripheral and placental parasitemia were determined as well as haemoglobin level. New born...
were weighted and also examined for congenital abnormality and potential adverse events.

Results: Out of 410 pregnant women enrolled, 378 have delivered: 189 in each treatment arm. The mean gestational age was 26.06 ± 3.3 weeks. Primigravidae and secundigravidae represent 15.3 and 14.6%, respectively. The two Groups were comparable at enrolment regarding anaemia and peripheral parasitemia. During the follow-up period, women in SP group were less likely to get malaria infection and malaria attack ($p = 0.005$), but no statistical difference was found in anaemia prevalence ($p > 0.05$). At delivery, the prevalence of anaemia were higher in CQ group (39%) compared to SP group (28.1%); ($p = 0.003$). The two groups were comparable regarding their peripheral and placental parasitemia, and the rate of low birth weight. These findings at delivery are different from the efficacy study results (Kayentao et al., 2005), the reason will be discussed. The rate of singleton stillbirth (4.1%) and preterm delivery (4.2%) was not statistically significant between groups. Anaemia was associated with age ($p = 0.003$) and parity ($p = 0.001$); primigravidae and young women (<20 years) were more affected.

Interpretation: Preliminary results of this community based trial indicate a reduction of malaria adverse effect with SP compared to CQ, therefore support WHO and NMCP efforts for the implementation of IPT/SP for the malaria prevention in pregnancy.

543C

Influence of malaria infection on the calcium profile of pregnant women – implications of parity and gestational age [MIM-JP-1349980]

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Introduction: To date, malaria control has focused on reduction of man-mosquito contact, rapid treatment, and development of a malaria vaccine. Clearly, additional low-cost and effective means to assist in the prevention and treatment of malaria are needed. The primary objective of this study was to prospectively assess the relationship between an important nutritional status (calcium profile) and burden of malaria during pregnancy.

Methods: A prospective study has been carried out on 208 pregnant women (78 malaria patients and 130 non-malaria patients). All subjects were going through medical consultation at Laquintinie Hospital in Douala (Cameroon). Several procedures have been done on each woman: Pregnancy test, by immunochromatographic sandwich; malaria test (thin and thick blood film, prepared directly from capillary blood); in order to estimate parasitemia, we counted the percentage of parasitized red cells in a thin film and the number of parasite against white cells in a thick film; The determination of calcemia by colorimetric method (at 610 nm) using serum from venous blood collection. All results were analysed by SPSS statistical software.

Results: Infected pregnant women (37.5%) had high parasitemia (100–350,000 parasites/$\mu$L). For 4% of them (in their first pregnancy) it was severe malaria. Almost 18% of malaria infected pregnant women had hypocalcemia, against 2.31% for non-malaria pregnant women ($P = 0.0006$); Women’s age, parity, and gestational age have been found playing an important role in the interaction. Significant correlations were obtained between parasitemia and calcemia ($r = -0.8079; P = 8.10–12$), and between gestational age and calcemia on malaria infected pregnant women ($r = 0.2693; P = 0.0034$).

Interpretation: The impact of the malaria infection on calcium profile is clear, but this influence decreases with age. Therefore, nutritional modulation of malaria infection can be an option to assist others malaria prevention and treatment methods.

544A

L’influence de la famille dans la prise en charge du paludisme chez la primipare au Sud Cameroun [MIM-BR-53499]

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Introduction: Afin d’analyser les modalités de prise de décision qui peuvent influer sur le recours à un traitement préventif ou curatif des femmes, nous

Methods: Pour mesurer l’impact de l’entourage familial, nous avons enquêté pendant 3 mois, à l’hôpital et au domicile de 70 primipares/primigestes et auprès de parents (15 entretiens) participant à la prise en charge de la santé de la primipare. Ces enquêtes ont été complétées par des entretiens avec différents soignants auxquels avaient recouru les infirmières: professionnels de santé à l’hôpital, tradipraticiens/guérisseurs, vendeurs de médicament dans la rue ... L’enquête a été réalisée au cours du premier semestre 2004, à Kribi, ville moyenne située sur la côte atlantique du Sud Cameroun ou le paludisme sévit en permanence.

Results: Il nous apparaît que la primipare est soumise constamment à l’influence de sa famille (mère, belle-mère, tantes, grandes sœurs belles-sœurs) à cause de son statut de cadette sociale, son désenclavement matériel et son expérience limitée de la grossesse. Un autre déterminant de cette soumission est la culpabilisation dont elle est victime, car la grossesse vient d’évoquer aux yeux de tous qu’elle a déjà une génialité active, et constitue de ce fait une transgression du statut de l’enfant au statut de femme. Par conséquent elle est conseillée, orientée et guidée dans la prise en charge de sa santé. En outre, certaines des manifestations de l’affection palustre que les représentations communes tiennent "pour compagnon de la grossesse", n’ont pas le statut de paludisme et sont alors considérées comme des affections bénignes. Ce statut coutumier et bénin de la maladie palustre explique en partie les négligences diverses, les retards de traitements ou leur prise partielle, les associations de médicaments modernes et de médicaments dits traditionnels et les refus de soins en situation d’accès palustre.

Interpretation: La famille est un acteur déterminant dans le choix et la conduite des soins. La qualité de soins que reçoit la primipare dépend donc de l’interaction avec sa famille et des représentations liées au lien paludisme-grossesse et aux différents recours.

545B Mapping of antigenic sites on the PFEMP1 protein VAR2CSA involved in placental sequestration [MIM-AS-345015]
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Introduction: In areas endemic for malaria, the main burden of disease is on young children and pregnant women. The major effects of pregnancy-associated malaria (PAM) are severe maternal anaemia and low birth weight of the offspring. The infected erythrocyte (IE) binds to placental tissue to avoid being filtering through the spleen. The receptor on the surface of the infected red blood cell that enables the parasites to bind to chondroitin–sulphate-A in the placenta was recently discovered and named VAR2CSA.

Methods: Overlapping 30-mer peptides covering the exon1 of 3D7 VAR2CSA were synthesized as solid phase peptide synthesis (SPPS) with a stepwise addition of the different amino acids attached to a solid resin. The long peptides were synthesized with a cysteine at aa position 15 allowing some secondary structure. This approach allows identification of antigenic sites that cannot be mapped using short, linear peptides (Pepscan systems, The Netherlands). The exon1 of VAR2CSA is 2676aa and correspondingly 442 overlapping peptides were synthesized. For identification of antigenic epitopes we screened the peptide array with (1) rabbit sera (2) female pregnancy sera and (3) antibodie eluted from IE.

Results: We have previously shown that var2csa transcription is highly associated with CSA binding. We have also shown that antibodies to VAR2CSA are only acquired during pregnancy in women exposed to placent malaria and that the presence of the antibodies in pregnant women is predictive of a favourable birth outcome and we have demonstrated that antibodies against VAR2CSA reacts specifically with the surface of parasites binding CSA in vitro. Here we validate that rabbit sera generated against VAR2CSA are protein- and domain specific and several specific epitopes are identified. Using individual Ghanaian female pregnancy sera we show that antigenic sites are dispersed throughout the whole VAR2CSA sequence, with a significantly
higher number of epitopes in three distinct domains. We incubated CSA selected parasites in sera from pregnant women and eluted the surface reactive antibodies. Antibodies were eluted from both homologous and heterologous parasite lines to identify conserved epitopes. Using these eluted antibodies on the Pepscan array we identify major and minor conserved epitopes that appear to be surface expressed.

**Interpretation:** We have identified several epitopes of VAR2CSA that are accessible for antibodies on the surface of the infected erythrocyte. A chimeric construct containing all these epitopes is being evaluated.

**546C**

**Epidemiology of Plasmodium falciparum and P. vivax infection in placenta and umbilical cord blood in central India [MIM-NS-95172]**

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**Introduction:** Sinton (1935) described the vulnerability of the pregnant women and her unborn child to malaria in India. Since then the enormous problem of malaria in pregnancy had been little recognized in India. This pilot study aimed to describe the prevalence of placental malaria and to define the effects on maternal anaemia and pregnancy outcome to further define the burden of malaria in pregnancy in an area where the population is exposed to seasonal transmission of *P. falciparum* and *Plasmodium falciparum*.

**Methods:** All pregnant women with or without clinical symptoms giving birth at the district hospital Mandla between October 2002 to January 2003 and in civil hospital Maihar, from May to December 2004 were the subjects of this study. A malaria clinic of the Malaria Research Centre (ICMR) was established for this study in the Obstetrics and Gynaecological wards of the district hospital Mandla and civil hospital Maihar. Peripheral blood was collected by finger prick during labour. Each placenta was incised from maternal surface and a small quantity of blood was pipetted for preparation of placental thick and thin smears. Following the delivery of the baby, a malaria smear was prepared from the cord blood and the baby’s weight was recorded.

**Results:** A total of 209 pregnant women aged 18–45 years who came for delivery were screened for malaria at Mandla. Only 30 had malaria parasites in placental smears (14.4%). *P. falciparum* comprising 26(87%) and remaining mixed infections of *P. falciparum* and *P. vivax*. The peripheral smears were positive only in 11 of 209 pregnant women (5.3%), of which seven were *P. falciparum*. Umbilical cord blood smears were also positive in 5.3%. Delivery of low birth weight infants was significantly (P < 0.025) more common in women who had placental parasitaemia (2.19 ± 0.76 kg) than in women without placental infection (2.37 ± 0.31 kg). Parasitaemia women had lower mean (S.D.) hemoglobin concentration 9.6 (±0.87) versus 10.4 g/dl (0.52) in non infected women (p < 0.0001). A total of 590 women aged 19–40 years were screened for malaria at Maihar. Placental parasitaemia was found in 10.8% women. *P. falciparum* 84.3%, *P. vivax*, 12.5% and remaining were mixed infections (3.1%). The prevalence of malaria parasites in peripheral smear was 7%. Umbilical cord blood smear was also positive in 4% subjects. Mean birth weight of babies born with infected placenta was 2.47 ± 0.44 kg as compared to birth weight of babies born without placental infection 2.6 ± 0.44 kg (p < 0.05).

**Interpretation:** We found little effect of parity and all women were at equal risk of malaria. Further studies are required in areas of different endemicity to assess the burden of malaria during pregnancy to examine prevention and intervention opportunities.

**547A**

**Amodiaquine (AQ), sulphadoxine–pyrimethamine (SP) used singly and in combination (AQ + SP) in the treatment of falciparum malaria infection in pregnancy [MIM-HT-64074]**

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**Introduction:** MIP is potentially fatal to mother and fetus with outcomes that depend on the woman’s immu-
nity, which is influenced by the local malaria transmission profile. In endemic areas maternal anaemia and low birth weight are the main outcomes particularly in primigravidae. Prevention of such outcomes is threatened by widespread falciparum resistance to chloroquine and uncertainty over safety of alternatives in pregnancy necessitating urgent search for alternative safe, efficacious treatment options.

Methods: Pregnant women of all parities attending antenatal sessions at St. Theresa’s Hospital in Nkoranza, Ghana with gestational age of 16 weeks and above were screened for malaria antigens with OptiMAL dipsticks. Nine hundred pregnant women with positive antigen tests confirmed microscopically were enrolled into a four-arm randomised, double blind clinical trial of chloroquine, amodiaquine, sulphadoxine pyrimethamine and amodiaquine + sulphadoxine pyrimethamine combination. We measured their effects on the prevalence of peripheral parasitaemia, and the levels of haemoglobin, bilirubin, liver transaminases and white cell count on days 14 and 28 following treatment. In addition participants’ reports of side effects were recorded and monitored during follow-up visits.

Results: Chloroquine usage as indicated by history of ingestion is 51.3% and by antimalarial ELISA urine dipsticks was 59.4 and 48.7% at zero and 100-fold dilution, respectively, at enrolment. Women who had taken chloroquine up to 2 months before enrolment had slightly higher parasite densities than those who had not. Women presenting with symptoms at enrolment had higher parasite densities than women who were asymptomatic. Higher parasite density was associated with high bilirubin levels at enrolment but not with level of alanine and aspartate aminotransferases. Parasite prevalence on Days 14 and 28 post treatment were 3 and 11%, respectively. Multi gravid women had higher haemoglobin levels at enrolment and had more improvement in their haemoglobin level post treatment. Weakness, dizziness and vomiting were the commonest side effects reported post treatment. itching to chloroquine was the commonest (86.4%) side effect reported at enrolment but became the fourth commonest reported after treatment. No-one had WBC <2000/µl following treatment. About 11 and 14% of women had raised AST above twice the upper limit of normal on days 14 and 14, respectively, which fell to 9% on Day 28. However, about 4% of women had raised ALT on Days 0 and 14, which fell to 2% on Day 28.

Interpretation: The drug codes will be broken in April 2005 when the post partum follow up of all participants is completed. Comparisons of the efficacy and safety of the drugs will be presented at the conference in November.

S458B Malaria in pregnancy; promoting and scaling up the implementation of the strategy in southern Africa [MIM-DT-72360]

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(1) World Health Organisation, Southern Africa Inter-Country Malaria Control Programme; (2) World Health Organisation, Regional Office for Africa; (3) National Malaria Control Centre, Zambia; (4) National Malaria Control Programme, Zanzibar; (5) National Malaria Control Programme, Namibia; (6) National Malaria Control Programme, Angola; (7) National Malaria Control Programme, Mozambique; (8) National Malaria Control Programme, Tanzania; (9) National Malaria Control Programme, Zimbabwe

Introduction: Malaria in pregnancy remains a major cause of morbidity and mortality in southern Africa. Interventions directed towards malaria in pregnancy vary depending on the intensity of malaria transmission. Those countries with stable malaria transmission embrace all the three components of MIP, viz; good case management, use of IPT and ITNs. However, in those countries with unstable transmission case management and personal protection remain the main intervention areas.

Methods: Aim of paper is to highlight the adoption, implementation and scaling up of malaria in pregnancy in countries of southern Africa. Eight of the countries in southern Africa are implementing MIP strategy. The other countries are only looking at effective case management and personal protection. The process of adoption of MIP in most of the countries involved partnership participation in the development of the policy strategy and followed by consensus meeting to agree on the way forward for MIP Sensitization of MIP strategy to the health workers and involvement of RH for
the implementation of MIP was a crucial process as delivery of MIP is done by ANC workers. Results: As data collection for MIP in most countries is rather lacking a deliberate process of developing indicators that can be collected via the HMIS was initiated. In addition, the antenatal card was modified to reflect the delivery of IPT as DOT, nutritional supplements and ITN use. For the early adopter countries, Tanzania, Malawi and Zambia, coverage of both IPT and ITNs is at scale and coverage indicators are constantly improving. The involvement of RH in the implementation of MIP has strengthened integration between malaria and RH units. It has also supported the first line health worker in managing pregnant women in a holistic manner. Major challenges of MIP include inadequate personnel and commodities at ANC units. The concept of integrated information gathering is also a big challenge. Scaled up implementation for MIP is possible as shown by early adopter countries. Impact on country wide implementation on maternal and peri-natal morbidity and mortality remains. Other challenges include SP stock-outs, the unavailability of nets at ANC and the capturing of MIP data correctly within the HMIS.

Interpretation: Finally the increased resistance of \textit{P. falciparum} implies the intervention may be short-lived and urgent need for alternative drugs is critical.

549C
Rapid assessment of malaria burden in unstable malaria transmission of Mali [MIM-KK-120224]
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Introduction: Because of the adverse impact of malaria during pregnancy in Mali, the National Malaria Control Program supported by WHO had pushed towards the implementation of Intermittent Preventive Treatment using sulfadoxine–pyrimethamine for malaria prevention during pregnancy. As a replacement of chemoprophylaxis, IPT implementation had started in Mali since 2001. After 4 years of implementation, there is a need to conduct a rapid assessment survey to orient NMCP interventions for pregnant women. Methods: We have conducted a cross sectional study in unstable malaria transmission area of Mali from November to December 2004. The aim was to determine by rapid assessment the burden of malaria during pregnancy in order to guide the national malaria control program about decisions concerning malaria control during pregnancy. The study was conducted in two different health centers of Bamako where malaria transmission is seasonal with a peak in October to November. Women were recruited after their first trimester during antenatal visit and also at delivery. Peripheral parasitemia was determined during antenatal visit while hemoglobin was preformed only during antenatal visit. Placental parasitemia, low birth weight were also determined.

Results: A total of 236 women were enrolled during pregnancy while 184 women were enrolled at delivery. More than 65% of women had used malaria prophylaxis and Chloroquine had been cited by 60 and 72% of women, respectively at antenatal clinic and delivery. Only 4 and 6% of pregnant women were under IPT with SP and less than 60% of women used impregnated bed net. The proportion of women with anemia and severe anemia were 61.6 and 7.3%, respectively, and both were associated to peripheral parasitemia. The prevalence of peripheral parasitemia during antenatal visit was 6.5% and was associated to women’s age, gestity, and chimioprevention. Placental and cord infection at delivery were both 1.1%. The incidence of singleton low birth weight was 8.2% and the proportion of premature delivery was 7.7%.

Interpretation: Although malaria prevalence was lower, the prevalence of anemia and severe anemia were found to be higher. Prevention should be focused on IPT using sulfadoxine–pyrimethamine and impregnated bed net delivered at antenatal clinic.
Malaria in pregnancy in KwaZulu-Natal, South Africa [MIM-JT-67496]

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Introduction: The adverse effects of malaria in pregnancy are well documented and the measures required to prevent these effects are well known. In South Africa, there is no evidence of malaria in pregnancy and there is no specific policy available on prevention of malaria in pregnant women.

Methods: A study was undertaken during 2004–2005 to determine the burden of malaria in pregnant women in KwaZulu-Natal province, South Africa. In addition, this information was required to determine whether IPT as recommended by the WHO will be applicable to South Africa. Data was collected in three primary health care facilities in Umkhanyakude Health district, which carries the highest burden of malaria in KwaZulu-Natal. These data included demographic details, routine antenatal care attendance, history of and measurement of current malaria status and anaemia levels.

Results: Of the 1003 women recruited at antenatal care clinics, 26.5% were teenagers, 35.6% functionally illiterate and almost all of them were unemployed and unmarried. None of the women tested for malaria were positive. Based on history, 1.9% women mentioned that they had been infected with malaria during their pregnancy. Anaemia was measured in 402 women, 38% of these were anaemic and 1.7% severely anaemic. Data collection is still ongoing and a detailed analysis of the data has not been done.

Interpretation: The low burden of malaria in pregnancy can be attributed to the aggressive malaria control in the study area. These control efforts have resulted in a dramatic decrease of malaria cases by more than 90% in the general population between 2000 and 2005. This suggests that pregnant women have benefited greatly from these malaria control measures. Therefore, the findings provide no basis for recommending IPT for prevention of malaria in pregnancy in KwaZulu-Natal.

Analysis of the repertoire of placental parasite ligands of \textit{P. falciparum} using var2csa knock-out parasites [MIM-BG-461560]

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Introduction: Pregnant women are susceptible to severe \textit{P. falciparum} infections resulting from the massive adhesion of infected erythrocytes to chondroitin sulfate A (CSA) present on placental syncytiotrophoblasts. Epidemiological studies strongly support the feasibility of an intervention strategy to protect pregnant women from disease. Different parasites molecules have been associated with adhesion to CSA. We investigated the role of the \textit{P. falciparum} var2csa gene in the CSA-binding phenotype.

Methods: In order to investigate the role of var2csa in \textit{P. falciparum} IE adhesion to CSA, we established parasite lines with a disruption in the var2csa gene. Insertional disruptant mutants were generated by double-crossover homologous recombination of the pHTK-var2csa transfection construct, resulting in the replacement of the var2csa DBL4 domain with the hDHFR expression cassette. FCR3 parasites were transfected with pHTK-var2csa and selected on WR99210 and ganciclovir to obtain FCR3-var2csa mutants. The mutants were cloned and genetically characterized by PCR analysis, Southern Blotting and size-fractionated chromosomal DNA. The capability of the FCR3-var2csa mutants to cytoadhere to CSA and CD36 was examined before and after reselection on CSA. The mutants were cloned and genetically characterized by PCR analysis, Southern Blotting and size-fractionated chromosomal DNA. The capability of the FCR3-var2csa mutants to cytoadhere to CSA and CD36 was examined before and after reselection on CSA. Equal numbers of erythrocytes infected with trophozoites of the FCR3-var2csa 1F1 and 2A5 mutant clones or control parasites were seeded on Petri dishes coated with different molecules. FCR3-CSA and FCR3-CD36 were used as controls. Whereas FCR3-CSA IE bound in high numbers to CSA but not to CD36, no adhesion to CSA was observed for 1F1, 2A5 and FCR3-CD36 IE. In contrast, 1F1, 2A5 and FCR3-CD36 IE adhered strongly to CD36. No cytoad-
hesion to BSA and chondroitin sulfate C was observed. Total RNA was isolated from ring and trophozoite stage parasites to investigate var gene expression in the FCR3-var2csa mutants and the parental FCR3 parasites selected for a CSA- or CD36-binding phenotype. Whereas a full-length var2csa transcript (13 kb) was observed in the FCR3-CSA parasites, a non-functional truncated transcript (7 kb) was detected in the mutant clones 1F1 and 2A5. Using a semi-conserved varT11.1 exon II probe, larger transcripts of around 9kb were identified in ring stage RNA of FCR3-CD36 and in the two mutant clones, showing that full-length var genes are transcribed in the CD36 binding FCR3-var2csa mutants. 

Interpretation: These results show that disruption of the var2csa locus result in the expression of full-length var genes mediating IE cytoadhesion to CD36. Further data will be present on the capability of the FCR3-var2csa mutants to recover cytoadherence to CSA.

27: Vector/parasite relationships and population genetics

Posters 552–557

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

552C

Différenciation génétique des populations d’Anopheles funestus en Côte d’Ivoire par utilisation de marqueurs isoenzymatiques et microsatellites [MIM-AA-441112]

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Introduction: Anopheles funestus is an important vector of the malaria parasite in Côte d’Ivoire. Il est présent dans toutes les zones géographiques, mais la structure génétique des populations naturelles est inconnue dans ce pays. La présente étude, vise à identifier les espèces du complexe Anopheles funestus et à étudier la structure des populations d’Anopheles funestus sensu stricto des régions de savane soudanienne, savane arborée et de forêt.


Results: L’analyse de 380 échantillons par PCR spécifique d’espèce a permis d’identifier 3 espèces du complexe: An. leesoni (0.3%), An. rivulorum-like (1%) et An. funestus s.s (98.7%) qui est l’espèce prédominante. Deux à 2.6 allèles par locus ont été observés pour les tests isoenzymatiques, tandis qu’une moyenne de 9.4 à 11.1 allèles par locus ont été observés pour l’analyse par marqueurs microsatellites. L’analyse génétique des populations consécutives aux tests isoenzymatiques et microsatellites montre que chacune des populations est en équilibre d’Hardy-Weinberg, après correction de Bonferroni. Aucun déséquilibre de liaison n’a été observé entre les différentes paires de loci. La comparaison entre les populations issues des différentes zones géographiques, montrent même après application du test de Bonferroni, des valeurs d’indices Fst significatives (p < 0.05), de 0.3864 et de 0.0903 aux loci IDH1 et HK2 pour les isoenzymes et de 0.1537, de 0.0313 et de 0.0121 aux loci AF3, FunD et FunG pour les microsatellites. De même sur l’ensemble des loci, l’indice Fst de 0.1360 pour les isoenzymes et de 0.0198 pour les microsatellites reste significatif.

Interpretation: Ces indices élevés montrent l’existence en Côte d’Ivoire d’une structuration entre populations de savane soudanienne, de savane arborée et de forêt.
Uneven distribution of Plasmodium oocysts in field populations of malaria vectors from Cameroon

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Introduction: Huge genetic diversity has been demonstrated in both malaria parasites and their anopheline mosquito vectors in Africa. Within the frame of a large ongoing study aiming at unravelling genotype to genotype interactions between natural malaria vectors and the parasites they transmit, we compared the oocyst index (proportion of mosquitoes infected by Plasmodium oocysts) and load (mean number of oocysts per infected midgut) between vectors sampled from highly endemic sites in Cameroon.

Methods: Entomological surveys were carried out in two sites within the equatorial forest domain of South Cameroon (Simbock and Nyabessang). Adult mosquitoes were captured inside human dwellings. Anophelines specimens were identified according to morphological characters. Midguts were dissected, stained using orcein-red and Plasmodium oocysts were detected by standard microscopy. Oocysts were counted and the dissected midguts were stored individually in ethanol for further laboratory processing (single-oocyst species identification and microsatellite genotyping in progress). Corresponding mosquito carcases were stored as well, their DNA was extracted and species and molecular forms were determined using standard PCR-based diagnostic tools.

Results: Variable numbers of Plasmodium oocysts were detected on the midguts of An. funestus, An. gambiae (both M and S molecular forms), An. moucheti and An. nili specimens collected in the peri-urban locality of Simbock. The oocyst index was highest for An. gambiae (2.75%), N = 312, and An. funestus (1.43%). In the rural locality of Nyabessang, the oocyst index was 2.1% for An. gambiae (M molecular form only, N = 144), 1.8% for An. moucheti (N = 1,473) and 0.82% for the recently described An. ovengensis (N = 243). The oocyst load ranged from 1 to 50. As observed in Simbock, their was an inverse relationship between oocyst index and mean oocyst load amongst anophelines species, with An. moucheti showing the highest mean oocyst load (8.2 oocysts/infected midgut), followed by An. ovengensis (5.0) and An. gambiae (1.3).

Interpretation: Our results, although preliminary at this stage, reveal a reverse trend between the oocyst rate and mean oocyst load amongst sympatric malaria vector species that may appear crucial in our understanding of anophelines vector efficiency for malaria.

Population structure of the malaria vector Anopheles funestus (Diptera: Culicidae) in Madagascar and Comoros

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Introduction: During the later 1980s, malaria, transmitted mainly by Anopheles funestus, caused more than 100,000 deaths in Madagascar. The large diversity of natural environments and the presence of rice fields across this island, provides a huge number of different habitats for this anopheline species, that preserved it from the insecticide campaigns.

Methods: In order to develop adequate vector control strategies, microsatellites were used as markers for a study of the population structure of Anopheles funestus on Madagascar and Comoros, located at 250 km northwest of Madagascar, in the Pacific Ocean. Mosquitoes were collected in four different localities on Madagascar and one on Comoros. All samples were analysed using GENEPOP v3.3 software.

Results: According to the results, there was a significant genetic differentiation between all samples from Madagascar and that from Comoros (FST > 0.1043). Respecting to the Madagascar mosquito samples, it was found that there were no significant genetic dif-
ferences between samples that were collected at the east coast population, and in the highlands, respectively (FST < 0.0100). Supporting the idea that gene flow exists between both areas. By contrast, the west coast sample exhibited significant genetic differences with regard to all Madagascar samples (FST > 0.0306 from west coast to highland, for instance). The data, in addition, showed that there was no effect of intensive chemical control (DDT) on the population structure of An. funestus, according to highland samples.

**Interpretation:** Overall, this data could suggest evidences that populations from Madagascar and Comoros are genetically isolated. Furthermore, analyses of Madagascar samples give an interesting approach about genetic structure of An. funestus in the island.

555C

*Etude de la dynamique de population des formes moléculaires M et S d’* An. gambiae s.s. à Dielmo, Sénégal [MIM-NO-86380]*

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**Introduction:** Le complexe An. gambiae a la plus grande aire de répartition et assure le rôle le plus important dans la transmission du paludisme. La techniquede l’identification par PCR a permis d’identifier les différentes espèces du complexe An. gambiae s.l. et, au sein d’ An. gambiae s.s. de caractériser deux formes moléculaires S et M. Les analyses moléculaires effectuées à l’échelle du continent africain ont montré jusqu’ici que les flux de gènes entre les formes M et S étaient très réduits, quelles que soient les régions, témoin d’un phénomène de spéciation en cours.

**Methods:** L’étude que nous avons initié a pour objectifs de comparer la dynamique de transmission et la susceptibilité de ces deux formes moléculaires (S et M) à *P. falciparum*, dans un foyer sénégalais où les deux formes M et S sont sympatriques. L’échantillonnage des moustiques a été effectué mensuellement entre juillet et décembre 2004. La saison des pluies s’étendant de juillet à octobre. L’identification a été faite d’abord morphologiquement, puis par PCR-RFLP. La parturité a été déterminée par lecture des ovaires. Les repas sanguins ont été recueillis sur du papier buvard.

**Results:** Au total, 1110 An. gambiae s.l ont été capturées et 945 An. gambiae s.l. identifiées par PCR, parmi lesquels An. arabiensis a représenté 19.15%, la forme S 44.23% et 34.28% pour la forme M. 22 hybrides M/S ont été trouvés. La densité moyenne agressive a été de 2.31 piqûres par homme par nuit (PHN) pour An. arabiensis; 5.41 et 4.18 PHN respectivement pour la forme S et M. An. arabiensis est l’espèce la moins abondante, son pic d’agressivité est août (4.6 PHN). Les formes S et M se succèdent. La forme moléculaire S est beaucoup plus abondante en août (17.58 PHN pour la forme S vs. 5.08 PHN pour la forme M), alors que la tendance s’inverse en septembre période où les pluies sont bien installées, la forme moléculaire M est très majoritaire (17.66 PHN pour la forme M vs. 7.08 PHN pour la forme S). Le taux de parturité moyen a été de 73.1% pour An. arabiensis, 74.7% et 81% respectivement pour les formes M et S.

**Interpretation:** Le nombre d’hybride est resté très significativement inférieur à ce qu’on aurait eu si les croisements étaient panmictiques. Ces dynamiques de population traduisent vraisemblablement des adaptations à des biotopes et conditions climatiques différents.

556A

*Genome-wide analyses of transcriptome diversity between field and lab strain Anopheles gambiae* [MIM-GD-0]

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**Introduction:** Our current knowledge on mosquitoes’ molecular makeup is strongly biased by the experimental model that mainly involved highly inbred lab strains of Anopheles gambiae. The environmental conditions of the lab strains and field mosquitoes differ dramatically and have caused divergence of major physiological systems. The knowledge of these dif-
ferences is essential in order to validate the relevance of lab-based studies for the vectorial capacity in nature.

Methods: Our study employed microarray based global gene expression analyses to compare transcriptomes between naïve and immune challenged (bacteria and fungi) lab and field strain mosquitoes. Both Affymetrix and Agilent Technologies microarray platforms were used. Field mosquitoes of the Anopheles gambiae S and M molecular forms were collected in areas surrounding Yaoundé, Cameroon. The TIGR MIDAS software was used for microarray data analysis.

Results: A. gambiae transcript responses to experimental challenge with bacteria and fungi show significant regulation for approximately 2% of the mosquito transcriptome and affected genes represent a variety of functional classes that include: immunity, apoptosis, stress response, detoxification, metabolism, blood digestion, olfaction and others. Transcript responses to different microbial elicitors are exceptionally specific with limited overlap. This study identifies several transcripts that have not been linked directly to immune response in A. gambiae previously; their infection responsiveness and sequence features do however suggest implication in defense reactions. Comparative analyses on the JHSPH lab strain and the OCEAC M and S lab strains and field M and S strains show significant degree of transcriptomic divergence both at the naive and immune responsive level. Lab strain, filed strain and field species specific expression signatures suggest divergence of various physiological systems such as immune responsive, energy metabolic and stress responsive/detoxification systems. These differences are most likely relating to adaptations to the different environmental conditions and microbial flora.

Interpretation: A. gambiae mount specific transcript responses to immune challenge with different pathogens. A. gambiae transcriptome varies between lab and field adapted mosquitoes, as a result of differences in environmental conditions and microbial exposure.

557B Population genetic structure of the malaria vector Anopheles moucheti in Cameroon

MIM-CN-315324
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Introduction: Anopheles moucheti is a major vector of human malaria in forested areas of Central Africa. High population densities are often recorded in the vicinity of slow moving streams and large rivers where its larvae develop, but adult mosquitoes hardly penetrate surrounding forests. Despite its epidemiological importance, few studies have been carried out on this mosquito. We used recently developed microsatellite DNA markers to explore the population genetic structure of An. moucheti in Cameroon.

Methods: Mosquitoes were collected in the villages of Simbock, Olama and Nyabessan. All three villages belong to different hydrographic networks and were distant 50–200 km. A total of 184 female specimens were genotyped at 11 microsatellite loci. Genetic variability parameters (allelic frequencies, heterozygosity, number of alleles per locus) were assessed for each locus in each population. Genotype frequencies were tested against Hardy–Weinberg expectations for each locus in each population and overall. Differentiation between populations was examined by F-statistics and tested using the exact test of genotypic differentiation. Statistical significance levels were adjusted using the Bonferroni procedure to take into account multiple tests.

Results: All microsatellite loci tested revealed highly polymorphic with a number of distinct alleles per locus ranging from 9 to 17 and mean heterozygosity across all loci varied from 0.698 in Nyabessan to 0.731 in Olama. Hardy–Weinberg predictions were significantly rejected (P < 0.05) for 9 out of 11 loci when considering the pooled samples as belonging to one single gene pool. All deviations were associated with positive F’s values, suggesting heterozygotes deficits indicative of a Wahlund effect (pooling of separate gene pools). Hardy–Weinberg expectations were restored at most loci in all three geographic populations, suggesting random mating at this geographical scale. High amounts of genetic differentiation were observed between populations. Mean Fst estimate based on the whole dataset.
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(3 populations, 11 loci) was 0.157 and was highly significant ($P < 10^{-4}$, Fisher’s combined probability test). Highly significant genetic differentiation was revealed between all pairs of populations, with pairwise $F_{st}$ estimates across all loci ranging from 0.132 to 0.162 ($P < 0.05$).

**Interpretation:** Recently available microsatellite loci revealed useful markers to assess genetic differentiation between geographical populations of *An. moucheti* in Cameroon. Studies at a more refined geographical scale are underway.

**28: Roll back malaria**

**Posters S58-S85**

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

**558C**

Progress toward Abuja ITN targets for rolling back malaria in sub-Saharan Africa [MIM-MA-9984]

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**Introduction:** A pillar of the RBM strategy is to foster the use of ITNs to prevent malaria. The Abuja target calls for 60% of children under five and pregnant women to sleep under an ITN. Using data collected by the USAID-funded NetMark Program, we look at progress toward that target by showing changes in patterns of ITN awareness, ownership and use in three countries where data were collected in both 2000 and 2004, supplemented where appropriate by data collected in 2004 from two other countries.

**Methods:** NetMark conducted household (HH) surveys in Nigeria, Senegal, and Zambia in 2000 and again in 2004. In 2004, surveys were also conducted in Ghana and Ethiopia. The same stratified multistage sampling strategy and instruments were used in each country. Respondents were women 15–49 with a child under 5. Sample size was 1000–2000 per country per wave. In net-owning HH, questions were asked to determine the treatment status of each net owned (never treated, ever treated, currently treated). HH members were enumerated and linked to a specific net to enable calculation of the percent sleeping under nets and ITNs. Questions on awareness, knowledge and other ITN-related topics were also asked.

**Results:** Awareness: Awareness rose considerably in all countries: from 7% to 60% in Nigeria; 70 to 97% in Senegal, 51 to 88% in Zambia. Ownership: A far larger proportion of HH owned nets in 2004 than in 2000, and nets owned were more likely to be treated. In Nigeria, net ownership rose from 12 to 27%; ITN ownership from 0 to 9%. In Senegal, net ownership rose from 34 to 56%; ITN ownership from 8 to 39%. In Zambia, net ownership rose from 27 to 50%; ITN ownership from 4 to 34%. However, coverage within countries varied greatly by site and sometimes by urban-rural. Use: the proportion of children under five sleeping under an ITN the previous night ranged considerably, with some countries making substantial progress and others minimal. In Nigeria the percent of under-fives sleeping under an ITN was 0 in 2000 and 3% in 2004. In Senegal the comparable figures were 5 and 24%; in Zambia they were 4 and 17%. The proportions also varied within country by site and sometimes by urban-rural. Use by pregnant women was comparable. In Nigeria the percent of pregnant women sleeping under an ITN was 0% in 2000 and 4% in 2004; in Senegal the figures were 6 and 31%; in Zambia they were 1 and 13%.

**Interpretation:** Countries are at very different stages with regard to Abuja targets and some have made tremendous strides, but overall, countries are still far short of Abuja targets of 60% of vulnerable groups sleeping under a net.

**559A**

Epidemiological assessment of malaria among nomadic Fulani herdsmen in South Eastern Nigeria [MIM-OA-44646]

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**Introduction:** In Nigeria, malaria accounts for more than 200,000 deaths annually with about 60 million people experiencing bouts of malaria at least twice within six months. The status of parasitic infections...
amongst the nomadic Fulani Herdsmen in South Eastern Nigeria, which has not been investigated before due to their migratory habits, was done to enrich epidemiological data available on malaria.

Methods: In Nigeria, malaria accounts for more than 200,000 deaths annually with about 60 million people experiencing bouts of malaria at least twice within six months. The status of parasitic infections amongst the nomadic Fulani Herdsmen in South Eastern Nigeria, which has not been investigated before due to their migratory habits, was done to enrich epidemiological data available on malaria.

Results: Out of the 709 nomads used in this study, 78 (11.0%) were found infected with malaria parasites. *Plasmodium falciparum* was recorded in 42.3% of the infected study population. *P. malariae*, *P. vivax* and *P. ovale* was found in 24.4, 7.7 and 5.1%, respectively of the study population. A mixed infection of *Plasmodium falciparum* and *P. malariae* was observed in 20.5% of the study population. *P. falciparum* was significantly more prevalent than the other species (*P* < 0.05).

Malaria infection was significantly higher in the males than the females. No child below the age of 3 years was found infected. The 6–10 age group had the highest prevalence. Malaria parasitaemia was higher in males than females. A total of 378 huts out of the 406 examined had mosquito bed nets. About 91% of the nomads were aware that mosquitoes were responsible for malaria.

Interpretation: Roll back malaria intervention strategies appeared to be more effective amongst the nomadic Fulanis.

560B

Studies on home treatment of children with malaria and bednet use in Ebonyi State, South Eastern Nigeria [MIM-JA-195908]

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Introduction: Malaria remains a leading cause of morbidity and mortality in children under 5 years of age in most tropical Africa including Nigeria. This study was aimed at determining the Home management of febrile children under 5 years and to investigate the use of bednets by children in the rural parts of Ebonyi State, South Eastern Nigeria.

Methods: Structured pre tested questionnaires were given to mothers/care givers in order to provide information on their appropriate care-seek behaviour as well as the use of bed nets by children. Bednets were examined and their conditions recorded.

Results: Out of the 300 recently febrile children under 5 years of age, 525 received care at a health facility, 36% received an anti malaria drug at home and 12% received neither. Only 9% of children consulted a community Health worker while 4% consulted a traditional healer during their illness. Of the 177 recently febrile children taken to a health facility, 97% received medications, 84% were given tablets or syrups while 65% received an injection. Of these treated at home, 43, 46 and 6% were given syrups, tablets and injections respectively.

The most frequently administered drug was chloroquine. Some of the children were under dosed. Only 4% of the children under 5 years of age slept under a bednet. Treatment of the bednets was rarely observed implying that treated bednets were not been used.

Interpretation: Early and appropriate treatment of malaria detected in children by caregivers may help prevent complications in malaria. There is need for intensive health education of care givers.

561C

Malaria control in pregnancy in Ghana: From research to policy [MIM-VB-34730]

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Introduction: Malaria in pregnancy is a significant health problem in Ghana, contributing significantly to maternal and infant morbidity and mortality. The issue however had not received the needed attention.

Methods: We have completed two major field trials on intermittent preventive treatment in pregnancy, using sulfadoxine–pyrimethamine (SP) and in vivo studies on anti-malaria drug resistance.
Results: In the first trial, mean birth weight for sulphadoxine–pyrimethamine was 3.09 kg (CI: 3.02–3.20), chloroquine – 2.99 kg (CI: 2.91–3.06) and routine care – 2.93 (2.86–2.99). The F-test for means for combined primigravidae and secundigravidae was 4.38 (p = 0.01) and for primigravidae alone was 4.77 (p = 0.009). In the second trial, early SP (given from 4 months of pregnancy) gave significantly higher birth weight than early CQ in primigravidae (P = 0.005). In secundigravidae, the trend for early and late SP (given from 7 months of pregnancy) was similar to that observed for primigravidae. The results however showed significant differences in birth weight between early and late SP (P < 0.05). In vivo studies showed that resistance was evolving rapidly (for Chloroquine – 52%, SP – 42% at day 28). Based on these findings, Ghana has developed a new IPT policy with three SP doses starting after quickening and spaced at least 4 weeks apart. This has been rolled out in at least 20 districts initially and expected to scale up nationally by the end of 2005.

Interpretation: Ghana has used local evidence to develop new IPT policy. In view of evolving SP resistance, there is need for research on new IPT drugs in addition to assessing the impact of IPT on pregnancy outcome.

562A
Four malaria success stories: How malaria burden was successfully reduced in Brazil, Vietnam, India, and Eritrea [MIM-LB-282658]

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Introduction: In the last decade, four countries—India, Brazil, Vietnam, and Eritrea— have seen dramatic and well documented reductions in malaria morbidity and mortality. To provide other malaria-affected countries with lessons learned from these four programs, each country was assessed to determine common factors that likely contributed to the success of control efforts.

Methods: Data was collected through review of extant published and unpublished literature and interviews with key program staff and partners involved in design and implementation of control activities in these four countries. Interviews sought both objective and subjective information on what made the program successful. Epidemiologic, technical, programmatic, policy, and political aspects that might have contributed to these successes were probed. Recurrent themes were extracted from the reports and interviews to develop a set of common success factors.

Results: Multiple common success factors were identified in these four country programs, including: (1) active involvement of all levels of government, (2) participation of communities and local authorities, (3) a targeted technical approach using a package of effective tools, (4) data-driven decision-making based on good surveillance and operational research, (5) strong program leadership capable of navigating past bureaucratic hurdles, (6) sufficient and flexible funding with decentralized control of finances, (7) skilled technical capacity at national and sub-national levels, (8) pro-active technical and programmatic support from partner agencies and donors.

Interpretation: If the goals of Roll Back Malaria (RBM) are to be achieved in other malaria-affected countries, governments and their partners must take the lessons from these four country successes and work to strengthen their programs in all of these key areas.

563B
Malaria in ntouessong primary and nursery school children: An epidemiological survey [MIM-AN-278939]

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Introduction: Malaria is endemic throughout Cameroon with children under 5 years of age being the most affected. The epidemiology of malaria, however, remains poorly understood in many areas. This information is fundamental for developing control strategies. The malaria situation in Cameroon has worsened due to an increase in resistance of the parasite to anti-malarial drugs and vectors to insecticides. Thus, we conducted an epidemiological study in the southern-forested Cameroonian village of Ntouessong.
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Methods: In May 2004, finger-pricked blood samples from pupils in Ntouessong village were obtained for microscopic detection Plasmodium parasites and to determine the PCV. Parasite DNA was extracted and PCR amplified to identify the Plasmodium species. Antibody titers against crude malaria extract was determined by indirect ELISA. During 208 human nights for 6 months, mosquitoes collected indoors were sorted, female anophelines identified morphologically, and species of the Anopheles gambiae complex identified using PCR. Mosquito infectivity was determined using a double monoclonal antibody sporozoite ELISA.

Results: The 285 pupils were divided into three age groups; 2–5 years, 6–9 years and 10–15 years. The overall prevalence of slide-positive malaria was 88.8%, with Plasmodium falciparum being the most predominant species (84.2%). The overall average parasite density was 1730 parasites/μl of blood, with the highest average density of 4609 parasites/μl in 2–5 year olds. However, only three of the children had fever and clinical anaemia. The PCV ranged from 29 and 41%, while the gametocytic index was 0.4%. Antimalarial antibodies were detected in 77.0% of the children with the highest titres recorded in older and slide-negative children (p < 0.05). In total, 390 female Anopheles vectors of human malaria were collected consisting of An. nili (56.9%), An. gambiae (24.6%), An. funestus (17.2%) and An. moucheti (1.3%). An. nili was the most abundant and aggressive (1.07 b/p/n) species, while An. gambiae s.s was the only member of the An. gambiae complex found. An. funestus and An. gambiae had the highest sporozoite rates of 68.7 and 54.2%, respectively, with most vectors infected by P. falciparum (59.1%). An. nili had the highest mean daily entomological inoculation rate of 0.37ib/p/n, with higher values observed during the wet seasons.

Interpretation: Our study shows that children below 5 years of age tend to have large numbers of parasites without presenting with fever. P. falciparum is the predominant parasite species with An. nili being the primary malaria vector.

564C
Comparison of Hb and PCV for the measurement of anaemia in malaria-endemic settings

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Introduction: Prevalence of anaemia is best determined by measuring haemoglobin (Hb) concentration however, haematocrit or packed cell volume (PCV) is a common alternative because of its logistical simplicity. A standard threefold conversion between the two measures (Hb = PCV/3) has been used to define cut-offs for estimating the prevalence of anaemia. Given the increasing use of anaemia as an impact indicator in malaria intervention studies, we investigated the comparability of Hb and PCV.

Methods: Concurrent data on Hb and PCV were available for individuals from three malaria endemic areas (Navrongo, Ghana; Kwemasimba, Tanzania; Dielmo, Senegal). We used the Bland & Altman method to compare Hb and PCV/3 by plotting the difference between the two measures against their average. Further linear regression analyses were undertaken to identify factors associated with the difference between the two measures. A linear regression of the difference of the measures on their average enabled us to describe the line of best agreement and 95% limits of agreement for these two measures over a range of values.

Results: There was a clear and consistent bias between the two measures, with Hb less than PCV/3 in 78% (1558/1988) of observations. This difference was non-uniform, decreasing with the average measure in all three studies. Linear regression was carried-out separately for each database, and revealed significant variation in the mean difference between the two measures by correlates of malaria exposure. Overall, mean difference was greater in individuals with concurrent Plasmodium falciparum (Pf) infection, greater in the wet than dry season, peaked in children 6 months to 4 years,
and was greater in males than females in the same age group. Mean difference did not vary significantly with Pf infection or sex in the Dielmo data, although 72% samples were Pf positive. For children 0–4 years, we defined a linear relationship between difference and average of the two measures to obtain a “line of best agreement” and “95% limits of agreement” between Hb and PCV. We predicted Hb values from PCV and suggest that in malaria endemic settings, a cut-off of Hb < 5 g/dl is equivalent to PCV <20% and a cut-off of Hb < 8 g/dl is equivalent to PCV < 27%. However, a cut-off of PCV <33% is still a good approximation to Hb < 11 g/dl.

**Interpretation:** The relationship between Hb and PCV appears to be modified by exposure to malaria. Hb should be the measurement of choice for malaria intervention studies. PCV using standard cut-offs may underestimate the prevalence of moderate and severe anaemia.

**565A**
Southern Africa Malaria Control: A subregional model for building leadership and capacity for scaling up Roll Back Malaria control in Southern Africa [MIM-NC-334308]


World Health Organisation, Southern Africa Inter-Country Malaria Control

**Introduction:** The WHO-AFRO Southern Africa Malaria Control Program (SAMC) was set up in 1997 in response to OAU Declaration on Malaria Prevention and Control in the context of African Economic Recovery and Development and in response to repeated calls from SADC member countries to intensify efforts to improve the access, coverage and quality of malaria control interventions.

**Methods:** The launch of RBM movement in Southern Africa in 1998 put further demand on the WHO leadership and on the capacity at the WHO country and WHO inter-country levels to scale up delivery of malaria control interventions. Between 1998 and 2001, inclusive, marked the initial phase that was characterized by consultation, consensus, partnership building and strategic planning. Supported by core WHO funding and complemented by financial support from partners such as DFID and AusAID, SAMC has now established itself as a major partner to the national malaria control programs of the SADC region.

**Results:** Since 2000, SAMC has been spearheading the AFRO-RBM activities within the sub-region towards achieving the 60% 2005 Abuja targets and contributing towards the MDGs through development of malaria institutions and capacity in SAMC countries through the development of national malaria control units and programs within the Ministries of Health. The DFID supported implementation schedule included capacity building at ICP/MAL/SAMC, procurement of equipment and preparation of work plans by 2001, thereafter was the scaling up phase of interventions until 2004. The end of 2004 then saw the end of scaling up phase and the beginning of the consolidation phase, 2005–2006. SAMC country support has resulted in a remarkable increase in national and international investment into malaria control. All countries except one have well-established national malaria control units at all levels. The SAMC team and its supporting network of consultants and institutions have now become fully established resulting in major increase in country technical support delivery. Country teams at both national and WHO country levels have remarkably expanded resulting in expanded and improved access, coverage and quality of delivery of malaria control interventions.

**Interpretation:** Focal points at both country and SAMC for technical areas of work have been put in place. The RBM external evaluation has recognized SAMC as a working model for increasing support for countries in the next phase of scaling up in other sub-regions.

**566B**
Worm infections can worsen malaria: Towards a new means to roll back malaria [MIM-PD-408800]

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**Introduction:** Helminthic infections might adversely affect the clinical outcome of malaria infections. This suggests that helminths could influence the acquisition of immunity against Plasmodium and alter the assessment of efficacy of malaria control intervention, includ-
ing vaccine trials. We show the deleterious impact of helminths on clinical malaria in four different study sites and suggest that the treatment of helminths would offer an affordable, strongly effective and novel means to roll back malaria.

Methods: Three investigations were carried out in Senegal, West Africa. In Dielmo (220 inhabitants), 13 of the 80 children were infected by intestinal helminths at the time of study. In Mlomp, a cohort of 201 children (3–14 years old) was followed up for 5 years. In the two villages, each febrile episode was actively recorded so as to precisely identify the occurrence of each malaria attack. In Richard–Toll, 512 children (5–15 years old), were enrolled and tested for contamination by Schistosoma mansoni and for occurrence of malaria attacks determined by active detection. In a fourth study, in Madagascar, two cohorts of 2–15 years old children were closely monitored for 2 years for malaria attacks, one being treated against helminth infections.

Results: In Dielmo, the relative risk of clinical malaria was lower in helminth-free children as compared to children carrying Ascaris, Ancylostoma or Trichuris. This reduction was similar in magnitude to that associated with the sickle-cell trait (RR = 0.65, p = 0.003 versus RR = 0.57, p = 0.06, respectively). In Mlomp, compared to 79 age-matched children free of worms, the incidence of malaria attacks was 1.72-fold higher in 55 children co-infected with intestinal helminths (p = 0.0215). In Richard–Toll, a 2.25-fold higher incidence of malaria attacks was recorded in patients with the highest contamination by Schistosoma. The pattern was more complex for low or medium egg loads. In the absence of schistosomiasis, the relative risk of malaria was similar to that observed in worm-free individuals in Dielmo (RR = 0.59). In Madagascar, over a 2-year follow-up, a 60% decrease in malaria incidence, parasite densities, and spleen rates was observed among the children treated against helminths (n = 36), as compared to the non-treated and age-matched control children (n = 21). In these different studies, there was no indication for shared exposure to helminths and malaria, and no case of malnutrition, which could have constituted confounding factors.

Interpretation: An increased susceptibility to Plasmodium was found in wormy individuals both in distinct malaria endemic areas and with different species of worms. These studies suggest that the treatment of helminth infections could improve the malaria status.

567C

Bilan de la résistance de Plasmodium falciparum aux antipaludiques de première ligne au Cameroun

MIM-VF-49158

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Introduction: Les programmes de lutte contre le paludisme doivent s’appuyer sur des données fiables et constamment mises à jour pour être efficaces et appropriées. Ces données sont d’autant plus importantes que la situation de la chimiorésistance de Plasmodium falciparum change rapidement, à la fois dans le temps et dans l’espace.

Methods: Le Programme National de Lutte contre le Paludisme du Cameroun (PNLP) a retenu dans son plan d’action de mettre à jour la cartographie de la chimiorésistance de P. falciparum aux antipaludiques en vue d’adapter si nécessaire la politique nationale de prise en charge des cas. Le Laboratoire de Santé Publique de l’OCEAC, en collaboration avec le PNLP, a ainsi réalisé de 1999 à 2004, 28 enquêtes dans neuf des dix provinces du pays avec le protocole standard de l’OMS.

Results: Le bilan de ces enquêtes montre que la chloroquine n’est plus efficace au Cameroun, le taux d’échec ayant largement dépassé le seuil de 15%. Cependant, son efficacité paraît conservée à l’Extrême-Nord (13%). Pour ce qui est de l’amodiaquine (AQ), les résultats de 7 enquêtes montrent que cette molécule conserve une bonne efficacité: 0-4% de taux d’échec. En revanche, les taux d’échec de la sulfadoxine–pyriméthamine atteignent déjà les niveaux qui méritent une attention particulière (14% à Mango et Kelbi). Ces différents résultats ont conduit le PNLP à adopter une nouvelle politique de traitement des formes non compliquées du paludisme à P. falciparum au Cameroun en 2002 avec l’amodiaquine pour une période transitoire de 2 ans. En 2004, la combinaison artésunate-amodiaquine a été...
officiellement adoptée pour remplacer l’amodiaquine en monothérapie.


568A
Monitoring and evaluation of implementation of the Abuja declaration: Progress made on the Abuja targets in Southern Africa [MIM-KG-13105]
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The World Health Programme Inter Country Programme on Malaria, Harare, Zimbabwe

Introduction: Malaria is a major cause of death in many of Southern African countries, especially in countries such as Angola, Malawi, Tanzania, Zambia, Mozambique, Comoros and Madagascar. Since 2001, increased coverage of interventions has achieved results in terms of Abuja indicators and decreased morbidity and mortality. Increased scale up of interventions is expected to further improve on the indicators as the Southern Africa maintains the accelerated efforts in malaria control as 2010 approaches.

Methods: Information presented in this paper originates from reports provided by countries on the program made on achieving their malaria control goals and submitted to the (WHO) Inter Country Programme on Malaria for Southern Africa. These results provide tangible results and answers to all involved in malaria control in Southern Africa and are an encouragement to all countries who have been vigorously engaged in the fight against malaria.

Results: Implementation of malaria control interventions has been scaled up in all countries of the region with Malawi scaling up ITN distribution and sales from under 30,000 to over 1,000,000 (in 2004) ITNs yearly. Other countries have also seen increased distribution and selling of ITNs. In terms of IRS, six countries in the region have increased the number of households/units sprayed per year. The operational coverage for IRS also improved in Botswana, Swaziland, South Africa, Swaziland, Zambia and Namibia. The percentage of the population protected ranges from approximately 3% in Zimbabwe to 95% in the Republic of South Africa and Swaziland. Progress on the key malaria Abuja indicators shows access to IPT ranging from 20% in Zimbabwe to 93% in Malawi. Access to treatment within 24 h ranges from 27% in Tanzania to over 95% in South Africa. Antimalarial drug policy changes have been made in Botswana, Mozambique, Namibia, Tanzania, Zambia and Zimbabwe. ITN coverage in U5s and pregnant women has also improved in the region. Morbidity and mortality has since seen some encouraging results and decreases in mortality have been experienced in some country, more especially when unit population figures are used.

Interpretation: Southern African countries have shown commitment in reducing the malaria burden in the region. However, challenges still remain especially in the areas of case management and programme strengthening to achieve impact.

569B
Experiences and results of the World Health Organisation Inter-Country Programme on malaria for Southern Africa in monitoring and evaluation [MIM-KG-368640]
K. Gausi, S. Katikiti, S. Murugasampillay
WHO/ICP Malaria for Southern Africa (SAMC)

Introduction: By many estimates, malaria is still the number one killer in many of Southern African countries. Countries of high burden include Angola, Malawi, Tanzania, Zambia, Mozambique, Comoros and Madagascar. The Southern African Inter-Country Programme (SAMC) of the World Health Organisation has shown considerable steps in supporting ministries of health in the region in monitoring and evaluation of malaria control.

Methods: Countries in the region were supported by SAMC in quantifying the achievements the countries are making on the Abuja Targets as well as generating information for decision making at programme level. Information below is based on empirical evidence observed during implementation of a WHO initiative of providing technical assistance to countries from a sub-regional centre able to provide this assistance when required by countries in a timely manner. The unit supported all countries in the region in monitoring and evaluation.
Results: Swaziland is using the GIS system in mapping households which use ITNs. Health Mapper is used for processing routine data and mapping it for decision making, population surveys and health facility surveys were supported in four of the countries. Population based surveys were conducted in all countries. All countries improved their routine data collection systems and dissemination was also enhanced. Results attained during the life of the project has shown improved use of monitoring and evaluation tools by ministry of health malaria control programmes, increased demand for availing information to managers of malaria control programmes and more integration of information collection tools for these programmes in all countries.

Interpretation: The sub regional mode of technical support provision for WHO has improved demand for and use of information on malaria in various forms and has helped in these countries report to their national RBM partnerships on progress of implementation.

Clinical presentations and outcome of pediatric admissions with intention to treat malaria in Korogwe district hospital, Tanzania [MIM-SG-90414]


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Introduction: Malaria is the leading cause of morbidity and mortality in Tanzanian hospitals. The most susceptible individuals are children below five years and pregnant women. The frequency of severe malaria and the clinical manifestations change with transmission intensity. In this study, we describe the clinical features on admission and clinical outcome in children admitted to a hospital, where transmission intensity varies from low to very high in the catchments area.

Methods: After informed consent demographic and clinical data was collected and disease severity was assessed utilizing modified WHO criteria for severe malaria. Haemoglobin level was measured using Haemocue and blood slide was examined by microscopy. Diagnosis on admission and at discharge, treatment received and clinical outcome were also recorded.

Results: A total of 1589 children below five years of which 47.33% were infants were admitted to the Hospital from the outpatient clinic with a clinical diagnosis of malaria. Features on admission were pallor 97.8%, deep breathing 9.9%, unable to localise pain 6.5% and jaundice 2%. Severe malaria was diagnosed in 39.9% of children. For those with blood slide (BS) results, 48.5% had malaria parasites. A total of 36 patients died of which 87.9% had severe malaria with anaemia on admission. Respiratory distress on admission that was positively associated with death (odds ratio = 3.82, p < 0.001). Children with history of convolution in the ward also had significantly higher odds of dying (odds ratio = 8.36, p < 0.001). Many children, who according to the national guidelines were where treated for malaria on clinical suspicion turned out to have a negative slide reading. The mortality among patients with a negative BS was not significantly different from the mortality among those with a positive BS results (0.002, p = 0.967).

Interpretation: Anaemia due to severe malaria was the most frequent cause of malaria deaths in young children. Provision of safe and prompt blood transfusion is a major challenge. Improvement of diagnostic services and management in under-fives is highly needed.

Serum levels of vitamin a, zinc and calcium among malaria patients in Douala, Cameroon [MIM-IG-102645]

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Introduction: Vitamin A, zinc and calcium are essential micronutrients required for a proper functioning of the immune system and for growth. Their deficiency in patients living in malaria endemic areas such as Cameroon can therefore worsen the outcome of disease in target groups.

Methods: The investigation was carried out on 96 children under 6 years in Douala. Forty-four malaria
patients were compared to 52 controls. HPLC and colorimetric methods were used to measure serum vitamin A, zinc and calcium levels.

**Results:** Serum Vitamin A and calcium concentrations were significantly low ($P < 0.01$) among malaria patients (respectively, $0.8 \pm 0.4 \mu$mol/l and $81.3 \pm 23.7$ mg/l) as compared to controls ($1.1 \pm 0.6 \mu$mol/l and $96.3 \pm 16.7$ mg/ml). We found that Vitamin A, calcium and zinc concentrations were lower than normal levels in 52.27%, 52.27% and 27.27% patients, respectively.

**Interpretation:** This study suggests that there is a significant decrease in blood concentration of Vitamin A, calcium and zinc in children suffering from malaria. Dietary pattern should be ameliorated to provide enough bioavailable quantities of these nutrients to support physiological needs and to compensate their loss. Furthermore, their metabolism in parasite should be additionally investigated.

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**572B**

**Use of Personal Digital Assistants (PDAs) with Global Positioning Systems (GPS) in large-scale community household surveys [MIM-AH-47960]**

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**Introduction:** Personal digital assistants (PDAs) with global positioning systems (GPS) were preprogrammed to enable their use in community-based surveys. Main objectives were to assess their usefulness in (a) establishing a statistically valid sampling methodology, (b) improving data quality and (c) expediting the dissemination of results.

**Methods:** A PDA-based system was used to: (1) rapidly map all household units in the enumeration area (village) with multiple PDAs, (2) beam data between PDAs and select a statistically valid sample of all households within the enumeration area at field level, (3) beam list with selected households back to multiple PDAs and split teams to navigate back to the selected households, (4) conduct an interview tailored to the respondent (preprogrammed skip patterns), (5) and enter data using entry screens with logical and range restrictions, (6) safely store entered data including the generation of automatic back-up files, (7) send data to a laptop or desktop PC to enable rapid aggregation and preliminary analyses, and (8) provide data for spatial analyses.

**Results:** This technology has been used as part of a multi-disciplinary evaluations of the Togo 2004 National Child Health Campaign, over 40 PDAs equipped with GPS technology were used in a large scale community-based household survey to evaluate post-campaign ITN coverage levels. During a two week field survey over 2000 households were sampled within 120 enumeration areas from 12 districts throughout the country. Following a 3 day training, this PDA technology proved user-friendly, accurate and suitable for field conditions. Data collected were aggregated, analysed, and presented at both national and international level within days after the end of field work. In this presentation we will discuss the methodology, software, logistics, data quality, training requirements and costs involved.

**Interpretation:** We propose this approach as an alternative to the standard EPI cluster sample methodology for fast, but statistically valid, large-scale community-based surveys.

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**573C**

**Knowledge, attitude and perception of malaria among ANC women in Lagos, Nigeria [MIM-NI-36365]**

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**Introduction:** Pregnant women especially primigravidae are at increased risk of parasitaemia during pregnancy, with adverse effects for both mother and child particularly in endemic areas. Educational campaign targeted at women of childbearing age will curtail the scourge of malaria in pregnancy. We assessed the knowledge and perception of pregnant women to malaria infection and its management.

**Methods:** From the weekly antenatal care visits at the Lagos Island Maternity Hospital, 350 pregnant women were randomly recruited into this study.
was used to determine the level of anaemia and parasitaemia was determined with thick and thin giemsa stained blood smears. The level of awareness and knowledge of the women with respect to their social status was assessed. We also studied the effect of the “EKO free medical treatment” an outfit of Lagos State Government on the outcome of malaria in pregnancy.

Results: The prevalence of peripheral blood malaria parasitaemia was 27.4% (96/350) amongst the pregnant women and 88.5% (85/96) of the parasite positive subjects were infected with *P. falciparum* and the rest (11.5%) were infected with *P. malariae*. PCV ranged between 20% and 40% (mean of 34.2%) and 25.7% (90/350) were anaemic with PCV < 33%. Parasitaemia ranged between 32 and 1000 parasites/μl blood with a mean of 281.8 parasites/μl blood. We found an association between malaria prevalence and occupation ($X^2 = 5.60$, $P = 0.032$) and this association was not influenced by parity. Also the prevalence of malaria was highest among the least educated mothers.

Interpretation: Improvement in knowledge and education of childbearing age-mothers will impact malaria control.

574A The impact of childhood malaria chemoprophyaxis on cognitive abilities and educational outcomes 14 years later: A randomized trial in The Gambia


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Introduction: Associations have been found between malaria infection and poor cognitive ability but causality has not yet been demonstrated through preventative trials and the long-term impact has not been investigated.

Methods: One thousand two hundred sixty-eight children aged 3–59 months received malaria chemoprophylaxis (MaloprimR) or placebo for between one and three malaria transmission seasons from 1985 to 1987 as part of a randomised controlled trial. At the end of the trial prophylaxis was provided for all children under 5 years of age living in the study villages. Children were traced in 2001 and their cognitive abilities, educational history, height and weight assessed. A statistical analysis plan specified the primary endpoints cognitive function, school enrolment, and highest education level attained, and a secondary analysis examining intervention effects according to the number of years of post-trial prophylaxis received.

Results: Five hundred and seventy-eight trial participants were traced (290 prophylaxis group and 288 placebo group) in 2001 when their median age was 17 years 1 month (range 14 years 9 months to 19 years 6 months). In an intention-to-treat analysis adjusting for covariates, there was no significant difference overall in cognitive abilities between intervention and placebo groups, but there was a significant interaction between intervention group and the duration of post-trial prophylaxis, with cognitive ability somewhat higher in the intervention group among children who received little or no post trial prophylaxis. Among the children who received no post-trial prophylaxis cognitive score was 0.2 standard deviations higher in the intervention group (95% CI $= -0.03$ to $0.5$, $P = 0.08$), and among children who received less than 1 year of post-trial prophylaxis, the effect was 0.4SD (95% CI $= 0.1$–$0.8$, $P = 0.01$). The intervention group had 0.52 more years of schooling (95% CI $= -0.041$–$1.089$, $p = 0.069$). School enrolment was similar in both groups.

Interpretation: The results suggest that malaria prophylaxis may have improved cognitive function and further confirmatory studies would be useful.

575B Malaria control in Vietnam and the perspective for the year 2010

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Introduction: There are 80 million population in Vietnam among which 42 millions are living in the malaria endemic areas where the main vectors are *An. minimus*,...
An. dirus, and An. sundaicus and the main parasites are P. falciparum and P. vivax. The malaria eradication programmes were implemented 1958–1975 and from 1976 to 1990 its strategy was shifted to the malaria elimination. Since 1991 till now the malaria elimination strategy of the country was shifted to the malaria control programme.

Methods: The objectives of the malaria control programme are to reduce morbidity, mortality, no big outbreaks and develop sustainable factors for malaria control. The main solutions are to concentrate the investment and technical guidance for rolling back malaria in the high endemic areas and high risk groups. For control of malaria vectors, ICON 10WP for IRS, ICON 2.5 CS for ITNs have been used. Artemisinine Combination Treatment (ACT) and another antimalaria drugs have been used. From 1991 to 2004, the number of malaria cases, of death and malaria outbreaks were down from 1,091,300; 4646 and 144 in 1991 to 128,662; 24 and 0 in 2004 with reduction rates of 88.8%; 99.5% and 100%, respectively.

Results: The directions for malaria control for 2005–2010 are to continue rolling back malaria, develop sustainable malaria control factors, and make malaria be no longer a major health problem in parallel with social economy development of the country by the year 2010. The objectives are to reduce morbidity by 50% (0.8/1000p.), mortality by 50% (0.02%/100,000p.) and no outbreaks. The main solutions are to strengthen the quality of malaria epidemiological surveillance basing on stratification of malaria zones, health information system from central to communal levels, the quality of supervision, diagnosis, treatment and vector control; socialization of malaria control, health service system at all levels and to develop scientific researches, IEC and internal cooperation.

576C
A study of specific antibodies to a polymorphic Plasmodium falciparum antigen and of parasite antigen genotypes in children in Buea, Cameroon [MIM-HK-2350]

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Introduction: The genetic diversity of malaria parasites is increasingly being considered in epidemiological studies on transmission of infection and acquisition of immunity as well as in population genetic tests to identify polymorphisms that are adaptive. The aim of the present study was to test for associations between the presence of particular msp 1 block 2 genotypes and the specificities of antibodies in children within the same blood samples.

Methods: A total of 244 children (aged 4–16 years and of both sexes) were studied in April/May 2002, the peak transmission season in Buea, Cameroon. A 5 ml sample of venous blood was collected from each child and the name, age, gender and axillary temperature was recorded. Serum was separated from the blood and stored at –200 °C. Antibody reactivities were analysed by ELISA using an array of recombinant antigens representing different sequences from the polymorphic block 2 region of the merozoite surface protein 1 (MSP1), and the blood samples that were slide-positive for P. falciparum were genotyped for msp1 block 2 alleles.

Results: Overall, a total of 141 out of 244 (58%) children were slide positive for malaria. An analysis of parasitaemia shows that there was a steady decrease in proportions with higher parasite densities (>500 parasites/µl) from the youngest to the oldest age group, in both Molyko and other schools, but the decrease was more apparent and statistically significant (p < 0.05) in those from the other schools. Genotypes of msp1 block 2 were successfully amplified and scored in 122 out of the 141 slide positive blood samples (most of the remaining 19 had low level of parasitaemia). The mean number of clones per individual decreased slightly with age, ranging from 2.18(±0.21) in the youngest age group to 1.74(±0.166) in the oldest age group, but the difference was not significant. Individuals with higher levels of parasitaemia (>500 parasites/µl) had a slightly higher mean number of clones compared to those with lower parasitaemia, i.e. 2.33(±0.112) compared with 1.80(±0.10), but again the difference was not significant. There was a consistently positive association between the presence of each genotype of msp1 block 2 in the blood at the time of sampling and the presence of antibodies of the corresponding specificity in a child’s serum.

Interpretation: At a population level, antibody prevalence does not simply reflect prevalence of parasites,
but rather may be due to differences in the incidence of past infections. Antibody specificities are to some extent determined by current parasite infections.

577A  
La moustiquaire imprégnée d’insecticide à l’épreuve des conceptions populaires du paludisme et de l’organisation de l’espace domestique—Burkina Faso [MIM-PL-2620]

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Introduction: Le paludisme est une maladie en croissance (OMS 2003). Cependant divers moyens de prévention sont mis en œuvre pour sa prévention. S’interroger sur la perception et les pratiques d’utilisation de ces moyens de prévention devient une nécessité. La moustiquaire imprégnée d’insecticide, un de ces moyens de prévention connait de très faibles taux d’utilisation qui s’explique par les conceptions populaires du paludisme et par la difficulté à intégrer la moustiquaire au sein de l’espace domestique.

Methods: La méthodologie utilisée pour la production des données est exclusivement qualitative. L’entretien approfondi, l’observation directe et la photographie sont les outils utilisés. L’entretien individuel a concerné la désignation du paludisme par le savoir populaire et la perception de l’étiologie. L’observation directe et la photographie ont porté sur l’organisation de l’espace domestique et sur le mode de gestion des literies.

Results: La moustiquaire imprégnée d’insecticide est présentée par la santé publique comme le moyen qui permet d’éviter les piqûres de moustiques et par conséquent de prévenir le paludisme. Cependant la cause du paludisme telle que définie par le savoir médical ne rejoint pas toujours les étiologies populaires. Pour les individus, le moustique n’est pas la seule cause du paludisme mais une des causes. La moustiquaire est un objet matériel dont l’utilisation fait appel à des notions de fiabilité, d’utilité, de mode d’utilisation (facile/difficile), de résistance (opposée à la fragilité) de besoin ressenti, en un mot à des logiques rationnelles. La moustiquaire de part sa caractéristique monofonctionnelle (elle est censée rester à tout moment à la place où elle est fixée) et son mode d’utilisation (à priori individuel et pour le couple) s’insère difficilement dans un espace domestique socialement construit sur le principe de la multi-fonctionnalité. De plus la moustiquaire imprégnée d’insecticide n’est pas considérée par les individus comme un moyen de lutte efficace contre le paludisme car de leur avis, elle ne protège pas des causes climatiques et alimentaires du paludisme.

Interpretation: La moustiquaire imprégnée d’insecticide confrontée aux conceptions populaires du paludisme et aux conditions rationnelles d’utilisation a du mal à s’intégrer au vécu quotidien des individus même si son efficacité scientifique est incontestable.

578B  
Quality of malaria diagnosis in health facilities in Tanga Region, Tanzania: A situation analysis [MIM-KM-37292]

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Introduction: Despite problems of drug resistance in Tanzania, malaria remains a curable disease if patients have access to early diagnosis and prompt treatment. Unfortunately, the current economic strategies have failed to improve the quality of laboratory services which would save lives and prevent wastage of resources. This study was conducted to appraise the performance of health laboratory services and to assess the quality of malaria diagnosis in three districts of Tanga region.
Abstracts / Acta Tropica 95S (2005) S1–S506
S473

Methods: Total quality management was used to assess the current malaria diagnostic pattern and the involvement of laboratory facilities in the diagnosis. To assess the quality of laboratory results on blood smear examinations for malaria, a reference laboratory was established at the regional hospital with the assistance of the regional technician who carried out the assessment. External quality assessment was the main method for assessing the accuracy of the laboratories in processing blood smears and detection of malaria parasites by microscopy. Interviews and observations were used for assessing inventory and maintenance condition of laboratory equipment as well as the professionalism of laboratory personnel.

Results: There was high level of disagreement between the results provided by facility microscopists and those scored expert microscopists from the study team. High level of understaffing was also revealed whereby all 37 facilities involved in the study had only 87 laboratory personnel of all cadres and majority of them were under qualified. The National Guidelines on Laboratory Personnel stipulates that all laboratories in the country must be headed by qualified technologist with at least a Diploma in Laboratory technology. However, findings of this survey show that 20% (7/35) of the laboratories were headed by the laboratory attendants with or without formal training, 51.4% (18/35) headed by laboratory assistants and only 28.6% (10/35) were under qualified laboratory technologists. Most of the facilities in the three districts had few essential equipment with only 48.6% (17/35) of the facilities having a maximum of five types of equipment and only 5.7% (2/35) had 12 types of equipment irrespective of their functional state. Microscopy was the main method employed for examination of both thin and thick blood films for malaria parasites.

Interpretation: Malaria diagnosis is compromised by poor training and skills of personnel, and inadequacy of equipment and reagents. Thus, malaria diagnosis should be scale-up together with other control activities to meet the targets of Roll Back Malaria in Africa.

579C

Strengthening community based malaria control and poverty alleviation in Kanyemba; Mashonaland Central Province in Zimbabwe [MIM-SM-10552]


Introduction: Chapoto ward is situated 200 km to the North of Guruve Center and 400 km North of Harare. The Ward has an estimated population of 2611 and 820 households. Malaria and other communicable diseases are common cause of ill health in this area. There is high infant mortality due to childhood illnesses with high HIV levels in the area. In view of these problems, WHO and the Rotary International decided to assist the community in poverty alleviation and health improvement.

Methods: A consultative meeting was held between the Kanyemba community, the Ministry of Health and Child Welfare, Rotary International and World Health Organization. Resources were mobilized and distributed to the Kanyemba community. Various projects were initiated which include goat keeping, poultry, net sewing, integrated malaria and tsetse control and improving water and sanitation supplies.

Results: There was a notable increase in the IRS coverage after the training of the local spray persons. The village health workers were given bicycles and drugs hence were accessing all the villages in the area. There was an increase in mosquito net coverage due to the readily availability nets produced by the net sewing group. Net re-treatment centers were established and this resulted in an increase in nets being re-treated. The goat and poultry projects resulted in members generating more funds to sustain their families. Thirty-six tsetse targets were set up and the community tasked to maintain the traps. A survey by the Tsetse control department in 2003 indicated a reduction in tsetse density in the area. In general, there has notable improvement in the health status of the Kanyemba community.

Interpretation: If communities are supported with adequate resources, they can contribute significantly
The power of radio [MIM-PM-408460]


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Introduction: Radio programmes can convey information about public health more effectively if they combine entertainment with education. The use of radio as a tool for improving public knowledge attitudes and practice in relation to malaria was evaluated in The Gambia through the creation and national broadcasting of a radio soap opera including public health messages in the storyline, and through cross-sectional surveys to measure malaria knowledge, attitudes and practice before and after the broadcasts.

Methods: The drama, Bolongodala, chronicles day-to-day lives in a typical but fictional rural Gambian community. The characters model and reinforce positive practices to prevent and manage malaria. Twenty-six episodes were broadcast twice weekly in Mandinka from 3 July to 4 February. A phone-in programme was broadcast to obtain audience feedback. In one rural community radio clubs were established, listening patterns monitored, and bednets made available at subsidised cost, women caring for children under 5 years were identified in a census, and a systematic sample selected for interview before or after the series using a structured questionnaire to assess knowledge attitudes and practice in relation to malaria.

Results: Calls to the phone-in programme were received from listeners in all seven administrative divisions in the country. In the evaluation village, 75 women were interviewed before the broadcasts and 81 afterwards. 49% of children under 5 years slept under a treated or intact net before the broadcasts, and 69% afterwards (OR 1.3, 95% CI 2.3–3.8). Average percentage score for women’s knowledge about malaria before the broadcast was 60%, and after the broadcasts 74% (change of 14% (95% CI 8–22%)), while for questions about practice in relation to malaria treatment and prevention, percentage scores were 67% before the broadcasts and 81% afterwards (change 14% (95% CI 10–17%). These changes reflected more appropriate answers to questions about health seeking behaviour for a sick child, household use of treated nets and prioritisation of children and pregnant women for the use of treated nets after the broadcasts.

Interpretation: Radio drama can be an effective medium for health promotion in malaria. A longer time frame and more formal study design would be needed to determine if reported changes are sustained and have an impact on malaria morbidity and mortality.

Use of community owned resource persons (CORPs) in management of malaria in villages with different transmission intensity, North eastern Tanzania [MIM-BM-37562]


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Introduction: Home management of malaria has been developed to increase access to prompt and effective antimalarial treatment by children with uncomplicated malaria in high-endemic areas in Africa. Use of trained CORPs and mothers have been shown to have a significant impact in management of uncomplicated malaria. This longitudinal study therefore aimed at monitoring CORPs in management of uncomplicated malaria in two communities with different transmission intensity.

Methods: This longitudinal study was conducted in two villages, Mkokola (2008 inhabitants) and Kwamasimba (1849 inhabitants). Malaria transmission intensity is high in Mkokola (P. falciparum point prevalence among 2–4 years: 81%) and medium in Kwamasimba (point prevalence among 2–4 years olds: 21%). In each village, two trained CORPs were instructed to treat individuals with symptoms of
uncomplicated malaria with the national first line anti-malarial drug (sulfadoxine/pyrimethamine). Before treatment was given a blood smear was collected and clinical information obtained. Patients with symptoms of severe malaria or other illness were referred to the district hospital or the nearest health facility.

Results: During January to December 2004, 2694 patients reported to the CORPs, 1502 from Mkokola and 1192 from Kwamasimba. Of all individuals who reported to the CORPs, 96.9% from Mkokola and 99.5% from Kwamasimba had history of fever and were treated for malaria. About 50% of the treated patients in Mkokola and 40% of the patients in Kwamasimba had a positive blood smear. In adults over 20 years of age, these percentages were 29 and 24% for Mkokola and Kwamasimba, respectively. Sixty-three (2.34%) of the patients were referred to other health facilities.

Interpretation: The health service provided by the CORPs was highly appreciated by the villagers, and the rate of over treatment was similar to what has been reported at many district hospitals in the region.

582C
Studies on malaria and mosquito vectors in rural farmers of North Central parts of Ebonyi State, Nigeria [MIM-BN-22001]
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Introduction: The effects of malaria are partially noticeable in rural areas where malaria frequently strikes during the period when the need for agricultural work is greatest. The knowledge of the behaviour of local species of mosquitoes is indispensable before control can be effected. The objective of this study was to investigate the prevalence of malaria and mosquito vectors involved in its transmission in 12 rural farming communities of North Central parts of Ebonyi State, Nigeria.
Methods: All finger prick blood smears were stained with Giemsa stain and examined by microscopy. Adult mosquito collections by spray-catch methods were done in three houses in each village weekly for indoor resting mosquitoes.
Results: Of the 2212 persons examined by microscopy technique, Plasmodium falciparum was the most dominant species (93.5%) of malaria parasites. On average, 84% of the rural farmers were infected with Plasmodium parasite mostly pregnant women and children under 6 years. Infections varied significantly among communities and sexes ($P < 0.05$). Persons in these areas rarely use bednets. Anopheles mosquitoes were found breeding in swampy rice farms, ponds, water logged areas, cassava and yam fields. A total of 2886 Anopheles mosquitoes were collected indoors. Of these, A. gambiae s.l. and A. funestus accounted for 96.6% and 3.4%, respectively of the total Anopheles mosquitoes collected. Other mosquito vectors of arboviral infections were recorded.
Interpretation: Environmental modifications, persistent health education and effective Roll back malaria strategies are needed in these rural areas to prevent infections of pregnant mothers, children and possible future outbreak of water borne diseases in them.

583A
Scaling up home management of malaria in the context of accelerated child survival and development programme in upper east region, ghana [MIM-EO-3080]
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Introduction: Malaria is a leading cause of childhood morbidity and mortality in Ghana. Since June 2003, a partnership of Ghana Health Service, KNUST, Ghana Red Cross and UNICEF has been implementing home management of malaria (HMM) in the context of accelerated child survival and development (ACSD) in the Upper East Region, which has some of the poorest socio-economic indicators.
Methods: The tools used were: selection of community-based agents (CBAs), training in ACSD, case management of malaria using prepacked chloroquine and diarrhoea using ORS, community education, support-
ive supervision and monitoring. CBAs presently work in six districts comprising 1200 communities.

**Results:** By December 2005, the partnership had trained, deployed and provided drugs and logistics for home management of malaria, diarrhoea and referrals for acute respiratory infections for 2400 CBAs; 2 per community region-wide. Behaviour change communication campaigns on the 16 key household practices have also been actively promoted through CBAs, mothers clubs and community organisations. Initial reports indicate universal access to prompt and appropriate treatment of malaria and diarrhoea in the communities. ITN use has also been promoted as part of ACSD. Health facility reports indicate reduced admissions for cerebral malaria and anaemia requiring blood transfusion. These reports will be validated in a cross-sectional survey in June 2005.

**Interpretation:** In Upper East Region of Ghana, the Abuja target on HMM (60%) has been exceeded with 100% access to malaria treatment. Integrating HMM in the context of ACSD programmes is a practical and synergistic approach to promote child health in rural Ghana.

**584B**

**Personal digital assistants for data entry at the point of collection in a large household survey in southern Tanzania [MIM-KS-213869]**


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**Introduction:** For surveys, double entry of printed questionnaires and data cleaning often take substantial amounts of time. As part of an effectiveness evaluation of a new malaria control tool, we surveyed over 21,000 rural households in southern Tanzania. Information on child health and survival was collected using hand-held computers (PDAs) rather than paper-based questionnaires. This approach enabled us to give feedback on survey findings to local leaders only 2 days after completion of field work.

**Methods:** The questionnaire was first printed on paper before transfer to Palm m130 PDAs using PEN-DRAGON FORMS v4.0. Interviews were guided by the PDA, with built-in skip patterns, error messaging, range and consistency checks as data was entered during the interview. A small pilot study was done before the main survey which involved 13 teams each of one supervisor and seven interviewers, very few of whom had prior computer experience. Each person was provided with a PDA with solar and mains charging devices. Each supervisor downloaded data from their team’s PDAs onto a laptop computer every day, from which summaries of the day’s work were available for quality control. Car chargers were available for the laptop and PDAs.

**Results:** The handheld device was well accepted by both interviewees and interviewers, and data quality was enhanced. At the end of 7 weeks of field work, all survey data was available on a single CD within 24 h. Preliminary findings were shared with local leaders 2 days after completion of field activities. Data cleaning took two people 4 weeks, after which information was available for analysis on 21,529 households.

**Interpretation:** Our use of PDAs eliminated the usual time-consuming, error-prone process of data entry and validation. PDAs are a promising tool, particularly for surveys where most responses can be pre-coded.

**585C**

**Influence du sérodiagnostic des salmonelloses sur la prise en charge du paludisme à douala [MIM-LL-465206]**

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**Introduction:** Le paludisme et les salmonelloses sont endémiques au Cameroun. La similitude de leurs manifestations cliniques entraîne un recours fréquent au test de Widal pour le diagnostic des salmonelloses. La prolifération récente de personnes se déclarant atteintes de typhoïde nous a fait étudier la qualité des analyses biologiques utilisées.

**Methods:** Le paludisme et les salmonelloses sont endémiques au Cameroun. La similitude de leurs manifestations cliniques entraîne un recours fréquent au test de Widal pour le diagnostic des salmonelloses. La prolifération récente de personnes se déclarant atteintes de typhoïde nous a fait étudier la qualité des analyses biologiques utilisées.

**Methods:** Au cours de cette étude, une enquête a été menée afin de vérifier les rapports entre l’incidence du paludisme et celle des salmonelloses à l’Hôpital Laquintinie de Douala. 51 échantillons de sérum
humains testés Widal-positifs dans cet hôpital ont été analysés par la méthode sur lame et la méthode sur plaque de microtitration à l’aide de trois suspensions antigéniques de fabrications différentes.

**Results:** Les cas d’hospitalisations pour paludisme et pour salmonelloses ont présenté une évolution similaire au cours de l’année d’investigation. Les corrélations entre les titres d’agglutinines O et H étaient significativement faibles lorsque le test Widal sur lame était comparé au test sur Widal sur plaque ($r=0.226$ et $r=0.196$ respectivement). La qualité des suspensions antigéniques a également influencé les résultats obtenus (0.61 < $r$ < 0.92).

**Interpretation:** Notre étude suggère que de nombreux cas de paludisme sont traités comme des cas de typhoïde à cause de faux tests de Widal positifs à Douala. La prise en charge de ces paludéens pourrait s’améliorer si la qualité des analyses et des suspensions antigéniques utilisées pour diagnostiquer les salmonelloses était meilleure.

30: Innate immunity

**Posters 586–600**

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

586A

**Effect of host factors on malaria infection in pregnancy [MIM-OA-151925]**

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**Introduction:** Pregnant women are more susceptible to malaria infection than non-pregnant women. Some of the host factors responsible for this could be IgG level, G6PD deficiency and gravidity. This work was designed to study effect of anti MSP1 (19), antibody IgG level, G6PD deficiency and gravidity on the prevalence of malaria infection in pregnancy and also to determines the effects of malaria infection on haemoglobin (Hb) level.

**Methods:** Two hundred pregnant and 80 non-pregnant women were recruited into this study at Ade Oyo state maternity hospital, Ibadan, Nigeria. Pregnant women were grouped into primigravidae and multigravidae. Serum sample for the subjects were analysed for antibodies specific to MSP1(19) by ELISA, G6 PD deficiency was determined by fluorescent spot test, while haemoglobin (Hb) level was determined spectrophotometrically. Thick blood smears were prepared for parasite identification and quantification. Data was analysed by Student’s ‘t’ test. $P$ value less than 0.05 were considered statistically significant.

**Results:** Prevalence of malaria infection and parasite density were significantly higher among pregnant than non-pregnant women ($P$ < 0.02). Among pregnant women, primigravidae were more infected than multigravidae: The IgG level was significantly reduced among pregnant women than non-pregnant women, which was also reduced among primigravidae than multigravidae ($P$ < 0.05). The level of IgG was significantly higher among non-pregnant malaria positive than malaria positive pregnant women ($P$ < 0.05). Malaria positive primigravidae had lower IgG level than malaria positive multigravidae. All the G6 PD deficiency individuals studied were malaria negative, while 75% of G6 PD normal were malaria positive. 80% of malaria positive pregnant women were severely anaemic, while all malaria positive non-pregnant women were moderately anaemic.

**Interpretation:** The rate of malaria infection is a function of IgG level. Primigravidae were more infected with malaria. G6PD-deficiency confers advantage on malaria infection over G6PD normal individual. All those who were malaria positive were anaemic.
Spleen enlargement and genetic diversity of *P. falciparum* infection in two ethnic groups with different malaria susceptibility in Mali, West Africa [MIM-SB-291560]


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**Introduction:** The nomadic Fulani population has lower malaria morbidity, parasite prevalence as well as higher spleen rate than other ethnic groups with similar exposure. The genetic diversity of *P. falciparum* may reflect level of immunity. Asymptomatic multiclonal infections were associated with lower risk for subsequent clinical malaria. The aim of this study was to assess the diversity of *P. falciparum* infections in two ethnic groups as a potential factor explaining differences in malaria susceptibility.

**Methods:** The study was performed in the district of Koro, a mesoendemic area in Mali where the Fulani and Dogon live in sympatry. The study included 237 age-matched individuals (median age 7 years) from the Fulani and the Dogon, respectively, participating in a cross-sectional survey. Clinical malaria was defined as fever and presence of *P. falciparum* by microscopy, or a high parasite load (>10,000 parasites/μL) in absence of fever. Spleen enlargement was established on the basis of palpation. Genomic DNA was prepared by a new Tris EDTA buffer-based method from filter paper samples. Numbers of parasite clones within a single host were defined by PCR-based genotyping of the *P. falciparum* merozoite surface protein 2 (msp2).

**Results:** The peak *P. falciparum* prevalence was similar in the two ethnic groups, 55% in the Fulani and 58% in the Dogon, respectively. The parasite prevalence was higher in the Dogon individuals above 10 years, 29% compared to 12% in the Fulani (P=0.003). In both ethnic populations parasite densities were highest in the youngest ages (<5 years), followed by successive decrease until adulthood. The parasite densities were higher in the Dogon in young individuals (0–15 years) (P=0.034), but not in adults (P=0.066). There was no difference in number of *P falciparum* genotypes, with mean values of 2.25 and 2.11 (P=0.503) in the Dogon and the Fulani, respectively. Spleen enlargement was more prevalent in the Fulani and was apparent already in the youngest age groups. Spleen rates in children aged 2–9 years were 75% in the Fulani and 44% in the Dogon (P<0.001). Spleen rate increased with parasite prevalence, density and number of co-infecting clones in asymptomatic Dogons, spleen rate being highest in individuals infected with 2–3 clones. Moreover, splenomegaly was higher in individuals with clinical malaria in the Dogon, odds ratio 3.67 (95% CI, 1.65–8.15, P=0.003), an association not found in the Fulani, 1.36 (95% CI, 0.53–3.48, P=0.633).

**Interpretation:** Difference in malaria susceptibility was not explained by the number of *P. falciparum* clones. Pronounced spleen enlargement to multiclonal infections and disease were found in the Dogon, whereas the Fulani already appeared to have functional spleens.

Interethnic differences in antibody responses to malarial antigens but not to non-malarial antigen in sympatric tribes living in West Africa [MIM-EI-119816]

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**Introduction:** The relative resistance to malaria in the Fulani tribe as compared to that in neighbouring tribes has been associated with their higher levels of antibody to malaria antigens. It is unknown if these inter-ethnic differences can be ascribed to specifically enhanced anti-malarial responses or to a generally hyper-reactive immune system in the Fulani. We have investigated tribal levels of total serum IgG and IgM.
as well as of antibodies to malarial and selected non-malarial antigens.

**Methods:** The study areas are located in the Mopti area in Mali and in the vicinity of Ouagadougou, Burkina Faso. Blood donors were randomly selected and samples from Mali, Fulani (n = 36) and Dogon (n = 47), were collected at the end of the transmission season. Samples from Burkina Faso, Fulani (n = 92) and Mossi (n = 88), were collected during the peak of the transmission season. The levels of antibodies to malarial and non-malarial antigens (measles, rubella, T. gondii, H. pylori and M. tuberculosis) were determined by ELISA. Total IgG and IgM concentrations were determined by immunodiffusion. Statistical analyses of differences between the different ethnic groups were performed by Mann–Whitney test, a p-value less than 0.05 was regarded as significant.

**Results:** Despite differences in the endemicity, the Fulani of Burkina Faso and Mali both showed similar IgG and IgM anti-malarial antibody levels and these were significantly higher than those in the neighbouring tribes, Mossi and Dogon, respectively. The IgG responses were dominated by IgG1 and IgG3 antibodies, the levels of which were significantly higher in the Fulani than those in the other tribes. While Fulani of both countries showed significantly higher levels of total IgM (3.5 and 3.7 mg/ml) than their neighbours (Mossi, 2.5 mg/ml and Dogon, 2 mg/ml, p = 0.004 and 0.001), no significant differences were seen in total IgG between the tribes in either country. The levels of antibodies against measles and T. gondii antigens were significantly higher in the Fulani, but no significant differences in the levels of antibodies reactive with rubella or H. pylori were seen in any of the countries. Furthermore, while the Fulani had significantly higher IgG concentrations against M. tuberculosis antigens in Mali (p < 0.001), no such difference was seen between the tribes in Burkina Faso (p = 0.425).

**Interpretation:** Our findings suggest that the relative resistance to malaria found in the Fulani, appears not to be a reflection of a general hyper-reactive immune system in this tribe, but rather to specifically enhanced anti-malarial responses.

**S89A**

Plasmodium-infected erythrocytes inhibit Toll like-receptor (TLR)-dependent signaling in dendritic cells [MIM-DC-0]

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**Introduction:** Two molecules isolated from *P. falciparum* induce activation of antigen-presenting cells through toll-like receptor (TLR)-dependent mechanisms: hemozoin (TLR9) and glycosylphosphatidylinositol (GPI) (TLR2, TLR4). However, incubation of *P. falciparum* or *P. yoelii*-infected erythrocytes with dendritic cells (DC) does not induce activation and inhibits LPS-induced maturation. We have investigated DC responses to TLR ligands after incubation with *P. yoelii* in vitro and during infection in mice.

**Methods:** To investigate if Plasmodium blood-stage parasites induce DC maturation, we incubated DC (wild-type or MyD88−/−) with different ratios of *P. yoelii* schizonts/DC, or with schizont lysates, and analyzed expression of CD80 and CD86 by FACS, and production of IL-12 and TNF-alpha by ELISA. To study regulation of DC responses to TLR ligands we pre-incubated DC for 18 h with *P. yoelii* schizonts and then stimulated cells with one of the following: zymosan (TLR2), polyI:C (TLR3), LPS (TLR4), Flagellin (TLR5), Loxoribin (TLR7) or CpG (TLR9). To investigate TLR-mediated responses in malaria infection, we injected LPS or CpG into mice, infected or not with *P. yoelii*, at different days of infection, and the next day removed DC from spleen (CD11c+). **Results:** Different ratios of *P. yoelii* schizonts/DC (from 1 to 50/DC) did not activate expression of costimulatory molecules by DC, and also did not activate production of either TNF-alpha or IL-12. Schizont lysates only induced a low increase of CD86 expression, without secretion of TNF-alpha or IL-12. Such partial activation induced by lysates was found to be independent from MyD88, the main adaptor molecule which mediates TLR-dependent signaling. Incubation of DC with infected erythrocytes from both *P. falciparum* and *P. yoelii* inhibits responses to posterior stimulation with LPS, a TLR4 ligand. (Urban et al., 1999. Nature; Ocana-Morgner et al., 2003. JEM). We have now extended this observation to TLR2, TLR3,
TLR5, TLR7 and TLR9. Responses to all TLR ligands tested were strongly inhibited by P. yoelii at a ratio of 30 schizonts/DC. Lower ratios induced partial inhibition of TLR-induced activation. When LPS or CpG are injected into P. yoelii-infected mice, TLR stimulation of spleen DC in the first 7 days post-infection (p.i.) induces an increase in expression of co-stimulatory molecules, while at 10 days p.i. a considerable degree of inhibition of TLR-mediated DC maturation is observed.

**Interpretation:** While GPI and hemozoin purified from Plasmodium activate DC, our results suggest that, when whole infected erythrocytes are present, other factors appear to modulate the TLR-dependent DC responses, both in vitro and during late infection in mice.

**590B**
Alpha+-thalassaemia, but not sickle cell trait, is clearly associated with malaria endemicity in North-Eastern Tanzania [MIM-AE-197866]
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**Introduction:** Populations living in malaria endemic areas develop different protective mechanisms against the disease. Apart from immunity, innate human resistance, such as inherited genetic haemoglobinopathies, are thought to be associated with a selective advantage against malaria. Here we investigated the distribution of the two most common haemoglobinopathies in sub-Saharan Africa, sickle cell haemoglobin and alpha+-thalassaemia, in relation to altitude and malaria endemicity in North-Eastern Tanzania.

**Methods:** Cross-sectional surveys were conducted in eight villages arranged in two altitude transects (300–1600 m) in Western Usambara and South Pare mountains in North-Eastern Tanzania. In this malaria endemic region, Plasmodium falciparum prevalence and density correlate inversely with altitude. Six hundred samples from children under five were screened for sickle cell trait and alpha+-thalassaemia. DNA from blood samples was extracted and the frequency of the common alpha-globin deletion in Africa, alpha-3.7, was detected by PCR. Furthermore, the genotypic frequency of haemoglobin A, S and C polymorphisms were determined by a new simple high-throughput method using sequence specific oligonucleotide probes (SSOPs) and ELISA-based technology.

**Results:** The genotypic frequency of heterozygote alpha+-thalassaemia was inversely correlated with altitude and parasite prevalence. It increased significantly from 19% to 20% in the two high-altitude villages, to as high as 47% and 48% in the two low-altitude villages, respectively in both transects (Chi² for trend 20.1, p < 0.00001). The prevalence was higher in the Wasamba ethnic group compared to the Wapare (Chi² = 3.2, p = 0.07). These data suggest that the presence of alpha+-thalassaemia in these populations most likely results from a selective advantage against malaria. However, and in contrast to previous studies from other areas of Tanzania, the prevalence of the sickle cell trait was very low (4%) in all villages, irrespective of altitude, suggesting only a minor role in relation to innate resistance to malaria. The new sickle cell haemoglobin screening method clearly distinguishes homozygotes and heterozygotes and has the advantage of being more rapid and less tedious compared to the commonly used restriction-enzyme digestion techniques.

**Interpretation:** Our findings show a strong association between the alpha+-thalassaemia genotype and malaria endemicity, but not with the sickle cell trait, as well as providing a new method for rapidly screening large samples for haemoglobin A, S and C genotypes.

**591C**
High levels of red blood cell surface-bound, complement regulatory proteins are associated with severe malarial anaemia in Ghanaian children [MIM-GH-120496]
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Abstracts / Acta Tropica 95S (2005) S1–S506

591A
Haptoglobin polymorphism and CD163 expression in relation to malaria severity [MIM-KK-3984]
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Introduction: CD163 is an acute phase-regulated monocyte/macrophage membrane receptor expressed late in inflammation. It is a natural soluble plasma glycoprotein with potential anti-inflammatory properties, possibly linked to the individual’s haptoglobin (Hp) phenotype. CD163 is involved in the haptoglobin-mediated removal of free haemoglobin (Hb) from plasma. High levels of soluble CD163 late in a malaria episode may therefore down-regulate inflammation and curb disease severity.

Methods: The relationships between soluble CD163 (sCD163) levels, malaria severity and haptoglobin phenotype expression were assessed in a hospital-based cross-sectional study. Plasma samples were obtained from paediatric malaria patients who reported at the Department of Child Health, Korle-Bu teaching Hospital, during the 2002 and 2003 malaria seasons. Patients were categorized into uncomplicated malaria (UM), cerebral malaria (CM) and severe malarial anaemia (SA). Hp phenotypes were determined by native PAGE of haemoglobin-supplemented plasma and levels of sCD163, TNF-α, IL6, IL10 and the Th1 immune marker neopterin were determined in plasma by sandwich ELISA.

Results: The median sCD163 level was higher in UM (11.9 µg/mL) than in SA (7.7 µg/mL) (P = 0.010) and CM (8.0 µg/mL) (P = 0.031). sCD163 level was also higher in UM (11.9 µg/mL) than in complicated malaria (SA and CM) cases (7.9 µg/mL) (P = 0.007). sCD163 levels were positively correlated with plasma levels of TNF-α (r = 0.316, P < 0.001) and decreased
to normal levels after treatment and recovery. A unit increase in TNF-$\alpha$ levels corresponded to a 47-fold increase in plasma sCD163 levels. Though sCD163 levels were not different amongst the four Hp phenotypes determined, Hb levels were significantly higher in Hp2-1/2-2 (10.3 g/dL) than in Hp1-1 (5.85 g/dL) individuals ($P=0.006$). Odds ratio analysis also showed that Hp 1-1 individuals were 8.7 times more likely to have severe malaria than Hp2-1/2-2 individuals (95% CI, 1.34–56.23). sCD163 levels showed a significant negative correlation with levels of neopterin, especially in complicated malaria cases ($r=-0.358$, $p=0.027$). No differences were found between categories for the cytokines measured.

**Interpretation:** High sCD163 levels in UM suppress excessive inflammatory cytokine production. Association of Hp polymorphism with severe malaria may be due to differences in Hp molecules and their subsequent distribution in body fluids.

**593B**

**Activation of innate immune responses by hemozoin involves recognition by Toll-like receptor (TLR) 2**

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**Introduction:** Little is known about the interaction between the innate immune system and malaria. Toll-like receptors (TLRs) are critical components for the recognition of pathogens. During infection, rupture of erythrocytic schizonts is associated with clinical symptoms and the production of cytokines. Previous studies suggested a potential role for hemozoin in inducing proinflammatory responses during malaria. The goal of our study is to study and whether recognition of hemozoin by cells involves TLRs.

**Methods:** Hemozoin was synthesized using previously published protocols. Human PBMC were stimulated with increasing concentrations of hemozoin (10–100 ng/ml). Cytokine production was measured in the supernatant. In addition, stimulations were performed in the presence of an anti-TLR2 mAb or isotype-matched control mAb (50 ng/ml). HEK293 cells that stably express TLR2, TLR2/CD14, TLR4/MD2, TLR9 or an empty plasmid vector were used in an NF-$\kappa$B/luciferase reporter assay. Thioglycolate-elicited peritoneal-exudate macrophages and bone-marrow derived dendritic cells were obtained from C57Bl/6 wild-type mice and TLR2-gene deficient (TLR2$^{-/-}$), TLR4$^{-/-}$, TLR9$^{-/-}$ and MyD88$^{-/-}$ (adapter molecule involved in TLR responses) mice and used for stimulation experiments.

**Results:** Upon exposure to synthetic hemozoin, NF-$\kappa$B was activated only in HEK cells expressing TLR2/CD14. In contrast, all of the cell lines responded to their known TLR ligands. In human PBMC, hemozoin induced a strong inflammatory cytokine response, producing TNF concentrations of 1–2 ng/ml. In the presence of an anti-TLR2 antibody, the TNF response was inhibited by 30–50%, while addition of the isotype control mAb had no effect. While purified plasmacytoid dendritic cells (strongly TLR9 positive) failed to respond to hemozoin, highly purified CD14$^+$/TLR2$^+$+/TLR9$^{-}$ peritoneal monocytes produced abundant cytokines. We devised a binding assay using purified fusion proteins consisting of the Fc domain of IgG and the ectodomain of TLR2 or TLR4. We observed the direct binding of the ectodomain of TLR2, but not TLR4, with hemozoin. The involvement of TLR2 was confirmed in experiments with mouse macrophage cells: hemozoin-induced TNF production was almost completely absent in cells from MyD88$^{-/-}$ mice, and strongly reduced in cells from TLR2$^{-/-}$ mice. In contrast, the response in cells from TLR4$^{-/-}$ or TLR9$^{-/-}$ was unchanged.

**Interpretation:** Hemozoin is a potent activator of inflammatory responses. Our data show that synthetic hemozoin activates cells of the innate immune system in a TLR2– and MyD88– dependent manner.
**594C**

Sickle cell trait carriage: Different imbalanced distribution of IgG subclass antibodies to *P. falciparum* glurp and msp2 antigens in Gabonese children [MIM-dM-202725]

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**Introduction:** Plasmodium falciparum malaria remains a significant cause of human suffering, and most malaria-related morbidity and mortality occurs in children living in sub-Saharan Africa. But Polymorphism in the beta-globin gene (hemoglobin S) has been associated with protection against severe forms of malaria.

**Methods:** Plasma samples from Gabonese children with and without sickle cell trait and harbouring *P. falciparum* infections, were investigated for the presence and levels of parasite antigen-specific IgG and IgG subclass antibodies for a recombinant protein containing the conserved region of glutamate rich protein (GLURP). This investigation were done by ELISA (enzyme linked immunobsorbent assay).

**Results:** We reported increased levels of total IgG (*p* < 0.0001), IgG1 (*p* = 0.009) and IgG3 (*p* < 0.03) to GLURP with age. Cytophilic antibodies (IgG1 and IgG3) predominated with no difference in the antibody titers between HbAA and HbAS children, but GLURP-specific IgG4 antibodies were detected at significantly (*p* = 0.05) lower levels in HbAS children.

**Interpretation:** Our data show that the IgG2 and IgG4 distribution differs to the hb type and Ags in asymptomatic infections. This may have possible implication for the clearance and elimination of malaria parasites and for protection against severe malaria.

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**595A**

Clinical and haematologic indices in *Plasmodium falciparum*-infected children with the sickle-cell haemoglobin gene in western Kenya [MIM-RO-94127]

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**Introduction:** The high frequency of the sickle-cell haemoglobin gene (HbS) in *Plasmodium falciparum* malaria endemic areas is thought to arise from the selective advantage against mortality in heterozygous individuals (HbAS). Since our recent findings in children with malarial anaemia (MA) show that parasitemia and haemoglobin (Hb) levels are not strongly associated, the relationship between the HbS gene and malaria disease outcomes was determined.

**Methods:** To examine the association between the HbS gene and MA disease outcomes, haematological and clinical indices were examined in children (<3 years of age) with the HbAS and HbAA genotypes during acute disease. Children (*n* = 240) were recruited as part of an ongoing longitudinal prospective study in a malaria holoendemic area of western Kenya. Haemoglobin (Hb) genotypes were assessed by haemoglobin electrophoresis of fresh red blood cell (RBC) haemolysates. Haematological indices were determined by complete blood counts, parasitaemia was determined by Giemsa-stained blood films, and reticulocyte counts were determined by new methylene.

**Results:** There were 45 children with HbAS and 195 HbAA in the cohort, with the HbAS group being significantly younger than the HbAA group (*p* = 0.015). The HbAS group had a lower proportion of children with SMA than the HbAA group (*p* = 0.023). Levels of parasitaemia and gametocytes were not significantly different between the groups. The HbAS group had higher numbers of RBC (*p* = 0.004), Hb concentrations (*p* = 0.014), and Hct (*p* = 0.026), and lower mean corpuscular volume (MCV, *p* = 0.004) and mean corpuscular haemoglobin (MCH, *p* = 0.025) than...
the HbAA group. When divided according to age (less than/equal to 6 months), the HbAS group had a significantly higher number of RBCs ($p = 0.002$), Hb concentrations ($p = 0.003$), Hct ($p = 0.005$), MCV ($p = 0.009$), and monocytes ($p = 0.01$) compared with the HbAA group. Among children aged greater than 6 months, the HbAS group had significantly lower numbers of RBCs ($p = 0.016$) and significantly lower MCV ($p = 0.027$) and MCH ($p = 0.034$) than the HbAA group. Hb concentrations were non-significantly higher in the HbAS group in children greater than 6 months of age.

Interpretation: Findings presented here illustrate that the HbS gene protects against MA in young children residing in a holoendemic area of malaria transmission primarily through red blood cell related mechanisms and not reductions in parasite burden.

596B Genetic control of the antibody response induced by Plasmodium falciparum GLURP protein in naturally exposed individuals from Brazilian endemic area [MIM-LP-33850]


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Introduction: The Plasmodium falciparum Glutamate-Rich Protein (GLURP) is an antigen considered to be one of the leading malaria vaccine candidates. Immuno-epidemiological studies performed in high transmission areas have shown a significant association of high levels of GLURP-specific antibodies with low parasite densities and protection against clinical malaria. In this study we evaluated the antibody response against GLURP in individuals living in Brazilian endemic area with low levels of transmission.

Methods: This study was undertaken in rural villages situated near Porto Velho, the capital of Rondonia State, in the Brazilian Amazon. Serum samples were obtained from 187 exposed individuals. Recombinant proteins corresponding to the N-terminal non-repeat region GLURP94—489 (R0) and the C-terminal repeat region GLURP705—1178 (R2) of the protein were used in ELISA. In R0-positive individuals, the samples were tested for IgG response against peptides P3, P4, P5, P8, P9, P10, P11 and S3 covering known and predicted B-cell epitopes, and in R2-positive individuals, the samples were tested for IgG response against S4 peptide, corresponding to the R2 repeat unit. HLA-DRB and DQB low resolution typing was performed by PCR with sequence-specific primers.

Results: The results showed that most individuals presented IgG against R0 (67%) and R2 (79%) GLURP regions. The peptides S4 from R2 (53%) and P11 from R0 (49%) were identified as immunodominant B-cell epitopes and induced higher levels of antibodies. The number of GLURP peptides recognized and the levels of IgG against S4 and P11 peptides demonstrated a positive correlation with age and time of exposure in studied area as opposed to a negative correlation with the number of previous malaria episodes. The antibody responses against GLURP epitopes appear to be modulated by HLA class II antigens. The absence of anti-R0 response was associated with HLA-DR11 and HLA-DQ7 and the absence of anti-R2 response was associated with HLA-DR12. We also observed positive and negative associations between HLA and the immune response against GLURP-derived peptides (positive: P3 with DR4 and DQ8; P4, P8 and P9 with DR13; P10 with DR8; P11 with DR8 and DQ4; negative: S4 with DR7). Interestingly, the GLURP immunodominant B-cell epitopes in individuals from this Brazilian endemic area are distinguishable from those of African endemic areas.

Interpretation: Considering the importance of GLURP as a vaccine candidate the concern of the influence of class II allele frequencies in ethnically diverse populations may be important before vaccine trials are conducted among people naturally exposed to malaria.
Familial aggregation of cerebral malaria and severe malarial anemia in population living in Bamako [MIM-IS-11928]

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Introduction: Falciparum malaria causes more than one million deaths each year among African children. The predominant manifestations of severe malaria in African children are cerebral malaria (CM) and severe malarial anemia (SMA). As a first step toward a family-based approach to identify the environmental and genetic pathways that contribute to severe malaria, we tested whether it aggregates within families.

Methods: Family history of CM and SMA was explored during face-to-face interviews with parents using a standardized questionnaire. Logistic regression was used to determine whether CM and SMA aggregate within individuals and within families. The pattern of familial aggregation was then expressed as familial odds ratios that were adjusted for relevant risk factors. All analyses used estimating equations methods.

Results: This study was of 2811 inhabitants of Bamako (Mali), clustered in 407 nuclear families. The probands were 136 children with severe malaria (CM only, 47.8%; SMA only, 23.5%; CM+SMA, 28.7%) and 271 healthy children from the community. Within-person association of CM and SMA was significant (odds ratio, 6.15 [95% confidence interval (CI), 2.62–14.41]). Over a lifetime, with each additional affected relative, the odds of a person contracting CM increased by 1.98 times (95% CI, 1.59–2.45), and the odds of having SMA increased by 1.91 times (95% CI, 1.05–3.47). Over a lifetime, for a child whose sibling had a history of CM, the odds of having CM were 2.49 times greater (95% CI, 1.51–4.10) than the odds for a child whose sibling had no such history.

Interpretation: Our data suggest strong familial aggregation of CM and SMA.

Risk factors for severe malaria in Bamako, Mali: A matched case-control study [MIM-IS-35802]

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Introduction: The aim of this case-control study was to identify epidemiological risk factors for severe malaria among children living in Bamako, a malaria-endemic area.

Methods: For this, 260 healthy community controls (individuals with no history of severe malaria) were matched to 130 patients with severe malaria. The matching criteria were age (±2 years), place of residence (adjacent to the house of the matching case) and length of residence (at least equal to that of the matching case). Among 130 patients, 62, 30 and 38 were cerebral malaria (CM), severe malaria anemia (SMA) and CM + SMA, respectively.

Results: Conditional multiple logistic regression analysis indicated that all examined independent factors associated with severe malaria are directly related to characteristics of the child’s mother, with the exception of the child’s own yellow fever vaccination history (odds ratio (OR): 1.93, 95% confidence intervals (CI (95%)) [1.10–3.37]). The following characteristics were all associated with a decreased risk of severe malaria: maternal education (OR: 0.52, CI (95%) [0.31–0.86]), the mother’s adequate knowledge about malaria (OR: 0.46, 95% CI (95%) [0.25–0.86]), her use of mosquito bed nets (OR: 0.53, CI (95%) [0.30–0.92]) and breast-feeding for at least 2 years (OR: 0.57, CI (95%) [0.33–0.94]). Conversely, chronic maternal disease (OR: 3.16, CI (95%) [1.10–4.37]) was associated with an increased risk of severe malaria.
Interpretation: These findings strongly support the hypothesis that maternal factors are central to the development of severe malaria in children.

599B
Associations between the IL-4 -590 T allele and prevalence of Plasmodium falciparum infection in the Fulani of Mali [MIM-MV-209195]
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Introduction: Ethnic-based difference in susceptibility to malaria is well established. Despite similar malaria transmission, The Fulani are less susceptible to malaria infection, have less malaria attack and prevalence of Plasmodium falciparum infection, and higher anti-malarial IgG and IgE antibody levels than their neighbours (the Dogon in Mali and the Mossi and Rimaïbé in Burkina Faso). Inter-ethnic difference in mounting immune response to malaria might be influenced by cytokine gene polymorphisms.

Methods: Asymptomatic subjects of ethnic groups, the Fulani and the Dogon, living in sympathy in a malaria mesoendemic area in Mali were recruited in this study. The allele and genotype frequency of IL-10-1087 A/G in 181 Dogon and 139 Fulani, and IL-4-590 C/T in 205 Dogon and 211 Fulani were determined using PCR with restriction endonuclease mapping. The data were correlated to ethnicity, spleen rate and parasite prevalence, density and diversity. Prediction of infection was modelled using logistic regression, controlling for factors speculated to influence parasite prevalence including IL-4 and IL-10 genotypes and spleen enlargement. Adjustment was made for age (grouped into <2, 2–3, 4–5, 6–7, 8–9, 10–15 and 15< years) and sex.

Results: A statistically significant inter-ethnic difference in the allele frequency and genotype inheritance pattern for both loci was noted (P < 0.0001). No association between IL-10 genotypes and spleen rate was noted in any of the listed communities. The TT genotype was the most frequent genotype in the Dogon (71%), whereas in the Fulani the TT genotype was at the lowest frequency (18%). No significant differences in infection rate, parasite density or diversity between different IL-4 genotypes were found within the Dogon. However, amongst the Fulani an association between infection rate and the T allele was confirmed by sex- and age-adjusted modelling (OR, 4.69; P = 0.0003). No intra-ethnic association between IL-4 genotypes and spleen rate was noted in any of the study groups. Known inter-ethnic difference in spleen rate was seen only between T carriers (TT and CT) from both groups (P < 0.0001). Within the Fulani the odds ratio for the prevalence of P. falciparum infection in T carrier individuals with enlarged spleen after adjustment for age and sex was 4.92 (P = 0.0002).

Interpretation: These results suggest an association between the IL-4-590 T allele and prevalence of P. falciparum infection in the Fulani from Mali and also a correlation of this allele and spleen enlargement.

600C
α+Thalassaemia protects against malaria-induced anaemia in children living on the coast of Kenya [MIM-SW-139920]
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Introduction: α+Thalassaemia has been selected to high frequencies in many tropical populations because it protects against death from malaria. Little is known about the haematological consequences of α-thalassaemia in malaria endemic Africa. We therefore aimed to investigate this question in children living on the coast of Kenya.

Methods: We investigated α-thalassaemia-specific haematological parameters both at steady-state (through cross-sectional survey) and during acute episodes of clinical P. falciparum malaria, in two cohorts of children. The first was an age cohort of 311 children aged <8 years old, which we followed by active surveillance for febrile events, while the second was a birth cohort of 2104 children, which we followed...
by passive surveillance for hospital admission with malaria and other diseases.

Results: 48.6% of the study population were heterozygotes and 16.8% were homozygotes for α-thalassaemia. At steady state, haemoglobin concentrations (95% CI; \( P < 0.001 \)) were 0.26 g/dl (0.12,0.40; <0.001) and 0.60 g/dl (0.41,0.8; <0.001) lower in heterozygotes and homozygotes than in normal subjects, respectively. However, this pattern was reversed during acute episodes of clinical \( P. falciparum \) malaria. For example, in children admitted to hospital with malaria, haemoglobin concentrations were significantly higher in thalassaemia heterozygotes and homozygotes than in normal subjects, by 0.84 g/dl (0.28,1.40; <0.001) and 0.97 g/dl (0.29,1.65; <0.001), respectively.

Interpretation: Prevention of malaria-induced anaemia may be one important mechanism by which \( \alpha \)-thalassaemia protects against malaria-death. The reduced capacity for malaria-infected \( \alpha \)-thalassaemic red blood cells to rosette represents one potential mechanism.

31: Vector control

Posters 601–623

Presenting authors of A-posters must be at their posters as follows—A-posters: Tuesday; B-posters: Wednesday; C-posters: Thursday

601A Action larvicide de bacillus thuringiensis var. israelensis (bti) sur anopheles et culex dans la ville de douala (cameroun) [MIM-AB-60492]

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Introduction: Les bioinsecticides ont l’avantage d’agir contre les vecteurs du paludisme tout en protégeant l’environnement mais ils demeurent peu utilisés en Afrique. Nous avons de ce fait étudié l’action de Bacillus thuringiensis var. israelensis (Bti) sur les larves d’anophèles et de culex dans la ville de Douala au Cameroun.

Methods: Les larves prélevées aux stades vieux dans les gîtes naturels ont été soumises à des tests d’inhibition effectués en laboratoire. Parallèlement, des mesures de paramètres physico-chimiques de leurs milieux de vie ont été menées, ainsi qu’une étude macroscopique et microscopique.

Results: L’efficacité du Bti dans l’élimination des larves de moustiques a été confirmée aussi bien chez les anophèles que chez les culex. Les résultats montrent également que l’effet résiduel du Bti s’estompe au bout de 3 jours. De même les concentrations fetales (LC50) ont été respectivement de 0.0075 mg/l pour les larves d’anophèles et de 0.05 mg/l pour les larves de culex, montrant une plus grande sensibilité des anophèles par rapport aux culex. La grande sensibilité des anophèles est en rapport avec les valeurs de pH et de température élevées qui caractérisent leurs gîtes, ainsi qu’avec des valeurs de la conductivité, du total de matières dissoutes et du potentiel redox plus faibles.

Interpretation: Notre étude permet d’envisager avec optimisme l’application du Bti dans les programmes de lutte antivectorielle impliquant les communautés locales au Cameroun en complémentarité avec l’utilisation des moustiquaires imprégnées.

602B Bulldozers against mosquitoes: Environmental management options for malaria control in North-Central Sri Lanka [MIM-EB-10245]

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Introduction: In Sri Lanka, malaria is a major public health problem. In some of the remote areas, access to health care and protective measures is hard and larviciding is applied as a supplementary strategy. In the dry zone in North-Central Sri Lanka the main malaria vector responsible for epidemics is Anopheles culicifacies. It breeds in riverbed pools of natural streams such as Yan Oya, which is used as a conveyance canal for downstream irrigation, but not maintained as such.

Methods: A critical stretch of Yan Oya was selected because of its vector breeding potential and clear association with malaria cases. Mosquito larval popula-
tions were monitored bi-weekly from 2000 till 2003; water levels twice a day. Hospital records were complemented with blood slides. In collaboration with the irrigation agency, environmental measures were developed and streambed profiles measured before and after intervention. In the dry season of late 2001 the streambed was cleared of fallen trees, rocks and other barricades, and leveled using heavy machinery and manual labor. Upstream reservoirs and hydraulic structures were repaired for better regulation of the water flow. Costs were shared between the irrigation agency and the project.

Results: Larvae of *An. culicifacies* and *An. varuna* were found at the stream margins before and after intervention at low flows. After intervention, larval abundance of *An. varuna*, a secondary malaria vector, increased at two of the sampling points, probably as a result of human activities upstream, such as the construction of temporary dams for irrigation. Natural precipitation and supplementary water releases from the upstream reservoirs increased the water level and reduced breeding levels down to a negligible level within two months. Abundance of *An. culicifacies* was recorded at very low levels throughout the post-intervention period. Low malaria incidence was reported for the year 2000, and no cases for 2001 and 2002, which might reflect the success of the intervention. However, overall malaria prevalence in the country was also low. Therefore, a systematic long-term monitoring program is underway to assess the true impact of the control measures. More than three years after the intervention, the rehabilitated stretch is still visibly different from the untouched part. Additional benefits of the intervention included increased efficiencies of water delivery and reduction of floods in the wet season.

Interpretation: Costs of environmental management like this are high for any health intervention, but benefits may stretch over several years and go beyond malaria control. Similar interventions appear feasible at streams elsewhere in South Asia.

603C

**Netprotect®, a new generation of long lasting impregnated mosquito nets [MIM-Cb-131325]**

C. Bogh

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**Introduction:** Long lasting impregnated mosquito nets (LLINs) are as effective as conventional insecticide treated nets (ITNs) in preventing mosquito bites. The shortcoming of the ITNs is that they lose their insecticidal effect after about 2 washes, whereas LLINs have shown to be effective for 15 or more washes. Recent studies have cast doubt on how wash resistant the LLINs actually are and how effective they are when used under field conditions. Addressing this issue is of real importance for disease control programs. We here present the first laboratory and field tests of a new generation of LLINs.

**Methods:** A new type of LLINs were designed to address the key shortcomings of earlier generations of LLINS. The primary improvements are directed at improved resistance to tare and ware, resistance to ultra violet light exposure and significantly improved capacity to withstand continuous washing under field conditions. Laboratory tests were done using WHO standard washing procedures in time accelerated experiments. Residual insecticidal testing was done using *Anopheles gambiae* using WHO standard test cones. Field tests were done using time series of bio assays using WHO standard test cones and a local malaria vector species from the test locations.

**Results:** The present study shows that a fine meshed net can be made with a controlled release technology that allows for rapid regeneration of the insecticide at the surface no more than 2 days after washing and without re-heating. These nets resist at least 35 laboratory washes and still remained 100% effective in killing exposed mosquitoes. The initial field test results demonstrate the nets increased resistance to tare and ware and how acceptable they are to the local populations. Further we present the initial data on the nets insecticidal effect when used under third world village conditions.

**Interpretation:** Our findings demonstrate that this new type of mosquito nets have much desired properties with regards to: durability, resistance to light exposure and routine washing.
**604A**
The use of impregnated curtains does not affect antibody responses against *Plasmodium falciparum* and complexity of infecting parasite populations in children from Burkina Faso [MIM-AB-123513]

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**Introduction:** In Burkina Faso, where malaria is hyperendemic and transmission intensity is very high, the majority of malaria related morbidity and mortality occurs in children less than 5 years of age. A control measure such as the use of insecticide-treated curtains (ITC) significantly reduces transmission of malaria infection. Concerns remain whether reduced transmission intensity may lead to a delay in the development of immunity in younger children and even to a partial loss of already acquired immunity.

**Methods:** In this study, the levels of *P. falciparum*-specific IgG subclasses, the number of infecting parasite clones determined by PCR-based genotyping of the msp2 gene and the parasite density were analysed in 154 asymptomatic children (3–6 years) living in 16 villages (eight with and eight without ITC) in the vicinity of Ouagadougou, the capital of Burkina Faso. In addition, the parasite inhibitory effects of Ig fractions, prepared from selected children, in cooperation with normal human monocytes were studied.

**Results:** Blood samples from asymptomatic ITC users showed a significant decrease in *P. falciparum* prevalence as well as in parasite density. However, no significant difference was observed in *P. falciparum*-specific antibodies or in parasite multiplicity of infection between the two groups. Furthermore, Ig fractions from children of both groups showed similar levels of inhibitory activity against autologous parasite growth both on their own and in cooperation with monocytes.

**Interpretation:** The data reported here indicate that the development of potentially protective humoral immune responses is not compromised in children living in villages equipped with ITC for vector control in a area of high malaria transmission.

**605B**
Intrinsic efficacy of three synthetic repellents (DEET, IR3535 and KBR 3023) against mosquitoes of public health importance [MIM-VC-91400]

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**Introduction:** The intrinsic activity of three synthetic repellents (DEET, IR3535 and KBR3023) was evaluated in the absence of a host against mosquitoes of public health importance (i.e. *Aedes aegypti*).

**Methods:** WHO susceptibility tests were used to assess the knock-down effect (KD50 and KD95) and mortality (CL50 and CL95) induced by each repellent on impregnated papers. In addition, irritability tests were used to compare the fly mosquito behaviour (time for “first take off” or FT50) for increasing concentrations of repellents (from 2 to 7%) over the distance (0, 2, 10, 20 and 40 mm).

**Results:** The results shown that DEET, contrary to IR3535 and KBR3023, showed insecticidal properties (lethal and knock-down effect) close to that induced by pyrethroids and DDT (KDT50 = 9.7 min at 7%; CL50 = 3.17%). All repellents provided significant irritancy as compared with untreated papers (relative irritancy or RI > 1, P < 0.05). Irritancy of DEET and IR3535 was maximum at 2% (12.3) and 5% (17.7), respectively. Surprisingly, the KBR3023 showed significantly lower irritant properties than DEET and IR3535 for a same range of concentrations (RImax = 3.7 at 6%). With increasing distance (from 10 to 20 mm), DEET remained the most irritant molecule against mosquitoes.

**Interpretation:** In the absence of a host, repellents significantly interfere with the mosquito behaviour. DEET also showed strong insecticidal properties which emphasis the fact that repellents do most probably affect different physiological systems in insects.
Evaluation of the efficacy and persistence of insect repellents DEET, IR3535, and KBR 3023 against Anopheles gambiae complex malaria vectors [MIM-AB-242138]

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Introduction: Protection against malaria transmission in Africa has focused on the evaluation of insecticide-treated materials (ITM). The level of protection offered by ITMs can be low when vectors bite early in the evening before people go to bed. In this case, a cheap, safe, efficient, and persistent repellent could provide additional protection. We conducted laboratory and field studies to evaluate the efficacy/persistence profile of three synthetic repellents against Anopheles gambiae complex malaria vectors.

Methods: Laboratory tests assessed the sensitivity to DEET, IR3535 and KBR 3023 of F1 progenies of wild-collected An. gambiae s.l. Mosquitoes from 2 to 7-day-old were exposed according to a ‘separate arms’ protocol to logarithmic dose increments applied on one arm of human subjects to evaluate the relative potency, and median effective dosages. Field tests were performed in a rural village near Ouagadougou, Burkina Faso. Eight human volunteers collected mosquitoes landing on their lower limbs where a repellent was applied. Four doses (0.1, 0.3, 0.6 and 0.8 mg/cm²) of repellents and a placebo were tested according to replicated Latin Squares. Logistic regression was performed to determine the efficacies, as well as the decay constant and half life characterizing the exponential loss of repellent with time. The ED95 for DEET, IR3535, and KBR 3023 were 94.3, 212.4, and 81.8 µg/cm², respectively. KBR 3023 showed the greater persistence, with a half-life of 4.1 h. Both DEET and IR3535 had half-lives of 2.9 h. ELISA detection of P. falciparum CSP protein in 842 An. gambiae s.l. indicated that CSP-positive mosquitoes were equally frequent in treated and control subjects.

Interpretation: KBR 3023 appears as the repellent having the most favourable efficacy/persistence profile for personal protection against An. gambiae complex malaria vectors. The repellents afforded a reduction in the number of infectious bites.

Prospects for mosquito larval control in Africa [MIM-UF-66319]

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Introduction: Today larval control remains a forgotten tool of malaria control. It is not part of the strategy of RBM, nor is it a central theme supported by major institutions engaged in malaria control. However, there is a growing interest in this field. The presented work is part of a project with the goal to identify eco- and epidemiological settings for which anti-larval methods
might be appropriate, and to determine the contribution it could make to reduce the burden of malaria in particular settings.

Methods: Pilot studies have been implemented for 4 years in a rural town in western Kenya collecting weekly data on larval habitat availability and mosquito larval and adult densities. Microbial larval control has been implemented for 2 years following an 18 months baseline survey. Following this pilot experience six separate sites in two different ecological settings have been selected for further studies: urban Dar es Salaam and western Kenyan Highlands. Routine surveillance takes place over 2 years. In the first year pre-intervention entomological and clinical data will be collected from all study communities. In the second year, half the communities will be randomly selected for larval control interventions, the remainder will serve as controls.

Results: In all three study areas mosquito larval habitats could be easily identified and are readily accessible for anti-larval measures. Entomological data collected pre-, during and post-intervention in a rural town in western Kenya showed that regular larviciding can reduce mosquito larval and adult abundance over 90% compared to pre- and post-intervention periods. Mosquito larval habitats in urban Dar es Salaam as well as in the western Kenyan Highlands are highly associated with human activities. Major sources of aquatic habitats suitable for Anopheles development are associated with agricultural activities implemented in formerly unused land.

Interpretation: The characteristics of mosquito larval habitats, their availability in the dry and rainy seasons, and their potential for mosquito larval control in the frame of malaria control programmes will be discussed for all three sites.
Field and laboratory evaluation of long-lasting insecticide treated nets [MIM-JG-19180]

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Introduction: Long-lasting insecticidal nets (LLINs) would eliminate the need for frequent retreatment of mosquito nets for the prevention of malaria but evaluation of these products is slow and difficult, delaying their approval, acceptance and implementation. We discuss the field and laboratory approaches adopted by CDC and KEMRI for evaluating LLINs in laboratory and field settings, including advantages and potential pitfalls of these approaches.

Methods: Five candidate LLINs were evaluated in the laboratory and the field. All nets were treated with either permethrin or deltamethrin. Nets were compared with a conventionally treated net (deltamethrin @ 25 mg/m²). For laboratory wash-resistance studies, nets were washed up to 20 times and tested in WHO cone tests. For field evaluations, nets were distributed to residents of Kisian village in western Kenya and each net was tested with a WHO cone test at 5–6 month intervals. A net was considered “failed” if mortality was below 50% in two consecutive tests. The durability of the insecticide treatment was compared among different nets by fitting a Cox proportional hazards model controlling for the cumulative number of washes.

Results: The PermaNet retained significant biological activity after 20 washes and up to 2 years in the field. The DAWA net also retained some activity after repeated washing but exhibited wide variation in insecticide retention and biological activity among different nets. Bioassay mortality in all other nets declined to <10% after six washes. Results from the field evaluation correlated well with those observed in the laboratory wash resistance study with the PermaNet significantly less likely to “fail” compared to a conventionally treated net. Probability of “failure” of other nets, including a net approved by the WHO pesticide evaluation scheme, was not significantly different from a conventionally treated net. The relative performance of the nets in laboratory wash resistance studies was correlated with the longevity of insecticide in the field.

Interpretation: Only the PermaNet performed significantly better than a conventionally treated net. Further studies are needed to refine the evaluation methods and to determine entomological outcomes that are predictive of protection against malaria.

The use of DDT (dichlorodiphenyltrichloroethane) for malaria vector control: Past present and future [MIM-JG-224970]

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Introduction: Ever since the discovery that the Anopheles mosquito transmits the malaria parasite to man, vector control, has been one of the most effective ways of controlling the disease. Among the many ways to control malaria vectors, indoor residual spraying (IRS) has been one of the most enduring and effective methods. The method involves spraying a tiny amount of long-lasting insecticide inside houses where the female Anopheles mosquito rests.

Methods: There are several insecticides that can be used for IRS, however, DDT has been shown to be one of the most successful. Unfortunately it is also one of the most controversial and the controversy is largely unwarranted and tends to overshadow the chemical’s usefulness. DDT was first applied in disease control in 1944. It controlled a typhus epidemic in Naples, eradicated malaria in Europe and North America, led to the implementation of Malaria Eradication Programme by WHO freeing 1000 million people from the malaria risk and saved 500 million lives. In Southern Africa, DDT use in South Africa, Swaziland, Namibia, Botswana, Zimbabwe reduced malaria to near elimination.

Results: Recently, DDT reduced malaria cases by 80% in KwaZulu Natal, South Africa, and reduced malaria prevalence from 74 to <1% in Konkola Copper Mine in Zambia. However, historical indiscriminate excessive use of DDT in agriculture resulted in the build up of an environmental load of DDT and its subsequent ban in agriculture and the advent of the Stockholm
Convention. DDT is currently used in several malaria control programmes, mostly in southern and eastern Africa, but also in India. In Southern Africa, IRS utilising DDT is the principal vector control intervention. South Africa, Swaziland, Namibia, Madagascar and Mauritius, have maintained the use of DDT for malaria vector control. Zambia and Zimbabwe have introduced DDT for malaria control. Debate is ongoing in Kenya and Uganda on the reintroduction of DDT for malaria control. Due to increasing pyrethroid resistance, high cost and toxicity of carbamates and organophosphates, respectively, DDT will continue to be required for malaria vector control. The Stockholm Convention grants DDT an exemption for use in public health as long as effective and affordable alternatives are not available.

Methods: Training need assessment was obtained from analysis of a baseline survey previously carried out with community groups. Training exercises were designed and developed covering mosquito sampling methods, breeding habitat, mosquito life cycles, disease life cycles, and source reduction. In addition, mosquito scouts were trained on mapping and characterization of different larval habitats, effective facilitation, and communication skills, giving trainees the skills to make decisions about control options.

Results: Three training programmes were designed and implemented. They included trainer of trainers (TOTs), mosquito control action groups (MCAG) and mosquito scouts training. TOTs assisted the research team in design and implementation of other training activities in the community. Several MCAGs and mosquito scouts were trained. The study has shown that mosquito scouts can learn key aspects of the biology and management of vectors through field-based learning methods and that this stimulated communication and coordination. Post-training experience suggests that mosquito scouts and MCAGs coordinated their action plans and implementation on source reduction. Further, all the groups formed an umbrella body mandated to liaise with municipal council authorities, Ministry of Health, hoteliers, and other stakeholders in major control operations that require more resources and expertise.

Interpretation: Our results imply that community groups can be motivated and educated to play an active and competent role in the control of mosquitoes in their environment.

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Interpretation: Our results imply that community groups can be motivated and educated to play an active and competent role in the control of mosquitoes in their environment.
Introduction: Long-lasting insecticidal nets (LLIN) have been accepted as a new technology to achieve high levels of malaria prevention with insecticide treated nets (ITN). While standardized washing of LLIN is an accepted method to evaluate this technology, the ultimate evidence of their effectiveness will have to come from field tests under real-life conditions. We have carried out such field tests in Western Uganda comparing the second generation PermaNet® with conventionally treated nets.

Methods: In five villages 250 LLIN and 120 conventionally treated nets were randomly distributed to households. Physical conditions and frequency of washing were assessed through regular household interview surveys. Every 6 months a random sample of 40 nets was collected and net samples taken for analysis. Bio-assays were carried out with Anopheles gambiae s.s. (Kisumu strain) using a 3 min exposure conus test. Knock-down rate after 60 min and functional mortality rate after 24 h were assessed. Minimal effectiveness was defined as mortality >50% or knock-down >75% while optimal effectiveness was mortality >80% or knock-down >95%. Chemical residue of deltamethrine was measured with gas chromatography and expressed as mg/m².

Results: Baseline median deltamethrine concentration was 69.2 mg/m² for LLIN (target 55 mg/m²) and 42.8 mg/m² for conventionally treated nets (target 25 mg/m²). All nets showed optimal effectiveness in bio-assay. Average wash frequency was 0.2/month or 2–3 washes per year and this rate did not vary between types of nets nor over time. After 12 months median deltamethrine concentration on conventional ITN had dropped to 1.4 mg/m² (0.3–26.4), geometric mean mortality rate to 44.8% and knock-down rate to 59.5%. Therefore, after 12 months 42.5% of the conventional ITN still had optimal and 72.7% minimal effectiveness. In contrast, median deltamethrine concentration on LLIN was 55.8 mg/m² after 12 months and mortality and knock-down rates were 95.2 and 99.0%, respectively. After 24 months median deltamethrine concentration on conventional ITN had dropped to 5 mg/m² and had been washed immediately before collection. When re-examined 1 month later, these nets showed significantly better bio-assay results. Overall 97.5% of LLIN still had minimal effectiveness after 24 months.

Interpretation: Our data show that the 2nd generation PermaNet® had an excellent performance after 2 years of use in a rural community. The insecticidal effect on these nets can be estimated to last at least 3 years, the average life span of a polyester net.

613A

Bednets for malaria control in urban Accra, Ghana

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(1) International Water Management Institute, Accra, Ghana; (2) Liverpool School of Tropical Medicine, Liverpool, UK; (3) Noguchi Memorial Institute for Medical Research, Accra, Ghana; (4) International Water Management Institute, Colombo, Sri Lanka

Introduction: We conducted studies in urban Ghana to determine malaria transmission risk, impact of urban agriculture (UA) on malaria risk and to identify those groups most vulnerable to infection. In some communities the data showed strong associations between UA and parasitaemia, but the general high prevalence in the urban areas suggests that the most effective control tool is likely to be bednets with their documented community effect rather than a specific intervention targeted directly at UA.

Methods: The intervention was carried out in a community (with an adjacent community as control) around the main UA area in central Accra from May–December 2004. In the selected area households with children under 10 years and/or pregnant women were given a free net (control area 6 months later). From each area a cohort of 250 children was randomly selected. On month 0, 3 and 6 these children were seen in a mobile clinic. Malaria parasitaemia, haemoglobin (Hb) level and anthropomorphic measures and a questionnaire on bednet usage, travel to the rural area and socio-economic status were taken. On months 0 and 6 stool samples were obtained and examined for geohelminths. All children positive for geohelminths or malaria or with Hb < 8.0 g/dl were treated.

Results: Data collection finished in December 2004 and results are analysis ongoing. Preliminary results indicate parasitaemia prevalence was 2.4, 4.7 and 4.5% in
the intervention and 5.1, 3.1 and 7.2% in the control area at month 0, 3 and 6, respectively. The prevalence of anaemia was 48.1, 52.4, 43.8% in the intervention and 48.6, 63.2 and 48.9% in the control area at month 0, 3 and 6, respectively. The geohelminth prevalence was 12.78 and 14.8% at month 0 and 0.0 and 3.7% at month 6 in intervention and control area, respectively. Multivariate analysis is being done to identify which factors are important determinants for prevalence and how the intervention impacts on anthropomorphic measures. We hope the data will contribute to the discussion on use of haemoglobin as indicator for malaria control intervention in the urban setting.

Interpretation: The trial showed that such an intervention can reach most people in the urban setting including the vulnerable groups. Results will help determine whether the majority of malaria infections in the urban setting are imported or locally transmitted.

614B
Insecticide-treated nets: Closing the know-do gap
[MIM-CL-73200]
C. Lengeler
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Introduction: Insecticide-treated nets (ITNs) form the basis for vector control in sub-Saharan Africa (SSA). Over 110 completed trials in the 1980s and 1990s, demonstrated the impact of ITNs beyond doubt. Large gains in child survival and important reductions in clinical attacks can be achieved by the large-scale deployment of ITNs. Recently, good epidemiological evidence became available to show that no mortality re-bound was taking place in children protected from birth by ITNs.

Methods: By 2002, the Roll Back Malaria Partnership (RBM) had produced a strategic framework for the upscaling of ITNs in all endemic countries and large-scale programmes had been initiated in a number of countries. Currently three main areas are particularly important in view of reaching a high and sustained ITN use, especially in pregnant women and children.

Results: Firstly, the main operational problem has consistently proven to be the regular re-treatment (every 6–12 months) of the nets. Currently two brands of long-lasting insecticide treatment kits offers further the prospect of treating permanently existing nets. Secondly, different mechanisms for the country-wide distribution of ITNs have been explored, including assisted commercial distribution, social marketing and different models of distribution through health services. There is still scant evidence on their feasibility, cost-effectiveness and sustainability but each country will need to make strategic choices on the basis of its own situation. Finally, various avenues have been explored to subsidize ITNs to the main risk groups, pregnant women and very young children. Although the evidence base is also sparse, the pros and cons of each approach need to be carefully evaluated for each setting.

Interpretation: Many exciting ITN developments have taken place during the past 20 years. However, there is an urgent need to accelerate the speed of ITN deployment in all affected countries. Products and strategies exist and they must be put to good use.

615C
The Larvicidal activity of Abate and Lavex100 on Anopheles gambiae sensu lato mosquitoes
[MIM-NL-297950]
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Introduction: Mosquito larvae are not very mobile since their habitats are fixed and this is an advantage for high coverage of up to 90% to be achieved. Very little information is available on the interaction of mosquito larvicides with water containing different salt concentrations and this study aims to give an insight on the efficacy of Abate and Lavex100 under such conditions.

Methods: A laboratory study was carried out in order to measure the effect of Abate and Lavex100 on Anopheles gambiae sensu lato mosquitoes. Interaction of the two larvicides with pond and salt water was also measured. Pond water had a chloride concentration of 1.5 mg/l and salt water from Kamhoro had a chloride concentration of 400 mg per litre. Experiments were conducted in plastic containers with an open end having an area of 78.5 cm² and a volume of 250 ml.
**Methods:** Plants for this study were selected for, according to their reported traditional use in Africa. A number of taxa reported as treatments for fever were investigated. Using weighted criteria, species were prioritised, according to their potential for success. Various available plant parts were collected and extracts obtained through distillation. These extracts were tested for bioactivity against the larvae and adults of *Anopheles arabiensis*, the main vector of malaria in southern Africa. Once significant activity was obtained using whole plant extracts, bioassay-guided fractionation was used to identify the active compounds. Dose response assays were conducted for positive extracts, to determine the LD50 values.

**Results:** The 381 plant extracts evaluated consisted of 79 taxa representing 37 plant families. The extracts were obtained from different parts of the plant, namely roots, leaves, stem, fruit, flower, seeds, twigs and bark. Stringent criteria were established to determine a potential plant-derived insecticide. In testing against adult mosquitoes, five extracts exhibited mortality between 40 and 59%, demonstrating that toxicity against the target species does exist. Three hundred thirty-four extracts exhibited little or no activity with mortality between 0 and 19%. It was found that the susceptibility level for larvicidal activity of immature *Anopheles arabiensis* to the different plant extracts ranged from 0 to 99%. Dose–response assays revealed that certain extracts were effective at concentrations of 4 parts per million. Although taxa from the Plantaginaceae and Pittosporaceae families yielded the best overall insecticidal results, of all families tested the Asteraceae had the most representatives producing more than 25% mortality. Differences in activity were found to be dependent on the part of the plant used and also on the method of extraction.

**Interpretation:** Small quantities of chemicals found in plants can be highly effective in killing mosquitoes, especially the aquatic larval stages. The potential exists for large scale use of these chemicals in the control of malaria transmitting mosquitoes.
617B
Acquisition, use patterns and physical status of nets bought from the local market in Muheza District, North eastern Tanzania [MIM-RR-285945]
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Introduction: The Tanzanian Government has emphasized that the supply of insecticide treated bednets against malaria vectors should mainly depend on villagers buying nets and insecticide and considerable numbers of nets have been sold.

Methods: A survey was carried out in seven villages near Muheza outside the areas where people were provided with ITNs for village scale studies, to inspect nets on the beds or sleeping mats of all adults and children. Whether or not the nets were badly torn was recorded and the householder was asked whether the nets had ever been treated with insecticide.

Results: Analysis of 2412 houses inspected showed that coverage of people of all ages by any nets was only about 15%, most were badly torn, less than 10% were said to have been treated and in a considerable number of cases, children without nets were in the same house as people with untreated nets. In such cases, when the untreated nets were badly torn, extra biting would have been diverted to the children without nets, so the nets would have been harmful to the prospects of controlling malaria. However, the majority of the nets are now untreated and badly torn. Such nets would have neither a beneficial nor a harmful effect because mosquitoes can readily enter them to blood feed.

Interpretation: We conclude that effective control of malaria in rural areas will require organised, free provision of nets and assurance that they are insecticidal by use of long lasting insecticidal nets and/or a free re-treatment service.

618C
Evaluation of selected aqueous plant extracts as potent larvicides and/or repellents of malaria vectors [MIM-CN-174360]
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University of Buea, Buea, Cameroon

Introduction: Resistance of Anopheles mosquitoes to insecticides coupled with environmental hazards caused by these synthetic chemicals, as well as the toxicity of DEET to children when used as a repellent have made these methods of vector control less popular. There is thus increasing need for the search of botanicals as effective alternatives. We therefore evaluated aqueous extracts of some locally available plants as larvicides and/or repellents to malaria vectors.

Methods: To determine lethal concentrations, fourth instar larvae were put in water treated with various concentrations of each plant extract and mortality counts taken at definite time intervals. LC50 values were determined graphically. The consistency of larvicides was tested by introducing larvae weekly into cups treated once and semi-field evaluations by treating collected pond water containing larvae with lethal concentrations of each plant extract. Mortality counts were then taken 24 h after treatment. For repellency tests, human forearms treated with each extract were introduced into a cage containing neonate female Anopheles mosquitoes and number of bites recorded within 2 h. DEET served as the control.

Results: The extracts of Piper guineense and Ricinus communis were most potent with LC50 values of 1.8 and 3% of the stock solutions, respectively. Tephrosia purpurea was relatively less potent with LC50 value of 6.8%. R communis took a shorter time to kill 50% of larvae (T50=6h) closely followed by P. guineense (T50=8h). The T50 of T. purpurea was 34 h. Larvicidal activity declined over weeks with that of R. communis dropping to <10% in 5 weeks while those of P. guineense and T. purpurea took 4 weeks each. Under semi-field conditions, R. communis, P. guineense and T. purpurea significantly reduced number of third and fourth instars by 85, 80 and 60%, respectively. Extracts of P. guineense and Aframomum citratum, respectively, provided 39 and 57% protection from mosquito bites compared with the control. None was as good as DEET.

Interpretation: R. communis and P. guineense are potent mosquito larvicides; P. guineense also repels mosquitoes. Therefore, with further research extracts of these plants or their derivatives can be used in malaria vector control especially in poor rural communities.
619A
2 années d’étude d’acceptabilité, d’efficacité et de durabilité des moustiquaires Olyset (LLNs) dans les conditions de terrain en Afrique de l’Ouest [MIM-DR-35948]
R. Djouaka, J. Doanio, L. Toé, D. Dimi, M. Akogbeto
(1) CREC-Cotonou, Republic of Benin; (2) IPR-Bouaké, Côte d’Ivoire; (3) CM, Bobo Dioulasso, Burkina Faso
Introduction: Pour étudier les performances réelles des Olyset, nous avons pendant 2 années enregistré l’avis des utilisateurs. La présente évaluation a porté sur des Olyset utilisées et soumises aux pratiques quotidiennes des populations. L’étude est réalisée par le Réseau de Socio-anthropologie appliquée à l’Éradication du Paludisme en Afrique de l’Ouest. Ce réseau créé par le programme Pal+ du Ministère Français de la Recherche a pour but d’évaluer les performances des outils de lutte contre le paludisme.

Methods: La conception et la mise sur pied de cette étude repose sur 2 grandes orientations: évaluer le niveau d’acceptabilité des Olyset 6 mois après distribution ensuite, suivre leur efficacité et l’état de dégradation des fibres après deux années d’utilisation. Nous avons choisi deux types de sites: un site où la population dort volontiers sous moustiquaires et où le taux de couverture est supérieur à 50% (Kétouon et Vossa) et un site où le taux de couverture est inférieur à 25% (Toflo). Les Olyset ont été distribuées gratuitement aux femmes enceintes, aux élèves et à la population générale. Les outils de collecte d’informations quantitatives et qualitatives ont été élaborés. Les données a été analysées par Excel, Epi-info et Text base 8.

Results: Les résultats révèlent des taux d’utilisation très élevés (97.7 à 100%) dans les 3 groupes cibles, tant à Kétouon qu’à Vossa. A Toflo, on note une faible réceptivité et un grand nombre de transactions. L’appréciation est globalement bonne et une unanimité se dégage sur les divers avantages offerts par les Olyset. Toutefois, des réserves ont été faites sur la taille, le maillage et la texture des fibres. L’évaluation de l’impact du lavage sur l’efficacité et la durabilité des Olyset révèle une fréquence élevée de lavage dans les sites d’étude (2 à 5 lavages par mois). Ceci s’explique par une utilisation quasi permanente des moustiquaires: “Nous lisons et mangeons avec des lampions sous nos Olyset et en plus l’eau est disponible ici”. Après 24 mois d’utilisation, 50% des Olyset recensés possédaient des trous, des déchirures et l’efficacité semble avoir fortement baissé: “Olyset a diminué d’efficacité. J’entends le bruit des moustiques dans ma chambre alors que c’était différent au début; de plus, je ne trouve plus des moustiques morts au sol”. “Ma Olyset est devenue inefficace car c’est trouvé et les moustiques pénètrent facilement”. Les données provisionnelles montrent une baisse d’efficacité après une série de 10 lavages.

Interpretation: Tout en ressortant l’impact de la forte pression de lavage sur l’efficacité et la durabilité des ITNs, ces résultats ramènent sur table la problématique de définition du concept Long lasting (LLNs), sa contextualisation, et ses limites sur le terrain.

620B
Effect of Indoor Residual Spraying on numbers and sporozoite rates of A. funestus and A. gambiae s.s on Bioko Island, Equatorial Guinea [MIM-JK-38665]
B. Sharp, I. Kleinschmidt, F. Ridl, J. Kakulinski
(1) Medical Research Council, 491 Ridge Road, Durban 4091, South Africa; (2) One World Development Group, Gainesville, Florida, USA
Introduction: A comprehensive package of malaria control measures has been initiated on Bioko Island, Equatorial Guinea (EG), since February 2004, funded by Marathon Oil Company in partnership with the government of EG. The measures consist of indoor residual spraying (IRS) and case management including the introduction of improved diagnosis and effective treatment based on combination therapy.

Methods: Window traps were fitted to six houses at each of 16 sentinel sites in December 2003. Between February and July 2004, all domiciliary structures on the island were sprayed with pyrethroid insecticide. Daily window trap contents were collected and shipped to MRC (South Africa) laboratories. Householders completed checklists specifying the nights for which traps were operating. All specimens were morphologically identified; a sub-sample was specifically identified to species and for malaria infectivity using PCR techniques. Numbers of specimens per trap per night and proportions of infective specimens were calculated, comparing pre- and post spraying periods. The effects
of a number of factors on counts and on sporozoite rates were investigated.

**Results:** For *A. funestus* there was a significant and sustained reduction in numbers per trap per night in all areas after spraying. For *A. gambiae* there was a significant reduction in numbers per trap per night in all but one area after spraying, with significant between-site heterogeneity of effect. Numbers of *A. gambiae* rebounded with a significant upward time trend ($P < 0.001$) after spraying which continued up to the second spray round in January 2005. Sporozoite positivity in *A. gambiae* reduced from 7% pre-spraying to less than 2% post-spraying ($P = 0.023$). The pre-spraying sporozoite rate in *A. funestus* was 4.1%; the numbers of *A. funestus* post spraying were too small to adequately assess sporozoite levels. The total number of sporozoite infective mosquitoes exiting through window traps per night, reduced to about 20% of its pre-spraying level.

**Interpretation:** IRS achieved a significant reduction in numbers of infective mosquitoes leaving through window traps. Window traps are an effective means of continuous ongoing monitoring of relative change in malaria transmission parameters.

**621C**

**Analysing markets for insecticide-treated nets in Tanzania** [MIM-GS-60480]

G. Stephen, J. Multigan, K. Hanson, S. Ebenezeri, E. Mtawa

(1) Ifakara Health Research and Development Centre, Dar es Salaam, Tanzania; (2) London School of Hygiene and Tropical Medicine, London, UK

**Introduction:** Coverage of ITNs continues to lag despite the commitment of national governments to achieve 60% coverage of vulnerable groups by 2005. Debate continues on merits of alternative approaches to distributing ITNs such as commercial supply and a voucher scheme to target subsidies to the groups. These approaches aim at balancing goals of equity, efficiency and sustainability in ITN delivery. This paper presents the results from a study on commercial ITN market in Tanzania.

**Methods:** Twelve districts were purposively selected to represent variation in malaria transmission and economic development. In each district, 30% of wards were randomly selected and a six-monthly “retail census” was undertaken to identify shops selling ITN products (nets, insecticide, “bundled” nets and insecticide). Shops that reported selling ITN products were visited monthly for “retail auditing” to measure prices, sales volumes and characteristics of the supply chain. Information from ‘retail census’ was combined with information from the district level on the presence of other ITN related activities and the structure of the supply system, to analyse the development of the twelve markets over a twenty four month period.

**Results:** There some positive trends in availability of ITN products and the percentage of wards with at least one outlet stocking net kits has improved substantially since the start of the census rounds. However, there continues to be gaps in availability in non major trading centres compared to major trading centres. Price trend for insecticides shows a general decline over the period whereas prices for insecticide treated nets are relatively stable. Trends in ITN prices, availability and sales volumes commonly, among other factors, influenced by seasonality factor. Implications for understanding the opportunities and limitations of commercial sector ITN distribution are drawn, and the implications for national strategies for scaling up ITNs are considered.

**Interpretation:** Our findings show development in private sector involvement in ITNs distribution and declining and stable prices for insecticides and insecticide treated nets. However, there still ITN availability gaps in private sector in rural areas.

**622A**

**Control of malaria vectors and transmission in an urban area in Central Sri Lanka using the insect growth regulator, pyriproxyfen** [MIM-AY-437220]

A. Yapabandara

Regional Office, Anti Malaria Campaign, Matale, Sri Lanka

**Introduction:** An evaluation of pyriproxyfen as a larval control agent with the aim of reduce malaria vector density and incidence of malaria a study was conducted in Dambulla town in the dry zone of Central Sri Lanka. There are many pools were formed in the beds of streams which flow across the town during the dry season of the year. These are the major breeding
places of the primary malaria vector, Anopheles culicifacies and secondary vector, An. subpictus.

Methods: Adult mosquito collections were carried out using three standard methods and parasitological data were collected by the two government hospitals. The pools in the beds of streams and agricultural wells of two divisions of the town were treated with a granular formulation of the insect growth regulator, pyriproxyfen at the rate of 0.01 mg a.i./l at the end of pre-intervention year. Re-application of pyriproxyfen to the stream bed pools and agricultural wells was decided by field bioassays.

Results: The field bioassays indicated a single treatment of pyriproxyfen effectively inhibited the emergence of adult mosquitoes in the river bed pools for a period of 155 days. The treatment caused significant reduction of the adult An. culicifacies (51%) and An. subpictus (62%) in the post intervention year compared to pre-intervention year. Similarly, incidence of malaria was reduced in the post treatment villages and town about 53% of that of the pre-intervention year.

Interpretation: It is concluded that pyriproxyfen can be a very effective means of malaria control in urban situations, if all the possible vector breeding places in the area can be located.

623B
Progress in the insecticide treated mosquito nets implementation and success of net re-treatment in Malawi [MIM-JZ-249132]

(1) National Malaria Control Programme, Malawi; (2) World Health Organisation, Malawi Country Office, Malawi

Introduction: Malawi has the history of using the mosquito nets for privacy especially along Lake Malawi, however the Malawi Government adopted use of nets as a malaria intervention in 1995. During this time, the implementation was based on small projects with different pricing until 2002 when the Government scaled up the implementation national wide. Aim is to highlight progress in ITN implementation and the success story of net re-treatment in Malawi.

Methods: The scale up started with the development of ITN guidelines and later on the standardization of nets price. The nets were heavily subsidized at $0.5 for children under-five and pregnant women distributed through the health facilities, $1.0 for community based distribution and the full cost recovery for commercial sector. However, the re-treatment rates were still very low at 7%. The National Re-treatment campaign was initiated in 2003 in order to improve the re-treatment coverage. In process of scaling up ITNs distribution in Malawi, three major channels are being used. Heavily subsidized nets through the health facilities, subsidized nets through community based organizations and full cost through the commercial sector.

Results: The net re-treatment campaigns started in 2003 as a national event when all nets in the country are re-treated free. In Malawi approximately 43% of the households own at least one mosquito net compared to 13% as indicated in the 2000 DHS. Cumulatively over 2.3 million nets have been distributed through all channels. According the survey done in 2004, the nets usage is 35 and 31% for children under-five years and pregnant women, respectively. Some of the challenges faced by the programme is the limited supply of ITNs. Net re-treatment, coverage has increased from 7% in 2000 to 61% in 2004. This increase has been attributed to the free re-treatment that were being organized as nation wide campaigns.

Interpretation: The NMCP has achieved a lot in the ITN implementation. The programme has been successful raising the nets re-treatment from 7 to 61%. This is attributed to Government bold decision to heavily subsidize the price of the nets for the target groups.
Symposia/Side Meetings

1: The MARA project: Recent developments and an open discussion on applications

Monday 14 November 14:30–16:00—Mongosi Hall

Organized by: Medical Research Council of South Africa. Address: 491 Ridge Road, Overport, Durban 4091, South Africa. E-mail: craigm@mrc.ac.za (M. Craig)

The mapping malaria risk in Africa (MARA) project aims to support evidence-based decisions toward appropriate targeting, timing and planning of malaria control. At the last MIM conference in Arusha MARA distributed the MARA LITE CD which provides access to its continental prevalence database, maps of malaria, and estimates of populations at risk. This symposium will give an update on recent developments, such as the on-line MARA LITE, risk models developed recently and other data being collated (e.g. drug and insecticide resistance). This will be followed by an interactive session on the implications of various data sets for policy. Input from attending delegates, re what decisions can be made (better) in the light of available data, will contribute towards improving the MARA LITE product.

2: Operational research in Africa

Monday 14 November 14:30–16:00—Zingana Hall

Organized by: The Global Fund to fight AIDS, Tuberculosis and Malaria. Geneva Secretariat, 53, Avenue Louis-Casei, 1216 Geneva-Cointrin, Switzerland. E-mail: Bernhard.Schwartlander@TheGlobalFund.org (B. Schwartlander)

Specific details to be provided.

3: Capacity building: Lessons learned

Tuesday 15 November 09:00–12:00—Zingana Hall

Organized by: Fogarty International Center, NIH. Building 31 B2C39, Bethesda, MD 20892, USA. E-mail: sinab@mail.nih.gov (B. Sina)

The mission of the Fogarty International Center at the U.S. National Institutes of Health is to build scientific research capacity in developing countries to address global health problems. The Fogarty International Center primarily develops the capacity to tackle malaria through support for MIM and collaborative research training grants with U.S. universities. Lessons learned about building sustainable research capacity will be presented by Fogarty scientific staff and former Fogarty trainees who are currently malaria researchers in Africa. Approaches to monitoring and evaluating research capacity will be presented. Successful individual approaches to establishing malaria research careers in Africa through research training and re-entry grants will be described.

4: Home and community management of childhood febrile illness

Tuesday 15 November 09:00–12:00—Mongosi Hall

Organized by: Div International Health (IHCAR), Karolinska Institutet, Norrbacka plan 2, 17176 Stockholm. E-mail: karin.kallander@phs.ki.se (K. Kallander)

Malaria and pneumonia are the two biggest killers of African children. home management of malaria strategies using community health workers are now being introduced on a wide scale for more effective treatment of febrile illness in children. This symposium will review implementation experiences and effects of such programs, as well as implications for other febrile childhood illnesses. The potential to address also e.g. pneumonia and diarrhea using this approach will be discussed.

5: Malaria vaccine technology roadmap

Tuesday 15 November 09:00–12:00—Sapelle Hall

Organized by: Malaria Vaccine Initiative of PATH (MVI). 1455 NW Leary Way, Seattle, WA 98107. E-mail: sewart@malariavaccine.org (S. Ewart)

The malaria vaccine technology roadmap (TRM) uses a novel approach to provide a coherent framework through which to focus resources, facilitate partnerships, and identify multiple pathways to a viable malaria vaccine. In March 2005 a diverse group of stakeholders will convene to produce a draft Roadmap that can be vetted and built upon by the entire malaria vaccine community. They will discuss existing scientific and technological gaps, define R&D pathways,
and identify high-priority activities needed to accelerate malaria vaccine development. This symposium will present the draft Roadmap to the MIM community, and solicit their involvement in this ongoing process. Presenters may include scientists and representatives from MVI, the Wellcome Trust, and the Bill & Melinda Gates Foundation.

6: Scaling-up insecticide-treated nets in Africa

Tuesday 15 November 13:00–14:30—Zingana Hall
Organized by: NetMark, USAID Regional Partnership for Sustainable Malaria Prevention, 1825 Connecticut Avenue, NW Washington, DC 20009, USA. E-mail: malilio@aed.org (M.S. Alilio)

The special symposium will be hosted by USAID’s NetMark Project, a Regional Partnership for Sustainable Malaria Prevention managed by the Academy for Educational Development. This session aims to: (i) present the results of household surveys conducted by the NetMark project and; ii) create a forum for discussion of progress toward Roll Back Malaria’s Abuja targets for insecticide treated net (ITN) coverage in Africa. Panelists will present findings on net/ITN awareness, ownership and use from two rounds of national household surveys in Senegal, Nigeria and Zambia (2000 and 2004), and baseline surveys in Mali (2003), Ghana and Ethiopia (2004). Results show increases in awareness, coverage and use, suggesting the effectiveness of Roll Back Malaria’s strategic framework, which focuses on building sustainable and equitable use of ITNs. Presentation in this session will include: (1) Rolling Back Malaria in Sub-Saharan Africa: Progress toward ITN targets in six African Countries; (2) Intra-household net use: who sleeps under the net in Ghana, Ethiopia, Mali, Nigeria, Senegal and Zambia; (3) Trends in the price of mosquito nets in Nigeria, Senegal and Zambia; (4) Methodological issues in measuring ITN coverage in Africa. Following these presentations there will be a panel discussion by different stakeholders.

7: Partnering with communities to enhance malaria control

Tuesday 15 November 14:30–16:00—Sapelle Hall
Organized by: GlaxoSmithKline, 980 Great West Road, Brentford TW8 9GS, UK. E-mail: richard.p.south@gsk.com (R. South)

Malaria is responsible for up to three million deaths each year, but the people and communities most at risk are often poorly informed and inadequately equipped to respond. Three distinct projects, supported by the GlaxoSmithKline African Malaria Partnership, present data assessing the effects of comprehensive behavioral development and community mobilization approaches to improving the prevention and treatment of malaria at the community level. The projects – differing approaches – linking education with microfinance in West Africa; supporting the role of volunteer community drug distributors in Uganda; and community awareness and mobilization linked to strengthened formal healthcare services in Sudan, particularly target young children and pregnant women and also seek to improve access to essential commodities including ITNs and appropriate treatments.

8: Malaria and pregnancy in Africa

Tuesday 15 November 14:30–16:00—Mongosi Hall
Organized by: Rose F.G. Leke, University of Yaounde 1, and Diane Wallace Taylor, Georgetown University, USA

Plasmodium falciparum infections during pregnancy can have a major impact on the health of both the mother and the developing fetus. The increased susceptibility of pregnant women to malaria is due to the sequestration of parasites in the placenta and suppression of maternal antimalarial immune responses, but the overall contribution of each of these components remains unclear. This special session will focus on changes in malarial immunity in pregnant African women, including factors related to parasite sequestration in the placenta. An up-date of recent information and future areas for research will be discussed. Important questions that may be considered are as follows. Are pregnant women with the lowest level of immunity to P. falciparum the ones most likely to develop...
clinical episodes of malaria? Are both cellular and humoral antimalarial immune responses suppressed during pregnancy? What impact does HIV have on malarial immunity during pregnancy? How does placental malaria influence the transplacental transfer of antibodies to the fetus? Can a vaccine that prevents the sequestration of parasites in the placenta be developed? Does intermittent preventive treatment (IPT) have an impact on immunity to malaria in pregnant women? Short presentations will be given and then everyone in the audience will be encouraged to participate in the subsequent discussions. Support for the symposium was provided by the Maternal-Child Health Research Training program, Fogarty International Center.

9: MIM partnership symposium: Players, programmes, opportunities

Wednesday 16 November 09:00–12:00—Padouk Hall
Organized by: MIM and EU Commission, DG Research, Poverty-linked Diseases. Rue du Champs de Mars 21, B-1050 Bruxelles, Belgique. E-mails: andreas.holtel@cec.eu.int (A. Holtel), andreas.heddini@mim.su.se (A. Heddini)
A symposium open to interested African and other malaria researchers where MIM funders/active supporters present themselves, their present role in MIM and other MIM relevant programmes/organizations they may have/be involved in. Each funder/MIM partner would present, followed by short questions session.

10. Traditional medicines for malaria: Importance for public health

Wednesday 16 November 09:00–12:00—Zingana Hall
Organized by: Research Initiative for Traditional Antimalarial Methods (RITAM). Address: 36 Hare Close, Buckingham MK18 7EW, UK. E-mail: merlinwilcox@doctors.org.uk (M. Wilcox)
Malaria strikes hardest not just in poor countries, but in the poorest populations of these countries. These people rarely have access to modern medicine, and commonly rely on traditional medicines for the treatment of malaria. Several recent clinical trials have demonstrated that traditional preparations of herbal antimalarials can be clinically effective, especially in adults and older children in endemic areas. This symposium will discuss the role that traditional medicines can play in national malaria control programmes, and what further research is needed before they can be adopted in such programmes.

11: Defining the intolerable burden of malaria: Progress and perspectives, point-counterpoint

Wednesday 16 November 13:00–14:30—Mongosi Hall
Organized by: Fogarty International Center, National Institutes of Health. 16 Center Drive, Room 202, Bethesda, MD 20892. E-mail: jbreman@nih.gov (J. Breman)
Are there 1.0 million or 3.0 million malaria-related deaths annually? Four hundred and two million "acute malaria episodes" or 4.9 billion "episodes resembling malaria"? Is 90% of the burden really in Africa? How are these numbers derived, and how are the burdens calculated due to long-neglected malaria-related anemia, low birth weight, hypoglycemia, and cognition deficit? What contribution does malaria make to overall childhood deaths, and how are co-morbidity and co-mortality considered? Have the Roll Back Malaria Program and other initiatives defined these burdens accurately, and have the strategies and interventions used decreased them? Authorities on these topics from malarious countries, RBM, and research institutions globally will address these provocative questions. A publication is planned.

12: Role of non-artemisinins containing combination therapy in the management of malaria in Africa

Wednesday 16 November 14:30–16:00—Zingana Hall
Organized by: Pfizer, Pfizer Africa Middle-East Region. E-mail: Chris.Migom@pfizer.com (C. Migom)
Given the current focus of the Global Fund, WHO and other organizations on artemisinins-based anti-malarial combinations, concern has been raised in many countries because of artemisinins supply problems and costs. Consequently, interest is growing in non-artemisinins containing anti-malarial combination therapies, such as SP/amodiaquine. A review of the data on the safety & efficacy of non-artemisinincontaining combination therapies will be presented by Prof. Akin
Sowunmi (Nigeria) and Prof. Zul Premji (Tanzania). This will be followed by a discussion on the role of nonartemisinin-containing combination therapies in the management of malaria in Africa. The discussion will be moderated by Profs. Sowunmi and Premji.

13: Comprehensive evaluation of ITNs interventions in Togo: Model for MIM

Wednesday 16 November 14:30–16:00—Sapelle Hall

Organized by: Centers for Disease Control and Prevention (CDC). Atlanta, GA 30333, USA. E-mail: byh0@cdc.gov (W.A. Hawley)

CDC has assisted the Togo MOH in evaluation of the recently completed measles and malaria integrated campaign in Togo. In this massive and innovative effort, ITNs were distributed to all children under 5 in Togo in conjunction with measles and polio vaccination. Evaluation efforts include community coverage surveys with social and economic components (conducted by the MOH and CDC using innovative hand-help computer and GPS technology), a morbidity survey in a sample of over 2000 children looking at anaemia, fever, and parasitemia (conducted by the MOH and Liverpool School of Tropical Medicine), and a strong hospital based surveillance program (carried out by the MOH, CDC and WHO). The different investigators will present their findings together in the program.

14: Intermittent preventive treatment in infants (IPTi)

Thursday 17 November 09:00–12:00—Mongosi Hall

Organized by: Intermittent Preventive Treatment in Infants (IPTi) Consortium. Centre de Salut Internacional, Hospital Clinic, Universitat de Barcelona, Rossello 132, 2-2, 08036 Barcelona, Spain. E-mail: aegan@ub.edu (A. Egan)

Studies have shown that intermittent preventive treatment in infants (IPTi) reduces malaria and anaemia in the first year of life by up to 60%. IPTi has the potential to become a major tool for malaria control in Africa as it could be given at the time of routine vaccinations, such as through the Expanded Programme for Immunization (EPI). The IPTi Consortium, and others working on IPTi, will present results from a number of trials being conducted in Africa testing the safety and efficacy of IPTi in a range of different epidemiological settings. The IPTi Consortium present and discuss the concept of developing a consortium to rapidly address the remaining research questions, and discuss the next steps for taking research into policy and implementation.

15: BioMalPar – Network of Excellence on basic malaria research – one year and a half after its launch

Thursday 17 November 09:00–12:00—Sapellé Hall

Organized by: BioMalPar Secretariat at: Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris, Cedex 15, France sylvieg@pasteur.fr (S. Gratepanche)

Chair: Marita Troye-Blomberg Stockholm University.

The symposium will give an outline of the network and activities as follows:

- The BioMalPar PhD symposium and cluster 4 research activities – Sylvie Gratepanche (BioMalPar Project Manager)
- Malaria in the mosquito: Parasites, Proteomes and Perspectives - Fotis C. Kafatos Division of Cell & Molecular Biology, Imperial College London, UK
- Trafficking of STEVOR to the Maurer’s clefts in P. falciparum infected Erythrocytes – R.E. Sinden, Parasite Cell Biology, Imperial College, London, UK
- Pathology and Pathogenesis – Michael Lanzer – Hygiene Institut, Abteilung Parasitologie, Universitätsklinikum Heidelberg, Germany
- Hitting the malaria life cycle early on: Attenuated Plasmodium liver stages – David Roberts1, David Modiano2: 1Oxford University, Oxford, United Kingdom; 2 University “La Sapienza”, Rome, Italy
- Malaria development in the mosquito requires unusual protein kinases with stage-specific, essential functions – Kai Matuschewski1 and Stefan H.I. Kappe2. 1Department of Parasitology, Heidelberg University School of Medicine, Heidelberg, Germany. 2Seattle Biomedical Research Institute, Seattle, USA.
- Malaria development in the mosquito requires unusual protein kinases with stage-specific, essential functions – Kai Matuschewski1 and Stefan H.I. Kappe2. 1Department of Parasitology, Heidelberg University School of Medicine, Heidelberg, Germany. 2Seattle Biomedical Research Institute, Seattle, USA.
functions - Oliver Billker, Imperial College London, UK.

16: Neurotoxicity of artemisinin drugs in malaria treatment
Thursday 17 November 09:00–12:00—Zingana Hall
Organized by: Institute for OneWorld Health. 50 California Street, Suite 500, San Francisco, CA 94111, USA. E-mails: tdiagana@oneworldhealth.org (T. Dialoga), bpalmer@oneworldhealth.org (B. Palmer)
Artemisinins are rapidly acting antimalarials effective against multidrug-resistant falciparum malaria and widely used throughout the tropics. In animals, these compounds selectively damage areas of the brainstem nuclei, especially those involving hearing and balance. The dose, route of administration and pharmacokinetic profile of these drugs influence the extent of neurotoxicity observed in animals. In contrast, there is no clinical evidence of neurotoxicity upon exposure to these drugs in man. Yet, the clinical data assessing artemisinin neurotoxicity is limited. The panel of experts for this symposium will discuss their pharmacological and clinical experience with these drugs and the neurotoxic potential in man, and particularly in children and pregnant women.

17: Systematic and standardized template for cost-effectiveness assessment of malaria intervention
Thursday 17 November 13:00–14:30—Sapellé Hall
Organized by: Swiss Tropical Institute, Medical Research Council (MRC), Durban, South Africa. Address: Socinstrasse 57, P. O. Box CH-4002, Basel, Switzerland
The topic of a systematic and standardized template for cost-effectiveness assessments of malaria interventions will be discussed. There are currently many discussions going on about the best way to implement ITN programmes, and there is also increasingly a discussion as to whether ITNs or indoor residual spraying (with DDT or other insecticide) is the best way forward in highly endemic areas in sub-Saharan Africa. One major consideration in this debate is the cost and cost-effectiveness of the different options. A real problem is that every programme calculates these in a different way and that comparisons are virtually impossible.

These issues have been highlighted repeatedly in different working groups and meetings. Our group (STI and MRC Durban) is currently (1) carrying out a systematic cost assessment of both interventions and (2) working out a standardized template for cost assessment. The session aims to get wider consensus from health economists and programme people on the standard template.

18: ACTs and fixed-dose ACTs for malaria treatment
Thursday 17 November 14:30–16:00—Sapellé Hall
Organized by: Dafra Pharma NV, Slachthuisstraat 307, 2300 Turnhout, Belgium. E-mail: hjh@dafra.be (H. Jansen)
Discussion on ACT’s and fixed dose ACT’s for malaria treatment and related topics. Chairman of the meeting will be prof Doumbou Ogoubara of Bamako, Mali. Collaboration of several leading professors in the area is ascertained.

19: MTIMBA. Lessons from the field
Thursday 17 November 14:30–16:00—Mongosi Hall
Organized by: MIM Project “Malaria Transmission Intensity and Mortality Burden across Africa (MTIMBA)”, funded by MIM/TDR. Manhiça Health Research Centre, P. O. Box 1929, Maputo, Mozambique. E-mail: rthompson@tvcabo.co.mz (R. Thompson)
The MTIMBA Project was initiated with the ultimate objective of exploring the relationship between overall and malaria-attributed mortality and the intensity of malaria transmission in sites with different level of transmission throughout West, East and Southern Africa, and identifying other contextual factors that could affect the relationship. For three years (2001–2004) overall mortality was estimated based on data form the Demographic Surveillance Systems functioning in the participating sites. Cause specific mortality was determined by Verbal Autopsy from which malaria-attributed mortality was estimated. During the same period, malaria transmission intensity was continuously monitored by measuring the entomological inoculation rates (EIR) on mosquitoes collected using light traps. Limited human landing catches were
carried out in order to calibrate the light trap collections. This symposium, organized by the co-PIs of the project aims at presenting the overall results of the project. Specifically, we will present: (a) estimates of overall and malaria specific mortality; (b) results of the entomological monitoring, including EIR estimates and results of the calibration of light traps with human landing catches; (c) contextual factors that can have an influence in the relationship between mortality and malaria transmission intensity, namely, main malaria control activities in the sites. The results of the exploratory analysis of the relationship between mortality and malaria transmission intensity will be presented followed by a discussion of the analytical methodology and the rationale on which it was based. Issues regarding comparisons between regions, sites and intra-site analysis will also be presented. The role of the type of research platform created in the context of this project in monitoring and evaluation of the global efforts for malaria control.