Abstracts

1 Comparison of two strategies for the delivery of IPTc in an area of seasonal malaria transmission [MIM16701601]
Kalifa Bojang

Intermittent preventive treatment in children (IPTc) has been shown to be effective in reducing the incidence of malaria. However, it is uncertain how IPTc can be most effectively delivered. Thus, there is a need to find ways in which the IPT principle could be applied to protect children in areas where the main burden of malaria is in children. During 2006 malaria transmission season, the catchment population of 26 reproductive and child health (RCH) trekking clinics were randomly allocated to receive IPT from the RCH trekking team or from a village health worker (VHW). Treatment with a single dose of sulfadoxine/pyrimethamine (SP) plus three doses of amodiaquine were given to study subjects at monthly intervals during September, October and November. The incidence rate of malaria in children in the VHW arm was 3.66 per 1000 child days at risk while that for RCH arm was 6.51 per 1000 child days at risk. Mean hemoglobin concentrations at the end of malaria transmission season was similar in both groups (10.3 g/dl in RCH arm vs. 10.4 g/dl in VHW arm). However, more children in the VHW arm received three doses of SP plus AQ compared to the RCH arm (75% vs. 48%). The intervention resulted in a substantial reduction in clinical attacks of malaria in both delivery arms. However, compliance was significantly better in VHW compared to the RCH. Thus in areas of seasonal malaria transmission, VHW delivery provides a better option than RCH delivery.

Email address for correspondence: kbojang@mrc.gm

2 Establishing a surveillance system to measure childhood mortality and drug related adverse events in an Intermittent Preventive Treatment implementation area in Senegal [MIM16690603]

A simplified Demographic Surveillance System (DSS) was established in three health districts in Senegal to measure the impact of seasonal IPT for malaria on childhood mortality and for monitoring the incidence of serious adverse events. The DSS covers a population of 602,000 served by 54 health posts in three districts, and includes the existing Niakhar DSS area. The size of the population was determined in order to be able to measure the impact of IPTc delivery on all-cause mortality among 94,386 children under 5 years (3–59 months) over a period of 3 years (2008–2010). Births, deaths and migrations, ITN use and occurrence of serious illness are recorded in 6-monthly rounds. Verbal autopsies are performed on all deaths in the population served by 12 of the health posts. Mothers of 208,156 children under 10 years were issued with a DSS card bearing details of all the children in their care. This card is used to record any health interventions delivered at village level (Vitamin A, Mebendazole, malaria IPT). Child identification is recorded by health staff whenever the child visits a health facility to allow tracking of all hospital admissions and outpatient visits and recording of all vaccinations. No serious adverse events attributed to IPT drugs were reported during IPT delivery by nine health posts. Logistics of project management will be described and preliminary results from the first round of surveillance will be presented including estimates of childhood mortality rates. Results will be compared with those for the Niakhar DSS area where in 2007 31% of under five deaths were attributed to malaria. Measres used to improve reliable data collection from health posts will be described.

Email address for correspondence: baeh@ird.sn

3 Impact of zinc and vitamin A supplementation on malaria incidence and nutritional status among children under 5 year in Burkina Faso [MIM16690268]
T. Zékiba Zéba, I. Zongo Sorgho, N. Rouamba, S. Diabaté, J.B. Ouédraogo

Zinc and vitamin A can help to resist to malaria, and improve nutritional status. Our study aimed to assess the impact of zinc–vitamin A on the incidence of malaria and nutritional status among children ≤5 years. We used a randomized, double blind, placebo-controlled intervention trial. 320 children were randomly assigned to one of two intervention groups: 10 mg Zn/6 d/week plus 200,000 IU vitamin A for the supplemented group and zinc placebo plus 200,000 IU vitamin A for the placebo group. Children were followed up for malaria detection for six months. Microscopic examination of blood smear was done in the case of fever (temperature ≥37.5°C). The profile of malaria immunoglobulin G (IgG) anti-msp1 and IgG anti-csp has been assessed in the two intervention groups using ELISA method. Anthropometric data were performed, at baseline and at the end of the study in two cross-sectional surveys, and were transformed into z-score indexes. Plasma zinc was used to assess zinc status. Malaria and fevers attacks were respectively 23% (p = 0.009) and 13.3% (p < 0.001) lower in supplemented group. In the supplemented group, a significant increase in the IgG anti csp (p = 0.034) and IgG anti-msp1 (p = 0.008) were noted. We found a reduction from 35.31% to 10.17% cases of zinc deficiency in the supplemented group, but no significant difference relative to placebo group (p = 0.4), Zinc supplementation in well vitamin A status condition improves immune system thus may protect against malaria and can help to resolve nutritional deficiencies.

Email address for correspondence: bado.bi@yahoo.fr

4 Malaria morbidity and drug policies in Dielmo and Ndiop, Senegal: A 18-year longitudinal study [MIM15082129]

Malaria is the first cause of morbidity in tropical Africa but much incertitude persists about the impact of drug policies on the
incidence of the disease. From 1990 to 2008, we monitored the incidence of malaria morbidity in Dielmo and Ndiop, Senegal, by daily clinical surveillance of the study population and by blood testing of patients with fever. We also monitored malaria transmission, drug use, drug resistance, mosquito net use, and the prevalence of asymptomatic malaria infections. During the study period, four drug policies were successively deployed for the first line treatment of malaria attacks: oral Quinimax® (QX: 1990–1994), chloroquine (CQ: 1995–2003), amodiaquine + sulfadoxine/pyrimethamine (AQ–SP: 2004–2006), and artesunate + amodiaquine (AS–AQ: 2006–2008). In Dielmo, an area of intense perennial transmission, the incidence density of Plasmodium falciparum malaria was attacks 1.3-, 2.1- and 3.5-fold higher during the period of CQ administration than during treatment periods of QX, AQ–SP and AS–AQ, respectively. In Ndiop, an area of moderate seasonal transmission, the incidence density of P. falciparum malaria attacks decreased 3.2-fold between the QX and AS–AQ periods. In both villages, parasite prevalence also decreased. There were no impregnated bed-nets in the village during the study period and bed-net use remained unchanged. Adequate drug policies may dramatically reduce the incidence of clinical malaria and the prevalence of infection.

Email address for correspondence: trape@ird.sn

5 Pilot implementation of intermittent preventive treatment in infant against malaria through expanded program of immunisation in remote rural areas in the southern Senegal [MIM16689725]


Intermittent preventive treatment in infant (IPTi) is the delivery of antimalarials during the routine expanded program of immunisation (EPI). This study aimed to measure its feasibility and the prevalence of malaria, anaemia and the resistance molecular markers of sulfadoxine pyrimethamine (SP). This pilot implementation was done in 2007 on a cohort of 17,000 infants (0–11 months) who received a half tablet of SP at time of Pentavalent 2. Pentavalent 3 and vaccines against measles (VAM). A comparative cross-sectional survey (IPTi versus control) before and after the intervention measured malaria prevalence anaemia and resistance molecular markers. The acceptability was evaluated by questionnaires, interviews and focus group. The coverage (SP/antigens) reached rapidly a sustainable rate of 98%. The coverage of Pentavalent 1, Pentavalent 3 and VAM increased significantly from 17%, 14% and 15% respectively after intervention compared to control area. After 43,109 doses of SP only two cases of moderate adverse effects were noti-

6 Synergistic effects of home-management and intermittent preventive treatment of malaria on malaria morbidity in children aged less than 5 years [MIM16689218]


Home management of malaria (HMM) and intermittent preventive treatment (IPT) are interventions proposed to reduce clinical episodes of malaria in children aged less than five years. This study aimed to determine whether implementing an IPT schedule alongside a HMM programme adds significantly to the reduction in the incidence of clinical episodes of malaria than would have been achieved by a HMM programme alone. We introduced a HMM programme using amodiaquine plus artesunate combination into 13 rural communities of Ghana in the first quarter of 2007 following a cross-sectional morbidity survey. The programme was run by volunteers who received a two weeks training on record keeping, recognition of symptoms of complicated and uncomplicated malaria and how to manage them in children under five years old. In a randomly selected 6 of the 13 communities, children with no measured fever or history of fever in the past 24 h received AQ+AS-IPT in May, July and September of 2007 and post-intervention surveys conducted in November 2007 and April 2008. Visits to trained community volunteers on account of measured fever or history of fever were counted as malaria episodes. Preliminary results show that the incidence of malaria episodes was similar among the two arms (IRR = 0.98 [0.42–2.26]; p = 0.96) and differences in post-intervention mean haemoglobin and parasite density between the groups were not significant. Implementing an IPT schedule alongside a HMM programme did not enhance the reduction of clinical episodes of malaria compared to HMM alone.

Email address for correspondence: harry.tagbor@lshtm.ac.uk

7 Mathematical modelling for malaria elimination [MIM16598535]

Lisa J. White, Richard J. Maude, Wirichada Pan-Ngum, Somphob Saralamba, Ricardo Aguas, Nicholas P.J. Day, Nicholas J. White

The control of malaria infection has been mainly targeted at reduction in morbidity and mortality while protecting the longevity of chemotherapy. The sparing use of new malaria drugs is often recommended in order to minimize the selective pressure on the parasite population for resistant mutants. This is a reasonable argument if the aim is to accept the continued presence of malaria and control its most deleterious effects. However, recent initiatives stating eradication as the new aim of malaria control require a sea change in strategy design. Elimination strategies require that transmission rather than disease is targeted. Therefore, extending the lifespan of chemotherapies is in direct conflict with rapid and effective elimination. Here we use a simple mathematical structure to demonstrate the potential of combining multiple strategies that applied singly would not necessarily result in elimination, but applied in combination have the potential to achieve this aim within a realistic timeframe. We demonstrate how quite different controls (such as vaccination or ITNs) prevent the propagation of drug resistance in a similar way to combination therapy, but at the population rather than individual level. We conclude that although instantaneously many elimination strategies exert a higher selective pressure for resistance compared to modest disease control strategies, the cumulative pressure is lower from successful elimination strategies. We predict in many cases, even a failure to eliminate with the inevitable increase in infection would
result in lower cumulative morbidity and mortality than if the attempt were not made.

Email address for correspondence: lisa@tropmedres.ac

8 Blackwater fever an important cause of acute renal failure in congolese pediatric population [MIM16691070]

Ntetani Michel Aloni, Mbiaja Joseph Bodi, Ngandu Lukute, Ndosimao Nsibu

Blackwater fever (BWF) is characterized by acute intravascular haemolysis occurring after the re-introduction of amino-alcohols, in residents in Plasmodium falciparum endemic area. We retrospectively studied 52 children with acute renal failure (ARF) following BWF after a malaria attack, at the Paediatric Nephrology Unit at the University Hospital of Kinshasa. Patient with ARF who had history and clinical finding suggestive of blackwater fever were included in this study, between January 2000 and December 2007, 94 children with ARF were seen. Of these, 52 (55.3%) had BWF. BWF was associated with quinine ingestion in 51 (98.1%) children and lumefantrine in 1 (1.9%). The male:female ratio was 1:5. Thirty-four (65.4%) had impaired renal function <50 ml/min/1.73 m² calculated by Schwartz formula and 24/34 (70.6%) were oligo-anuric, 6/34 (17.6%) patient had a severe hyperkalaemia (K > 6.5 mequiv./l), 17/34 (50%) with severe metabolic acidosis and 5/34 with diluted hyponatraemia (Na < 130 mequiv./l). Of the cohort, 19/34 (55.9%) required peritoneal dialysis. The mortality rate was 17.3%. Of the survivors, 42 (80.8%) had completed recovery of renal function. The oligo-anuria, advanced uremia, late reference and poverty were bad prognostic factors in simple univariate analysis. BWF is an important cause of ARF. Patients who do not succumb to ARF, peritoneal dialysis and treatment with antimarialars bring about recovery of renal function. A rational use of amino-alcohols is necessary.

Email address for correspondence: nephropedcuk@yahoo.fr

9 Malaria and helminthic infections alongside haemoglobin levels amongst school children in b ello sub-division cameroon [MIM15762274]

Anna Njunda

Malaria is one of the most deadly disease while helminthes cause the most prevalent parasitic infections. These two infections co-exist, with anaemia being an overlapping symptom. Aim: To investigate the impact of malaria and helminth co-infection on anaemia amongst school children in Bello, Cameroon. 112 apparently healthy school children aged 3–11 years were enrolled into the study. There were 40 (35.7%) from the nursery section and 72 (64.3%) from the primary section. Amongst these 53 (47.3%) boys and 59 (52.75) girls. With parent’s consent, blood films were made from finger prick samples and stained with 10% Giemsa. Fresh stool sample were collected from each child and imme-

mediately examined for helminthes and protozoan. All 112 had malaria parasite with concentrations ranging from 1600 p/ul to 11,200 p/ul and a mean parasitaemia of 5785 p/ul. High malaria parasitaemia (>5000 p/ul) was found in 71.2% of nursery school children as compared to 50% of those in primary school. There was no significant correlation between malaria parasitaemia and gender. Malaria and helminthes co-infection was recorded in 38.3% of the children. Intestinal helminthes included Ascaris lumbricoides (25.6%), Trichuris trichuria (5.1%) and the protozoan E. histolytica (36%). Haemoglobin concentrations were negatively correlated with levels of malaria parasitaemia (r = -1; P = 0.04) but positively correlated with age (r = +1;). Of the 54 (48.2%) children found to be anaemic, 52 (96.2%) had mild anaemia and 2 (3.3%), severe anaemia symptomatic malaria is still endemic amongst school children in Bello and is significantly associated to anaemia.

Email address for correspondence: ann_njunda@yahoo.com

10 A biallelic dinucleotide microsatellite repeat (TA repeat) at −906 in the macrophage inflammatory protein-1alpha (MIP-1α) promoter is associated with enhanced susceptibility to severe malarial anaemia and functional changes in MIP-1α production [MIM16613937]


Macrophage inflammatory protein (MIP)-1α (CCL-3) is an important immunoregulatory mediator that suppresses erythropoiesis. We previously demonstrated that (MIP)-1α is elevated in children with enhanced severity of Plasmodium falciparum (Pf) malaria characterized by overlapping sequelae of hyper-parasitaemia and mild-to-moderate anaemia. A biallelic dinucleotide microsatellite repeat (TA repeat) at −906 in the MIP-1α promoter is associated with clinical outcomes in autoimmune disease. Since this variant has not been investigated in malaria, the role of MIP-1α −906TA in conditioning susceptibility to severe malarial anaemia (SMA, Hb < 6.0 g/dL), high-density parasitaemia [HDP, Pf parasites ≥10,000/μL] and MIP-1α production were investigated in children with Pf malaria (n = 465) from Siaya District, western Kenya. Complete haematological and parasitological indices were determined. Microsatellite repeats were genotyped by PCR with fluorescence-labelled primers and alleles were scored with GeneMapper software. Circulating MIP-1α were measured by a hu25-plex bead assay. Allele frequencies in the population were: 26.2% (TA)2; 41% (TA)3; 76.6% (TA)4; 29.0% (TA)5 and 0.4% (TA)6. Multivariate logistic modelling controlling for age, gender, sickle-cell trait, HIV-1 and bacteraemia showed that the (TA)3 allele was associated with enhanced susceptibility to SMA (Hb < 6.0 g/dL) (OR, 2.73; 95% CI; 1.01–7.34, P = 0.047). In addition, median (Q1-Q3) circulating MIP-1α (pg/mL) was elevated in (TA)3 carriers [139.7 (128.1–266.0)] relative to non-(TA)3 carriers [109.5 (72.0–155.8), P = 0.048]. The MIP-1α −906(TA)3 allele enhances susceptibility to SMA, at least in part, through increasing MIP-1α production.

Email address for correspondence: mugogwe@yahoo.com

11 A functional COX-2 gene promoter polymorphism (−765G>C) is associated with susceptibility to high density parasitemia among young children living in malaria endemic region of western Kenya [MIM16613849]


In malaria holoendemic regions such as western Kenya, Plasmodium falciparum malaria manifests primarily as severe anemia and/or high density parasitemia (HDP) in infants and young children. Effector molecules such as cyclooxygenase synthase (COX-2) derived prostaglandin E2 have been associated with severe falciparum malaria outcomes. Studies have associated COX-2 promoter polymorphisms with various inflammatory and autoimmune diseases. However, the role of COX-2 promoter variants in conditioning
malaria disease outcome remains to be determined. We investigated the association between COX-2 −765G>C and severe malarial anemia (Hb < 6.0 g/dL), malarial anemia (Hb < 8.0 g/dL) and HDP (parasites ≥10,000/μL) in children resident in P. falciparum holoendemic transmission area. Children with acute malaria (n = 532, aged <3 years) were enrolled at Siaya District Hospital in western Kenya. Complete blood counts were determined with an automated hematology analyzer and Giemsa-stained slides used to determine parasite densities. COX-2 −765G>C variants were genotyped by PCR and RFLP analysis. The COX-2 genotype distribution was 39% GG, 47.8% GC and 13.2% CC, with allele frequencies of G = 0.63 and C = 0.37. Multivariate logistic regression analysis, controlling for confounding factors, demonstrated that relative to homozygous G genotype, heterozygosity predisposed to increased risk of developing HDP (OR = 1.45, 95%CI 0.98–2.16, p = 0.059). Analysis using dominant genotype in the population (GC = 47.8%) indicated a trend towards protection against HDP by GG variant, but did not reach significance (OR = 0.97, 95%CI 0.46–1.02, p = 0.065). These results demonstrate that variation at position −765 on the COX-2 gene may modulate susceptibility to HDP in young children living in regions of high P. falciparum transmission intensity.

Email address for correspondence: sbonuke@gmail.com

12 Complement regulatory protein levels, immune complex binding capacity, and complement susceptibility of red cells from children with sickle cell trait or normal adult hemoglobin in a malaria endemic area of western Kenya [MIM16729946]
Walter Otieno, Joash R. Aluoch, Benson Estambale, José A. Stoute

Severe anaemia is one of the most serious complications of Plasmodium falciparum malaria that occurs predominantly in children in the first 3 years of life and is an important cause of childhood morbidity and mortality in sub-Saharan Africa. Malaria infection leads to the formation of immune complexes that can activate complement and lead to the production of pro-inflammatory cytokines by interaction with macrophages. Heterozygous sickle cell trait individuals (HbAS) are relatively protected from severe manifestations of malaria such as anaemia. The underlying mechanisms of this protection are however not well understood but are thought to be due to several mechanisms. Given the important role played by immune complex deposition in the pathogenesis of severe malarial anaemia, this study investigated whether there are differences in IC binding capacity between erythrocytes from heterozygous sickle cell trait individuals and normal individuals that could partly explain the protection from severe anaemia in individuals with sickle cell trait. We used flow cytometry to measure red cell complement regulatory protein levels, C3b deposition, and immune complex binding capacity of children with AS hemoglobin or AA hemoglobin. The analysis of these results is in progress and will be presented at the conference.

Email address for correspondence: wotieno@wrp-ksm.org

13 Complement utilization in children with severe malarial anemia [MIM16426269]
Nancy Nyakoe, Ronald P. Taylor, John N. Waitumbi

The complement system plays important roles in both innate and adaptive immunity and complement can be activated and depleted during malaria infection, thus potentially compromising overall immune defenses. Activation of complement also leads to production of potent pro-inflammatory mediators such as C3a and C5a, which may explain the genesis of pro-inflammatory cytokines seen in children with severe malarial anemia (SMA). In a case control study, we compared the levels of complement hemolytic activity (CH50) in cases of SMA and in asymptomatic controls with malaria infections. The CH50 in SMA (16 ± 10 U/mL) were below normal (34–70 U/mL) and were half the levels in controls (34 ± 8.2 U/mL (P = 0.001, paired t-test). The levels of C3a were 10 times higher than normal (normal ranges = 257–690) in both the cases (mean = 3489 ± 650 ng/mL) and in controls (3852 ± 555 ng/mL), indicating a high degree of complement activation in both groups. Similar trends were obtained for C4a and C5a. PCR detection of C4 null genes (C4AQ0 and C4BQ0) found 5 homozygous individuals for C4BQ0 (1 case and 4 controls), but no patients expressing the C4AQ0 allele in either group. Collectively, these results indicate: (1) Profound uncompensated utilization of complement in patients with SMA. (2) Equal formation of pro-inflammatory complement fragments in cases and controls, indicating that the pro-inflammatory cytokines commonly seen in children with SMA cannot be accounted for by these anaphylatoxins. (3) Complement deficiency observed in SMA does not appear to be associated with genetic defects.

Email address for correspondence: nnyakoe@wrp-ksm.com

14 Elevation of HMGB1 in Ugandan children with Plasmodium falciparum malaria [MIM16697361]
Bernard N. Kanoi, Thomas G. Egwang

High mobility group box 1 (HMGB1) protein, originally described as a DNA-binding protein that stabilizes nucleosomes and facilitates transcription, can also be released extracellularly by monocytes and macrophages stimulated by GPI, TNF-α, or IL-1. Extracellular HMGB1 has been demonstrated to act as a proinflammatory mediator when actively secreted. We sort to determine whether it is released extracellularly in malaria. We carried out a nested study within a case–control study of severe malaria in Apac Hospital in Northern Uganda. The presence and quantity of HMGB1 was determined by a quantitative ELISA. There were elevated levels of HMGB1 in the children with symptoms of severe malaria. HMGB1 levels were significantly much higher (p < 0.001) in severe malaria 185 ± 60 ng/mL (mean ± SD) than asymptomatic controls 36 ± 46 ng/mL. The levels were also significantly associated with those of other chemokines; RANTES, MCP1 and MIP 3α. These data supports the school of thought that malaria is fundamentally a systemic inflammatory disease. Thus in malaria, as in inflammation in general, HMGB1 may be an important amplification signal in disease pathogenesis. This makes this protein a site of prospective therapeutic anti-inflammation intervention, using the already approved anti-HMGB1.

Email address for correspondence: bkanoi@gmail.com

15 Erythrocyte CR1/CD35 inhibits TNF-α; production by restricting immune complex uptake by macrophages [MIM16690204]
Michael O. Odera, José A. Stoute

Children suffering from severe malaria have elevated concentrations of TNF-α, increased levels of circulating immune complexes (ICs) and decreased levels of CR1/CD35 on their erythrocytes. We postulated that erythrocytes can serve a dual role during malaria infection. Red cells bind C3b opsonized ICs via CR1 and restrict stimulation of macrophages. However, under some circumstances such as during slow circulation in sequestered capillaries, IC-bearing
red cells can stimulate macrophages to produce pro-inflammatory cytokines. Using anti-CR1 monoclonal antibody, CR1/CD35 levels of cryopreserved erythrocytes from a cross-sectional study in a malaria endemic area in Kenya was determined by flow cytometry and categorized as low, medium and high expressers. 45 individuals were selected and IC binding capacities determined by flow cytometry. Using in vitro model system, macrophages were stimulated with erythrocytes and pre-opsonized BSA – anti-BSA ICs, loaded erythrocytes, and positive and negative controls. Supernatants were harvested and TNF-α ELISA was performed. Results: Data indicated that IC binding capacity was influenced by CR1 and was complement dependent. Erythrocytes inhibited IC-induced TNF-α production by macrophages and in a manner proportional to CR1 levels. Also, erythrocytes loaded with ICs stimulated macrophages and in a manner proportional to CR1. ELISA was performed. Results: Data indicated that IC binding capacity was influenced by CR1 and was complement dependent. Erythrocytes inhibited IC-induced TNF-α production by macrophages and in a manner proportional to CR1 levels. Also, erythrocytes loaded with ICs stimulated macrophages to release TNF-α. Erythrocyte CR1 may act as a dynamic buffering system which prevents ICs from stimulating macrophages to release TNF-α which is implicated in the pathogenesis of severe malaria. However, CR1 enables erythrocytes to soak in ICs and in the process able to stimulate the secretion of TNF-α by macrophages. Email address for correspondence: moderawrpksm.org

16 Impact of artemisinin-based combination therapy on malaria transmission in Mali [MIM16762172]


Most African countries have now changed their first line malaria treatments from monotherapies to artemisinin-based combination therapies (ACTs). ACTs are known to decrease the rate of gametocyte carriage and gametocyte density in a treated population. However, the impact of ACT treatment on gametocyte infectivity and malaria transmission is less clear. During a randomized controlled Phase IV trial in Bougoula-Hameau, Mali, we compared the infectivity of post-treatment gametocytes to Anopheles gambiense. Patients with falciparum uncomplicated malaria were randomised to one of the three treatment arms (artemether lumefantrine, AR-L; artesunate-amodiaquine, AS/AQ; artesunate-sulfadoxine-pyrimethamine, AS/SP) and followed for 28 days. Gametocyte carriage was assessed by microscopy before and after treatment. Whenever gametocytes were found, starved mosquitoes were direct-fed and maintained in the laboratory for 8 days. The presence of oocysts was determined and the number estimated by dissection on day 8 post-feeding. Before treatment 5.8% (n = 172) of oocyst-positive mosquitoes were oocyst positive at day 8 vs. 30.2% (n = 252), 40.2% (n = 174) and 8.0% (n = 601) of oocyst-positive mosquitoes direct-fed after AR-L; AS/AQ and AS/SP treatments, respectively. AR-L and AS/AQ significantly increased gametocyte infectivity to anopheline mosquitoes (p < 0.0001) while AS/SP had no impact on infectivity in this setting (p = 0.2). These data show that the impact of ACT treatment on malaria transmission and consequently on the spread of resistance may vary from among ACTs. The implications of these observations for large-scale ACT deployment in Africa will be discussed. Email address for correspondence: bfofana@mrtcbko.org

17 Role of lipid profile on pathogenesis of malaria [MIM15880172]

Olusegun Akanbi

Malaria in pregnancy has been associated with a range of deleterious effects in women and their baby. Increase in lipid profiles during malaria infection has been shown to contribute to pathological effect of malaria in adult. This study assesses the effect of lipid profiles on the pathogenesis of malaria in pregnancy. A total of 114 pregnant women were studied. Blood samples were collected into EDTA and plain bottles to determine haematological and serum lipid profile status of the individuals respectively. Thick blood films were prepared and used for malaria parasite counts. Higher density lipoprotein (HDL), lower density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride, and total cholesterol levels were determined using standard colorimetric method. The mean parasite density was significantly higher (P < 0.05) in primigravidae (1046/μl) than secundigravidae and multigravidae (547.8/μl and 424.44/μl, respectively). The serum status of Total cholesterol, HDL, LDL and VLDL were significantly higher (P < 0.05) in malaria positive primigravidae than secundigravidae and multigravidae, while triglyceride was significantly higher in multigravidae than in secundigravidae and primigravidae. Serum lipid profiles were lower in malaria negative than malaria positive pregnant women. This study shows that the levels of lipid profiles were higher among malaria positive than in malaria negative pregnant women. There was also an increase in the lipid profile levels in malaria positive primigravidae when compared with that of secundigravidae and multigravidae. The increase in lipid profiles among malaria positive pregnant women is dangerous as this could contribute to atherosclerosis and other pathological effect in pregnancy. Email address for correspondence: s_akanbi@hotmail.com

18 Role of TCA cycle enzymes in Plasmodium falciparum [MIM15077401]

Mallory P. Earnshaw, Sylke Müller

Recent evidence suggests that the tricarboxylic acid cycle (TCA) of Plasmodium falciparum varies from the commonly held view of eukaryotic mitochondrial metabolism. During development in the red blood cells, the parasites use glucose as their main carbon source and ATP generation is through substrate level phosphorylation in the cytosol while mitochondrial metabolism seems to be negligible in these parasite stages with respect to energy generation. However, the parasite genome contains genes encoding all the enzymatic components for a complete TCA cycle but their roles for the parasite’s metabolism are enigmatic especially with the unusual situation that the pyruvate dehydrogenase is solely located in the parasite’s apicoplast and not in the mitochondrion. This scenario suggests that the TCA cycle may have alternate functions for the parasite’s survival which are specific adaptations to life within the human host. Methods: This project aims to elucidate the function and importance of the first three enzymes in the TCA cycle through the use of genetically modified parasites and analyzing the resulting changes in metabolic flux. Results: It is expected that the deletion of the first three genes of the TCA cycle will have an impact on the metabolic flux of labeled metabolic precursors and give insights into their roles for the parasite’s metabolic integrity. Email address for correspondence: m.earnshaw.1@research.gla.ac.uk

19 SURFINs of Plasmodium falciparum [MIM16646443]

Fingani A. Mphande, Ulf Ribacke, Fred Kironde. Gerhard Winter, Mats Wahlgren

In its effort to survive the human immune system, Plasmodium falciparum uses several derived parasite antigens most of which are
expressed at the surface of the infected erythrocyte (IE). Recently SURFINs a new family of antigens encoded by the surf multi-gene family has been reported (Winter, Kawai et al., 2005). One member of the family, SURFIN4.2 was found present both at the IE-surface and at the merozoite apex. Here bioinformatic tools were used to study the structure of a second surf gene surf4.1. To investigate the expression of surf genes PCR and Q-PCR were employed and Northern and Western blots were used to confirm the size of surf4.1 and SURFIN4.1, respectively. Localisation of SURFIN4.1 was determined using immunofluorescence assay (IFA). While the surf4.1 was found present in one copy by Q-PCR in some parasites (3D7AH1, 3D7S8, 7G8, K1) six copies of the gene were identified in FCR3 and FCR3S1.2. surf4.1 was found transcribed in the schizont stages of the parasite beginning 32 h post-infection and throughout the schizont stages with the level of transcription peaking at 44 h. The levels of transcript correlated with the number of gene-copies in FCR3 and 3D7S8. surf4.1 was found to encode a polypeptide of ∼Mr 250,000 Da (SURFIN4.1) present within the parasitophorous vacuole (PV) and around free merozoites as merozoite associated material (MAM) but not at the IE-surface. The results suggest different SURFINs to be expressed at distinct time-points and carry diverse functions in the intra-erythrocytic cycle.

Email address for correspondence: finganiannie@yahoo.com

20 A codon-harmonised approach for the improvement of heterologous expression of two plasmoidal proteins [MIM16393634] Esmaré Human, Abraham I. Louw, Lyn-Marie Birkholtz

The folate and polyamine biosynthetic pathways are both involved in DNA synthesis. Polyamines are essential polycationic molecules required for cell growth and proliferation, while the polar folate derivatives serve as coenzymes/carriers for one-carbon transfer reactions. The folate pathway has been exploited in the fight against malaria with antifolate drugs, but the polyamine pathway is currently not targeted in malaria. In order to characterise new drug targets in these pathways, we investigated the influence of codon-harmonisation on the expression of bifunctional drug targets of \( P. falciparum \): \( S \)-adenosylmethionine decarboxylase/ornithine decarboxylase (AdoMetDC/ODC) complex that regulates the biosynthesis of polyamines within the parasite. The inhibition of AdoMetDC induces polyamine depletion within the parasite that ultimately results in cell cycle arrest. Functional genomics provide a global picture of metabolic systems and was used as the strategy to chemically validate the regulatory function of AdoMetDC in polyamine metabolism. Plasmodium parasites were treated with the AdoMetDC specific inhibitor, MDL 73811. Parasites were harvested at three different time points for transcriptome and proteome analyses. Microarrays were done using custom made Agilent slides. Proteome studies included one- and two-dimensional gel electrophoresis, and differentially expressed proteins were identified by tandem MALDI-TOF MS. Transcriptome data revealed differentially expressed genes and were validated by real-time PCR. Proteomic data confirmed some of the transcriptome data. One-dimensional SDS PAGE combined with reverse phase LC–MS revealed a further 48 proteins that could not be detected with 2D gels. These proteins play a role in transcriptional- and translational regulation. Both the transcriptome and the proteome of inhibited \( P. falciparum \) parasites revealed inhibitor-induced differences. These differentially expressed genes and proteins include polyamine specific proteins as well as regulated proteins in other essential pathways within the parasite. Polyamine and methionine specific compensatory mechanisms were identified that will be used to reveal the mode of action due to inhibition of AdoMetDC in malaria parasites.

Email address for correspondence: salome.smit@tuks.co.za

21 A functional genomic approach to investigate the effect of polyamine depletion induced by the inhibition of \( S \)-adenosylmethionine decarboxylase in the human malaria parasite [MIM16698298] Salome Smit, Abraham I. Louw, Lyn-Marie Birkholtz

Polyamine metabolism is essential to parasite survival and differs from that of eukaryotic cells. Plasmodium polyamine metabolism includes a uniquely bifunctional \( S \)-adenosylmethionine decarboxylase/ornithine decarboxylase (AdoMetDC/ODC) complex that regulates the biosynthesis of polyamines within the parasite. The inhibition of AdoMetDC induces polyamine depletion within the parasite that ultimately results in cell cycle arrest. Functional genomics provide a global picture of metabolic systems and was used as the strategy to chemically validate the regulatory function of AdoMetDC in polyamine metabolism. Plasmodium parasites were treated with the AdoMetDC specific inhibitor, MDL 73811. Parasites were harvested at three different time points for transcriptome and proteome analyses. Microarrays were done using custom made Agilent slides. Proteome studies included one- and two-dimensional gel electrophoresis, and differentially expressed proteins were identified by tandem MALDI-TOF MS. Transcriptome data revealed differentially expressed genes and were validated by real-time PCR. Proteomic data confirmed some of the transcriptome data. One-dimensional SDS PAGE combined with reverse phase LC–MS revealed a further 48 proteins that could not be detected with 2D gels. These proteins play a role in transcriptional- and translational regulation. Both the transcriptome and the proteome of inhibited \( P. falciparum \) parasites revealed inhibitor-induced differences. These differentially expressed genes and proteins include polyamine specific proteins as well as regulated proteins in other essential pathways within the parasite. Polyamine and methionine specific compensatory mechanisms were identified that will be used to reveal the mode of action due to inhibition of AdoMetDC in malaria parasites.

Email address for correspondence: salome.smit@tuks.co.za

Molecular chaperones such as heat shock protein 70 (Hsp70) prevent protein aggregation, and facilitate protein folding. Heat shock proteins are thought to play an important part in the life-cycle of \( P. falciparum \), and PfHsp70 has attracted much attention as a chaperone. We hypothesize that the PfHsp70 protein network would consist of both potential co-chaperone and substrate partners. Here we discuss our findings on the structure–functional characterization of PfHsp70 to further understand the nature of its role in parasite survival and pathogenicity. Bioinformatic studies were carried out to illustrate the predicted interactome of PfHsp70. The in silico (homology model) of PfHsp70 was carried out to establish the organization of its subdomains in space. PfHsp70's ability to suppress heat-induced aggregation of a model substrate, malate dehydrogenase (MDH), was assessed in vitro. Analyses of protein–protein interaction data revealed that PfHsp70 potentially occupies an intersectional position both in protein folding and trafficking pathways. Based on a predicted three-dimensional model of PfHsp70, solvent accessible residues potentially implicated in interactions with co-chaperones such as Bag-1, HspBP1 and Hsp40 were identified. In vitro studies showed that PfHsp70 was able to suppress the heat-induced aggregation of malate dehydrogenase.
(MDH), and that this chaperone activity was abrogated when key residues involved in substrate binding were mutated. The proposed interaction network of PfHsp70 suggests that this protein associates with a diverse range of functional partners. The ability of PfHsp70 to suppress MDH aggregation is further evidence of its possible role in the modulation of proteins of parasitic origin that are important for its survival and pathogenicity. In addition, the PfHsp70-co-chaperone interface represents an attractive target for the development of novel inhibitors of the malaria chaperone machinery.

Email address for correspondence: ashonyai@pan.uzulu.ac.za

23
Use of ICT Mal Pf rapid diagnostic test cassettes for polymerase chain reaction (PCR) analysis of Plasmodium falciparum RNA analysis in Zambia [MIM16759386]
Moonga B. Hawela, Berlin Londono, Thomas Eisele, Joe Keating, John M. Miller, Elizabeth Chizema-Kawesha, Donald Krogstad

Malaria diagnostic tools play an essential role in cost-effective malaria case management, especially as malaria parasitemia levels decline. In Zambia, ICT Mal Pf rapid diagnostic tests (RDTs) are widely used in field activities and research studies and offer an opportunity for greater diagnostic confirmation in areas not well served by trained microscopists. Further, under field conditions, where diagnostic confirmation is preferred with PCR-based analyses, we examined the ability of ICT Mal PF RDT cassettes to perform PCR-based RNA extraction. Among 31 children age 0–5 years tested with ICT Mal Pf RDTs, a sensitivity of 96% and specificity of 80% was determined against standard filter paper-based PCR analysis. These results indicate that RDT cassettes are useful and similar in sensitivity and specificity to standard filter paper preparations for P. falciparum RNA extraction. Using these techniques can simplify PCR-based case confirmation and RNA extraction under field conditions.

Email address for correspondence: mshawela@yahoo.co.uk

24
Eosin-5-maleimide and lectin binding are modified in Plasmodium-infected RBCs [MIM16655074]
Nii ayite Aryee

Intra-erythrocytic development of the malaria parasite, Plasmodium falciparum (P. falciparum) proceeds through well-defined stages in the red blood cell (RBC). It is yet to be fully understood how interactions between the malaria parasite and RBC membrane components affect the structural organization of the RBC membrane, and what the full functional outcomes are for the RBC. Using a combination of lectins of different saccharide specificities, eosin-5-maleimide (EMA), a marker of conformational change in Band 3, and flow cytometry, we set out to ascertain whether binding sites for these ligands were available in P. falciparum-infected red blood cells. A specific affinity-matrix that would facilitate binding and selection of interacting RBC transmembrane protein(s) with biotinylated-wheat germ agglutinin (WGA) was used to determine cell surface receptor affinity for WGA. We identified WGA as a likely cell surface ligand for Band 3. Lectin binding was diminished in P. falciparum-infected RBCs. This modification in binding did not however affect an EMA binding site in transmembrane protein, Band 3 of infected RBCs. We support the hypothesis that modifications occur in RBC surface receptors following malaria parasite invasion. These modifications are as result of conformational changes in RBC surface receptors. We propose that these parasite-induced alterations in the RBC do not affect an ecto-domain of Band 3 to which EMA binds. Although WGA could be a target for Band 3, WGA binding to Band 3 is abolished in P. falciparum-infected RBCs.

Email address for correspondence: drnaryee@yahoo.co.uk

25
Comparison of field-based xenodiagnosis and laboratory assays for estimating malaria parasite infectivity to mosquitoes in Western Burkina Faso [MIM16691051]
Louis Clement Gouagna, Germana Bancone, Frank Yao, Bienvenue Yameogo, Rock Dabité, Jean Bosco Ouedraogo, David Modiano

Several techniques have been used to study host infectiousness, including the experimental membrane and direct skin feeding. Only few studies have compared the relative efficacy of the membrane feeding method with the straightforward xenodiagnosis of indoor resting mosquitoes that have fed on available hosts. As allowed within the constraints of the approved ethical protocol, randomly selected children slept individually in different sentinel houses over nights (12 nights/child) from 6 p.m. to 6 a.m. in Soumouso, a village 35 km from Bobo Dioulasso. Following each night, indoor resting and blood fed Anopheles sp. mosquitoes were collected and kept alive in the insectary under ambient conditions. Oocyst prevalences were subsequently determined on day 7 postfeeding. In parallel, the infectiousness of the same children was estimated based on whole-blood membrane feeding procedure using An. gambiae that emerged from field-collected larvae cohorts. Data from 80 children aged 4–15 years were analysed. After controlling for the background infection in host seeking mosquitoes, the xenodiagnosis gave significantly higher infection rate (23.6% vs. 10.4%) (p < 0.05) and oocyst counts (7.4 + 3.2 vs. 3.92 + 1.8) (p = 0.02) than the membrane feeding assay. Overall, the prevalence of oocysts in wild An. gambiae collected after they had fed on hosts, correlated with the range of values expected for host infectiousness in the membrane feeding assay. This study suggests that the xenodiagnosis proves to be an even more convenient, precise and powerful way to estimate host infectiousness.

Email address for correspondence: gouagna@ird.fr

26
Open-label randomised trial of a fixed dose combination of artemisinin and naphthoquine for treating uncomplicated falciparum malaria in Calabar, Nigeria [MIM16205673]
Martin Meremikwu, Friday Odey, Ambrose Alaribe, Angela Oyo-Ita, Emmanuel Efa, Eyam Eyam, Emmanuel Onyenuche, Bisi Odewale, Chioma Oringanje, Vivian Asiegbu, Chukwutem Chukwuka, Emmanuel Ezedinachi

This was an open label, non-comparative study of a fixed-dose combination of artemisinin (125 mg) and naphthoquine (50 mg) for treating uncomplicated Plasmodium falciparum malaria among adolescents and adults in Calabar, South-east Nigeria. Adolescents (>15 years) and adults with uncomplicated P. falciparum malaria were enrolled if they met inclusion criteria and gave informed consent. Patients were randomly assigned to three dosage schedules: (A) 700 mg (4 tablets) single dose; (B) 700 mg 12-h × 2 doses; and (C) 1400 mg (8 tablets) single dose. Patients were followed up for 28 days, with clinical, parasitological, haematological and biochemical assessments. Adverse events were followed up until resolution. A total of 121 patients were enrolled into the study and 108 completed the study. The overall 28-day cure rate was 90.3%. Day 28-cure rate was 96.6% in group B (700 mg × 2 doses),
27 Auditory function following antimalarial treatment in patients with uncomplicated \textit{Plasmodium falciparum} malaria: A prospective, randomized, three-arm study \cite{MIM16644455}

Anne-Claire Marrast, Mailis Virtanen, Clemencia Barón, Verena Walter, Laurel Fisher, Gabriel Carrasquilla

Following the observation that artemisinin derivatives adversely affect the auditory pathways in the brainstem in animal models, a number of studies in humans have been performed. These studies have mainly been retrospective, only focused on pure-tone threshold data to determine the integrity of the auditory pathway, and did not employ adequate controls. This was the impetus behind the current study which will assess the auditory safety of artemether–lumefantrine (A–L) in patients with acute, uncomplicated \textit{Plasmodium falciparum} malaria, using auditory brainstem response (ABR) to detect damage to auditory brainstem pathways. In this prospective, open-label trial, performed on the Colombian Pacific coast, patients aged 12 years or older were randomly assigned in a 3:1:1 ratio to either A–L \( N=159 \) or atovaquone–proguanil \( N=53 \) or artesunate–mefloquine \( N=53 \). Study participants entered a treatment phase of 3 days followed by a 39-day follow-up period. Presence of auditory changes compared to baseline were investigated at days 3 (1 h after last dose of study medication) 7, 28 and 42 following treatment initiation. At each of these days, audiometric tests (e.g. tympanometry, pure-tone air conduction thresholds) and ABR measurements were performed. The primary assessment is ABR at day 7. An increase in ABR Wave III latency of greater than 0.30 ms will be considered an auditory nerve abnormality. A 15% or higher incidence rate of patients showing abnormal auditory function will be considered clinically relevant. Results are not available yet. The relevance of final results in the treatment of falciparum malaria will be discussed.

Email address for correspondence: anne-claire.marrast@novartis.com

28 Clinical efficacy and safety of amodiaquine + artesunate (Arsumac) and artemether–lumefantrine (Coartem) against uncomplicated malaria in Northern Cameroon \cite{MIM16683916}


Malaria treatment policy changed to artemisinin based combinations in 2004 in Cameroon against a backdrop of 6–18% resistance observed with amodiaquine. We set out to investigate the efficacy and safety of amodiaquine + artesunate (AQ + AS) and artemether–lumefantrine (AL) in a Guinea Savannah with lowlands in northern Cameroon. Three hundred and twenty children aged 6–120 months with acute uncomplicated \textit{P. falciparum} malaria were enrolled for the study in two peripheral clinics in northern Cameroon. They were randomized \((240 \text{ in AQ + AS and 80 in AL})\) to receive weight based doses of each drug over three days. The 2003 W.H.O protocol for evaluating efficacy of antimalarial drugs was used to classify treatment outcomes. Parasite genotyping was used to distinguish re-infections from recrudescence. Severity of adverse events was scored using common toxicity tables. 209 and 71 children completed the follow up in the AQ + AS and AL arms, respectively. Crude adequate clinical and parasitological response by day 14 were 98.3\% for AQ + AS and 100\% for AL, and by day 28, these figures were 96.9\% and 96.1\%, respectively. PCR-corrected cure rates by day 28 were 98.6\% for AQ + AS and 97.3\% for AL. These results show a non-significant superiority of AQ + AS over AL. A SAE of a 17 months infant resulted in death in the AQ + AS arm with decreased neutropenia and scarification marks. This was considered unlikely drug-related. Both drugs are efficacious and well tolerated in the study area. This study demonstrates the non-inferiority of AQ + AS over AL in northern Cameroon.

Email address for correspondence: alinn200uk@yahoo.com

29 Phase III pivotal trial of pyronaridine artesunate versus artemether lumefantrine in acute uncomplicated \textit{Plasmodium falciparum} malaria \cite{MIM16689311}

Antoinette Tshefu, Oumar Gaye, Kassoum Kayentao, Ricardo Thompson, Kirina Bhatt, Sanie Sessay, Dorina Bustos, Emiliana Tjitra, George Beddu Addo, Claude Oeuvravy, Chang-Sik Shin

A phase III comparative, double-blind, double dummy, randomised, non-inferiority, multi-centre clinical study was conducted to assess the efficacy and safety of fixed dose formulation of pyronaridine/artesunate tablet (PA; 180:60 mg) versus artemether/lumefantrine tablet (AL; 20:120 mg) in 1272 children and adult patients with acute uncomplicated \textit{P. falciparum} malaria. Patients were randomised in 10 sites in Africa and South-East Asia to receive a 3-day course of either PA od or AL td. The primary efficacy endpoint was D28-PCR-corrected adequate clinical and parasitological response (ACPR). Secondary efficacy endpoints included: D42 PCR-corrected-ACPR, D28 and 42-cruude ACPR, parasite clearance time. Safety was assessed with 12-lead ECG, clinical laboratory evaluations for haematology, biochemistry and urinalysis. In the efficacy evaluable population, the PCR-corrected ACPR was 99.5\% and 99.2\% at D28 and 93.2\% and 88.1\% at D42 with PA and AL, respectively. Results demonstrate non-inferiority of PA to AL at D28 with a 5\% margin and superiority of PA over AL at D42. The crude ACPR was 98.9 and 97.2 at D28 (non-inferiority) and 88.6\% and 83.4\% at D42 with PA and AL (superiority), respectively. Median parasite clearance time for PA and AL was 23.9 h and 24.0 h. Treatment with PA or AL was well-tolerated. The adverse events profiles of PA and AL were similar with a majority of mild events (only 1.2\% of severe events) and no drug related serious adverse events. This pivotal trial comparing PA to AL demonstrated high level efficacy safety and tolerability of the treatments in \textit{P. falciparum} malaria patients in Asia and Africa.

Email address for correspondence: rosignolr@mmv.org

30 Phase III pivotal trial of pyronaridine artesunate versus artemether lumefantrine in pediatric patients with acute uncomplicated \textit{Plasmodium falciparum} malaria \cite{MIM16689330}

Kassoum Kayentao, Louis Penali, Kirina Bhatt, Antoinette Tshefu, Michael Ramharter, A. Tiono, Dorina Bustos, Stephan Duparc, Chang-Sik Shin

A phase III comparative, open-labelled, randomized, non-inferiority, multi-centre clinical study was conducted in pediatric patients to assess the efficacy and safety of a fixed dose granule
31 Safety and tolerability of combination antimalarial therapies for uncomplicated falciparum malaria in Ugandan children [MIM16689840]

Catherine Maiteki-Sebuguzi, Prassana Jagannathan, Vincent M. Yau, Tamara D. Clark, Denise Njama-Meya, Bridget Nzurubara, Ambrose O. Talisuna, Moses R. Kamya, Philip J. Rosenthal, Grant Dorsey, Sarah G. Staedke

Combination antimalarial therapy is recommended for the treatment of uncomplicated falciparum malaria in Africa; however, some concerns about safety of new regimens remain. We compared the safety and tolerability of three antimalarial regimens. A longitudinal, single-blind, randomized trial was conducted between November 2004 and May 2007 in Ugandan children. Upon diagnosis of the first episode of uncomplicated malaria, participants were randomized to amodiaquine + sulfadoxine-pyrimethamine (AQ+SP), artesunate + amodiaquine (AS+AQ), or artemether-lumefantrine (AL). Participants received the same regimen for all subsequent episodes and were actively monitored for adverse events. Of 601 children enrolled, 382 were diagnosed with at least one episode of uncomplicated malaria and were treated with study medications; 1120 study treatments were administered. At 14 days of follow-up, AQ + SP treatment was associated with a higher risk of anorexia, weakness, and subjective fever than AL treatment, and a higher risk of weakness, and subjective fever than AQ treatment. Treatment with AL was associated with a higher risk of elevated temperature. Considering children under five, those receiving AQ + SP were at higher risk of developing moderate or severe anorexia and weakness than those receiving AL (anorexia: RR 3.82, 95% CI 1.59–9.17; weakness: RR 5.40, 95% CI 1.86–15.7), or AS + AQ (anorexia: RR 2.10, 95% CI 1.04–4.23; weakness: RR 2.26, 95% CI 1.01–5.05). In Ugandan children, all three regimens are safe, but AQ + SP is less well-tolerated, particularly in younger children. As newer antimalarial regimens are deployed, data on their safety and tolerability will be essential.

Email address for correspondence: cmaiteki@yahoo.com

32 Phase III pivotal trial of pyronaridine artesunate versus mefloquine plus artesunate in patients with acute uncomplicated Plasmodium falciparum malaria [MIM16691620]

Ronnatrai Rueangweerayut, Chiraong Uthaisin, Duong Socheat, Tran Quang Binh, Halidou Tinto, Louis Penali, Neena Valecha, Salim Abdulla, Nong Thi Tien, Isabelle Borghini Fuhrer, Chang-Sik Shin

A phase III comparative, open-labelled, randomized, non-inferiority, multi-centre clinical study was conducted to assess the efficacy and safety of fixed dose formulation of pyronaridine/arthesunate tablet (PA: 180:60 mg) versus mefloquine (250 mg tablet) plus artesunate (100 mg tablet) (M + A) in 1271 children and adult patients with acute uncomplicated P. falciparum malaria. Patients from six sites in South-East Asia and three in Africa were randomized (2:1) to receive a 3-day course of either PA or M + A once-a-day. The primary efficacy endpoint was D28-PCR-corrected adequate clinical and parasitological response (ACPR). Secondary efficacy endpoints included: D42 PCR-corrected-ACPR, D28 and 42-crude ACPR, parasite clearance time. Safety was assessed with 12-lead ECG, clinical laboratory evaluations for hematology, biochemistry and urinalysis. In the efficacy evaluable population, the PCR-corrected ACPR was 99.2% (PA) and 98.1% (M + A) at D28. Non-inferiority of PA to M + A was demonstrated, using a 5% non-inferiority margin. At D42, the PCR-corrected ACPR was 88.3% and 88.8% with PA and M + A, respectively. The crude ACPR was 98.7% and 96.7% at D28 (non-inferiority) and 88.4% and 88.8% at D42 (non-inferiority) with PA and M + A, respectively. Non-inferiority of PA to M + A was demonstrated in the intent-to-treat population at all timepoints. Treatment with PA or M + A was well-tolerated. The adverse events profiles were similar with a majority of mild events (only 0.6–1.1% of severe events) and no drug related serious adverse events. This pivotal pediatric trial comparing PA to AL demonstrated high level efficacy and no drug related serious adverse events. This pivotal pediatric trial comparing PA to M + A demonstrated high level efficacy, safety and tolerability of the treatments in P. falciparum pediatric patients in Asia and Africa.

Email address for correspondence: rossignolr@mmv.org

33 Comparative efficacy of sulfadoxine–pyrimethamine + amodiaquine vs. sulfadoxine–pyrimethamine + artesunate vs. sulfadoxine–pyrimethamine alone on uncomplicated falciparum malaria in Mali [MIM16697822]

I. Traoré Zoumana, Sangare Cheick Papa Oumar, Beavogui Abdoul Habib, Hama Maiga, Tekete Mamadou, Ouologuem Dinkorma, Dara Antoine, N’Dong Christelle, Traore Oumar Bila, Doumbo Ogobara, Djimde Abdoulaye

Artemisinin-based combination therapies are now first line drugs in malaria treatment in Africa. However, their deployment to remote areas remains a challenge. The purpose of this study was to investigate the efficacy of the combination of sulfadoxine–pyrimethamine (SP) + amodiaquine (AQ), two drugs readily available and affordable. From 2004 to 2005, we carried out an open-label randomized trial of the efficacy of sulfadoxine–pyrimethamine + artesunate (SP + AS), sulfadoxine–pyrimethamine (SP) alone and sulfadoxine–pyrimethamine + amodiaquine (SP + AQ) for the treatment of uncomplicated malaria in two Malian savannah villages: Kollé and Bancoumana. 736 children under five years of age with uncomplicated malaria were included and followed for 28 days according to WHO 2003 protocols. MSP1, MSP2 and microsatellite CA1 were used to distinguish true recrudescence from new infections (molecular correction). These children were randomized between SP + AQ (n = 244), SP alone
A vaccine against pregnancy-associated malaria based on a single DBL domain of VAR2CSA [MIM16669902]

Madeleine Dahlbäck, Morten A. Nielsen, Mafalda Resende, Sisse B. Ditlev, Vera V. Pinto, Pernille Andersen, Gorm Andersen, Thor G. Theander, Ali Salanti, Ditlev, Vera V. Pinto, Pernille Andersen, Gorm Andersen, Thor G. Theander, Ali Salanti, Madeleine Dahlbäck, Morten A. Nielsen, Mafalda Resende, Sisse B. Ditlev, Vera V. Pinto, Pernille Andersen, Gorm Andersen, Thor G. Theander, Ali Salanti

Pregnancy-associated malaria is a major cause of maternal and offspring morbidity and is characterized by the selective accumulation of parasite-infected erythrocytes (IE) that bind to chondroitin sulfate A (CSA) in the placenta. This adhesion is mediated by a unique *Plasmodium falciparum* protein named VAR2CSA, which is a large protein consisting of six DBL domains. The major challenge for PAM vaccine development is to define smaller parts of VAR2CSA, which induce antibodies that inhibit the CSA-binding of IE in placental tissue. Two strategies were employed to define parts of VAR2CSA that can be included in a vaccine, using recombinant fragments of VAR2CSA produced as secreted proteins in a baculovirus expression system: 1. Define which domain(s) of VAR2CSA bind CSA in an ELISA-based system and use these domains to induce inhibitory antibodies. 2. Generate antibodies against a large panel of VAR2CSA constructs and test these antibodies for inhibition of IE binding to CSA. The results show that CSA-binding of single DBL domains is not exclusive for VAR2CSA since the control DBL domains of non-VAR2CSA origin also bind. This observation suggests that binding of single DBL domains does not predict the functional capacity of the whole protein. Consequently, using CSA-binding of single VAR2CSA domains as a criterion for choosing which region(s) to include in a vaccine can be questioned. Instead, our second approach has generated promising results showing that the DBL4e domain from our large panel of VAR2CSA constructs can induce antibodies that inhibit 90–100% of the IE binding to CSA. These results are promising for PAM vaccine development and show that it is feasible to design a vaccine based on a single domain of VAR2CSA.

Email address for correspondence: dahlback@sund.ku.dk
(3) Positive correlation between stimulatory indices and duration after last malaria attack means higher levels of this cytokine mediates immune protection against malaria supporting the idea that this cytokine mediates immune protection to this disease

Email address for correspondence: ambelema@yahoo.com

37 Asexual blood-stage antibody responses in children vaccinated with RTS,S/AS02A in Mozambique [MIM16674377]

Joe Campo, Jahit Sacaral, Augusto Nhambamba, John Aponte, Caterina Guinovart, Eusebio Macete, Pedro Alonso, Carlota Dobaño

RTS,S is a subunit vaccine based on the *P. falciparum* circumsporozoite protein and is currently in advanced stages of clinical trials. Long-lasting protection has been observed in one of two cohorts of children receiving RTS,S/AS02A vaccination at 1–4 years of age in a randomized, controlled trial in a rural area of Mozambique. This study aimed to investigate a hypothesis of vaccine-induced “natural boosting” of blood stage immunity to describe mechanisms of long-lasting protection with RTS,S vaccination. Additionally, this study aimed to detect continued evidence of protection up to 5-years after first vaccination. A cross-sectional survey of both vaccine cohorts was conducted at the 5-year timepoint from first vaccination. Parasitaemia was measured by microscopy. Sera collected a various timepoints during follow-up are assessed for blood stage immunogenicity against MSP-142, AMA-1 and EBA-175 by multiplexed Luminex assays, and functional antibody responses against live blood-stage parasites are assessed by growth inhibition assay and variant surface antigen (VSA) FACS assay. Primary analysis of results are presented as blood stage immunogenicity against recombinant asexual erythrocytic stage antigens based on antibody units measured against a control standard. Acquisition of functional inhibitory antibodies to asexual erythrocytic stage is presented as percentage of growth inhibition. VSA data are reported in mean fluorescent intensity. The contribution of “natural boosting” in the context of partially protective vaccines is important to the overall understanding of mechanisms of vaccine-induced protection as well as general understanding of acquired immunity to malaria in African children.

Email address for correspondence: joe.campo@cresib.cat

38 Assessment of the ability of antibody reagents with specificity against VAR2CSA to inhibit binding of *Plasmodium falciparum* isolates from pregnant women to chondroitin sulfate A (CSA) [MIM16674341]

Pamela A. Magistrado, Ali Salanti, Daniel Minja, Lea Barfod, Tina Dobrilovic, Davis John, Mafalda Resende, Madeleine Dahlbäck, Vera Pinto, Christentze Schmiegelow, Martyna Gasowski, John Lusingu, Raimos Olomi, Martha Lenenge, Lars Hviid

Pregnancy-associated malaria (PAM) causes maternal anemia, low birth weight and stillbirths due to the accumulation of *Plasmodium falciparum*-infected erythrocytes (IE) in the placenta via VAR2CSA on the IE surface and placental chondroitin sulfate A (CSA) interaction. CSA-selected parasites and fresh placental isolates transcribe high levels of var2csa and express the VAR2CSA protein on the IE surface. Our previous study has shown that polyclonal antibodies against recombinant VAR2CSA domains on the genetic background of the 3D7 and FCR3 clones can cross react with parasites transmitted in endemic areas. Moreover, antibodies raised against the DBL4 domain from FCR3–VAR2CSA can inhibit adhesion of the homologous FCR3 parasite to CSA in vitro. In this study we assess the capacity of FCR3–DBL4 antibodies to inhibit placental isolate-adhesion to CSA. *P. falciparum* isolates were obtained from pregnant women in Korogwe, Tanzania. Late-staged parasites were allowed to bind to CSA immobilized on plates with and without VAR2CSA antibodies raised in rats. Binding inhibition was assessed by counting bound parasites after washing. VAR2CSA surface expression was confirmed by antibody staining and flow cytometry. We show for the first time that FCR3–VAR2CSA–DBL4 antibodies can inhibit CSA binding of fresh *P. falciparum* isolates from pregnant women. The existence of cross reactive anti VAR2CSA antibodies and their ability to inhibit CSA binding is encouraging for the efforts to develop a PAM vaccine. Studies are on going to define epitopes targeted by the protective antibodies as well as the mode of action of the inhibitory IgG.

Email address for correspondence: pemlamagistrado@yahoo.com

39 CD8+ T cell responses to novel liver stage antigens of *P. falciparum* in naturally exposed individuals [MIM15894075]

C.M. Okafor, C. Speake, V. Malkov, G. Siwo, K. Urszula, D.M. Koelle, M. Fried, P. Duffy

Only radiation-attenuated sporozoites have been experimentally shown to consistently protect human and animals against subsequent infection with viable parasite. This finding is also an indicator of the plethora of antigenic targets in the *Plasmodium* liverstage proteome that may play crucial roles in the development of this protective immunity. However research on the immunobiology of the liver stage parasites has encountered a lot of difficulties due to the constraints in studying the biology of the liverstage. We have identified 20 highly upregulated and liver stage-specific proteins in Plasmodium falciparum. From these, over 540 9-amino acid long CD8 targeting epitopes have been predicted based on dominant HLA allele in the East-African population and validated bioinformatics software. We then tested the ability of these epitopes to induce recall responses in vitro and studied the epitope specific CD8+ response in IFN-γ ELISPOT and Intracellular cytokine staining assays using PBMCs from individuals naturally exposed to malaria infection. Data of the immune response from our on-going experiment show that malaria exposed individuals recognized over 40% of the predicted epitopes with coverage in 18 of the 20 proteins that we have found to be liver stage-specific. We anticipate that this study will yield a number of new vaccine targets that can be used alone or in synergy with RTS,S, the current and only existing *P. falciparum* liver stage directed vaccine candidate.

Email address for correspondence: cmfokafor2001@yahoo.com

40 Design of second generation Pan-reactive Apical Membrane Antigen-1 (AMA1) vaccine against *Plasmodium falciparum* malaria [MIM16679822]

Sheetij Dutta, Joshua Clayton, Lisa Dlugosz

Diversity of Apical Membrane Antigen-1 remains a hurdle in the critical path to its development as a malaria vaccine. We are serotyping *Plasmodium falciparum* isolates to determine the AMA1 population structure using growth inhibition assay (GIA). To develop a monovalent AMA1 vaccine we are mapping the cross-reactive inhibitory epitopes of AMA1 and then testing protein engineering strategies to preferentially present these epitopes to the immune system. Chimeric AMA1 proteins displaying the structural elements of *P. falciparum* AMA1 on an immunologically
non-reactive *P. berghei* AMA1 scaffold are being produced. These include the three domains of AMA1 (1, 2, 3, 1+2 and 2+3), the hydrophobic trough, the conserved and polymorphic face, the C-terminal processing site and the domain-2 loop. Growth inhibition assays are being done to serotype AMA1 and to map the cross-reactive epitopes using the chimeric proteins as reversal agents. In parallel protein engineering strategies are being tested to dampen or enhance the immunogenicity of AMA1 epitopes. A small loop (C1L) appears to be a critical determinant of AMA1 serotype. At the domain level the cross-reactive epitopes map to inter-domain boundaries of domains 1+2 and 2+3. Although, alanine mutagenesis did not work, heterologous protein-boosts showed promising results for inducing antibodies towards novel inhibitory epitopes. The work on the project is ongoing and the final results will be presented at the meeting. Lessons from structure based vaccine design against malaria can serve as a guide to develop smarter vaccines against HIV.

Email address for correspondence: sheetij.dutta@amedd.army.mil

### 41 Double blind, randomized, phase Ib study to evaluate the immunogenicity, safety, and efficacy of Plasmodium falciparum vaccine candidate, Merozoite Surface Protein-3 (MSP3) adjuvanted in aluminium hydroxide versus VERORAB in health [MIM16778809]

Mahamadou S. Sissoko, Kourane Sissoko, Abdramane H. Sall, Hamidou Traoré, Mariam Sekou Traoré, Nana Kodo, Drissa Guindo, Bourema Kouriba, Boubacar Traoré, Amagana Dolo, Renion Saye, Issaka Sagara, Mahamadou Aly Thera, Alassane Dicko

*Malaria vaccine RTS,S/AS01 in 5–17-month-old children in Kilifi, Kenya* [MIM16667602]

Ally Olotu, Trudie Lang, Preeti Vansadia, Terrell Carter, Patricia Njuguna, Ken O. Awuondo, Neema Mturi, Barbara Savarese, Tonya Villafana, Kevin Marsh, Philip Bejon

We previously described the efficacy of RTS,S given with the Adjuvant System (AS01E), in 5–17-month-old children in Kilifi (Kenya) and Korogwe (Tanzania). Adjusted vaccine efficacy against the first or only episode of clinical malaria was 52.9% (95%CI 28–69%), *p* < 0.001 by Cox regression. Long term efficacy is as yet undescribed for RTS,S/AS01E, although efficacy against clinical malaria in 1–4-year-old children in Mozambique with RTS,S/AS02A was sustained at 28.9% (95%CI 8–45%) over 18 months. A multi-centre Phase III study of RTS,S/AS01E is underway. Following unblinding of the Phase Ib study at a mean of 8 months post vaccination, we undertook extended follow up at one site (Kilifi), using active and passive surveillance for episodes of clinical malaria. We will present analyses of the efficacy of vaccination against first or only episode of clinical malaria, and for multiple episodes of clinical malaria. We anticipate that 20 months of follow up data will be available. We will discuss the implications of these data for use of RTS,S/AS01E.

Email address for correspondence: aolotu@kilifi.kemri-wellcome.org

### 43 Satisfactory safety and immunogenicity of MSP3 malaria vaccine candidate in Tanzanian children aged 12–24 months [MIM16778833]

John Lusingu, Samwel Gesase, Salum Msham, Filbert Francis, Martha M. Lemnge, Seth Misago, Samwel Sembuche, Acleus Rutta, Daniel Minja, Method Segeja, Samwel Bosomprah, Simon Couzens, Ramadhani Noor, Roma Chienghi, Pierre Druilhe

A blood stage malaria vaccine candidate, Merozoite Surface Protein-3 (MSP3), produced as a long synthetic peptide has been shown to be safe in non-immune and semi-immune adults, henceforth, the need for further studies in children. We conducted a phase Ib dose escalating study to assess safety and immunogenicity in children aged 12–24 months in Korogwe, Tanzania. Double-blind, randomized, controlled, dose escalation phase Ib trial in which two groups of children were given different doses of the MSP3 antigen (15 µg or 30 µg). In each group, children were randomly allocated to receive MSP3 or the control vaccine administered at 0, 1, and 2 months. The primary endpoint was safety and reactogenicity within 28 days post vaccination. Blood samples collected at different time points for immunological response measurements. Primary analysis was up to 84 days post-vaccination. 45 children were enrolled, 15 in each of the MSP3 groups and 15 in the control (Engerix B) vaccine group. No difference in reactogenicity between the two MSP3 groups and Engerix B. Grade 3 adverse events were infrequent, only 5 were detected, transiently and all resolved. No SAE reported was related to MSP3 vaccine. Both MSP3 doses elicited predominantly strong cytophilic subclasses of IgG responses to MSP3; isotypes involved in the monocyte-dependant mechanism of *P. falciparum* parasite-killing. Immunisation induced a seroconversion in all vaccinees. MSP3 malaria vaccine candidate was safe, well tolerated and immunogenic in children residents of malaria endemic community aged 12–24 months.

Email address for correspondence: jpalusingu@yahoo.co.uk
44 Health-seeking behaviour and the cost of presumptive diagnosis compared to rapid diagnostic testing in malaria treatment in South East Nigeria in the era of combination therapy [MIM14983940]


Appropriate diagnosis and medication, based on methods cost effective to the community, is essential to efficient malaria management programs. **Objective:** To determine the health-seeking behavior and the cost of presumptive diagnosis as compared to Rapid Diagnostic Testing (RDT) in malaria treatment in South East Nigeria in the era of Combination therapy. Data was obtained by interviewer administered questionnaires, capillary blood samples for RDT and microscopic smear for malaria parasitaemia from urban and rural communities in Enugu-East LGA. Out of 635 respondents studied, 195 (30.7%) (116 urban vs. 79 rural) sought medical care in the month preceding the study. Doctors managed 44.4% urban and 50% rural cases. Presumptive treatment for malaria was 59.6% urban and 79.2% rural. Sulfadoxine/pyrimethamine (36% urban vs. 32% rural) and chloroquine (15% urban vs. 30% rural) usage were relatively high. The prevalence of malaria parasitaemia from results of RDT and microscopy were 31.7% and 36.8%, respectively, thus RDT sensitivity of 86.3%, specificity of 92.6% and positive predictive value of 87. The average cost of a single malaria test with either RDT or microscopy is (US$ 1.57), while adult dose of Artemisinin-Based Combination Therapy (ACT) is (US$ 5.5). Although doctors handled most cases, the practice of presumptive treatment is still high and use of ACT low. There was relatively low malaria prevalence for an endemic area, good RDT sensitivity and specificity, low cost of malaria investigation compared to costly adult dosage of ACT. Therefore, RDT usage will reduce over-diagnosis of malaria and cost, and the use of ACT should be promoted. The study was funded by the UNICEF/UNDP/World Bank/WHO special Programme for Research and Training in Tropical Diseases (TDR) ID No A50076. Email address for correspondence: kakatatits@yahoo.co.uk

45 Community participation in malaria management in Mali [MIM15394737]

Sorayya Khan

The continued burden of malaria in the world has led to renewed international initiatives to combat malaria. Increasingly, these have espoused community participation as a crucial strategy against this preventable disease. This study examines the processes of participation in malaria management in three rural communities in the Mopti region, an area characterized by high morbidity and mortality from malaria and inadequate provision of health services. A quantitative and qualitative methodology was applied in these communities over 10-months in 2007. Data gathered through a survey of 300 households, in-depth interviews with diverse stakeholders, give insights into local knowledge, attitudes and practices of malaria, and participation in local socio-political frameworks. Initial findings suggest that, despite a decentralized health system, a limited transfer of financial and human resources affect the provision of community health services. Socio-economic and structural factors impinge heavily on the participation capability of the majority in the communities. A low level of awareness of malaria transmission and the influence of cultural norms in the existing system of knowledge, affect social inclusion and access to health services. Despite extensive debates on the benefits of participation, there has been a lack of in-depth analysis on participation in malaria control. This study provides an understanding of how community participation can improve malaria management in rural Mali, by examining the mechanisms enabling and constraining participation. It will also contribute to the theoretical debates on whether community participation is always beneficial, or whether externally driven efforts to reduce the burden of malaria are more effective. Email address for correspondence: sorayya.khan@rhul.ac.uk

46 Evaluation of awareness, accessibility and use of malaria control interventions in Ogun State, Nigeria [MIM16465711]


Awareness and use of malaria control interventions was evaluated among at-risk groups in Nigeria with a year to the deadline of RBM targets and more than half the time to MDGs deadline now past. It was a survey of 262 women attending antenatal clinics and 233 mothers of under-five using questionnaire in Ogun State, Nigeria. 32.7% and 23.0% of 495 respondents knew about home management of malaria (HMM) (33.0% mothers of under-five vs. 32.4% pregnant women) and ACTs (26.2% mothers of under-five vs. 20.2% pregnant women) respectively. Only 30.3% had received health education on HMM. For malaria treatment, 48.3%, 22.6%, 18.0% and 0.6% preferred analgesics, sulfadoxine–pyrimethamine, chloroquine and ACTs, respectively. Age and education influenced their awareness of HMM and ACTs (p < 0.05). While 45.5% (46.4% mothers of under-five vs. 44.7% pregnant women) knew ITN/LLIN, only 23.6% (27.9% mothers of under-five vs. 19.8% pregnant women) used ITN/LLIN. Reasons for ITN/LLIN non-use included: “didn’t know” (71.3%), “prefer house spraying” (9.0%), “no money” (7.4%), “causes heat” (3.5%), and “unavailable around” (2.1%). 47.3% of women attending antenatal clinics (32.4% private vs. 57.3% public) knew about IPT, while 43.5% (30.5% private vs. 52.2% public) had received at least one dose. Results showed poor awareness and low use of malaria control interventions in study communities. Efforts need be intensified to make adequate information and materials relating to the different malaria control interventions more available and accessible at the community level. This is important if the RBM/MDG targets are to be realized in Nigeria. Email address for correspondence: oakadeneye@yahoo.co.uk

47 Immediate neuropsychological and behavioral benefits of computerized cognitive rehabilitation in Ugandan pediatric cerebral malaria survivors [MIM16522122]

Paul Bangirana, MS, Bruno Giordani, PhD, Robert O. Opoka, MMED MPH, Chandy C. John MD, MS, Connie Page, PhD, Michael J. Boivin, PhD, MPH

Our earlier studies on Ugandan children surviving cerebral malaria showed cognitive deficits mainly in attention and memory. We now present the first study in sub-Saharan Africa to investigate the feasibility and potential benefits of computerized cognitive rehabilitation training on neuropsychological and behavioural functioning of children surviving cerebral malaria. A randomized trial in which 65 children admitted 45 months earlier with cerebral malaria were recruited at Mulago Hospital, Kampala, Uganda. For eight weeks, 32 of the children received weekly training sessions using Captain’s Log cognitive training software and the other 33 were assigned to a non treatment condition. Pre- and post-intervention assessments were completed using CogState,
a computerized neuropsychological battery, measuring Visuomotor Processing Speed, Working Memory, Learning, Attention and Psychomotor Speed and the Child Behavior Checklist measuring Internalising Problems, Externalising Problems and Total Problems. Pre-intervention scores were similar between both groups. The intervention group performed better than the non-treatment group at post-intervention on Visual Spatial Processing Speed (mean (standard error); 0.26 (0.02) vs. 0.11 (0.02), p < 0.0001; on a Working Memory and Learning task 0.20 (0.01) vs. 0.12 (0.12), p < 0.004 and on Internalising Problems 14.56 (1.06) vs. 15.53 (1.04), p = 0.011). Analysis of the difference between the pre- and post-intervention scores showed a significantly better improvement on these same tasks and in Psychomotor Speed. Computerized cognitively training long after the cerebral malaria episode has immediate benefit on some neuropsychological and behavioral functions in African children. The long-term benefit of this intervention needs to be investigated.

Email address for correspondence: pbangirana@yahoo.com

48 Are malaria treatment expenditures catastrophic to different socioeconomic and geographic groups and how do they cope with payment? A study in southeast Nigeria [MIM16669130]
Obinna Onwujeke, Kara Hanson, Benjamin Uzochukwu, Hyacinth Ichoku, Edith Ikehe, Cyril Onwughalu

The study determined inequities in the household income depletion resulting from malaria treatment expenditures, the sacrifice of basic household needs (catastrophe) and the differences in payment strategies among different socioeconomic and geographic groups in southeast Nigeria. Data was gathered through pre-tested, structured questionnaires from a random sample of 2250 householders in rural and urban parts of southeast Nigeria. The level of catastrophic malaria treatment expenditure was computed as the percentage of average monthly malaria treatment expenditure divided by the average monthly non-food household expenditure, using a threshold of 5%. The average cost to treat a case of malaria was 796.5 Naira ($6.64) for adults and 789.0 Naira ($6.58) for children. The monthly malaria treatment expenditure as a proportion of monthly household non-food expenditure was 7.8%, 8.5%, 5.5% and 3.9% for the most poor, very poor, poor and least poor SES groups, respectively. Malaria treatment accounted for 7.1% and 5.0% of non-food expenditures for rural and urban dwellers, respectively. More than 95% of the people financed their treatment through out-of-pocket payment (OOP). There were socioeconomic and geographic inequities in the financial burden resulting from malaria treatment. The treatment expenditure depleted more of the aggregate income of the two worse-off SES (Q1 and Q2) and of the rural dwellers. Government and donor agencies should institute the abolition of user fees for malaria, the transition from OOP to pre-payment mechanisms and the improvement of physical access to appropriate malaria treatment services.

Email address for correspondence: onwujeke@yahoo.co.uk

49 Financial impact of new antimalarial combination on health insurance [MIM16670180]
A. Souares, G. Savadogo, D. Parmar, A. Sie, R. Sauerborn

Increased resistance of Plasmodium falciparum to chloroquine has prompted national malaria programs to develop new policies in several African countries. The costs of artemisinin-based combination therapy (ACT), compared to chloroquine, is about five times higher for adults and about the same for children below five years for whom treatments are strongly subsidized. A year after the introduction of ACT as first-line treatment in Burkina Faso, we examined the financial impact of its increased costs on local community-based health insurance (CBI). The study was conducted in ten rural health facilities in Burkina Faso where CBI has been implemented since 2004. We compared antimalarial prescriptions and their cost before (2007) and after (2008) the implementation of ACT by using information from registers filled by nurses and drug providers. 1789 patients (44.4%) received an antimalarial treatment in 2007; among them 56.1% had amodiaquine and 25.5% sulfadoxine–pyrimethamine. In 2008, 85.9% received ACT and the others quinine. The average cost of antimalarial treatment almost doubled between 2007 (250 CFA) and 2008 (491 CFA). The deficit of the health insurance was 7885$ which amounted to 170.8% of premium income in 2008 compared to 25.8% in 2007. The cost of the new ACT treatment regime imposes a significant financial burden on households and also on the health insurance scheme. Mechanisms for increased and sustained financing are urgently required to both maintain the sustainability of the health insurance scheme without increasing premiums and allow families to obtain ACT in case of malaria.

Email address for correspondence: souares@uni-heidelberg.de

50 Studies on visual instructions to improve patient understanding of malaria treatment through public and private health providers in East Africa [MIM16673222]
Ane Haaland, Vicki Marsh

Malaria control case management depends on prompt effective treatment; assumes full courses are taken in appropriate doses. However, this depends on patients’ decision to follow provider and/or package instructions. Adherence is main challenge: to replace symptoms relief strategy with disease treatment understanding. Comprehension of instructions is one component, providers’ communication methods another. Four studies in East Africa 2004–2008 developed and tested materials to support appropriate use of antimalarial drugs in formal and private retail sectors, and education materials with providers. Studies used flexible action research methodology. Formative research using semi-structured interviews developed and tested preliminary versions (n = 263). Final versions were tested (n = 368), using checklists. purposive sampling with >80% mothers 18–45 years; 30% had 0–3 years schooling, 40% 4–6 years, 30% 7+ years. Semistructured interviews and observations used with 140 providers. Quantitative data analysis: simple proportions calculated for key outcome measures related to understanding. Qualitative analysis combined inductive and deductive approaches common trend of comprehension shown: (i) 85–94% of respondents with 4+ years schooling and 57–70% with 0–3 years understood dosage instructions correctly; (ii) reasons for adherence understood (2 studies) by 72–85% with 4+ years and 52–55% with 0–3 years; (iii) 82–97% preferred instructions including visual explanations for reasons for adherence. Objects understood; concept of meaning and use of instructions better understood by respondents with 4+ years schooling. Most with low schooling remembered instructions when shown once. Providers give short dosage information, but hardly explain adherence. Tested visual instructions increase comprehension of correct treatment.

Email address for correspondence: ane.haa@online.no
51 A decision analytic approach to assessing policy tradeoffs in malaria control in Tanzania [MIM16684927]

Randall Kramer, Zachary Brown, Katherine Dickinson, Dohyeong Kim, Leonard Mboera, Marie Lynn Miranda, Clifford Mutero

The Malaria Decision Analysis Support Tool (MDAST) is an integrated framework that allows policy makers to systematically examine potential impacts of alternative malaria control strategies on health, environmental, and social outcomes. Our objective is to develop an initial application of MDAST in Tanzania for promoting evidence-based malaria control policy making. A prototype MDAST, employing the Analytica® 3.1 software, is used to provide a platform for decision makers to optimize across different malaria control strategies. In the prototype, we synthesize findings from an array of malaria-related studies and conduct “value-of-information” analyses to identify priority research areas. The prototype is refined through original data collection assessing national-level policy makers’ perceptions of key challenges in current malaria control strategies. The prototype MDAST allows policy combinations to be compared across user-selected epidemiological and socioeconomic metrics. Contributions of the MDAST include: joint assessment of vector control and disease management in a single decision analysis framework; assessment of tradeoffs between malaria outcomes and non-malaria-related outcomes of malaria policies (e.g. human health and environmental quality risks associated with use of insecticides in vector control). Results from stakeholder consultations indicate strong support among policy makers for the use of decision analysis in setting malaria control policy in Tanzania. By addressing social and institutional factors influencing malaria control choices and outcomes, MDAST can provide an effective means for decision makers to jointly optimize vector and disease management strategies. Looking forward, MDAST can be used to promote evidence-based malaria policy, through the identification of key knowledge gaps.

Email address for correspondence: kramer@duke.edu

52 Willingness to pay for Rapid Diagnostic Test (RDT) for Malaria in the Southeast, Nigeria [MIM16689766]

Eric Obikeze, Benjamin Uzochukwu, Obinna Onwujeke, Chima Onoka, Soludo Eze, Maduka Ughasoro, Emeka Agbata

Malaria ranks among the major killer diseases in Africa. In Nigeria, there is current policy change that makes Artemisinin-based combination therapy (ACT) the first line drug for malaria treatment. However, the cost of ACT is very high hence the need for proper diagnosis before malaria treatment. This study therefore investigates the willingness to pay for Rapid Diagnostic Test (RDT) for malaria in the Southeast Nigeria. Respondents were purposely selected from two rural and two urban communities in Enugu, Southeast, Nigeria. Pre-tested interview administered questionnaires were used to elicit information from household care givers or their representatives using the bidding game question format. Principal component analysis (PCA) was carried out to determine the socioeconomic status of respondents. The results showed that 431 (38%) of the respondents were willing to pay $3.42 for malaria diagnosis using RDT, whilst 702 (62%) respondents were willing to pay $1.71. The mean willingness to pay for RDT was more than the unit cost of the test, showing a net benefit. This makes RDT a worthwhile intervention for malaria diagnosis in the Southeast, Nigeria.

Email address for correspondence: ericobikeze@mail.com

53 ace-1 duplication in Anopheles gambiae: A challenge for malaria control [MIM14964836]

Luc Djogbénou, Pierrick Labbé, Fabrice Chandre, Nicole Pasteur, Mylène Weill

Insecticide resistance is a rapid and recent evolutionary phenomenon with serious economic and public health implications. In the mosquito Anopheles gambiae s.s., main vector of malaria, organophosphates and carbamates resistance is mainly due to a single amino acid substitution in acetylcholinesterase 1 (AChE1). This mutation entails a large fitness cost. However, a resistant duplicated haplotype of the gene encoding AChE1 (ace-1) recently appeared in A. gambiae. Using molecular phenotype data collected from natural populations from West Africa, we investigated the frequency of this duplicated haplotype by statistical inference. This inference is based on the departure from Hardy–Weinberg phenotypic frequency equilibrium caused by the presence of this new haplotype. The duplicated haplotype reaches a frequency up to 0.65 in Ivory Coast and Burkina Faso, and is probably present in Benin. It was generated by a single genetic event and its current distribution suggests that it is spreading. Unfortunately, the spread of this less costly resistance haplotype is potentially a major threat to public health, as it may impede A. gambiae control strategies, and thus increases the risk of malaria outbreaks.

Email address for correspondence: luc.djogbenou@ird.fr

54 Analysis of signal transduction pathways in pathogenesis of Plasmodium falciparum malaria [MIM15030743]

Foussenyi S. Touré Nduou, Anthony Siau, Odile Owé-Missi-Oukem-Boye, Ulrick Bisvigou, Ousmane Moussa, Christophe Rogier, Paco Pino, Dominique Mazier, Sylvie Bisser

Plasmodium falciparum infection can lead to a life threatening disease. However, the pathogenetic mechanisms of severe manifestations are not fully understood. Here we investigated whether P. falciparum-parasitized red blood cells (PRBC) with clinical malaria can induce endothelial cell (EC) apoptosis in vitro. In all 45 subjects tested, PRBC that cyto-adsorbed to HLEC could be found albeit to a variable degree. In contrast, PRBC that induce HLEC apoptosis was found only in 9 subjects. Interestingly, apoptosis was significantly associated to the presence of neurological signs (P = 0.02) but there was no significant association between apoptosis and the severe malaria clinical status as a whole (uncomplicated versus severe malaria; P = 0.11). The degree of cyto-adherence was higher among individuals with apoptosis (median, 25–75% interquartile interval: 937, 610–1811 PRBC/mm² HLEC) than individuals without apoptosis (median, 25–75% interquartile interval: 437, 130–830.5 PRBC/mm² HLEC). This difference was significant (Mann–Whitney U-test, P = 0.017), however, the correlation between the degree of cytoadherence and the capacity to induce apoptosis was not significant (rho = 0.14, P = 0.37) suggesting a qualitative rather than a quantitative relationship. Analysis of the whole transcriptome from apoptogenic versus non-apoptogenic P. falciparum revealed 59 genes putatively associated with the induction of EC apoptosis. These genes were mainly composed of enzymes and only 10 surface antigens. Silencing of parasite...
gene expression with specific double-stranded RNA was performed on 8 selected genes; 5 of these, termed “Plasmodium apotosis-linked pathogenicity factors” (PALPFs), were found to be linked to parasite apotogenicity. Strategies of using these new pathogenicity factors as anti-malaria drugs or vaccine targets will be discussed.

Email address for correspondence: foussenyi@yahoo.fr

55 Transcriptional regulation in field isolates of Plasmodium falciparum [MIM15300469]

Margaret J. Mackinnon, Jin-Liang. Moses M. Kortok, Kevin Marsh, Peter R. Preiser, Zbyněk Bozdech

Mechanisms for differential regulation of gene expression may underlie much of the phenotypic variation and adaptability of malaria parasites. Here we describe transcriptional variation among field isolates of Plasmodium falciparum. We find that genes coding for parasite protein export to the red cell cytosol and surface, and sexual stage proteins, are up-regulated in field isolates compared to long-term laboratory isolates. Much of this variability is associated with the loss of small or large chromosomal segments, or other forms of gene copy number variation that are prevalent in the P. falciparum genome. Expression levels of genes inside these segments was correlated to that of genes outside and near the segments. This form of cis-acting co-regulation is strongly suggestive of epigenetic mechanisms that involve locus repositioning in transcriptionally permissive zones of the nucleus, as occurs for the large virulence-determining gene family, var. Transcriptional control of clusters of adaptive genes may be a way that the parasite can readily modify its genes in response to its highly heterogeneous and strongly selective host environment.

Email address for correspondence: mmackinnon@kilifi.kemri-welcome.org

56 Surveying genetic diversity in Plasmodium falciparum [MIM15434502]


Genetic diversity enables Plasmodium falciparum to evolve drug resistance and escape host immunity. Genome-wide analysis of this genetic variation will enhance our understanding of how the parasite thwarts control efforts and assist in the development of new interventions. We designed two tools to assess single nucleotide polymorphisms (SNPs): molecular barcoding and Affymetrix arrays. The molecular barcode surveys specific SNP variants, including common variants to identify parasite types and drug resistance markers. The Affymetrix array surveys 17,000 SNP variants across the malaria genome to assess population structure and find genetic markers. We performed a genome-wide association study (GWAS) for drug resistance using a global set of 48 P. falciparum culture-adapted isolates that were hybridized to the array and surveyed for their resistance to 10 antimalarial drugs. The molecular barcode can identify individual parasites and detect important drug resistance loci. The array data reveals population structure within and between continental populations of parasites. Using drug resistance phenotypes for the genotyped parasites, we identified two well characterized drug resistance loci—pfcr and dhfr. We also identified two novel candidate loci for drug resistance: one on chromosome 2 associated with chloroquine resistance and another on chromosome 10 associated with halofantrine resistance. Tools developed from genomic data can uniquely identify parasites and assess known drug resistance or other genetic markers of clinical significance. GWAS can identify drug resistance loci and these methodologies can be applied to other phenotypes of clinical importance to help develop intervention strategies and diagnostic tools.

Email address for correspondence: svolkman@hsph.harvard.edu

57 Phylogenetic and bioinformatics analysis of cytochrome P450 supergene family from Anopheles gambiae [MIM16098052]

B.P. Niranjan Reddy, A.P. Dash, K. Raghavendra

India is the one among the countries which depends on insecticides to control the spread of malaria. However in the recent times, the increased insecticide resistance to DDT, organophosphates and pyrethroids is reported in India. As there is no any genomic information available for this medically important vector of India, here we studied Anopheles gambiae genome for CYP gene(s) organization (supergene family involves in detoxification mechanisms) with an objective to isolate and characterize the involvement of CYP genes in Anopheles culicifacies insecticide resistance. The cytochrome P450 Coding (CDS), expressed and protein sequences of An. gambiae were downloaded from the public domains. The sequences were analyzed in terms of mapping of P450 genes (Gene name; Gene IDs; UniGene ID) on to An. gambiae chromosomes, multiple sequence alignment, phylogenetic tree construction, construction of BLOCKS and sequence logos, GC% calculation, and the Principal Coordinates Analysis. Out of 104 total genes ∼62% of genes are from CYP3 (40) and CYP4 (45) clans only. The chromosomes 2R (42) and 3R (30) consists ∼69% of genes while 70% (45) CYP4 clan genes are on 2R and 3R. There are no single gene localized onto 3L of CYP2 and mitochondrial clans. In CYP4 clan CYP4 family and CYP325 family shares ∼64% and ∼35% genes of total 45 genes. While in CYP3 clan CYP6 family owns majority of genes contributes 75% of total genes and CYP9, CYP329 shares 22.5% and 2.5% of genes respectively. The CYP2, mitochondrial, CYP3, and CYP4 clans consists of 6, 6, 3 and 2 subfamilies respectively. Out of 104 CYP genes 34 (∼32%) genes have no single EST representative. While 40, 19, 8 and 3 have ≤5, <10, ≤20 and >20 ESTs have representations respectively. Currently, we are in the process of isolating the CYP450 members from the genome of An. culicifacies s.l. using the degenerative primers that were designed using the BLOCKS constructed specific to CYP450 in this study, with an aim to analyze the role of monooxygenases in development of insecticide resistance in India.

Email address for correspondence: bp.niranjanreddy@gmail.com

58 Low linkage disequilibrium in Anopheles gambiae s.l. populations [MIM16559142]

Caroline Harris, Isabelle Molrais, Didier Fontenille, Anna Cohuet

In the malaria vector Anopheles gambiae, understanding diversity in population biology and genetic components of important phenotypes like resistance to malaria infection is crucial to develop new malaria transmission blocking strategies and requires the study of polymorphism. Linkage disequilibrium determines the density of Single Nucleotide Polymorphisms (SNPs) to be genotyped to represent the majority of haplotypes present. Here, we...
aim to determine linkage disequilibrium in *A. gambiae* populations in genes potentially involved in mosquito immune responses against pathogens. We analyzed fragments containing exons and introns of four immune related genes (Gambicin, NOS, REL2 and FBN9) distributed on *A. gambiae* genome in natural populations of seven species of the complex. We used already published and new sequences. Genes were cloned and sequenced for 8–16 individuals per population. Detected polymorphisms allowed the measurement of linkage disequilibrium between SNPs was very low: at a distance of less than 200 bp, SNPs were rarely linked to each other. The linkage observed in the *A. gambiae* could be the result of large population sizes and high recombination rates. These results are of great interest in the development of large scale polymorphism studies for population genetics and association studies. It indicates that very fine scale SNP detection will be required to detect association to phenotypes of interest in malaria transmission and to give a general view of genome polymorphism to decipher vector immunity, *Anopheles–Plasmodium* interactions, vector behavior, etc.

Email address for correspondence: anna.cohuet@ird.fr

### 59

**The variant-ome of *Plasmodium falciparum* [MIM16598603]**

Alfred Cortés, Núria Rovira, Zbynek Bozdech

A major challenge for the development of vaccines against asexual blood stages of *Plasmodium falciparum* is the variant expression of many of the parasite proteins exposed to the immune system. Variant expression also occurs for non-exposed proteins. In spite of the critical role of variant expression in *P. falciparum* biology, it is currently unknown how widely it occurs in the parasite's genome. We have obtained subclones from five culture-adapted cloned parasite lines. RNA will be prepared at six different time points for each of the parental parasite lines and subclones. RNA samples will be analyzed by microarray. Genes that are expressed in some individual parasites within the clonal population but silenced in others will be identified by this approach. For two parasite lines, we have clones from before and after passage through sexual cycle, which we predict may reset the program of expressed and silenced genes. Preliminary analysis of a pilot experiment has revealed several new variantly expressed genes. While genes that are co-regulated along the life cycle are not found together in chromosomes, some variantly expressed genes cluster together, suggesting the existence of hotspots for co-regulated variant expression. Our complete dataset will provide a comprehensive list of variantly expressed genes in *P. falciparum*, providing an estimation for the extent of this phenomenon. This information will be incorporated into PlasmoDB, pointing to genes under variant expression as not essential and indicating that the parasite has alternative options for the processes in which these genes participate.

Email address for correspondence: alfred.cortes@irbbarcelona.org

### 60

**Genetic polymorphism in odorant binding proteins (OBP-olfac) genes between incipient species of the African malaria vector *Anopheles gambiae* [MIM16645626]**


Olfaction plays a critical role in the host-seeking behaviors of insects, and may promote assortative mating between incipient species. We assessed the level of genetic variability and divergence in gene sequences and encoded peptides of an Odour Binding Protein (OBP), among sympatric specimens of the M and S form of *An. gambiae* and *An. arabiensis*. Mosquitoes were sampled in Dielmo (Senegal) from July to November 2007 and were identified morphologically as *An. gambiae* s.l. Genomic DNA was extracted from legs and analyzed by PCR to determine the species and molecular forms. The candidate OBP, LIM, localized on chromosome 2L, was PCR-amplified using primers designed from the Pest strain genome. PCR products were cloned, sequenced and analyzed in Mega4 and DNAsp. Out of 224 *An. gambiae* s.l. collected, 38% (*n* = 87) were S form, 11% (*n* = 24) M form, 42% (*n* = 94) *An. arabiensis* and 1% (*n* = 3) M/S hybrids. A 1601 bp of OBP gene (six exons and five introns) was obtained from four mosquitoes in each of the three taxa. The analysis of DNA sequence revealed a high number of polymorphisms (*Pi* = 0.016; *Hd* = 0.82) and significant genetic structure (*Fst* = 0.98, *P* < 0.01). Among the 48 parsimony informative mutations observed, eleven were fixed between the M and S forms and distributed over the six exons of OBP. Eight of them were replacement substitutions, encoding different peptides in the M and S. Consistence of this pattern at a wider geographical scale and the impact of replacement substitutions on the structure and function of the protein will be discussed.

Email address for correspondence: pierre.kengne@mpl.ird.fr

### 61

**Polymorphisms in *Anopheles gambiae* immune genes show association to malaria resistance [MIM16649418]**

C. Harris, I. Morlais, F. Rousset, L. Abate, D. Fontenille, A. Cohuet

In nature *Anopheles* mosquitoes are found to have varying levels of resistance to the malaria parasite, a characteristic known to be under strong genetic control. Understanding the molecular basis of their immunity will give essential insights for novel malaria control strategies. This study aims to uncover single nucleotide polymorphisms (SNPs) associated with mosquito resistance to malaria in natural vector/parasite combinations through genotype to phenotype associations. *Anophelengambiae* M form mosquitoes from Cameroon were experimentally infected with a sympatric wild *Plasmodium falciparum* isolate. The number of oocysts/midgut at day 8 post blood meal was counted giving each mosquito a quantitative phenotype. These mosquitoes were then genotyped for selected SNPs in known immunity genes and statistical tests applied to determine association (genotype/phenotype). 6 out of 157 SNPs show an association to phenotype, located within or upstream of SNAKeLike, TOLL6, SP PPO activate, CLIP84, AgMDL1 and CEC1. These 6 SNPs were then tested for association in 2 subsequent infections (same mosquito colony infected with different local wild parasite isolates) where 2 out of the 6 SNPs showed association in the second infection and 1 in the third. No SNPs showed association in all three infections. This study reinforces the importance of genetic variability in mosquito immunity. Associated SNPs and the genes in which they lie deserve further attention for their role in the response to malaria. As the associated SNPs do not show association to all three parasite isolates it suggests their role in immunity is parasite genotype specific.

Email address for correspondence: caroline.harris@mpl.ird.fr
63 Physical and insecticidal deterioration of bednets after 3–6 months household use in rural Ethiopia [MIM16675936]

Stephen C. Smith, Aprille Brackery, Paul Emerson, Tekola Endeshaw, Patricia Graves Estefinos Bir

A total of 200 PermaNet™ 2.0 long-lasting insecticidal bednets (LLINs) were collected from households in four zones in Ethiopia 3 or 6 months after distribution to assess physical and insecticidal deterioration after short-term use. Distribution dates of 169/200 LLINs could be confirmed. Overall, 57.4% of the LLINs showed damage: holes of at least 0.5 cm in the longest axis, were found at a rate 3 per net used for 3 months, and 6 per net used for 6 months. Large holes (greater than 10 cm in longest axis) were found at a rate of 0.03 per net after 3 months and 0.20 after 6. No attempts to repair damage to any of the nets was evident. The level of deltamethrin in 166/169 of the LLINs was in the range expected for new nets, while all nets performed well in bioassay. This study shows that although these nets retained insecticide, and were insecticidal, physical deterioration starts early and proceeds rapidly, potentially causing a premature reduction of protective efficacy. It is recommended that education campaigns stress the importance of bednet care and repair, as a means of extending the useful life of LLINs.

Email address for correspondence: jdoannio@yahoo.fr

64 Mosquitoes and bed nets: Testing the spatial positioning of insecticide on nets and the rationale behind combination insecticide treatments [MIM15902204]

R.M. Oxborough, F.W. Mosha, J. Matowo, R. Mndeme, E. Festion, J. Hemingway, M. Rowland

The recent development of pyrethroid resistance of operational significance in Anopheles gambiae is a major threat to the control of malaria in West Africa. The ‘2-in-1’ bed net, in which the top is treated with a non-pyrethroid insecticide and the sides with pyrethroid, has been proposed as a way of maintaining efficacy. For this to serve as a tool for resistance management the Anopheles mosquito must contact both the top and sides of the net. The interaction between mosquitoes and insecticide was explored by restricting the insecticide to particular surfaces and then testing the nets in experimental huts under simulated field conditions. There was no significant difference in mortality between nets treated with pyrethroid on the top only (39.2%), sides only (39.6%) or all surfaces (39.7%), thus indicating that Anopheles arabiensis contacts both top and sides during host-seeking behaviour. Blood feeding data indicated the insecticide used on the sides of the net may be more important in preventing mosquito biting than that on the top. These results support the rationale behind the 2-in-1 net. With the scaling up of ITN coverage and the need to preserve the pyrethroids more consideration should be given to switching from pyrethroid-only nets to combination nets. The results also indicate that spatial heterogeneity in insecticide distribution over the surface of the net, as produced using home-treatment insecticide kits, may not reduce the overall efficacy of nets if mosquitoes contact a variety of surfaces during host seeking.

Email address for correspondence: oxandbull@hotmail.com

65 The development of a new Long Lasting Impregnated net [MIM17179901]

Rosemary Peter, Walter Focke, Walter van Pareen, Vincent Nel

Malaria, a disease that is often fatal puts 3.3 billion people at risk annually in 109 countries and territories around the world. The greatest burden of disease is experienced in Africa where at least 90% of the 1 million deaths that occur annually are recorded. In addition to this the disease creates a heavy economic burden in endemic countries that contributes to the cycle of poverty and limits economic development. In Africa alone this is estimated to be about 12 million USD dollars per annum in direct losses and clearly considerably more in lost economic growth. Currently malaria can
be prevented, treated and diagnosed with a variety of tools and interventions. One of the primary tools used for prevention are the Long Lasting Insecticide treated nets (LLINs). At present there are two types of LLIN that are distributed, namely polyester nets (PES) which have the insecticide coated onto them during the manufacturing process and which last approximately three years and polyethylene nets (PE) into which the insecticide is incorporated during the manufacturing process. There are a variety of pros and cons associated with each of these nets. It has therefore been suggested that the ideal net would therefore be a polypropylene net (PP) which would combine the best properties of both the PES and PE nets. Chemcity, a division of Sasol in conjunction with the University of Pretoria has developed a suitable PP net. The presentation will report on the development of this net along with the results that have been achieved in terms of the requirements for WHOPES Phase 1 testing. The methods as described by the World Health Organisation in “Guidelines for Laboratory and field testing of Long Lasting Insecticidal Mosquito Nets” were used as well as the Median Knock Down test (Skovmand et al., 2008) were followed. Results showed that the nets had an excellent regeneration time and that they withstood repeated washing well. The PP net has shown that it performs well in the laboratory. Currently field test are determining both acceptability and long term efficacy.

Email address for correspondence: rose@nexcorp.co.za

66 A modeling approach to evaluate distribution strategies for universal ITN coverage [MIM16523140]
Albert Kilian
With the shift of focus for ITN distributions in Africa from being targeted to pregnant women and children to sustained, universal coverage as a step towards elimination there is urgent need to better understand the potential of various distribution strategies. A simple compartmental model was developed that uses number of households, existing net crop at time 0 and annual net output by net type to predict coverage with at least one net or ITN. It builds on the empirical relationship between net ownership and mean nets per household to translate net crop into coverage. To account for loss of nets over time a non-linear loss function was included allowing for two net types; one with a 3-year median survival the other with 5 years. Testing various distribution strategies reveals that large scale campaigns are the only way to rapidly achieve large coverage increases. However, repeated campaigns in 3- to 5-year intervals without interim continuous distributions will result in a strongly varying ITN coverage with dips as low as 40% pre-campaign. In contrast, one initial campaign followed by continuous distributions leads to sustained high ITN coverage. In most – but not all countries – this continuous supply could be achieved through ANC and EPI services plus 20% of households obtaining a new ITN from other sources such as the commercial market. A mixed model distribution strategy is the most promising approach to achieve sustained high ITN coverage.

Email address for correspondence: a.kilian@malariaconsortium.org

67 Monitoring and evaluating the impact of Indoor Residual Spraying in Zambezia Province, Mozambique [MIM16691315]
M. Coleman, S.J. Mabunda, V. Ramdeen, N. Morris, I. Seochran, S. Coetzee, I. Klienschmidt
During the past 3 years, the National Malaria Control Program with support from partners has scaled-up IRS activates in Zambezia Province, Mozambique. As part of an initiative to develop new tools for the monitoring and evaluation of disease control programs the Innovative Vector Control Consortium (IVCC) funded a Malaria Decision Support System (MDSS) to conduct monitoring and evaluation activities in the area. Nineteen sentinel sites were established in 2006 in the IRS areas. At each sentinel site 6 exit window traps were installed and relative mosquito density, sporozoite rates and insecticide resistance monitored. A household survey is carried out annually and rapid diagnostic tests are used to determine parasite prevalence in 6-month- to 15-year-olds. The 2008 survey estimated parasite prevalence at 22% which represents a 38% decline in the proportion of positives compared to 2007, and a 30% decline compared to 2006. This follows high IRS coverage in these areas. There has been an impact on the species density and sporozoite rates where IRS activities have been carried out resulting in reduced transmission. As yet no insecticide resistance has been detected. The MDSS tools for monitoring and evaluations allows for impact assessment of a malaria control program. As scale up of malaria control is occurring in the region and elimination becomes a realistic milestone in some areas, the MDSS tool is essential to support such activities.

Email address for correspondence: mcoleman@liverpool.ac.uk

68 Indoor residual spraying for preventing malaria—A Cochrane review [MIM16690531]
Bianca Pluess, Frank C. Tanser, Sarah Donegan, Christian Lengeler
Prevention of malaria on a large scale is achieved through two main vector control interventions: indoor residual (insecticide) spraying (IRS) and insecticide-treated nets (ITNs). While there is no doubt that IRS is effective in reducing malaria, this effect has never been properly quantified. The health effects of IRS were summarized and quantified in the frame of a Cochrane review. Studies considered for the review had to be either Randomized Controlled Trials (RCTs), Controlled Before-and-After studies (CBA), or Interrupted Time Series (ITS). They had to include children and adults living in malarious areas and be carried out with one of the WHO recommended insecticides Out of 132 identified studies covering all endemic areas of the world, only 4 RCTs, one CBA and 1 ITS met the stringent criteria of the Cochrane Collaboration. IRS was shown to reduce significantly malaria incidence but not prevalence in unstable malaria settings. In stable malaria settings, no significant difference was seen between the study groups. For both endemicity situations, the basis of quality (quantifiable) evidence was very narrow. The Cochrane review confirms the health impact of IRS, but also points out to the need to better quantify that impact in order to allow evidence-based discussions on the best way forward for national upscaling of vector control. No quality evidence was available on the combination of IRS and ITNs.

Email address for correspondence: bianca.pluess@unibas.ch

69 Indoor use of carbamate treated plastic sheeting in combination with long lasting insecticidal nets to control pyrethroid resistant malaria vectors [MIM16671122]
A. Djentonin, F. Chandre, T. Balder, K.R. Dabiré, J. Chabi, J.-M. Hougard, M. Akogbeto, V. Corbel
Recent findings in Benin showed that pyrethroid resistance in Anopheles gambiae can reduce the efficacy of insecticide treated nets (ITN) and indoor residual spraying (IRS) recommended for malaria vector control. In this context, we have tested a new
strategy based on a combination of long lasting insecticidal net (LLIN) and carbamate treated plastic sheeting (ITPS) to improve personal protection and “killing effect” against pyrethroid resistant mosquitoes. Experimental hut trial following WHO phase II procedures was carried out in Burkina Faso (Kou Valley) where An. gambiae M and S forms are sympatric and exhibit high level of pyrethroid resistance. Efficacy of LLIN (Permanet® 2.0) alone and either in combination with ITPS (bendiocarb, 400 mg/m²), or in combination IRS (bendiocarb, 400 mg/m²) were compared in phase II trial. 1374 An. gambiae were collected during the 2 months of evaluation. The blood feed inhibition was 43.4%, 58.1%, 56.3% with LLIN, LLIN + ITPS and LLIN + IRS respectively, suggesting that LLIN remains effective in term of personal protection against pyrethroid resistant mosquitoes. Low mortality rates were observed with the LLIN (44.0%), IRS (42.4%) and ITPS (52.5%) whereas both combinations killed significantly more mosquitoes (72.6% and 66.4% for LLIN + ITPS and LLIN + IRS). The results suggested that the association LLIN + ITPS (or LLIN + IRS) is a promising alternative to control pyrethroid resistant mosquitoes. A phase III trial is currently evaluating this strategy at community level in Benin to assess the people acceptability and the efficacy of these combinations based on entomological, parasitological and clinical parameters.

Email address for correspondence: armeldj@yahoo.fr

70 Integrated mosquito control using community based adaptive techniques in Urban Malindi, Kenya [MIM15901326]

Joseph M. Mwangangi, Samuel C. Kahindi, Janet T. Midega, Lydia W. Kibe, Joseph Nzovu, Peter Luethy, John Githure, Charles M. Mbogo

Mosquito control is a major component of malaria prevention programs. This study evaluated the use of integrated mosquito control measures in urban Malindi, Kenya from June 2006 to December 2008. The urban and peri-urban area of Malindi Town was mapped and categorized into cells of 1 km². A total of 16 1 km² cells were selected based on presence of households within the cells. In each cell, integrated mosquito control activities were undertaken which included scaling up the use of long lasting insecticide treated bednets, treatment of larval habitats with Bacillus thuringiensis (Bti) bio-larvicides, environmental management, community education and neighborhood mosquito campaigns. Community groups in Malindi under the umbrella body of PUMMA (Punguza Mbu Malindi) performed mosquito control activities within the town with the help of mosquito scouts who are Community owned resources persons. A total of 10,000 long lasting insecticide treated Bednets were distributed to pregnant women and children under 5 years. About 200 habitats were eliminated through the active participation of community groups. During the baseline survey (June 2006), the mean Anopheles and Culex mosquito larvae per dip were recorded as 11.52 and 102.46 respectively. After larval control using adaptive IVM, the Anopheles and Culex larval densities reduced significantly during the study period to 0.01 and 1.99 larvae per dip respectively by December 2007. The overall Anopheles and Culex larval reduction in the larval habitats was 55.05% and 74.57%. For the adult Anopheles mosquitoes there was a 23.87% reduction while for the Culex mosquitoes the reduction was 75.43% after the implementation of the IVM. The application of community adaptive techniques is useful in reducing the mosquito larval densities in a wide range of habitats which have a direct impact of adult mosquito populations.

Email address for correspondence: jmwangangi@kilifi.kemri-wellcome.org

71 Laboratory assessment of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae (Hypocreales = Clavicipitaceae) against Anopheles funestus (Diptera: Culicidae) [MIM16430120]

Joel C. Mouatcho, Basil D. Brooke, Bart G.J. Knols, Lizette L. Koekemoer, Maureen Coetzee

The increasing spread of insecticide resistance and environmental insecticide toxicity has raised the prospect of using entomopathogenic fungi as alternative forms of malaria vector control. This study investigated the potential of fungus species Beauveria bassiana and Metarhizium anisopliae against three colonies of An. funestus strains: pyrethroid resistant (FUMOZ-R), insecticide susceptible (FANG) and the baseline (FUMOZ). Levels of fungal infectivity in blood fed and unfed FUMOZ-R females were also determined. Female mosquitoes were exposed to fungal spores for 3, 6 or 24 hours inside cages each containing a hair roller treated with B. bassiana I93-825 or M. anisopliae ICPE30. Mortality was recorded daily following exposure. Sporulation was assessed on cadavers 2–4 days post mortality. Survival was analysed using STATA 10.0 Software. Lethal time to kill 50% of mosquitoes (LT50) per cohort was also calculated. The LT50 values of treated versus control samples were compared using Student t-tests. Both fungus species were found to be pathogenic to all the strains tested. Mortality rates were significantly higher than the respective control samples using FANG, FUMOZ-R and FUMOZ (p < 0.05) treated samples for all exposure times. Mortality rates generally increased with an increase in exposure time and were higher at 12 days post exposure (±98%). The LT50 values for treatments were significantly lower (p < 0.05) than their respective controls for all exposure times. An average of 95% fungal infected mosquitoes sporulated between 2 and 4 days after death. The mortality rate was not significantly different between unfed and blood fed mosquitoes when exposed to either of the entomopathogenic fungi (p > 0.05). Entomopathogenic fungi represent a potential candidate to complement current malaria vector control methods. The presence or absence of insecticide resistance or blood meals is not likely to affect susceptibility to fungal infection in An. funestus.

Email address for correspondence: joelm@nicd.ac.za

72 Examining implementation experiences of public health interventions: Case studies from three private medicine retailer programmes in Kenya [MIM14962987]

Timothy O. Abuya, Vicki Marsh, Greg Fegan, Abdinasir Amin, Abdusalan Noor, Sassy Molyneux, Willis Simon Akhwale, Robert Snow, Lucy Gilson

The role of private medicine retailers (PMRs) in malaria control is well recognized. Past evaluations of PMR interventions focused on the impact on retailer knowledge and practices with little emphasis on implementation processes. This study aimed at determining factors influencing programme implementation in a Ministry of Health (MoH) programme in Kwaile district; a non-governmental organisation supported programme, Merlin, in Kisii Central district; and a social marketing approach supported through USAID/AMREF in Bungoma district. 26 focus group discussions with clients and PMRs, 19 in-depth interviews with district level implementers and document reviews were conducted. Data was analysed using Nvivo7 (QSR international). A thematic framework was developed and a range of analyses including stakeholder analyses. Finally, the scaling up of health care innovations and the diffusion of
innovations frameworks were used for interpretive analysis. Factors that facilitated successful implementation included; a good relationship between the resource team and the user organisation; a flexible management system, allowing localised decision-making in response to immediate contextual features; the selection and functioning of a core implementing team requires adequate consultation and capacity to support implementation. The study highlights the importance of adequate management of intervention including developing good relations between actors involved. Examining implementation experiences is important in understanding the influence of complex interactions between actors and the intervention in a dynamic context. It also points to the complexity of working with district health teams for innovative interventions, particularly where these are in competition with existing conventional programmes.

Email address for correspondence: tabuya@kilifi.kemri-wellcome.org

73
The implications of cost on acceptance of referral advice after pre-referral treatment of severe malaria with artesunate suppository at community level [MIM15000765]

Daudi Simba

The WHO recommends pre-referral dose of artesunate suppository as an emergency measure before referring a child with severe malaria to a health facility. However, referral advice is not always followed, posing a risk of mortality and increasing drug resistance. This study was done to determine factors influencing adherence to referral advice after pre-referral treatment with artesunate suppositories. A stratified random sample of 757 out of 2200 children treated with pre-referral artesunate suppositories were randomly selected and followed-up at home. Using a structured questionnaire, caretakers were interviewed about their knowledge on malaria, socioeconomic status and cost incurred at the referral facility. Data were merged with information on symptoms, time of treatment and action taken after pre-referral treatment. The odds of adhering to referral advice was three times greater for children with altered consciousness and/or convulsions compared to those with the other symptoms (OR 3.26, 95% CI 2.24–4.74, p < 0.001). The odds of adhering to referral advice were four to five times lower [OR = 0.25; 95% CI: 0.09–0.67, p = 0.006] for caretakers who reported paying than those who did not. In these villages, severity of symptoms was found not to influence adherence to referral advice. In the wake of introducing and rolling out the strategy the finding that caretakers of patients with severe symptoms adhered to referral advice is reassuring. However, for a successful implementation of the strategy patient charges for under fives should be waved in order to maximize adherence.

Email address for correspondence: dsimba@muhas.ac.tz

74
Acceptability of Rapid Diagnostics Test (RDT) and pre-packed drug (Coartem) through community based volunteers for uncomplicated malaria in under 5 years’ children, Home Management of Malaria (HMM) strategy in Jimma town, Ethiopia—Preliminary results [MIM15047255]

Morankar Sudhakar, Yihenew Alem, Ayalew Tegegn, Mirkuzie Woldie

This is a TDR/WHO funded study. In baseline survey mothers/caretakers of children preferred community based volunteers (CHVs) to provide diagnostic (RDT) and treatment services (Coartem) of malaria for their under five children. About 130 CHVs were recruited. After certification of CHVs by providing necessary training, they were deployed to their area providing storage box for RDT, Coartem, IEC materials and registration formats. CHVs visited every household to make community aware of the services they will be providing. CHVs were supervised and monitored in a monthly meeting providing feedback on submitted registration formats. These preliminary findings are of activities from October 2007 to September 2008. About 1875 children (49.9% male) were seen by the CHVs with median age of 22 months. About 85% of mothers/caretakers directly brought their febrile children to CHVs. About 51.7% of children tested positive to RDT and most (96.6%) mothers/caretakers agreed to take treatment (Coartem). About 96% of children put on treatment followed medication as advised. About 95% of children treated with Coartem improved illness and remaining were referred to health facility as some of them had side effects (1.8%) of the drug (dizziness (0.1%), loss of appetite (1.1%), abdominal pain and vomiting (0.1%). RDT negative children were referred to health facility after giving a dose of baby paracetamol. As CHVs are available close to their home, mothers/caretakers took their febrile child anytime to them. All the mothers/caregivers accepted RDT test. Coartem delivery seems feasible as majority of mothers/caregivers complied instructions.

Email address for correspondence: morankarsn@yahoo.com

75
Malaria SOS: A web-based data integration application for malaria surveillance [MIM15055399]

S.K. Yanow, S.E. Shokoples, S. Mukhi

Surveillance is an integral component of all malaria control efforts. Real-time web-based access to clinical, laboratory and epidemiological data provides a much needed mechanism for alerting local, regional and national authorities of potential malaria outbreaks or the emergence of drug resistance. Malaria SOS (System for Online Surveillance) is a Canadian initiative to integrate data on malaria from public health and reference laboratories across the country. Sources of data include laboratory diagnosis, clinical data from submitting physicians, and epidemiological data. A secure web-based system has been developed to enable case-by-case data entry that provides automated and interactive analysis capabilities including trending and user-generated data interrogation tools. The Provincial Laboratory for Public Health in Alberta, Canada, served as the pilot site for application development. The system includes data from malaria diagnosis (species, parasitemia), genotyping of P. falciparum strains, clinical symptoms, patient demographics, and travel history. Although the system has been designed using data from Canada, Malaria SOS can be accessible to users globally through the Internet and can be adapted as needed. With its capacity for data sharing and flexible interface, data can be integrated from a wide variety of users, from local/district laboratories, central public health facilities, clinical trials and sentinel surveillance sites. Malaria SOS leverages a mature and robust Canadian Network for Public Health Intelligence (CNPHI) framework providing secure web-based access to users around the world to a platform with rich set of data collection, analysis and reporting tools.

Email address for correspondence: shamir.nizar.mukhi@phac-aspc.gc.ca
76 Evaluating the impact of subsidized artemether–lumefantrine (AL) in the retail sector on coverage of prompt effective treatment of children under five [MIM15064775]

Beth B. Kangwana, Sarah V. Kedenge, Abdisalan M. Noor, Victor A. Alegana, Andrew J. Nyandigisi, Jayesh Pandit, Manya Andrews, Mbogo Bunyi, Greg W. Fegan, Simon Brooker, Catherine A. Goodman

Since 2006 Kenya has distributed its first line anti-malarial artemether–lumefantrine (AL) free through government facilities but the proportion of children with fever receiving AL remains very low. To improve treatment accessibility and quality, in December 2008 the Government, in collaboration with Population Services International, began piloting the provision of subsidized AL through trained retail providers in three districts in Western Province, supported by low literacy instructions and communications to improve treatment seeking behavior and adherence. We present the evaluation, focusing on the impact on the coverage of prompt effective treatment for under-5s. We undertook a pre–post randomized cluster controlled trial with baseline data collected mid-2008 and follow-up data in mid-2009. Nine intervention and nine control sub-locations were selected across the three districts. Three ‘communities’ within each sub-location were randomly selected using probability proportional to size, and 43 homesteads randomly selected per community. In each selected homestead we interviewed the household head and all care-givers of children under five at both baseline and follow-up. A total of 2322 homesteads were interviewed in each survey round. Data will be presented on the impact of the intervention on access to prompt AL treatment for under-5s, use of artesinin monotherapies, patient adherence to AL treatment, care-giver knowledge, and household costs. The impact of socio-economic status on these outcomes will be examined. The results will guide the development of strategies to increase access to AL in Kenya, and inform plans for the implementation of the proposed Global Subsidy for artesinin-based combination therapies.

Email address for correspondence: bkangwana@nairobi.kemri-welcome.org

77 A systematic review of the accuracy of rapid diagnostic tests for malaria in endemic areas [MIM15066396]

Cho-Min Naing, Katharine Abba, Paul Garner, Daw-Khin Win, Jon Deeks, Mala Maung, Piero Olliaro

Rapid diagnostic tests (RDTs) are being more widely used in the diagnosis of uncomplicated malaria. We are currently carrying out a Cochrane review assessing the accuracy of RDTs in detecting *P. falciparum* and *P. vivax* malaria in endemic areas, and investigating factors that may influence their accuracy. We have completed a protocol that specifies inclusion criteria; we will include all studies where the same blood samples taken from participants in malarial areas with symptoms of malaria, are tested for malaria using both an RDT and an accepted reference standard (microscopy or PCR). We will assess the quality of each study using modified QUADAS (Quality Assessment of Studies of Diagnostic Accuracy included in Systematic Reviews) criteria. We will present sensitivities and specificities for RDTs compared to reference standards, grouped by test antigen (HRP-2, aldolase and pLDH). If appropriate, we will also conduct meta-analyses by type of malaria parasite, and antigen targeted by the test. We will conduct sensitivity analyses using quality components. The review will contain at least 40 studies with assessment on at least 12 types of RDT. The anticipated comparisons are the four groups of RDTs. At the meeting we will present the preliminary, unpublished results.

Email address for correspondence: k.abba@liverpool.ac.uk

78 Using geographic information systems to improve the interpretation of malaria data from clinics in Kenya [MIM15080386]

Peter W. Gething, Abdisalan M. Noor, Victor A. Alegana, Simon I. Hay, Robert W. Snow

Reliable and timely information on the burden of malaria within the health system and the volume of resources used in its treatment is essential for effective planning and monitoring of service provision. Despite continued investment, the national routine data collection mechanisms designed to meet this need in Kenya remain inadequate: underreporting from facilities is widespread and national databases are largely incomplete meaning critical public health decisions are often based on crudely adjusted metrics with unknown reliability. We assembled two independent national health system data sets operated by the Kenyan Ministry of Health: monthly records of presumed malaria in outpatients from the health management information system (HMIS) and of consumption of the first line therapeutic artemether–lumefantrine (AL) from the AL tracking system operated by the division of malaria control. Both were triangulated with a recently updated comprehensive spatial database of clinic locations within a geographic information system (GIS). A space–time geostatistical modelling framework was constructed that could predict values in both databases that were missing in space and time and provide associated measures of uncertainty. Validation of our models showed that by aggregating facility-level estimates, we could quantify annually both the total burden of presumed outpatient malaria and the total requirements for AL in the public health sector to an acceptable level of precision at the national and provincial level. We were also able to cross-reference the two metrics to assess their relative fidelity and compare spatial–temporal patterns of malaria diagnosis and drug consumption. The construction of spatial databases of health clinics and the use of GIS and spatial–temporal geostatistical approaches allows improved handling of imperfect malaria data from health clinics leading to a more robust evidence base for critical health system decisions.

Email address for correspondence: peter.gething@zoo.ox.ac.uk

79 Characterization of antigen presenting cell subsets and their activation in parasitized and non-parasitized children belonging to different ethnical sympatric tribes in Mali [MIM16690295]

Arama Charles, Giusti Pablo, Boström Stefanie, Varani Stefania, Dara Victor, Traoré Boubacar, Dolo Amagana, Doumbo Ogobara, Troye-Blomberg Marita

Interethnic comparative studies have shown that Fulani are more resistant to *Plasmodium falciparum* malaria than other sympatric ethnic groups as reflected by less clinical symptoms, lower parasite rates and densities. Earlier studies have shown that the Fulani have higher levels of anti-malarial antibodies, higher number Th1 and Th2 cells and lower expression of FOXP3 and CTLA4. Despite monocytes and dendritic cells are crucial in initiating and regulating acquired immunity, little is known about differences in these cell subsets among ethnic groups that exhibit different malaria susceptibility. To address this, an immunological study was performed in Mali during the malaria season in October to November 2008 enrolling children aged 2–10 years with and without active
P. falciparum infections belonging to the Fulani and Dogon sympatric ethnic groups. Peripheral blood mononuclear cells (PBMCs) were collected and stimulated with toll-like receptor (TLR) ligands specific for different dendritic cell subtypes and supernatants were collected. The remaining PBMCs were fixed, frozen and stained for FACS analysis. In line with earlier data the Fulani exhibited higher serum levels of anti-malaria antibodies as compared with the Dogon. In addition the serum levels of IFN-gamma and IFN-alpha were higher in the Fulani. Presently we are evaluating the data regarding the phenotypic characterization, activation markers and co-stimulatory molecules on the different subsets of antigen-presenting cells. The results will be discussed with the aim to elucidate whether to higher immune reactivity to malaria antigens seen in the Fulani can be detected at the level of antigen-presenting cells.

Email address for correspondence: pablo@wgi.su.se

80 Phenotype and parasite inhibitory activity of blood monocytes in acute uncomplicated Plasmodium falciparum malaria [MIM16690649]

P. Chimma, C. Roussillon, P. Sratongno, K. Pattanapanyasat, J.L. Pérignon, D.J. Roberts, P. Drulilhe

The balance between inflammatory MO (CD14highCD16+ and CD14dimCD16+ subsets) is expected to be strongly regulated during P. falciparum infection, and might relate to the clinical outcome. We used flow cytometry to characterize the phenotype of blood MO isolated from 10 healthy (malaria exposed) individuals and 76 patients with acute uncomplicated malaria and studied their anti-parasitic activity by performing antibody-dependent cell-mediated (ADCI) test, with blinded counting of parasitaemias. Results showed that, at the time of admission, MO from patients had lower percentage of CD56+ and mIFN-γ+ cells but higher percentage of CCR2+/CX3CR1+ and CD16+ cells than healthy individuals. Patients were divided into two groups: with CD14highCD16+ MO dominant in group 1 (17/76 cases), or CD14dimCD16+ MO dominant in group 2 (59/76 cases). Group 1 and 2 patients MO had low direct inhibitory effect, but differed drastically in their ability to control growth of parasites by ADCI, the inhibitory indexes being on average 10 times higher in group 1 patients. CD16+, CD14high, CCR2+/CX3CR1+ and HLA-DR+ cells were necessary required for priming the classical MO to perform ADCI. In conclusion, patients with acute uncomplicated malaria attacks have a marked increase of circulating inflammatory MO, but differ according to the balance between the CD14highCD16+ and CD14dimCD16+ subsets.

Email address for correspondence: chimma@pasteur.fr

81 Modulation of the monocyte scavenger receptor CD36 expression and non-opsonic [MIM14954826]


The class B scavenger receptor CD36 on monocytes/macrophages plays an important role in innate immunity through opsonin-independent phagocytosis of P. falciparum parasitized erythrocytes (PE). Up-regulation of CD36 expression by peroxisome proliferator activated receptor gamma-retinoic-X-receptor (PPARY-RXR) agonists has been shown to enhance phagocytosis of PE. We explore for the first time the effect of curcumin on CD36-mediated non-opsonic phagocytosis of PE by monocytes/macrophages. Cultured human THP1 monocytes and PBMC were exposed to curcumin and CD36 and PPARγ expression evaluated by real-time PCR, flow cytometry and western blotting. Phagocytosis after curcumin treatment was assessed by microscopy. Curcumin increased CD36 expression in human monocytes at the mRNA and protein level and enhanced non-opsonic phagocytosis of PE. This increase in CD36 expression took place following the production of reactive oxygen intermediates (ROI) and could be inhibited by the antioxidant N-acetylcysteine. However, this effect was not abrogated by the PPARγ antagonist GW9662 indicating that CD36 expression on monocytes following curcumin exposure is independent of this nuclear transcription factor. We also demonstrate here that the nuclear related {erythroid-derived 2} factor 2 (Nrf2), a stress sensitive nuclear transcription factor, is an alternative PPARγ-independent pathway for CD36 induction by curcumin. The increase in CD36 expression and non-opsonic phagocytosis of PE induced by curcumin may play an important role in parasite clearance in vivo. This “host targeted approach” represents a novel strategy to complement the direct anti-parasitic effect of compounds with antimalarial activity and as such could be a valuable tool in limiting the emergence of drug resistant parasites.

Email address for correspondence: logonda@wrp-ksm.org

82 Expression of FcγRIII by monocytes from children with Plasmodium falciparum malaria: Association with severe malarial anaemia [MIM16755257]

A.L. Ogonda, A.S.S. Orago, M.F. Otieno, J.A. Stoute

Monocytes/macrophages play an important role in the innate immune response to malaria. Malaria infection leads to formation of immune complexes (ICs) that can interact with monocyte/macrophages by binding to their surface Fc gamma receptors. Fc gamma receptor III (FcγRIII, CD16) expressed on monocytes/macrophages can be cross-linked by immune complexes resulting in production of tumor necrosis factor alpha (TNF-α), a cytokine implicated in the development of severe malaria. FcγRIII can also mediate phagocytosis of antibody-coated infected and uninfected red cells which could contribute to the development of severe anaemia. Therefore, expression levels of FcγRIII may influence an individuals’ susceptibility to severe Plasmodium falciparum malaria. We investigated the expression of FcγRIII on monocytes of children with severe malarial anaemia, cerebral malaria, and their age and gender-matched uncomplicated malaria controls by flow cytometry at enrollment and after recovery from illness. In addition, we stimulated monocytes with BSA-anti-BSA immune complexes to determine the effect on the intracellular expression of TNF-α by monocytes. The expression of FcγRIII on monocytes was highest in children with severe malarial anaemia compared to all the other groups at enrollment. Furthermore, there was significant inverse correlation between haematocrit levels and FcγRIII expression levels on monocytes. The intracellular TNF-α expression by CD14+CD16+ monocyte subpopulation in response to immune complex stimulation correlated positively with their FcγRIII expression. These data suggest that children who are predisposed to SMA may overexpress FcγRIII during malaria infection which may lead to FcγRIII-mediated red cell destruction via erythropagocytosis and increased TNF-α production.

Email address for correspondence: logonda@wrp-ksm.org
Malaria and CD36: beta1 integrin stably associates with CD36 in mouse macrophages [MIM14965759]

Hani Kim, Kevin C. Kain

Innate immune responses are essential in controlling early malaria parasite replication and decreasing the risk of progression to severe and fatal disease. We have recently shown that a scavenger receptor, CD36, modulates innate immune response to malaria by regulating cytokine production and parasite clearance. In the present study, we examined potential signalling mediators downstream of CD36, as they may represent novel therapeutic targets. Specifically, we assessed the involvement of beta1 integrin (beta1-INT) as it is a known regulator of actin cytoskeleton, which is important during phagocytosis. Mouse peritoneal macrophages were harvested, and membrane proteins were solubilized in the presence of a non-ionic detergent, Brij 99. Immunoprecipitation was performed using an anti-mouse CD36 antibody, followed by western blotting using an anti-beta1-INT antibody. To assess the activation status of beta1-INT, the immune complex was subsequently probed with an antibody specific to talin, which binds to the activated form of integrins. Beta1-INT co-immunoprecipitated with CD36 in macrophages in the resting state. The beta1-INT was not associated with talin, indicating that it was in an inactive state when complexed to CD36. Beta1-INT forms a stable immune complex with CD36 in mouse macrophage phagocytes at steady-state, and the CD36-bound integrins are in an inactive state. Our findings suggest that integrins may participate in signalling downstream of CD36 in macrophages, thereby contributing to CD36-mediated phagocytosis and parasite clearance. Studies are underway to assess the involvement of other integrins, and their regulation when CD36 is activated by its ligands.

Email address for correspondence: hani.kim@hotmail.com

B cell activity in children with malaria [MIM16731990]

Jackson C. Korir, Ronald P. Taylor, John N. Waitumbi

Mature B cells express CD20 molecules on their surface that distinguishes them from antibody-producing plasma cells. The antigen-processing and presentation capabilities of mature B cells are enhanced by their ability to bind complement-opsonized immune complexes (IC) via the membrane bound receptor CD21 (CR2). B cell numbers and expression levels of both cell-associated and soluble CD21 (sCD21) are affected differently by different diseases and this has impact on the competency of B cells. In a case control study, we assessed how malaria affects B cell numbers and expression levels of CD21 in children presenting at Kisumu District Hospital, western Kenya with either severe malarial anemia (SMA) or uncomplicated malaria. Children with SMA had a higher % of CD20 B cells (26.8 ± 9.7 S.D.) as compared to their age and sex matched controls (20.9 ± 9.0 S.D., P = 0.025) probably as a result of polyclonal activation of B cells by malarial antigens. However, the median fluorescence intensity for CD21 on the mature B cells of children with SMA was much lower (251.9 ± 90.29 S.D.) than that of the controls (372.99 ± 140.82, P = 0.01). We think these results from the processing and removal of CD21, along with associated complement-opsonized IC, by fixed tissue macrophages of the mononuclear phagocytic system (MPS). In addition, the B cells of SMAs had higher levels of the complement split product C3dg (18.91 ± 11.66 S.D.) compared to controls (11.5 ± 7.33 S.D., P = 0.02), pointing to increased complement activation in the SMA group, a phenomenon that has been observed in severe malarial anemia. We also found that children with SMA had lower levels of sCD21 (223.70 ± 131.79 S.D.) compared to controls (341.43 ± 137.32 S.D., P = 0.003). This result indicates that, unlike in the normal enzymatic cleavage of B cell-associated CD21 and subsequent release of sCD21 into the bloodstream, in malaria CD21 is removed from B cells (by the MPS) as part of the C3dg IC and therefore not released into circulation. Since both membrane-associated and sCD21 play a critical role in humoral immunological responses, their reduced expression probably contribute to the pathogenesis of complicated malaria.

Email address for correspondence: jcheruiyot@wrp-ksm.org

Adjuvant induced immune responses in populations living in a malaria-endemic area [MIM16692157]

Jose Fernandes, Selidji Agnandji, Lucja Labuda, Adegnika Akim, B. Lell, P.G. Kremsner, M. Yazdanbakhsh

In the near future, adjuvanted malaria vaccines are likely to be available for the immunization of millions of sub-Saharan Africans. Currently, there are limited data on the immunostimulatory properties of adjuvants in populations living in malaria-endemic areas. For a better understanding of vaccine-induced immune responses and in order to assist the development of malaria vaccines, the immunostimulatory properties of potential malaria vaccine adjuvants on the immune system in a malaria exposed population need to be characterized. In the present study, we describe the cytokine production of peripheral blood monocyte cells (PBMCs) and purified B cells stimulated by various adjuvants. PBMCs and B cells were purified from 20 adults (10 exposed and 10 naive) and 10 children. These were then stimulated with the adjuvants alum, CpG, ISA 720 MPL, and QS-21, using LPS as positive control and medium negative control. Supernatants were collected on days 1, 2, 5 and 7 for multiplex cytokine analysis to measure the level of various Th1, Th2 and Th17 cytokines IFNg, IL-5, IL-13, TNFa, IL-2, IL-17, IL-21, IL-10, IL-6, IL-1b, IL-18, IL-23, MIP1a and IP10. In addition,
samples were lysed, fixed and cryopreserved to assess the level of intracellular production of CD4 (Th1, Th2, Th17) cells, CD8+ and regulatory T cells, plasmocytes and B cells memory characteristic markers. Results will be presented.

Email address for correspondence: josefranciscofernandes@yahoo.fr

87 Field evaluation of three commercial repellent formulations against malaria mosquitoes of a forest area in Cameroon [MIM16696621]

P. Nwane, J. Etang, C. Costantini, F. Batomen, C. Antonio-Nkondjio, R. Mimpfoundi, I. Morlais, F. Simard

Application of repellents to the skin is a common personal protection practice for preventing mosquito-borne diseases. Here, we tested the efficacy and persistence of three commercial repellent formulations against the bites of malaria vectors. The study was conducted in a suburban village near Yaoundé. Four target doses (0.1 mg/cm²; 0.3 mg/cm²; 0.6 mg/cm² and 0.8 mg/cm²) of each repellent i.e. 30% DEET (Buzz-Off™) and 25% IR3535 (Cinq-sur-Cinq™ and Prébutix™) or 90% ethanol as control were applied on the legs of volunteers who performed human landing catches to determine repellent efficacy. Effective dosages and persistence of each repellent were estimated by fitting a logistic plane model. A total of 2072 malaria mosquitoes were collected during 48 tests nights: An. gambiae s.s. (7.8%), An. funestus (8.3%), An. moucheti (45.8%), An. nili (18.7%) and An. ziemanni (19.2%). After 8 h exposure to mosquito bites, percentages of repellency provided by each of the three formulations were quite variable, ranging from 20 to 80%. Because of sample size constraints, the effective dosages and persistence were estimated only for An. moucheti. The median and 95% effective dose (ED50 and ED95) estimates of the IR3535-based repellents were lower than those of the DEET-based formulation. The estimated effective half-lives for the IR3535-based repellents were between 3.1 and 3.6 h. Our results confirm the heterogeneity in the response of malaria mosquitoes to insect repellents, showing the relevance of evaluating efficacy and persistence profiles of different formulations in specific environmental contexts.

Email address for correspondence: philino07@yahoo.fr

88 Why do African malaria vectors specialize on humans or cattle over alternative hosts? [MIM16696900]

Issa Lyimo, Edgar Mbehela, Kasian Mbina, Ally Daraja, Tanya Russell, Heather Ferguson

The human biting rate of Anopheles vectors critically determines malaria transmission intensity. Identifying the selective pressures that cause vectors to select humans could thus be useful for the development of malaria control strategies based on mosquito behaviour change. We conducted an experimental study of the African vectors An. gambiae and An. arabiensis to test whether their respective preference humans and cattle is correlated with the fitness benefits obtained from feeding on these hosts. During each night of experiments, one host of either human, cow, calf, goat, dog or chicken was placed inside an experimental hut within a semi-field system (SFS) at the Ifakara Health Institute (6 replicates /host). Two hundred females of either An. gambiae or An. arabiensis were then released within the SFS. The following morning, mosquitoes were recaptured and their feeding success determined. The feeding success of An. gambiae and An. arabiensis varied with host species, with the former feeding most on humans and the latter on cattle. Blood fed An. gambiae also acquired significantly more blood from humans than other species. In contrast, An. arabiensis obtained similar amounts of blood from humans and other animals. However, when human hosts were partially protected with a holed bed net, the few An. arabiensis that were able to feed on them acquired substantially more blood than from unprotected hosts. We hypothesize that the specialisation of An. gambiae and An. arabiensis for humans and cattle respectively is a product of enhanced blood acquisition efficiency on these hosts.

Email address for correspondence: i.lyimo.1@research.gla.ac.uk

89 The effect of Mosquito Magnet Liberty Plus™ traps on the human mosquito biting rate under semi-field conditions [MIM16227452]


Increasing insecticide resistance and dwindling number of approved chemical insecticides for mosquito control necessitates an urgent need for development and use of alternative and complementary methods. This study evaluated the efficacy of a mosquito trap, the Mosquito Magnet Liberty Plus™ (MM), in reducing human biting rates under semi-field conditions. Human landing catch was used to monitor mosquito biting rates when un-baited and Lurex3-baited MM traps were used. Different types of repellents were also tested to investigate their effect on the MM trap catch and the subsequent human biting rate. MM trap significantly reduced the human biting rate with both Culex quinquefasciatus and Anopheles gambiae sensu stricto. The MM trap catch did not increase when a mosquito coil was burned but did significantly increase when a skin repellent was applied to the human bait. Microencapsulated repellent ankle bands did not increase the MM trap catch with either Cx. quinquefasciatus or An. gambiae s.s. although its combination with the trap was more effective at reducing bites by Cx. quinquefasciatus. The absence of the commercial attractant, Lurex3, in traps significantly lowered the catch efficiency of Cx. quinquefasciatus even when the skin repellent was applied to volunteers. From the results, synergistic use of skin repellent and attractant-baited traps can significantly reduce the human biting rate of both nuisance biting mosquitoes and malaria vectors. Further work is required to investigate how this push–pull system would work in a field environment.

Email address for correspondence: jvnkit@yahoo.co.uk

90 Behavioral studies of the chromosomal forms of Anopheles funestus from Burkina Faso [MIM16679761]

N’Fale Sagnon, Malgaouenedé Yaméogo, Wamdaogo M. Guelbeogo

Cytogenetic and molecular studies on Anopheles funestus lead to the definition in Burkina Faso of two taxonomic units (Folonzo and Kiribina) with limited gene flow and contrasting degrees of chromosomal polymorphism. In despite of that, few is known on the role of these taxonomic units in malaria transmission. This study was carried out to elucidate the behaviour such as host preference, resting and feeding behaviour of these forms, in village where the two forms are sympatric. Resting behaviour was assessed by collecting outdoor and indoor resting mosquito from pit shelters and house respectively. Ovaries of all half gravid specimens were removed and fixed in carnoy for cytogenetic analysis. Corresponding carcasses were fixed in silicagel for origin of blood meal and infection status.
digestion of the male seminal fluids. Our studies identify genes role in the maintenance and function of stored sperm and in the females a number of mating-responsive genes likely to play a gering female post-mating responses. Furthermore, we detected the females and are important for sperm function and for trig- machinery associated with copulation in both males and females. performed a comprehensive analysis of the molecular and cellular perations relevant to the fertility of biology analyses to identify male and female factors and mech- females during copulations. However, such factors have not been identified yet and very little is known on the molecular mechanisms regulating the cascade of events shaping post-mating responses in females. We used a combination of microarray, proteomics and cell biology analyses to identify male and female factors and mech- anisms relevant to the fertility of An. gambiae mosquitoes. We performed a comprehensive analysis of the molecular and cellular machinery associated with copulation in both males and females. We identified a number of male proteins that are transferred to the females and are important for sperm function and for trig- gering female post-mating responses. Furthermore, we detected in females a number of mating-responsive genes likely to play a role in the maintenance and function of stored sperm and in the digestion of the male seminal fluids. Our studies identify genes and mechanisms regulating the reproductive biology of An. gamb- ia mosquitos, highlighting similarities and differences with Drosophila melanogaster. Our data inform vector control strategies and reveal promising targets for the manipulation of fertility in field populations of these important disease vectors. 

Email address for correspondence: f.catteruccia@ic.ac.uk

91 Molecular bases of post-mating behaviour in Anopheles gambiæae females [MIM16691937]

David W. Rogers, Francesco Baldini, Janis Thailayil, Flaminia Catteruccia

In Anopheles gambiae mosquitoes, similar to other insects, mat- ing induces a series of behavioural and physiological responses in females including changes in the flight activity rhythm, enhanced ovulation and oviposition, and induced refractoriness to further mating. Such post-mating responses are likely to be triggered by factors produced by the male accessory glands and transferred to females during copulations. However, such factors have not been identified yet and very little is known on the molecular mechanisms regulating the cascade of events shaping post-mating responses in females. We used a combination of microarray, proteomics and cell biology analyses to identify male and female factors and mech- anisms relevant to the fertility of An. gambiae mosquitoes. We performed a comprehensive analysis of the molecular and cellular machinery associated with copulation in both males and females. We identified a number of male proteins that are transferred to the females and are important for sperm function and for trig- gering female post-mating responses. Furthermore, we detected in females a number of mating-responsive genes likely to play a role in the maintenance and function of stored sperm and in the digestion of the male seminal fluids. Our studies identify genes and mechanisms regulating the reproductive biology of An. gamb- ia mosquitos, highlighting similarities and differences with Drosophila melanogaster. Our data inform vector control strategies and reveal promising targets for the manipulation of fertility in field populations of these important disease vectors.

Email address for correspondence: f.catteruccia@ic.ac.uk

92 Comparing prey behavioural responses to predation in the M and S forms of the African malaria mosquito, Anopheles gambiae [MIM16439417]

Geoffroy Gimonneau, Serge Morand, Marco Pombi, Roch Dabiré, Frédéric Simard

The M and S molecular forms of the African malaria mosquito Anopheles gambiae are considered incipient species. In West Africa, the M and S forms seem to breed preferentially in permanent habi- tats and temporary habitats, respectively. It was suggested that selection mediated by larval predation and competition promoted divergence between temporary and permanent freshwater habi- tats. To further explore this hypothesis, we conducted behavioural tests with immature stages of the M and S forms in the presence and absence of a widespread predator. Larval behaviour was assessed experimentally in the M and S form in absence or physical presence of the predator Anisop sp. (Notonectidae). Ethograms were realized using 15-s instantaneous scan censuses. Behaviours were catego- rized into activities (thrashing, browsing, filtering and resting) and positions (surface, wall, middle and bottom). For each larva, propor- tion of observations in each position or activity was converted into uncorrelated descriptors using PCA. Principal Components were analysed using MANOVA. In dual choice tests, the S form was more vulnerable to predation than the M form (61% vs. 39%, p < 0.001). Behavioural tests showed that the M form was more active than the S form, spending more time filtering and browsing (e.g. forag- ing behaviours). In presence of the predator, the M form reduced risky behaviours whereas the S form did not change its behaviour. Stronger predator avoidance together with superior competitive ability of the M form over the S form likely contributed to the adaptation of the former to more permanent breeding habitats.

Email address for correspondence: geoffrey.gimonneau@ird.fr

93 Swarming and mating behaviour of the M and S molecular forms of Anopheles gambiae in areas of sympathy, Burkina Faso (West Africa) [MIM16684767]


Anopheles gambiae, the African malaria vector, is subdivided into two incipient species identified as M and S molecular forms. Fac- tors that support the reproductive isolation between these forms are still until unknown. The present study investigates the swarm- ing and mating behaviour in both forms in areas of sympathy in Burkina Faso. Mosquitoes were sampled within the swarms during the rainy season from June to October 2007 at Soumouso (65% S form) and Vallée du Kour (70% M form). At least 5 swarms per month were sampled and mosquitoes PCR-analysed to determine their molecular form. Female’s insemination status was checked by dis- secting their spermathecae. In Soumouso, 3671 mosquitoes were collected from 32 swarms. Except in July and August when respec- tively 30% and 16.6% of swarms were composed of mosquitoes of both forms, all swarms were monospecific. In Vallée du Kour, 38,926 mosquitoes and 229 pairs caught in copula were collected from 22 swarms. All swarms were monospecific except one mixed swarm sampled in September. Of 100 couples analysed by PCR, 99 were M–M couples and one S–S. The insemination rate recorded in the swarms averaged 68%. Sperm extracted from each female was found to be of the same form as the female. These results indicate that the swelling and mating system involved mainly mosquitoes of the same form suggesting the existence of specific recognition factors. Further researches on the reproductive behaviour of An. gambiae s.l are of key importance in prospects of using genetically modified mosquitoes for malaria control.

Email address for correspondence: dabire_roch@hotmail.com

94 Can topical mosquito repellents prevent malaria in Africa? [MIM161394463]

P. Sangoro, J.E. Miller, J.A. Armstrong-Schellenberg, S.J. Moore

Insecticide-treated bed nets (ITNs) are considered the best way to prevent malaria in areas where Anopheles gambiae is a vector, as the mosquito bites late at night. However, high ITN coverage may force mosquitoes to feed earlier and reduce protective efficacy of ITNs. Several studies have shown significant malaria reduction among those who use ITNs and repellents versus ITNs only where evening malaria exposure occurs. This study tested whether repellents, used in combination with ITNs, can provide additional prevention from
malaria in an area of high ITN use in rural Tanzania. Data is collected using a household-randomized cluster-controlled trial measuring malaria incidence by passive case-detection among two arms of 453 households: (1) ITN users using 15% topical DEET repellent; (2) ITN users using placebo lotion, with 80% power, 95% confidence and 20% inter-cluster correlation. Every month, fieldworkers conduct compliance questionnaires at all households. Malaria testing is conducted through rapid diagnostic test at a local clinic. Clinical malaria incidence, household mosquito densities, geographical, housing and demographic parameters are included in Poisson regression, with test applied to the residuals of cluster-observations. The results of this study will be presented for the first time at the conference. The authors predict a significant reduction in clinical malaria episodes among all age repellent users when controlled for confounders. PSI proposes to distribute repellents via social marketing, provided malaria reduction is significant. User acceptance and ethical implications for mosquito repellents will also be discussed.

Email address for correspondence: jniller@psi.or.tz

95 Pregnancy induces malaria recrudescence in immunized mice [MIM16700230]
Rita Neres, Sabrina Epiphano, Lígia A. Gonçalves, Manuela Beirão Catarino, Carlos Penha-Gonçalves, Claudio R.F. Marinho

It is estimated that more than 50 million pregnancies per year occur in malaria endemic areas, and approximately half of these occur in sub-Saharan Africa, where P. falciparum transmission is most intense. Women living in areas with high endemicity or with stable malaria transmission experience fewer malaria symptoms during pregnancy and the most severe effects of the disease are related to the first and second pregnancies. In our model, pregnancy-associated malaria (PAM) in pre-exposed mice females does not require re-infection and suggest that malaria recrudescence during pregnancy can be caused by pregnancy-specific mechanisms. Females were infected with P. berghei ANKA and subjected to a chemotherapy treatment. More than half of the immunized females had recrudescent infections induced by pregnancy. Recrudescent and non-recrudescent pregnant females were followed until the third pregnancy, characterized for pathological and immunological parameters and their offspring was evaluated. The proportion of recrudescent females was higher among primigravidae as compared to multigravidae. In contrast, non-pregnant females never showed malaria recrudescence. Placenta pathology was observed specially among recrudescent females. Moreover, the results also suggest that PAM severity and pregnancy outcome injury were reduced with parity. The data indicate that exposure to recrudescent parasites causes PAM, leading to immunological and pathological modifications. We have observed that exposure to malaria in consecutive pregnancy recrudescences induces protection to placental malaria.

Email address for correspondence: crfmarinaho@gmail.com

96 Cellular immunological responses in pregnancy associated malaria [MIM16670864]
Mayke Oesterholt, John Lusingu, Martha Lemnge, Nadine Fievet, Stefania Varani, Marita Troye-Blomberg, Ali Salanti, Daniel Minja, Christentze Schmiegelow, Thor Theander, Achille Massougbodji, Nicaise Tuikue Ndam, Philippe Deloron, J. Adrian

Pregnancy-associated malaria (PAM) due to Plasmodium falciparum is detrimental to both mother and child. Ongoing anti-PAM vaccine development focuses on the induction of antibodies targeting VAR2CSA, a parasite-derived protein expressed on the surface of infected erythrocytes that sequester in the placenta, since naturally acquired anti-VAR2CSA IgG titres increase in a gender-specific and parity-related way, and PAM shows a concomitant parity-related decrease in incidence. These findings imply a protective function for antibody responses. In contrast, a defined role for specific T cell responses is unclear and remains largely unexplored. We are conducting a longitudinal, prospective study of 1000 pregnant mothers in Korogwe, north-eastern Tanzania. For a subgroup of 200 mothers with and without evidence of malaria, ex vivo frequencies of the T cell, B cell, monocyte, regulatory T cell and dendritic cell populations are being measured. Cytokine activity of isolated peripheral blood mononuclear cells is measured following stimulation in vitro with VAR2CSA-specific reagents and P. falciparum-infected red blood cells. Cord blood mononuclear cells isolated at delivery are assessed in a similar way in order to determine the extent of sensitization to P. falciparum antigens in utero. At the time of writing, inclusion into the study is ongoing and should be completed by the end of 2009. We will present and discuss data derived primarily from samples collected from mothers at inclusion. We will compare and contrast our cellular immunological findings with those from a study that is ongoing in parallel in southern Benin, where malaria transmission is more intense and perennial.

Email address for correspondence: maykeoesterholt@gmail.com

97 Phenotyping and levels of activation of dendritic cells (DCs) and monocytes in Pregnancy-Associated Malaria during a follow-up in Benin [MIM16693964]
Samad Ibitokou, Justin Doritchamou, Nadine Fievet, Mayke Oesterholt, Achille Massougbodji, Thor Theander, Stefania Varani, Marita Troye-Blomberg, Adrian Luty, Philippe Deloron

The STOPPAM consortium conducts 2 longitudinal cohort studies in pregnant women from Benin and Tanzania to evaluate the immunopathological consequences of P. falciparum infections during pregnancy. DCs are of particular relevance for pregnancy maintenance given their unique ability to induce both antigen-specific immunity and tolerance, and they are implicated in protecting the mother from infection without compromising fetal survival. Data on DCs in PAM are needed to understand the CMI implication in a vaccine design. In Comé, southwestern Benin, a longitudinal prospective study of 1000 pregnant mothers is going on. Pregnant women are enrolled <24 weeks of pregnancy and followed at each ANV until delivery. CMI is done in a subgroup of 200 women at inclusion and 200 at delivery: 100 mothers with active Pf infection detected by rapid diagnostic test matched for gravidity, gestational age with 100 mothers with neither Pf infection at inclusion nor a history of such earlier in the pregnancy. Ex vivo DC and monocyte phenotypes, and their level of activation (HLA-DR, CD86) after LPS activation are evaluated using flow cytometry. We will compare data on 200 women at inclusion and at delivery according the timing and pathology of malaria infection. From now, we made CMI exploration in 100 women at inclusion and in 10 at delivery. Results will be discussed on women having completed their follow-up before the end of September. At the time of the MIM congress all the women will be included in Comé.

Email address for correspondence: jdoritchamou@yahoo.fr
98 T cells IFN-γ responses in Pregnancy Associated Malaria during a follow-up in Benin [MIM16690219]

Bertin Vianou, Carine Agbowà, Nadine Fievet, Maybe Oesterhold, Stefania Varani, Marita Troye-Blomberg, Adrian Luty, Thor Théander, Ali Salanti, Nicole Tuijke Ndam, Achille Massougbodji, Philippe Deloron

The STOPPAM consortium conducts 2 longitudinal cohort studies in pregnant women from Benin and Tanzania to evaluate the immunopathological consequences of *P. falciparum* infections during pregnancy. T cell-mediated CMI may contribute to previous observed gravidity-related reduction in prevalence of PAM. In the immunomodulation pregnancy context, it is important to evaluate the IFN-γ secreting T cells specific responses to putative vaccine candidates, as VAR2CSA domains. In Comé, southwestern Benin, a longitudinal prospective study of 1000 pregnant mothers is going on. Pregnant women are enrolled <24 weeks of pregnancy and followed at each ANV until delivery. CMI is done in a subgroup of 200 women at inclusion and 200 at delivery: 100 mothers with active Pf infection detected by rapid diagnostic test matched for gravidity, gestational age with 100 mothers with neither Pf infection at inclusion nor a history of such earlier in the pregnancy. Ex vivo T cells phenotypes and IFN-γ responses to PHA and VAR2CSA domains are evaluated in maternal PBMC. We will compare data on 200 women at inclusion and at delivery according to the timing of malaria infection and the induced placent al pathology. From now, we made CMI exploration in 100 women at inclusion and in 10 at delivery. Results will be discussed on women having completed their follow-up before the end of September. At the time of the MIM congress all the women will be included in Comé.

Email address for correspondence: bertinosidase@yahoo.fr

99 Impact of placental malaria on neonatal Treg cells and *Plasmodium falciparum*-specific immune responses [MIM16645854]

Valérie Soulard, Martin Amadoudji, Catherine Fitting, Samad Ibitokou, Adrian J.F. Luty, Maybe Oesterhold, Achille Massougbodji, René-Xavier Perrin, Philippe Deloron, Antonio Bandeira, Nadine Fievet

Infants born to mothers with Placental Malaria (PM) are at increased risk for malaria in early life. Transplacental passage of soluble parasite proteins would tolerate the fetal immune system to *P. falciparum* (Pf) antigens. We analyzed the impact of PM on the frequency of neonatal Treg cells and Pf-specific responses. Frequency of CD4+CD25+CD127-Foxp3+ Treg cells was determined by flow cytometry on cord blood mononuclear cells (CBMC) from neonates born to PM-positive and PM-negative mothers. Pf-specific cytokine responses of CBMC after culture with live parasites were addressed by Multiplex analysis on supernatants. PM is associated with a decrease in Treg frequency in neonates born to primigravids. High Pf-associated placental inflammation correlates with low Treg frequency in neonates. PM is also associated with a decrease in neonatal Pf-specific pro- and anti-inflammatory cytokine responses. Increase of Tregs in Pf-infected adults has been associated with a less efficient control of parasitemia and with clinical malaria. Infants born to PM-positive mothers and having the lowest frequencies of Tregs at birth may be less susceptible to malaria in early life.

Email address for correspondence: zamamart@yahoo.fr

100 *Plasmodium falciparum* exposure in utero, maternal age and parity influence the innate activation of fetal antigen-presenting cells [MIM16559804]

Samad Ibitokou, Stefania Varani, Bertin Vianou, Valérie Briand, Stéphanie Louis, René Xavier Perrin, Achille Massougbogji, Anne Hosmalin, Marita Troye-Blomberg, Philippe Deloron, Nadine Fievet

Pregnancy-associated *Plasmodium falciparum* malaria is associated with immunological abnormalities in the newborns, such as hampered T helper (Th)1 responses and increased T regulatory responses, while the effect of maternal *P. falciparum* infection on fetal innate immunity is still controversial. In this study, we evaluated the immunophenotype and cytokine release by dendritic cells (DC) and monocytes in cord blood from 59 Beninese women with or without placental infection. Accumulation of malaria pigment in placenta was associated with a partial maturation of cord blood myeloid and plasmacytoid DC, as reflected by up-regulation of the expression of MHC class II but not of CD86 molecules. Cells of newborns of mothers with malaria pigment in placental leukocytes had significantly increased cytokine responses to a TLR9 ligand. In addition, parity and maternal age influenced the absolute number and activation status of cord blood antigen-presenting cells (APC). Lastly, maternal age, but not parity, influenced TLR3, 4 and 9 responses of cord cells. Our findings suggest that *P. falciparum* infection in pregnant women can modulate innate immunity in the newborn. Additionally, other factors, such as maternal age and parity should be taken into consideration when analyzing fetal/neonatal innate immune responses.

Email address for correspondence: ibitokou.samad@yahoo.fr

101 Pregnancy-associated malaria affects cord blood TLR ligand-induced cytokine responses [MIM16060559]

Ayôla A. Adegnika, Carsten Köhler, Selidji T. Agnandji, Sanders Chai, Lucja Labuda, Lutz P. Breitling, Dorrith Schonken, Eveline Weerdenburg, Saadou Issifou, Adrian J.F. Luty, Peter G. Kremsner, Maria Yazdanbaksh

Pregnancy-associated malaria is known to modify foetal immunity. Most previous studies have been cross-sectional in nature and have focused on priming of acquired immune responses in utero. In this context, the influence of timing and/or duration of placental infection with *P. falciparum* are unknown, and changes to innate immune responses have not been studied extensively. Pregnant women in Gabon where *P. falciparum* infection is endemic were followed-up by performing monthly clinical and parasitological examination from the second trimester to delivery. Cells of neonates born to mothers with *P. falciparum* infection acquired within 1 month of delivery had significantly altered responsiveness to TLR ligands such as LPS and Poly I:C in terms of IFN-γ and TNF-α production compared to cells of those born either to mothers free of *P. falciparum* infection, or to mothers who were successfully treated for malaria during pregnancy. An independent association between gravidity and neonatal TLR responsiveness was also discerned in our study. Therefore, *P. falciparum* infection history during pregnancy appears to have a pronounced effect on neonatal innate immune responses. The observed effects may have profound implications for the outcome of newly encountered infections in early life.

Email address for correspondence: aaadegnika@yahoo.fr
102
Cord cytokine levels predict cytokine profiles and malaria hospitalization risk throughout early life [MIM16779619]
Edward Kabyemela, Moses Gwamaka, Jonathan Kurtis, Marla Husnik, Bess Sorensen, Wonjong Moon, Theonest Mutahingwa, Michal Fried, Patrick Duffy

Some children have more frequent or severe malaria illnesses than others but the reasons for this are not completely understood. We hypothesized that TNF-α and IL 10 levels at birth reflect interindividual differences that persist through early life and influence malaria morbidity in early childhood. Cord and peripheral blood levels of TNF-α, TNF-RI, TNF-RII and IL-10 were measured in 805 newborns enrolled in a longitudinal birth cohort in Muheza, Tanzania between 2002 and 2005. Levels of TNF-α and the ratio of TNF/IL 10 in cord blood correlated with levels measured at routine visits later in childhood. High levels of TNF-α, and high ratio of TNF to IL 10 predicted decreased risk of severe malaria in these children. Compared to the lowest 25th percentile of the distribution of the cytokines levels, the highest (75th–100th percentile) quartile of TNF-α levels at birth was associated with a significant reduction in the risk of clinically severe malaria (OR (95%CI), 0.36 (0.18–0.73)). In particular, high levels of TNF-α decreased the risk of malaria hospitalization for moderate-severe anemia (0.37 (0.17–0.80)) or for respiratory distress (0.41 (0.17–0.94)). High TNF to IL 10 ratio in cord blood also decreased risk of malaria hospitalization (OR (95%CI), 0.50 (0.25–1.00)). These results confirm that interindividual differences in cytokine levels are related to differing risk of malaria syndromes in children. The relationship between TNF-α and protection against malaria may be related to the anti-parasitic effect of the cytokine.

Email address for correspondence: earkabyemela@yahoo.com

103
Effect of intermittent preventive treatment during pregnancy with sulphadoxine-pyrimethamine on maternal and fetal antibody responses against malaria [MIM16690559]
Elisa Serra-Casas, Clara Menéndez, Azucena Bardají, Llorenç Quintó, Carlota Dobaño, Betuel Sigaude, Alfons Jiménez, Pau Cisteró, Inacio Mandomando, Chetan Chitnis, Pedro L. Alonso, Alfredo Mayor

Intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP) has been adopted as policy by many countries in sub-Saharan Africa. However, data about the effect of IPTP on malaria-specific immunity is scarce and focus exclusively on maternal responses in HIV-negative primigravidae. IgGs, IgMs and IgG-subtypes against Plasmodium falciparum merozoite antigens and whole-parasite lysate were measured by ELISA in 302 peripheral and 258 cord plasma samples from Mozambican pregnant women participating in a randomised, placebo-controlled trial of IPTP-SP. IgGs against variant surface antigens (VSAs) expressed by a CSA-binding line and a pediatric isolate were measured in maternal samples by flow cytometry. Antibody levels were compared between intervention groups. Association of antibody responses with delivery outcomes and infant malaria morbidity during the first year of life was also assessed. The intervention did not significantly affect maternal and cord antibody levels of HIV-negative women at delivery. HIV-positive mothers receiving SP had significantly lower levels of peripheral IgGs against AMA-1 and VSAs, and lower cord IgGs against EBA-175 and parasite-lysate. High antibody levels were significantly associated with maternal infection and infant’s increased risk of a first malaria episode, but not with reduced maternal anemia, prematurity or low birth weight. IPTp may lead to a decrease of maternal and cord humoral responses among HIV-positive mothers at delivery. However, this reduction of P. falciparum-specific antibodies does not translate into an enhanced risk of malaria-associated morbidity in mothers and newborns.

Email address for correspondence: elserra@clinic.ub.es

104
One hundred malaria attacks since birth. A 18-year longitudinal study of malaria morbidity in Dielmo, Senegal [MIM15083152]

Malaria is the first cause of morbidity in tropical Africa but few of the patients come to the attention of any formal health system. As a result, much incertitude persists about the true burden of the disease and the range of individual differences in susceptibility to clinical malaria. From June 1990 to May 2008, we monitored malaria incidence in Dielmo, Senegal, by daily active surveillance. Malaria parasitaemia was systematically measured during fever episodes and during monthly cross-sectional surveys. We analyzed data of 81 children who born after June 1988 (maximum age at inclusion: 20 months) and were followed at least ten years. Diagnostic criteria for malaria attacks were based on fever with a parasite density higher than the threshold measured in this population for each year of age and Plasmodium species. The individual number of malaria attacks ranged from 99 to 8 for P. falciparum, from 16 to 0 for P. malariae, and from 6 to 0 for P. ovale. Three children presented more than 100 malaria attacks, with a maximum of 104 attacks in a child followed during 4970 days who presented 98 P. falciparum attacks, 3 P. malariae attacks and 3 P. ovale attacks. The median numbers of attacks was 43 for P. falciparum, 3 for P. malariae and 2 for P. ovale. The daily surveillance of endemic populations shows that the malaria burden is much higher than previously believed and that the susceptibility to the disease varies considerably according to individuals.

Email address for correspondence: ndiagne@ird.sn

105
A randomized clinical trial of the protective efficacy of trimethoprim-sulfamethoxazole prophylaxis against malaria in HIV-exposed children [MIM16209592]
Taylor Sandison, Jaco Homsy, Emmanuel Arinaitwe, Neil Vora, Abel Kakuru, Humphrey Wanzira, Victor Bigira, Julius Kalanya, Moses Kamya, Grant Dorsey, Jordan Tapper

Trimethoprim-sulfamethoxazole (TS) prophylaxis is used throughout Africa to prevent opportunistic infections in HIV-infected and HIV-exposed (HIV-uninfected children born to HIV-infected mothers) children. Observational studies suggest TS prophylaxis also protects HIV-infected children against malaria. In an area of high malaria transmission in Uganda, we are conducting the first randomized clinical trial of TS protective efficacy among HIV-exposed children. We enrolled 203 HIV-exposed infants and, per WHO recommendations, prescribed daily TS prophylaxis for each child from enrollment until confirmation of negative HIV status 6–8 weeks after breastfeeding cessation. Children were then randomized to discontinue or continue TS prophylaxis through age 2 years. Malaria was diagnosed when a child presented with fever and a positive thick blood smear. The association between TS use and malaria incidence was estimated as an incidence rate ratio (IRR) using negative binomial regression. Among 203 HIV-exposed

www.mimalaria.org
enfants enrôlés, 177 ont été randomisés pour continuer ou discontinuer le paludisme prophylaxie. Parmi les 93 enfants qui continuaient le TS, il y avait 182 cas de paludisme après 66,0 personnes*année (2,76 cas/personne*année). Parmi les 84 enfants qui ont cessé de chaîner le TS, il y avait 258 cas de paludisme après 59,1 personnes*année (4,37 cas/personne*année). Le paludisme prophylaxie yield a 39% reduction (IRR=0.61, 95%CI=0.47–0.79, p <0.001) in incidence in mère. Ces résultats indiquent que le paludisme prophylaxie is modestly protective against malaria in HIV-exposed children when continued beyond the period of HIV exposure. This degree of protection is substantially lower than previously reported in published observational studies, possibly due to the differences in antifolate resistance, transmission intensity, HIV infection, or other unmeasured confounders.

Email address for correspondence: tgsand@uwashington.edu

106 Immunity to febrile malaria in children: An analysis that distinguishes immunity from lack of exposure [MIM16665040]

Philip Bejon, George Warimwe, Claire L. Mackintosh, Margaret J Mackinnon, Sam M Kinyanjui, Jennifer N Musyoki, Pete Bull, Kevin Marsh

In studies of immunity to malaria, the absence of febrile malaria is commonly considered as evidence of "protection". However, apparent “protection” may be due to lack of exposure to infective mosquito bites or due to immunity. We studied a cohort that was given curative anti-malarials before monitoring began, and documented newly acquired asymptomatic parasitemia and febrile malaria during 3 months surveillance. With increasing age, there was a shift away from febrile malaria to acquiring asymptomatic parasitemia, without changing the overall incidence of infection. Antibodies to the infected red cell surface were associated with acquiring asymptomatic infection rather than febrile malaria or remaining uninfected. Bednet use was associated with remaining uninfected rather than acquiring asymptomatic infection or febrile malaria. These observations suggest that most uninfected children were unexposed, rather than “immune”. Had they been immune, we would expect the proportion of uninfected children to rise with age, and to be distinguished from children with febrile malaria by protective antibody responses. We show that removing unexposed children from conventional analyses clarifies the effects of immunity, transmission intensity, bednets and age. Observational studies and vaccine trials will have increased power if they differentiate between unexposed and immune children.

Email address for correspondence: pbejon@kilifi.kemri-wellcome.org

107 Réalité du paludisme urbain, à Dakar: paludisme infection et paludisme ressentii [MIM16676037]

A. Diallo, J.-Y. Le Hesran, S. Dos Santos, A. Ndonky, G. Koné, R. Lalou

Pour illustrer le poids du paludisme les chercheurs s'appuient sur le paludisme objectifé par le diagnostic parasitologique. Dans les dispensaires, le diagnostic est plus souvent présomptif. Mais, la population a aussi son propre diagnostic (autodiagnostic), basé sur la symptomatologie et souvent, se procure seul le traitement (automédication). Nous avons voulu mesurer dans la population à Dakar la fréquence de ces pratiques et évaluer la prévalence du paludisme-infection. Une femme et un enfant âgé entre 2 et 10 ans issus de 3000 ménages tirés au sort dans la région Dakaroise ont été interrogés sur l'autodiagnostic, l'automédication, la prévention en matière de paludisme. Une goutte épaisse a été réalisée pour chacun. L'analyse intermédiaire portant sur 418 femmes et sur 405 enfants (âge moyen 5,5 ans) montre que 118 (25%) mères ont déclaré un accès palustre dans l'année. 84 (71%) ont consulté au centre de santé et 20 (17%) se sont traités elle-même. Le taux de portage du plasmodium est de 4,06% chez les mères. Pour 29 (17%) enfants, les mères ont porté elle-même le diagnostic et ont directement traité par automédication. 4,69% des enfants héber- gent des plasmodium. 207 (44%) mères ont déclaré dormir sous moustiquaire et 175 (43,2%) pour les enfants. Le taux prévalence du portage asymptomatique indique une faible mais réelle endémicité palustre à Dakar. Toutefois, le paludisme ressentii est beaucoup plus important et amène à une surconsommation d'antipaludique. Il convient d'identifier les déterminants de cet autodiagnostic afin d'adapter les messages d'informations et optimiser la stratégie de prise en charge.

Email address for correspondence: diallaye@yahoo.fr

108 Serological markers can detect heterogeneity in malaria exposure in an area of very low transmission intensity in Somaliland [MIM16689209]

Teun Bousema, Randa M. Youssef, Jackie Cook, Jon Cox, Victor A. Alegana, Jamali Amran, Abdisalan M. Noor, Robert W. Snow, Chris Drakeley

Many parts of Africa are characterised by low malaria transmission intensity. Low endemic areas are suitable for malaria elimination efforts but assessing transmission intensity and evaluating control is difficult due to the low sensitivity of commonly used tools. We evaluated serological markers to detect variants in malaria exposure in low endemic Somaliland. Two cross-sectional surveys were conducted at the end of the dry and wet seasons in three villages located 7–15 km apart. Houses were mapped and parasite carriage was determined by rapid diagnostic test and examination of 200 high power microscopic fields. Antibody responses against P. falciparum and P. vivax MSP-1 and AMA-1 were determined by ELISA. P. falciparum transmission intensity was 0.11 (95% CI 0.04–0.30) infectious bites per person per year. No parasite carriage was determined in participants of the surveys in the dry (0/1178) or wet (0/1128) season. Antibody responses against P. falciparum or P. vivax were detected in 17.9% (179/1002) and 19.3% (202/1045) of the individuals, respectively. Reactivity against P. falciparum was significantly different between the three villages (p <0.001). In the largest village, P. falciparum seroreactivity was negatively associated with distance to the nearest river (OR 0.95, 95% CI 0.90–0.99, p = 0.02). Our data show that serological markers can detect differences between villages at very low malaria endemicity. Serological markers appear as a promising tool to detect spatial variation in malaria exposure and to evaluate malaria control efforts in areas where transmission has dropped to levels where microscopy has lost its discriminative value.

Email address for correspondence: chris.drakeley@lshtm.ac.uk

109 Pregnancy Associated Malaria (PAM) follow-up in Benin: An epidemiological survey [MIM16689806]

Gildas Gbaguidi, Bich-Tram Huynh, Nadine Fiever, Michel Cot, Blaise Guozo-Mévo, Achille Massougbodji, John Lusingu, Nicaise Tuikue Ndiam, Thor Theander, Marita Troye-Blomberg, Adrian Luty, Philippe Deloron

The effects of PAM on pregnant women (placental infection and anaemia), and their babies (low birth weight (LBW)) are well
known. LBW is one of the most important determinants of mortality in African infants. The STOPPAM consortium conducts 2 longitudinal cohort studies in pregnant women in Benin and Tanzania to evaluate the immunopathological consequences of \textit{P. falciparum} infections. The aim of the follow-up is to identify and characterise the \textit{P. falciparum} infections which are most harmful, and that need to be prevented. The timing of \textit{P. falciparum} infections during pregnancy is being monitored and the clinical consequences will be investigated. In Comé, southwestern Benin a longitudinal prospective study of 1000 pregnant mothers is going on. Pregnant women are enrolled ≤ 24 weeks of pregnancy and followed at each ANV until delivery. Clinical and biological investigations are done at each visit (on average 5) and 4 ultrasound investigations will be repeated. The follow-up of pregnant women should allow establishing the relationships between the timing of the peripheral infection during pregnancy and the newborn's birth weight. 500 women are included and 97 have delivered yet. Inclusion and follow-up are on going. The results will be based on women having completed their follow-up before the end of September. We will present general description of the study and first analysis on the women follow-up. At the time of the MIM congress all women will be included in Comé.

Email address for correspondence: gilmart2001@yahoo.fr

### 110 Changing malaria epidemiology and susceptibility to pregnancy associated malaria among a cohort of pregnant women in Korogwe, northeastern Tanzania [MIM16691590]

Daniel Minja, John Lusingu, Pamela Magistrado, Christentze Schmiegelow, Mayke Oesterholt, Charles Tunuka, Nicole Ndám, Marita Troye-Blomberg, Achille Massougbodji, Nadine Fievet, Phillip Deloron, Adrian J.F. Luty, Thor Theander, Martha Schmiegelow

In sub-Saharan Africa, malaria has been one of the major disease burden affecting pregnant women and underfives. Susceptibility to malaria during pregnancy is thought to be promoted by expression of parasite-specific proteins encoded by a large var multigene family. Of late, malaria prevalence has shown a declining trend in many areas which is thought to also follow a similar trend during pregnancy. The effects of malaria during pregnancy are low birth weight babies, maternal anaemia, stillbirths and increased morbidity and mortality among infants born to these mothers. We are longitudinally following up a cohort of 1000 pregnant women attending antenatal clinics at Korogwe District Hospital, northeastern Tanzania. Clinical, ultrasonographic, biochemical, haematological and parasitological parameters are being assessed. Venous blood is being collected to estimate malaria prevalence and other parameters. Sequential samples are being collected at inclusion, subsequent antenatal clinic visits and at delivery. Preliminary results from rapid diagnostic tests (RDT) and microscopy indicate a decline of malaria in the area. A similar trend has been reported elsewhere in children. Overall, in all samples assessed to date, the positivity rate was 11/406 (2.7%). Among the 208/1000 (20.8%) pregnant women recruited, 7/208 (3.4%) had a positive RDT at inclusion and 4/111 (3.6%) at the second antenatal clinic visit. Analyses will be based on assessment of the effect of IPTp, gestational age, transmission season and gravidity. Upon completion of the study, we will not only establish whether there is changing malaria epidemiology but also the extent of the malaria burden among pregnant women.

Email address for correspondence: minja@msl.com

### 111 Declining incidence of malaria in a cohort of children living in Kampala Uganda [MIM16697411]

Denise Njama-Meya, Bridget Nzurubara, Catherine Maiteki-Sebuguzi, Tamara D. Clark, Sarah G. Staedke, Moses R. Kamya, Philip J. Rosenthal, Grant Dorsey

There have been several recent reports of declining malaria incidence in Africa, however, there is limited data on factors associated with declining incidence in well described cohorts. Prospective cohort study of 690 children age 1–10 years at enrollment recruited from a geographically defined area of Kampala, Uganda using probability sampling. Children were followed for a period of 4 years (2005–08) for all of their health care needs. Malaria was defined as a fever and positive blood smear. Genotyping was used to distinguish new infections from recrudescence. Malaria incidence was defined as the number of new episodes of malaria per time at risk. Multivariate analysis was used to identify risk factors for malaria. At enrollment 6% of children reported ITN use. All children were given an ITN in 2006. Malaria incidence (episodes per person year) declined from 0.96 (2005) to 0.80 (2006) to 0.50 (2007) to 0.31 (2008). Malaria incidence was similar for children 1–8.5 years, but decreased by 28% (95% CI 9–45%) in children >8.5–14 years. ITN use was associated with a 29% (95% CI 16–40%) reduction in the incidence of malaria. 69% of the decline in malaria incidence from 2005 to 2008 could not be explained by increasing age or ITN coverage. We observed a 3-fold reduction in malaria incidence in a cohort of children living in Kampala Uganda over a 4 year period. Increasing age and ITN coverage only accounted for a minority of this decline, suggesting other factors were responsible.

Email address for correspondence: denise.meya@gmail.com

### 112 Distinct pattern of class and subclass antibodies in immune complexes of children with cerebral malaria and severe anaemia due to \textit{Plasmodium falciparum} infection [MIM16755079]

Erick K. Mibebe, Walter O. Otieno, Alloys S.S. Orago, José A. Stoute

\textit{Plasmodium falciparum} infection can lead to deadly complications such as severe malaria-associated anaemia (SMA) and cerebral malaria (CM). Children with severe malaria have increased levels of circulating immune complexes (ICs). The exact mechanisms underlying pathogenesis of severe forms of malaria are not fully understood. Children with severe malaria have increased levels of circulating ICs. The study investigated the quantitative and qualitative differences in antibody class/subclass of ICs in SMA and CM. 75 children with SMA and 32 children with CM were enrolled and matched to 74 and 52 control children respectively with uncomplicated symptomatic malaria. IC levels were measured using ELISA protocols and antibody classes/subclasses were identified. ICs were purified using polyethylene glycol precipitation. IgG IC levels were elevated in children with severe malaria upon enrolment; children with CM had the highest levels of any group. Conditional logistic regression showed a borderline association between IgG4-containing ICs and increased risk of SMA (OR = 3.11, 95% CI 1.01–9.56, \(P < 0.05\)). Total IgG-containing ICs (OR = 2.58, 95% CI 1.20–5.53, \(P < 0.02\)) and IgE-containing ICs (OR = 3.27, OR = 1.38–7.78, \(P < 0.01\)) were associated with increased risk of CM. These findings point to differences in the quantitative and qualitative characteristics of ICs in children with SMA and CM and give insight into potential mechanisms of disease, and suggest the class/subclass make up of these ICs as well as the role that they play in each may be distinct.

Email address for correspondence: emibebe@wrp-ksm.org
113 Failure of ACTs to suppress human infectiousness in a malaria holo-endemic area, Rufiji-Tanzania [MIM16757928]

Artemisinins are known to be gametocidal, killing the sexual stage of malaria parasites responsible for infecting mosquitoes. This is an added advantage that can, in principal, reduce the reservoir of parasites that infect mosquitoes. Two large rural sites in Tanzania were chosen to survey measures of human infectiousness in local vector populations. Kilombero was the control site using SP alone as the frontline anti-malaria provided by public sector health facilities while Rufii used Artemisin in combination with SP from March 2003 onwards. In both sites, parallel adult mosquito surveys were conducted from May 2002 to April 2004. Anopheline mosquitoes were dissected to assess oocyst prevalence and tested independently for the presence of sporozoites by ELISA. Logistic regression and analytical models of vector parasite biodemography were used to assess human population infectiousness. Controlling for site, species of mosquitoes, ACT use and year of mosquito collection, the use of ACT increased oocyst prevalence (OR [95%CI] = 3.7 [2.8–5.2], P > 0.001) while having no effect on sporozoite prevalence (OR [95%CI] = 1.09 [0.84–1.39], P = 0.512). Findings from the analytical models correcting for vector biodemography are consistent with increased and unchanged prevalence of oocysts and sporozoites, respectively. Our observations of increased oocyst prevalence hint that drug pressure selection for increased parasite virulence and infectiousness, as suggested by research with animal and theoretical models, may be occurring in human populations in Africa. ACT use alone appears ineffective for controlling human infectiousness in holo-endemic Africa, necessitating complementary vector control measures mosquito-to-human transmission.

Email address for correspondence: bjohn@ili.or.tz

114 Implementing and sustaining strategies for malaria control through the health system: Lessons from the Tanzania Net Voucher Scheme [MIM15090956]
C.O.H. Jones, Y. Sedekia, H. Mponda, T. Marchant, R. Nathan, K. Hanson

Evidence is accumulating on the effectiveness of strategies for delivering ITNs in achieving equitable intervention coverage. However, little attention has been paid to the factors that enhance or constrain successful strategy implementation or longer term sustainability. Financial sustainability is important, but broader institutional and political dimensions are also crucial to developing long term strategies for ITN delivery, particularly in the context of the recent drive towards malaria elimination. Key to this is the acceptability of the strategy among the public health community from global through to district and community levels. Since 2004 the Government of Tanzania has implemented a nationwide voucher scheme to increase coverage of ITNs among pregnant women and children under five. The scheme is implemented through a public–private partnership approach that is new to Tanzania. In-depth interviews were conducted with key stakeholders (National Malaria Programme, District Health Management Teams, Health Workers, community members and the partner NGOs) to investigate the factors enhancing and constraining effective implementation, the acceptability of the approach and the degree of institutionalization of the strategy. The results are used to identify the extent to which the strategy has evolved and become institutionalized in the health system, and to identify the key internal and external factors that affect the acceptability and sustainability of this delivery strategy. Findings are used to discuss the importance, particularly in light of the drive towards elimination, of identifying and managing the factors that enhance and constrain effective implementation of interventions and their sustainability over the longer term.

Email address for correspondence: caroline.jones@lshtm.ac.uk

115 Mapping the private sector supply chain to understand the retail prices and availability of antimalarials in five low-income countries in Africa [MIM15761839]
ACTwatch Study Team

In many low-income settings the private sector plays a crucial role in the delivery of antimalarials, often complementing the formal public health system. In the context of the introduction of a global subsidy to improve access to artemisinin-based combination therapies (ACTs) it is important to understand private sector supply chains and how they vary across countries, as these have an important impact on price and availability, and therefore equitable access. We undertook a supply chain survey collecting volume, price and mark-up data in a representative sample of private sector pharmaceutical wholesalers in 5 countries (Benin, Nigeria, Zambia, Uganda and Madagascar). In each country, approximately 275 structured interviews were conducted across 19 randomly selected sub-districts at various levels of the supply chain. We also conducted reviews of key documents and in-depth interviews amongst key stakeholders. Data were collected January–August 2009. We will present key similarities and differences in the structures of the private sector antimalarial supply chains across countries, and estimates of the volumes of antimalarials – particularly ACTs – flowing through them. Price structures of ACTs and other antimalarials will also be reported. The linkages between prices and supply chain structure will be discussed, and implications of the findings for interventions to improve access to ACTs through the private sector, such as a global ACT subsidy, will be explored.

Email address for correspondence: benjamin.palafox@lshtm.ac.uk
remains sub-optimal. Interventions to improve access to treatment and quality of care, including training private providers, community health workers, and an ACTs subsidy, are potentially cost-effective but will require substantial investments in health systems to become effective in the short and long terms. The findings can inform decisions on funding scaling up and research on malaria control, and help guide policy formulation and implementation. The effects of the Affordable Medicines Facility for Malaria (AMFm), a defining innovation in malaria case management, can be explored. Ultimately, decisions on ACT scale-up strategies must consider the specific local and national context; this work provides a framework for analysis and points to the most influential variables.

Email address for correspondence: valerie.crowell@unibas.ch

117

A snapshot of the price, availability and treatment-seeking behavior in the Democratic Republic of Congo [MIM16668643]

B.A. Atua (MD, MPH), A. Kutekemeni (MPH), L.T. Akulayi (MD, M Fam Med, MPH)

In 2005, Artemisinin Combination Therapies (ACTs) were introduced as the first line treatment for simple malaria in Democratic Republic of Congo. Despite this, it is estimated that only a quarter of children under five suffering from simple malaria receive this recommended treatment. Barriers to this treatment include availability, price, as well as poor treatment-seeking behaviors of caregivers of children under five. In light of the National Malaria Control Program strategies for better implementation of the new antimalarial policy in DRC, four complementary research studies were undertaken to identify different antimalaria drugs locally available. To determine the price and availability of these drugs, a stratified, panel study was conducted across 78 randomly selected health areas (HAs) in October 2008 and April 2009. Within each of the HAs, we conducted a census of all outlets with the potential to sell antimalarials. Outlets were audited using a modified Health Action International questionnaire to address the price, availability, and volumes of antimalarials. Data were collected using Personalised Digital Assistants. To address treatment-seeking behavior, a cross-sectional, population-based household survey was conducted in randomly selected enumeration areas in 2008. A total of 862 outlets were sampled, in which 12.1% were public health facilities, 71.2% “pharmacies”. Results will be presented on the price, availability, quality and volumes of antimalarials; including changes in price and availability. Cross-sectional data of treatment-seeking behavior among caregivers of children under five and antimalarial source and type will also be presented. Implications for interventions to improve access to antimalarials will be explored.

Email address for correspondence: lakulayi@psicongo.org

119

Integrated management of febrile illness in children: How much would confirmation of malaria contribute to improved treatment outcomes? [MIM16679405]

Frank Baiden, Seth Owusu-Agyei, Jane Bruce, Christopher Whitty, Daniel Chandramohan, Jayne Webster

With the shift to relatively expensive artemisinin-based combination therapy (ACT) for treatment, and growing recognition of the clinical over-diagnosis of malaria, momentum is growing for the introduction of confirmatory tests, specifically rapid diagnostic tests (RDTs). Confirmatory tests are one of a number of intermediate steps in the effective case management of a febrile child. We quantify the relative impact of each of the intermediate steps on the treatment outcomes. Structured observations and exit interviews (n = 1500) of management of febrile children were conducted in a representative sample of 5 hospitals and 10 health facilities from 6 districts of Brong Ahafo Region, Ghana. A randomly selected cohort of febrile children (n = 600), identified at exit interviews, was followed up to measure the adherence to treatment and health outcomes. A deterministic model of the intermediate steps (consultation and prescription, laboratory testing, post-laboratory consultation and prescription, dispensing, understanding of prescription, adherence to treatment) in the causal chain of health outcomes will be developed and the effect of each step will be quantified. This presentation discusses the relative effect of different health delivery system determinants on treatment outcomes of management of febrile children using IMCI or IMCI plus confirmative test for malaria. The findings will provide an evidence base for focusing interventions to improve effective case management to the steps within the causal chain that will yield the greatest impact.

Email address for correspondence: baidenf@yahoo.co.uk

120

Intermittent preventive treatment of malaria in pregnancy: The incremental cost-effectiveness of a new delivery system in Uganda [MIM16690065]

A.K. Mbonye, K.S. Hansen, I.C. Byghjerg, P. Magnusson

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

Email address for correspondence: kristian.hansen@ishtm.ac.uk

121 Malaria rapid diagnosis performance by community health workers, Zambia [MIM16690330]

Helen Counihan, Masela Chinyama, Steve Harvey, Hawela Moonga, Fred Masaninga, Elizabeth Chizema, David Bell

As countries move towards elimination of malaria, many malaria-endemic countries realise the importance of home management of malaria (HMM) in rural settings to reduce mortality in children. However, there are concerns about blood-safety and accuracy of diagnosis when community health workers (CHWs) use malaria rapid diagnostic tests (RDTs). This Zambia-based study tested whether CHWs can use malaria RDTs correctly and safely following a half-day proficiency-based training programme. 65 CHWs in Livingstone district received training and were then sent home with all materials necessary to implement HMM. CHW performance was assessed in their homes by observers at 3, 6, and 12 months post-training using a structured checklist. This included assessments to ensure test results were interpreted correctly and that safe procedures for handling blood and disposing of biohazardous waste were followed. Despite minimal supervision, most CHWs performed all essential steps correctly at 6 months, and performance remained very high at 12 months. Some individuals required extra support and refresher training, demonstrating that training works best with follow-up support and supervision. The main challenges were less correct interpretation of results or safety but more a lack of support from health facilities and RDT stock-outs. The study provides a model for other countries as they roll out community-based diagnosis and treatment of malaria. It demonstrates how a proven strategy of using a well-structured training and job-aid is important in enabling CHWs to use malaria RDTs accurately and safely in a sub-Saharan setting at community level.

Email address for correspondence: h.counihan@malariaconsortium.org

122 Potential cost savings by improving malaria diagnosis in low and moderate transmission settings in Tanzania [MIM16692781]

Jacklin F. Mosha, Lesong Conteh, Jane Bruce, Samwel Gesase, Ramadhan Hashim, Chris Drakeley, Brian Greenwood, Daniel Chandramohan, Roly D. Gosling

Over-diagnosis of malaria contributes to improper treatment, wastage of drugs and increased rates of drug resistance. We investigate the cost implications of over-diagnosis of malaria among children attending dispensaries in rural Tanzania. Estimation of over-diagnosis of malaria was obtained by comparing 3 data sources: from routine Health Management Information System (HMIS) registers, from cross-sectional RDT malaria surveys and surveillance from a clinical trial of Intermittent Preventive Treatment of malaria in infants (IPTi). The cost presented compare the IPTi study participants patterns of diagnosis with 2007 HMIS data for under fives. Drug and diagnostic costs are modelled using local and international prices. A comparison of the distribution of causes of morbidity diagnosed in under 5 years old children in low transmission site in the IPTi study 0.08% were diagnosed with malaria, 41.7% ARI compared to the HMIS data malaria 28.2% and ARI 26.3%. In high transmission site in IPTi study 9.8% children had malaria, 39.4% ARI compared to HMIS data malaria 44.3% and ARI 18.4%. The use of RDTs to ensure accurate outpatient diagnosis and subsequent treatment of malaria and non-malaria cases could save between 3 and 8% of a district drug budget in areas with high transmission of malaria, and in areas of low transmission by 13%. This study shows the cost effectiveness of improving the quality of diagnosis. It also suggests that respiratory tract disease and diarrhoea are still major causes of morbidity in Africa even in areas of high transmission of malaria.

Email address for correspondence: jfmosha@yahoo.com

123 Species identification errors and false negative results [MIM16669413]

P. Obare, E. Wagar, B. Ogutu, D. Walsh, C. Ohrt

Management of malaria cases is currently centered on microscopy. Although it remains the ‘reference standard’, incompetence of malaria microscopists diminishes its potential as a valid diagnostic tool. We explore effect of species errors on microscopy results. 104 participants attending malaria microscopy workshops organized by KEMRI/WRP, Malaria Diagnostics Centre of Excellence, Kisumu, Kenya between May and October 2008 were tested by way of pre- and post-tests with 20 known positive blood slides consisting of 10 P. falciparum, 5 P. malariae, 3 P. ovale and 2 P. vivax and densities between 1000 and 30,000 parasites/µL. Slides were examined for 5 min each, presence or absence of parasites recorded and species indicated. Accuracy of test outcome was evaluated based on positive or negative results and correct species identification. Of 2080 total observations generated in the pre-test, 23.4% (486/2080) were incorrectly reported as negative. 37.1% (386/1040) of P. malariae, 0.4% P. ovale and 0.4% P. vivax observations combined were false-negative results, significantly greater (p < 0.001) than 9.6% (100/1040) of P. falciparum reads incorrectly reported as negative. Post-test false-negative results decreased significantly (p < 0.001) to 0.4% (8/2080), with other species and P. falciparum contributing 0.6% (6/1040) and 0.2% (2/1040) respectively. Inability of microscopists to identify uncommon parasite species present on blood films increases false-negative results in routine laboratory settings. Training in species identification is critical with regard to reduction in false-negative results.

Email address for correspondence: pobare@wrp-ksm.org
124 The quality of paediatric malaria case-management under artemether-lumefantrine treatment policy in Kenya, Uganda and Zambia [MIM15050646]
Dejan Zurovac, Julius Ngigi, Willis Akhwale, James Tibenderana, Joan Nankahirwa, Micky Ndhlovu, Nawa Sipilanyame, Robert W. Snow

Case-management with artemisinin-based combination therapies (ACTs) is one of the recent, key strategies to control malaria in Africa. Yet, reports on translation of ACT implementation activities into clinical practice are scarce. Cross-sectional, health facility surveys, using a range of quality-of-care assessment tools, were undertaken between 2006 and 2007 in Kenya, Uganda and Zambia. Main outcome measures were health facility and health worker readiness to implement artemether-lumefantrine (AL) treatment policy, the quality of paediatric AL prescribing, counselling and dispensing in comparison to national guidelines, and factors influencing AL prescribing. 492 facilities, 594 health workers, and 2526 paediatric outpatient consultations were evaluated in three countries. Health facility and health worker readiness varied between countries: 60–89% of facilities stocked AL, 41–79% of health workers were trained on AL and 55–92% had access to guidelines. AL was prescribed to 26% of children in Kenya, 35% in Zambia and 66% in Uganda. No significant improvement was observed at facilities with AL in stock on the survey day. When AL was prescribed, 89–95% of children were prescribed correct weight-specific dose. Only in Zambia, the majority of AL counselling and dispensing tasks were performed for more than 50% of children. Routine AL implementation activities were rarely associated with better prescribing. Across all countries, the quality of AL case-management is not yet optimal. Strengthening weak health systems, rigorous testing of innovative quality improvement interventions and their effective translation into clinical practice should be high priority in all countries implementing ACT policies for malaria.
Email address for correspondence: dzurovac@nairobi.kemri-wellcome.org

125 Withdrawing antimalarials in febrile children with a negative Rapid Diagnostic Test is safe in moderately and highly endemic areas of Tanzania [MIM16672170]

To be on the safe side, clinicians tend to treat presumptively with antimalarials all febrile patients, regardless of age, availability of microscopy or endemicity. Objective: To evaluate in two uncontrolled settings the safety of withdrawing antimalarials in febrile children with a negative RDT for malaria. We recruited all children attending a dispensary for the first time with fever <48 h. RDT was performed and, if negative, no antimalarial prescribed. At day7, all patients were assessed at home if cured or not. When ill in-between or not cured at day 7, patients were retested for malaria and assessed at day 14. Among the first 600 children analyzed (median age 25 months), 198 (33%) had a positive and 402 (67%) had a negative RDT. 13/600 (2%) children could not be followed up. 569 (97%) were completely cured at day 7 and 581 (99%) at day 14. 3% and 8% of the initially positive and negative RDT children respectively attended spontaneously the dispensary in-between, 82% because of persisting fever. All children with an initial negative RDT had again a negative result, except one who became positive at day 4. Among the total 1000 children recruited, 4 children with an initial negative RDT were admitted with RDT/blood slide still negatives. Two died. Not giving antimalarial drugs in febrile children with a negative RDT result is safe. By using RDT as sole test for diagnosis, no patient was admitted or died because malaria was missed. Our study provides further evidence for a revision of the WHO policy of blanket antimalarial treatment in children.
Email address for correspondence: aggrey.malila2002@yahoo.co.uk

126 Affordability leads to dramatic uptake of effective malaria treatment for under-fives in Uganda [MIM16697373]
Ambrose Talisuna, Penny Grewal, Andrew Balyeku, Renia Coghlan, Jaya Banerji, Susan Mukasa, John Bosco Rwakimari, David Nahamya, George Jagoe

Artemisinin combination therapies (ACTs) are unaffordable and thus inaccessible to most Ugandans through the private sector. Yet the private sector is the first port of call for about 60% of all Ugandans seeking treatment in event of fever. The Affordable Medicines Facility, malaria (AMFM) provides a framework to close this critical affordability gap. The Ministry of Health Uganda and Medicines for Malaria Venture (MMV) initiated a pilot study to test whether ineffective treatments could be displaced by highly subsidized ACTs provided through the private sector. The study, similar to the AMFM design, was carried out in 4 high transmission districts and one control area. In addition, a number of supporting interventions were implemented to ensure large-scale availability and correct dispensing and use. Exit interviews were conducted at regular intervals at drug shops to document purchase of antimalarials. The subsidized ACT accounted for 43% of antimalarials purchased for children under 5 years from licensed drug shops. It halved the market share of chloroquine from 40% to 19% and of SP from 50% to 28%. Chloroquine and SP remained the key products purchased for children under five from unlicensed drug shops. There was no evidence of price gauging. Affordability is a precondition for improving access to effective treatment. It drives uptake of ACTs by drug shops and patients. However, other barriers also need to be addressed such as licensing criteria which limit availability of ACTs.
Email address for correspondence: banerji@mmv.org

127 Intermittent preventive treatment during pregnancy reduces neonatal mortality in Mozambique [MIM16690615]
Clara Menendez, Azucena Bardaji, Betuel Sigauque, Sergi Sanz, John Aponte, Pedro L. Alonso

It is accepted that malaria in pregnancy impacts neonatal survival mainly through a reduction in birth weight. However, there is no direct evidence to support this association. 997 of the 1004 live born babies (99%) to 1030 enrolled Mozambican pregnant women into a randomised, placebo-controlled trial of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) (500 in the placebo group, 497 the IPTp-SP group), were followed up until 12 months of age. There were a total of 58 infant deaths [35 (60.4%) born to women who had received placebo and 23 (39.6%) to women who received IPTp (p = 0.136)]. There were 25 neonatal deaths, of which 18 (72%) were babies born to women in the placebo group and 7 (28%) born to women who received IPTp (p = 0.041). Of the 25 neonatal deaths, 20 (80%) occurred in the first week of life, of which 15 (75%) were babies born to women in the placebo group and 5 (25%) in the IPTp group (p = 0.022). It has been observed for the first time that malaria prevention in pregnancy can reduced neonatal mortality. This indicates a direct negative effect of malaria
on neonatal survival. The protective effect of the intervention was concentrated in the first week of life. Mechanisms associated with higher malaria parasitemia at the end of pregnancy may explain the excess mortality. These results are of public health relevance to promote malaria prevention during pregnancy, and contribute in the understanding on how malaria may affect fetal and neonatal health.

Email address for correspondence: menendez@clinic.ub.es

128 Massive reduction of antimalarial prescription after Rapid Diagnostic Test implementation in Dar es Salaam, Tanzania [MIM16690331]

C. Lengeler, V. D’Acremont, J. Kahama-Marco, N. Swai, D. Mtasiwa, B. Genton

Presumptive treatment of febrile children with antimalarials leads to over-treatment and wastage of drugs, especially in moderate/low endemic areas. Laboratory-confirmed diagnosis should reduce antimalarial consumption, provided test result is taken into account by clinicians. We aimed to assess the effect of implementing malaria-RDTs as first-line diagnostic tool in routine management of febrile patients living in a moderately endemic area on prescription of antimalarials [artemether/lumefantrine (AL)]. After training health workers in 3 hospitals, 3 health-centers and 3 dispensaries, RDTs were introduced. Three similar health facilities without RDT implementation were used as controls. Consultation processes were observed before and 18 months after RDT initiation. Data on antimalarial use were extracted from ledger books of storage places in each health facility. When comparing consumption of AL during three months prior to RDT implementation with eighteen months post-initiation, there was a mean of 6-fold (range: 2–26) decrease in intervention and 1.7-fold decrease in control health facilities. Proportion of patients tested negative who were still prescribed antimalarials decreased from 67% to 7%. For 100 patients attending with medical problems, 57 antimalarial treatments could thus be saved. Among febrile patients, proportion of tested patients increased from 72% to 90%. Programmatic implementation of RDT in a moderately endemic area where microscopy is available, reduced drastically overtreatment with antimalarials. Properly trained clinicians with adequate support complied with the recommendation of not treating patients with negative results. RDTs used as first-line diagnostic tool have a huge potential for reducing inappropriate prescriptions and improve management of patients.

Email address for correspondence: christian.lengeler@unibas.ch

130 Ranking malaria factors to guide malaria control efforts in Africa highlands [MIM16689614]

Dismas Baza

Malaria is re-emerging in most African highlands. Understanding factors’ driving this change is needed to develop appropriate strategies for malaria control. A detailed conceptual model for malaria risk factors in the highland will be presented and the hierarchical importance of this factors evaluated based on the Burundi case Literature review describing potential malaria risk factors was used to build a conceptual model. The Classification And Regression Trees (CART), an unexploited statistical method was the basis to determine the relative impact of identified risk factors on malaria. Data used were collected through periodic surveys conducted in the highland province of Karusi, Burundi between 2002 and 2007. The conceptual model update what is known on highlands malaria. The CART methods showed that Anopheles density was the best predictor for high malaria prevalence in Burundi highlands. Lower rainfall, no vector control, higher minimum temperature and houses near breeding sites were associated by order of importance to higher Anopheles density. In the highlands of Burundi, the follow up of the residual Anopheles densities when rainfall is low could be helpful in predicting or in the early detection of epidemics. The conceptual model combined with the CART analysis could be a relevant decision support tool that may improve the prevention or control of malaria by identifying major risk factors.

Email address for correspondence: dismas.baza@yahoo.fr

131 A retrospective cohort study among pregnant women to measure the effectiveness of current malaria control guidelines on the prevention of malaria in pregnancy [MIM16729700]

Peter Ouma, Kephas Otieno, Simon Kariuki, Shi Ya Ping, Marta Ackers, Laurence Slutsker, Mary Hamel

Malaria in pregnancy causes maternal anemia and low birth weight, a risk factor for infant mortality. Strategies to prevent malaria in pregnancy include insecticide treated nets (ITNs) and intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP). In areas where both malaria and HIV are common, WHO recommends that HIV-infected women receiving daily cotrimoxazole (CTX) prophylaxis do not receive SP. CTX prevents malaria in non-pregnant adults and twice daily CTX treats malaria in children but there are no data showing whether daily CTX prevents malaria in pregnancy. We are enrolling women presenting to labour and delivery ward in two hospitals in western
Kenya. Participants are categorized based on their drug exposure during pregnancy as follows: HIV-infected women who took CTX, CTX + SP or took neither and HIV–uninfected women who took IPTp with SP or no SP, as determined by the questionnaire and ANC card. Our primary outcome measure is prevalence of placental parasitemia. Secondary outcome measures are prevalence of peripheral parasitemia and anaemia. Preliminary results of this ongoing study show the prevalence of peripheral malaria parasitaemia among 174 HIV-infected participants who took CTX was 9% (7/82), SP 10% (5/53), and neither 14% (1/7). Prevalence of peripheral parasitaemia among 557 HIV-uninfected who took SP was 12% (62/500), and HIV on neither 22% (14/65). Prevalence of placental parasitaemia may not correspond to peripheral parasitaemia and will be presented at the conference. Study data are expected to validate the current guidelines or recommend further evaluation of alternative strategies for malaria prevention during pregnancy.

Email address for correspondence: sgwer@kilifi.kemri-wellcome.org

132  
Continuous EEG monitoring and seizures in children with cerebral malaria [MIM15307236]

Samson Gwer (MBChB), Richard Idr0 (MMED), Godfrey Otieno (BSc), Edwin Chengo Dip, Mwanamvua Boga (BSc), Samuel Akech (MBChB), Kathryn Maitland, Piet (PhD) A. Kager (PhD), Fenella Kirkham (FRCPCH), Brian G.R. Neville (FRCPCH), Steve White (MD), Charles R.J.C. Newt

Multiple seizures are a risk factor for poor outcome in children with cerebral malaria. Continuous EEG (cEEG) in patients with other causes of coma found a high prevalence of non-convulsive seizures. The aim of this study was to describe features on cEEG in children with cerebral malaria, relate specific EEG features to outcome and compare clinical seizure detection with that of cEEG. Fifty-two children with cerebral malaria were enrolled to undergo cEEG monitoring. Clinical seizures were recorded on a standard proforma. EEG records were analyzed by at least 2 clinicians and non-congruent results re-examined. At discharge, neurological assessment was performed to detect neurological deficits. The background EEG was characterized by very slow generalized high amplitude waves. A total of 372 seizures (149 [40%] electroclinical and 223 [60%] electrographic) were detected in 20 (38.5%) children. Four children had 10 short-lived clinical seizure-like events in which epileptiform activity was not detected on cEEG. Most seizures occurred in 7 children admitted with status epilepticus. Fifty-six percent were of focal origin. Seizures involving the whole hemisphere were common among the seven with repeated seizures. A higher frequency of the background EEG on admission was associated with faster resolution of coma while background asymmetry, rhythmic runs and electrical status epilepticus were associated with poor outcome. Electrographic seizures and electrical status are common in children with convulsive status epilepticus due to cerebral malaria. Electrical status epilepticus is associated with poor outcome. An admission EEG may help determine prognosis.

Email address for correspondence: sgwe@kilifi.kemri-wellcome.org

134  
Effectiveness of quinine versus arteether-lumefantrine for treating uncomplicated falciparum malaria in Ugandan children: A randomised trial [MIM15927411]

Jane Achan, James Tibendenrana, Daniel Kyabagye, Fred Wabwire Mangeni, Moses R. Kamya, Grant Dorsey, Umberto D’Alessandro, Philip J. Rosenthal, Ambrose O. Talisuna

This study was conducted to compare the effectiveness of oral quinine with that of arteether-lumefantrine in treating uncomplicated malaria in children. Randomized, open-label effectiveness study at the outpatient clinic of Mulago Hospital, Uganda’s National Referral Hospital. 175 children aged 6–59 months with uncomplicated malaria were randomised to receive either oral quinine or arteether-lumefantrine administered by parents or guardians at home. Primary outcomes were parasitological cure rates after 28 days of follow-up unadjusted and adjusted by genotyping to distinguish recrudescence from new infections; secondary outcomes included adherence to study medication, presence of gametocytes, haemoglobin recovery and safety profiles. Using survival analysis, the cure rate unadjusted by genotyping was 95.9% for the arteether-lumefantrine group compared to 64.7% for the quinine group (HR 10.7, 95% CI 3.3–35.5, p = 0.001). In the quinine group 18 of 26 (69%) parasitological failures were due to recrudescence compared to none in the arteether-lumefantrine group.

www.mimalaria.org
The mean adherence to artemether-lumefantrine was 94.5% compared to 85.4% for quinine (p = 0.0008). Having adherence levels of ≥80% was associated with a decreased risk of treatment failure (HR 0.44, 95% CI 0.19–1.02, p = 0.06). Adverse events did not differ between the two treatment groups. The effectiveness of a 7-day course of quinine for the treatment of uncomplicated malaria in Ugandan children was significantly lower than that of artemether-lumefantrine. These findings call into question the advisability of the recommendation for quinine therapy for uncomplicated malaria in Africa.

Email address for correspondence: achanj@yahoo.co.uk

135

Efficacy and cost-effectiveness of malaria prevention in pregnancy in low and unstable transmission: Results of a randomised controlled trial [MIM16646779]

Richard Ndyomugyenyi, Kristian Schultz Hansen, Coll Hutchison, Daniel Chandramohan, Pascal Magnussen, Siân Clarke

Most intervention trials to control malaria during pregnancy have been conducted in areas of intense transmission. Less work has been carried out in areas of low transmission, where malaria is less frequent but the risk of spontaneous abortion and stillbirth is high due to limited maternal immunity. Data on cost-effectiveness in low transmission settings is also lacking. A randomised trial in the epidemic-prone highlands of SW Uganda, to compare the efficacy and cost-effectiveness of three strategies to control malaria during pregnancy: (1) intermittent preventive treatment (IPT); (2) insecticide treated nets (ITNs); and (3) IPT combined with ITNs. Maternal and birth outcomes were recorded for 5226 (90%) women. Peripheral parasitaemia at term was 10%, however there was no evidence of a difference between the three interventions on the risk of low birth weight or maternal anaemia. This was not attributable to poor compliance or differential loss to follow-up. Additional data on the incidence of abortion, stillbirth and neonatal death in this population, and the relative cost-effectiveness of the three interventions will be presented. Our findings indicate that in areas of low and unstable transmission the protective benefit of using IPT and ITNs in pregnancy is comparable, and no additional advantage is gained by using a combination of the two. The comparative cost of delivering each intervention, as well as the wider public health benefits of ITN use, may be more relevant considerations in formulating policy for malaria prevention in pregnancy in areas of low transmission.

Email address for correspondence: richardndyomugyenyi@yahoo.com

136

High frequency of Plasmodium falciparum CICNI/SGEEA and CVIET haplotypes without association with resistance to sulfadoxine/pyrimethamine and chloroquine combination in the Daraweesh area, in Sudan [MIM15093738]


Estimation of the prevalence of the molecular markers of sulfadoxine/pyrimethamine (SP) and chloroquine (CQ) resistance and validation of the association of mutations with resistance in different settings is needed for local policy guidance and for contributing to a global map for anti-malarial drug resistance. In this study malaria patients treated with SP alone (60) and SP with CQ (194) had a total treatment failure (TF) of 35.4%, with no difference between the two arms. The polymerase chain reaction–enzyme-linked immunosorbent assay (PCR–ELISA) method was used to identify polymorphisms in 15 loci in the dhfr, dhps and pfcrt genes in a subset of 168 infections. Patient informed consent was a prerequisite, and institutional and national ethical clearance from the Ministry of Health were obtained. Results revealed a similar frequency of all single nucleotide polymorphisms (SNPs) in the two arms, except dhps 581G, which was over-represented in infections that failed to respond to SP alone (TF). In all infections, a high frequency of dhfr CICNI haplotype (51I and 108N) was found, but without discrimination between the adequate clinical and parasitological response (ACPR, 75.6%) and TF (82.9%). Similarly, the dhps SGEEA haplotype (437G and 540E) (ACPR, 60.5%; TF, 65.9%) and the combined CICNI/SGEEA haplotype (ACPR, 50%; TF 55%) were not associated with TF. In contrast to other studies in Africa, the triple 51I/59R/108N mutation was rare (0.6%). In addition, the pfcrt CVIET haplotype (93%) was found to be associated with the CICNI/SGEEA haplotype.

Email address for correspondence: ishraga20@yahoo.co.uk

137

Hypoglycaemia in severe malaria, aetiological associations and relationship to quinine dosage [MIM15051232]

Gilbert Ogetii et al.

Hypoglycaemia is an independent risk factor for death in severe disease and may impact on long-term neurological impairment. A higher frequency of this complication in severe malaria was noted in adults treated with parenteral quinine compared to Artesunate. No similar data are available for children. In 2006 WHO recommendations for quinine regimen in children changed from 15 mg/kg loading (plus 10 mg/kg bd) to 20 mg/kg loading (plus 10 mg/kg tds). This increases fears that increased episodes of hypoglycaemia may result. To determine frequency of hypoglycaemia in children with severe malaria treated using the former and current parenteral quinine regimen. To establish factors associated with a hypoglycaemia episode. Case review of all admissions to the HDU with severe malaria treated using the former and current parenteral quinine regimen. To establish factors associated with a hypoglycaemia episode. Case review of all admissions to the HDU with severe malaria between April 2002 and March 2006 and those of children admitted since April 2006 when the new WHO regime was introduced. Over the first 48h of admission to HDU glucose level is monitored routinely 4 hourly. Hypoglycaemia, defined as a blood glucose ≤3.0 mmol/l, was detected using bedside monitoring devices. Clinical events immediately preceding or concurrent with each episode were recorded 955 with severe malaria received the old quinine regime and 246 received the new regime from April 2007 to October 2008. Earlier findings showed a higher prevalence (The data on the prevalence of hypoglycaemia for the two different regimes is work in progress and will report in full the findings at the MIM conference.) of hypoglycaemia episodes in the group treated with new quinine regime. We found that respiratory distress (18% and 23%), shock (33% and 33%), coma (58% and 61%), disruption of maintenance fluids and/or blood transfusion (37% and 31%) were associated with hypoglycaemia rather than seizures (9% and 10%) or posturing (7% and 10%), old and current regime respectively. The higher quinine dosage could be associated with higher frequency of hypoglycaemia episodes. More than half of the episodes were associated with either coma or circulatory failure. Disruption of maintenance fluids and/or blood transfusion was associated with a third of the hypoglycaemia episodes. Hypoglycaemia should be sought in unconscious children and those with features of circulatory failure, and also when there is disruption of maintenance fluids e.g. during transfusion.

Email address for correspondence: gogetii@kilifi.kemri-wellcome.org
138  
**A Participatory Action Research Programme aiming to make conducting malaria trials easier and guide the development of skills for African clinical trialists [MIM16168029]**

Trudie Lang

Clinical trials establish the evidence base for prevention and treatment of disease. They are critically important in resource poor settings because this is where there is greatest potential for improving health. This is especially true for malaria trials where sites need to engage constructively together through research to make locally run trials less daunting, expensive and cumbersome. A high level of activity in both malaria drug and vaccine trials. However disease management trials are equally important. To encourage more locally led trials we need to support researchers and provide skills and guidance in setting questions and operating protocols. We are undertaking a large programme of participatory action research around clinical trials in Tropical Medicine. This highly collaborative research effort combines situation analysis with specific research activities around key areas of trial conduct which will lead to the development of a range of open access tools, training resources and courses available for clinical researchers in resource limited settings. A website will enable free and open access. These data will help sites as can be applied to both locally led and externally sponsored trials. The sponsors can be confident that the tools and guidelines generated are compliant to regulatory requirements whilst having been locally derived and tested to be appropriate, acceptable and manageable. This programme cuts across all diseases to help sites develop broad interests and allow for sharing of best practice, as trials face similar issues. There are many opportunities for researchers to get involved.

Email address for correspondence: trudie.lang@ndm.ox.ac.uk

139  
**Developing an African framework for ethics and best practices in clinical research: The AfroGuide Project [MIM16773292]**

Francis P. Crawley

In March 2008 the Good Clinical Practice Alliance (GCPA) launched an African-wide project on ‘Developing Guidelines for Health Research in Africa (AfroGuide)’ at an international conference jointly organised by the United Nations Economic Commission for Africa (UNECA) and the African Union (AU). The AfroGuide Project addresses an urgent need to develop the legal, regulatory, and ethical frameworks for best practices in science and ethics in African clinical trials. The AfroGuide Project is constituted of a Steering Committee and Working Groups of Africa experts in health science and research ethics with the support of international collaborating partners. At a project organisation meeting organised by the UNECA, AU, and GCPA held in Addis Ababa in November 2008, three draft guidance documents prepared by the GCPA were reviewed: (1) An African Guideline for good clinical practice. (2) An African Declaration on Ethics in Health Research involving children. (3) An African Model Law on health research. The AfroGuide Project outcomes will contribute to the development of the regulatory framework, facilitating health research and human subjects protections in African clinical trials. The aim of this presentation is to engage the African and global malaria community in the discussion on developing best practices in the science and ethics of health research in Africa.

Email address for correspondence: fpc@gcpalliance.org

140  
**Feasibility assessment for the initialization and development of malaria-related computational chemistry research in Sub-Saharan Africa [MIM16647232]**

Liliana Mammino

The initialization and development of computational chemistry studies of molecules that can be promising as lead compounds for drug-development (including molecules identified from materials utilized in traditional medicine) can bring significant contributions to the overall capacity building for malaria research in African institutions. A feasibility assessment based on concrete experience is expected to provide benchmarks useful for analogous developments in other institutions. The initialization and development of computational chemistry studies of molecules that can be promising as lead compounds for drug-development (including molecules identified from materials utilized in traditional medicine) can bring significant contributions to the overall capacity building for malaria research in African institutions. A feasibility assessment based on concrete experience is expected to provide benchmarks useful for analogous developments in other institutions. Feasibility is realistic. The research capacity-building process can be designed and developed in close correspondence with the main stages of the investigation of biologically active compounds (from the study of a number of compounds of a given class sufficient to provide information enabling the further steps, through QSAR to the identification of lead structures), so that the capacity-building process practically coincides with a full research project realization. Feasibility is ascertained. The dominant dependence of the capacity-building process on human resources underlines the importance of networking and partnerships, to overcome drawbacks from scarcity of experts.

Email address for correspondence: liliana@univen.ac.za

141  
**Monitoring adherence to human subject protections in research protocols is a major challenge in post-conflict Liberia [MIM16869930]**

B.A. Robert Draper (BA), Edward G. Smith (M.Phil), Ellen George-Williams (MSN), Cecelia Morris (MSN), Jemee K. Tegli (BA), James N. Kollie (PhD), Stephen B. Kennedy (MD, MPH)

Operating an Institutional Review Board (IRB) in a post-conflict, resource-constraint setting is a complex enterprise that encompasses social, political, cultural and/or legal implications. The task of recruiting and retaining competent professionals for voluntary services on an IRB is a challenging process. IRBs lacked required incentives for its members; and as such, recruiting professionals of diverse expertise becomes a significant challenge. The interpretation and transcription of conventional Western instruments into feasible local ones that considers the social, traditional and cultural contexts, while retaining the scientific content and ethical standards, can be a perplexing factor. In addition, monitoring of research activities in these settings is even a daunting challenge. However, individual sacrifice and commitment to research can change the attitudes of professionals to serve on ethical boards in these countries. Therefore, securing the commitments of local experts to serve can have a lasting impact on the lives of subjects enrolled in research studies in developing countries. Within this context, the University of Liberia Institutional Review Board (UL-IRB) was established in 2005 to regulate ethics in social, behavioral and educational research in Liberia. It has a membership of seven (7) professionals with diverse expertise. These activities were funded by grant #s R01 HD 045133 (PI: Kennedy) from the National Institute of Child Health & Human Development (NICHD)

www.mmalaria.org
Malaria Clinical Trials Alliance, a program of the INDEPTH Network was launched in 2006 with two broad objectives; to facilitate the timely development of sufficient near-term capacity in Africa to conduct GCP clinical trials for malaria vaccines and drugs by Alliance partners and support, strengthen, mentor and network trial sites to facilitate their progression towards self-sustaining clinical research centers. The Alliance has been involved strengthening the capacity of 16 clinical trials sites in 10 African malaria endemic countries. We visited all the sites to perform a needs assessment and familiarize itself with the site leadership and research processes at the site. These sites were those that were already working with Malaria Vaccine Initiative and Medicines for Malaria Venture in malaria vaccines and drug development respectively. The Alliance has supported infrastructure refurbishment and organized workshops for GCP, pre-certification Association of Clinical Research Professionals exam and AMANET, malaria diagnosis, strategic management, and media training workshops. The Alliance has facilitated visits to political leadership within the countries the sites are located in increase visibility of the sites and lobby future government support. The site assessment outcomes and impact of Alliance support in strengthening clinical trials capacity will be discussed.

Email address for correspondence: bernhards.ogutu@indepth-network.org

Central Africa network on tuberculosis, HIV/AIDS and malaria (CANTAM) [MIM17470642]

Francine Ntoumi

In 2007, in response to an EDCTP call for the support of regional network of excellence, institutions from Cameroon, Congo, Chad and Gabon came together with the support of the University of Tubingen, the Multilateral Initiative on Malaria and OCEAC to establish the Central Africa Network on Tuberculosis, HIV/AIDS and Malaria (CANTAM) for the conduct of clinical trials. The strategy of CANTAM is to develop clinical research in the weakest institutions through the development or strengthening of collaborations and mentorship programs with the regional established institutions. The major operational objective of CANTAM is to strengthen research infrastructure in Brazzaville (BZV) and the University of Buea so as to provide additional regional capacity to carry out future malaria and HIV/AIDS clinical Phases I, II and III trials in compliance with ICH/GCP standards. In the first implementation phase, a consultative meeting on tuberculosis financially supported by French institutions was convened in Brazzaville. Invited experts from Africa (Cameroon, Republic of Congo, Gambia, Tanzania, Gabon) and Europe (Portugal, France, Germany) assisted regional TB groups in the development of a relevant strategy to initiate baseline studies and training programs in Congo and Cameroon. Additionally, CANTAM is actively networking with African organizations which have already developed educational and training programs on clinical trials like AMANET and MCTA. Open calls for the recruitment of trainees have been launched in Congo and Cameroon and the groups are in the process of selecting the best students who will be enrolled in sandwich programs with African and/or European partners. A poor research environment, lack of research culture in some cases and the great expectations from junior researchers and local authorities are part of the challenges that CANTAM will have to overcome so as to be able to move ahead; strengthening and not building local capacities.

Email address for correspondence: francine.ntoumi@amanet-trust.org

ISHReCA: Advocating for health research capacity in Africa [MIM17571947]


Africa bears the largest proportion of the world’s burden of disease that greatly contributes to the economic stagnation seen in many parts of the continent. While multiple socio-political factors contribute to the current status of health in Africa there is no doubt that good research is key to the development of urgently needed disease control tools. Yet research systems in many parts of Africa are either very weak or wholly neglected with low commitment of funding by domestic government. Undisputably, Africa lacks the critical mass of personnel necessary to undertake research, lead, manage and sustain strong research institutions. Massive efforts are therefore required to build and strengthen Africa’s capacity to do health research. Unfortunately, current research capacity building activities are fragmented and often aimed at meeting the needs of specific short term research programmes and therefore tend to focus on one aspect of capacity building generally personnel training while neglecting others such as infrastructural strengthening. Out of the need to address the issues mentioned above within a formal framework and the current drive among both African researchers and funding bodies towards more harmonise funding strategies in order to avoid duplication, the Initiative to Strengthen Health Research Capacity in Africa (ISHReCA), which is an African-led initiative was created. In this paper we present the vision, mission and roadmap of ISHReCA to make African researcher play a more central role in health research in the continent.

Email address for correspondence: masumben@gmail.com

The Declaration of Helsinki (2008): What researchers in Africa should note [MIM16755167]

Godfrey Tangwa

Another version of the Declaration of Helsinki (DoH) has recently been adopted in Seoul, South Korea, on 11 October 2008, by the 59th General Assembly of the World Medical Association. Although the buildup to this eighth revision of the DoH was not as noisily contentious and controversial as that of its immediate predecessor in 2000, the very same issues were at the background and it is already evident, given the various reactions to the new version of the Declaration, that these issues have not, perhaps cannot, be laid to rest once and for all. In this presentation, I will highlight what is new in the 2008 version of the DoH by comparison with the 2000 version and then draw attention to the underlying issues which have conditioned reactions and attitudes to Helsinki 2008. I will argue that the main contentious issues in this version of the DoH remain connected with Articles 29 and 30 of the 2000
version and that this explains why a body like the US Food and Drug Administration (FDA) has quietly withdrawn from the DoH in preference for the ICH-GCP. These issues particularly concern industrialized world research in the developing world, particularly in sub-Saharan Africa. I will conclude by underlining the de facto moral authority of the DoH around the world and pointing out that, in spite of possibly misleading language and inevitably ambiguous expressions, which are present in nearly all the paragraphs of the Declaration, that simple good will is enough to grasp the categorical ethical imperative in each of the guidelines of the Declaration as it is, without further complicating and obscuring them with needless rewordings and clarifications. In my understanding, the Declaration is not meant to be a thumb book of practical rules for medical research but rather a set of abstract ethical principles that can effectively guide practice and actions.

Email address for correspondence: gbtangwa@yahoo.com

146 Application of Good Clinical Practice (GCP) guidelines in African settings [MIM16779602]

Ramadhani Noor, Brenda Okech, Aceme Nyika, Roma Chilengi

The major accomplishment of the International Conference on Harmonisation ICH GCP lies in the fact that participating regions are able to accept a common set of data in a registration dossier whose data was generated through GCP trials. This has brought substantial gains in reducing the time that it takes to develop and register a product in several regions. Clinical trials done in Africa, like elsewhere, carry no exception under these standards. Increasing attention to the diseases of poverty coupled with other reasons contributing to relocation of clinical trials has resulted to increased funding and subsequent unprecedented increase in clinical trials in Africa. Unfortunately African institutions have lagged behind in the implementation of GCP. Not surprisingly so because, product development mainly focussed on diseases endemic to richer regions of the world. The African Malaria Network Trust (AMANET) has championed the cause of capacity building across Africa and successfully taken leadership for African driven malaria vaccine research efforts in the continent where GCP standards have been respected. This paper shall discuss application and compliance of these guidelines in Africa, potential abuses, role of pharmaceutical companies, ongoing regional efforts with special emphasis/highlights on AMANET experiences and challenges in the process of applying GCP principles in Africa. Invariably, AMANET has found that with some support, African research institutions have demonstrated sufficient GCP compliance despite prevailing challenges. This shows that capacity building can be achieved and calls for commitment and continued support to see more African Institutions rise to GCP compliance.

Email address for correspondence: ranoor@amanet-trust.org

147 Experiences with community engagement and informed consent in a genome-wide association study on malaria in Kenya [MIM16598204]

Vicki Marsh, Albert Mlamba, Dorcas Kamuya, Thomas Williams, Sassy Molyneux

Genome-wide association (GWA) research has the potential to increase understanding of disease mechanisms, improve diagnostic techniques and provide targets for the development of new vaccines and drugs. Ethical issues, including informed consent, have been raised for international biomedical research, and these are highlighted for GWA studies. Community engagement has been described as a potential way of meeting ethical challenges. This study aimed to describe researchers’ experiences of community engagement and informed consent during a GWA on malaria at a Kenyan research institute, and explore their implications for strengthening ethical research practice. Qualitative analysis of documentation from the planning, conduct and monitoring of community engagement activities for the project and quantitative analysis of project records of recruitment rates. Community engagement contributed to assessing acceptability, planning communication and responding to emerging issues but has not explored attitudes around less visible risks, such as archiving with open access; the role of field workers is critical, supporting the notion of a relations-based ethics; interpretation of recruitment rates is not straightforward. A fall in these rates may indicate increasingly informed choice, although this may vary with specific aspects of research; challenges for community engagement and informed consent for GWAS are similar to other types of research although has features that accentuate these. These challenges underline the need for systematic community engagement that draws on a range of methods, addresses different groups within the community and is sustained at least over the lifetime of the project.

Email address for correspondence: vmarch@kilifi.kemri-wellcome.org

148 Health Research Ethics capacity strengthening in Africa: Current situation and prospects [MIM16735147]

Aceme Nyika, Wenceslaus Kilama, Roma Chilengi, Godfrey Tangwa, Paulina Tindana, Paul Ndebele, Joyce Ikingura

The high disease burden, the emergence of new diseases, increases of drug resistance efforts to address the 10/90 gap and meet the UN millennium development goals have led to an unprecedented increase in health research funding and activities in Africa. Consequently, there is a need to strengthen ethical review capacity in Africa. With a grant from the Bill and Melinda Gates Foundation, the African Malaria Network Trust (AMANET) undertook a survey of 31 Ethics Review Committees (ERCs) across sub-Saharan Africa as an initial step to a comprehensive capacity strengthening programme. The number of members per committee ranged from 3 to 21, with an average of 11 members per committee. Members of 10 institutional committees were all from the same institution where the committees were based, raising prima facie questions as to whether independence and objectivity could be guaranteed in the review work of such committees. The majority of the committees (92%) cited scientific design of clinical trials as the area needing the most attention in terms of training, followed by determination of risks and benefits and monitoring of research. The survey showed that 38% of Ethics Committee members did not receive any form of training prior to or after joining. Overall, the survey identified areas of weakness in the operations of African ERCs. This paper flags the gaps identified in the ethical review process across Africa and gives an overview of a longitudinal capacity strengthening project by AMANET and other players.

Email address for correspondence: anyika@amanet-trust.org
149  
Iron supplementation for children in malaria-endemic areas: Systematic review and meta-analysis [MIM15030803]

Juliana U Ojukwu, Joseph Okebe, Dafna Yahav, Mical Paul, Cochrane Infectious Diseases Group

We aimed to assess the effects of iron supplementation on malaria and global outcomes among children living in malaria-endemic countries. Cochrane systematic review and meta-analysis. Individual and cluster randomized-controlled trials conducted in hypo- to holo-endemic malaria regions were included. We compared oral iron vs folic-acid vs placebo or no treatment among children <18 years. Fortification interventions were excluded. The primary outcomes were malaria-related events and death. Pooled relative risks (RR) are presented with 95% confidence intervals. Analyses were adjusted for clustering. The review is currently being refereed; due for publication in 2009. Seventy-two trials were included. When given for prevention or treatment of anaemia, iron did not increase the rate of clinical malaria, RR 1.03 (0.96–1.12), 13 trials, 21,105 children. Results were similar for non-anaemic children. Malaria parasitaemia was more frequent with iron, 1.13 [1.01–1.26], but there was no difference in trials with adequate allocation concealment. When given to treat malaria-related anaemia, parasitological failure was similar for iron vs. placebo, RR 0.98 (0.69, 1.39), 3 trials, 583 children. Iron did not increase mortality across all trials comparing iron vs. placebo, RR 1.13 (0.92–1.39), 27 trials, 26,290 children. Respiratory infections, hospitalizations, weight and height were not affected by iron administration. Iron + zinc increased diarrhoea, but not iron alone. The increase in haemoglobin afforded by iron was affected with co-supplementation with zinc or antiparasitics, but not by malaria endemicity. Iron does not increase the risk for clinical malaria or death. Recommendations regarding iron supplementation for children living in malaria-endemic areas should consider these results.

Email address for correspondence: julieojj@yahoo.com

151  
Exploring the difficult ethical choices in malaria vector research: Choosing between mosquito, science and humans [MIM16779332]

Paul Ndebele

Malaria vector studies are a very important aspect of malaria research as it assists researcher to learn more about the malaria vector. Research programmes in various African countries include studies that attempt various methods of controlling the malaria vector as well as mosquito colonies that are maintained by staff from the institutions. The choices that have been made during the past decade about how to build up and maintain mosquito colonies for malaria research in Africa reveal much about prevailing attitudes and assumptions with regard to research. The focus on the mosquito and the science of malaria research has led some researchers in African institutions to view humans in their study areas as well as those in their research teams as mere means to their research findings and consequently publications. Various cases from the field of malaria research in Africa collected during training workshops for malaria researchers as well as during interactions with malaria researchers in various institutions are described. Through these cases, the ethical choices inherent in the tension between science and respect for individuals are explored. This analysis of past and present choices has relevance to broader questions of human rights as they are part of the current emphasis on ethical research world wide.

Email address for correspondence: pndxexas@yahoo.com

150  
PABIN/SIDCER Ethical Review Committee Recognition Program in Africa [MIM16755798]

Abraham

The Pan African Bioethics Initiative (PABIN) promotes capacity building in ethical review of research protocols with the aim of protecting human study participants from harm and strengthening scientific research in Africa. PABIN works with interested African Institutional Review Boards (IRBs) or ethical review committees (ERCs) to strengthen the quality of review through improved understanding of international ethical principles, development and implementation of standard operating procedures and monitoring and evaluation of IRB/ERC performance. The primary requirement is full participation of committee members and self-assessment. This will be followed by: (1) Human Study Participant Protection Course, (2) IEC/IRB SOPs writing workshop and (3) Site Survey and Evaluation. IRB/ERCs would work with PABIN to meet quality standards in structure and composition, adherence to specific policies, completeness of the review process and post-review process and in documentation and archiving. An independent evaluation will be conducted by trained surveyors upon invitation by the IRB/ERC following self-assessment. Successful committees will be recognized by PABIN/SIDCER for a three-year period with continued monitoring to guarantee maintained excellence. The recognition program is a tool to promote quality standards in African research ethics committees. It has successfully been tested by FERCAP/SIDCER in South-East Asia and recent experience of PABIN/SIDCER in Africa shows promising results. The recognition program complements the efforts of various institutions and organizations that conduct ethics training workshops for researchers and provides a measurable output of capacity building in ethics.

Email address for correspondence: asefmaa@gmail.com

152  
Potential of methotrexate in the treatment of malaria [MIM15065977]

Alexis Nzila, John Okombo, Steven Murithi

Methotrexate (MTX) is used at high dose, up to 130–300 mg/kg (9–20 g per adult) for the treatment of cancer, and this use is associated with high toxicity. A low dose of MTX (LD-MTX), 0.1–0.35 mg/kg (7.5–25 mg per adult) weekly is used against arthritis in adults and in children, on a chronic basis. At this dose, MTX is safe and well-tolerated. We have provided evidence that MTX concentration <100 µM could inhibit parasite’s growth of laboratory reference strains, including multidrug resistant parasites, and this concentration can be achieved in vivo when LD-MTX is used. We report on the antimalarial activity of fresh Kenyan field isolates, and on the combination of MTX with other antimalarials. We used the in vitro system, based on the hypoxantine incorporation, to establish MTX activity against field isolates, and data were presented as concentrations that kill 50% of parasite amia (IC50). We also investigated the activity of MTX in presence of commonly used antimalarials lumefantrine, piperaquine, dihydroartemisinin. We tested MTX activity against 29 Kenyan fresh P. falciparum isolates. MTX is active against both pyrimethamine-sensitive and -resistant parasites, with IC50 <75 nM. The activity of MTX in presence of the tested antimalarial drug was additive, an indication that any of the tested drug can be combined with MTX. MTX is potent against current field isolates and that lumefantrine, piperaquine,
153 New mass spec-based screening methods used in CODFIN for the forensic investigation of suspected fake antimalarial drugs and bednets in less than a minute [MIM16670087]

Leonard N. Nyadong, Jose Perez, Glenn A. Harris, Dana Hostetler, Michael D. Green, Facundo M. Fernandez

The globalization of the world economy and the lack of appropriate regulations, law enforcement and adequate penalties have made counterfeiting an attractive source of income for criminals worldwide. In particular, drug counterfeiting has become a problem of epidemic proportions. Other products used to fight malaria, such as mosquito bednets have also been counterfeited. Faster, highly selective and more reliable methods for high-throughput authentication are urgently needed to assist in large scale quality surveys of antimalarial products. We present two new methods that we have been developing for the forensic analysis of antimalarial drugs within CODFIN (www.codfin.org) and insecticide-treated bednets, namely: Direct Analysis in Real Time (DART) and Desorption Electrospray Ionization (DESI) both coupled to mass spectrometry. These methods do not require sample preparation, are non-destructive and produce quantitative results in less than a minute. They allow identifying and quantifying active ingredients in fake drugs, and different insecticides embedded in bednets. Investigations into the composition of fake artesunate monotherapy collected during Operation Jupiter (PloS Med. 2008, 5, e32), revealed the presence of an astonishing variety of wrong active ingredients, including paracetamol, metamizole, sulphadoxine/pyrimethamine, chloroquine, chloramphenicol, artesinin, safrole and erythromycin, among others. More recent investigations into other antimalarial monotherapies and combination therapies have shown additional wrong active ingredients, such as sildenafil. Proof-of-principle results on the direct analysis of various bednets will also be presented. Counterfeiting of antimalarial drugs and insecticide-treated bednets is a serious threat to public health. Strong political will, backed by timely forensic information, are needed to combat this criminal trade.

Email address for correspondence: facundo.fernandez@chemistry.gatech.edu

154 Informing the evidence gap in Cambodia: Availability, price and provider practices of malaria diagnosis & treatment [MIM16689189]

Dianna Long, Sochea Phok, Diane Freeman, Chris Jones, Kheng Sim, Duong Socheat, Chea Nguon, Kate O’Connell

Prevalence of malaria is low in Cambodia at under 3%; however, resistance to recommended artemisinin-based combination therapies (ACTs) has been identified in the western border with Thailand. In October/November 2008, a study with the objective of monitoring levels and trends in the availability, price and volume of antimalarials, as well as to examine providers’ perceptions and knowledge of antimalarial medicines at different outlets was conducted. The study was a one-stage cluster design, with samples stratified by two malaria ‘Containment Zones’ which denote classifications of identified and suspected anti-malarial drug resistance. A census of all outlets with the potential to sell anti-malarials was conducted in each of the 38 randomly selected Health Catchment Areas. Each outlet was audited to explore the availability, price and volumes sold in the last week. A provider questionnaire addressing knowledge and behaviour was also administered. Totally 2528 outlets were audited those include 256 public health facilities, 95 type one pharmacies, 1152 village shop, 160 drug store and 856 others (pharmacy, clinical pharmacy, cabinet, and mobile providers). However, only 784 (31%) outlets reported with AM. Data on the price and availability of anti-malarials and diagnostic tests will be presented, as well as provider knowledge and perceptions. Results will be used to inform the existing evidence gap in Cambodia and will provide a baseline for the Affordable Medicines Facility for Malaria should Cambodia be accepted to participate in the pilot. The baseline data will be used to inform policy implications and suitable interventions.

Email address for correspondence: dianna@psi.org.kh


Diptiman Choudhury, P. Saha, S. Sau, G Chakrabarti

Malaria is a fatal infectious disease, caused by Plasmodium sp., causes a loss of 2.7 million lives each year. Among all, P. falciparum is the most deadly and account 95% of all death. This havoc is due to absence of effective drugs or vaccines. Now, most of the pathogens are resistant to available drugs. So, development of new drugs is required. Development of new drug demands a new target. P. falciparum is a protozoon & phylogenetically distant to human, hence have a lot of difference between genome and proteome profile. P. falciparum genes are AT rich having poly adenosine stretches, acting as transcriptional termination signal in heterologous expression system. Preference of codon is also markedly different from higher eukaryotes. Hence, to express P. falciparum protein in a heterologous system, codon change is required. Tubulin microtubule system, is a well established drug target for cancer therapy. Tubulin (αβ dimer), a conserved protein, upon lateral and longitudinal interactions forms microtubule, one of the important cytoskeleton protein, have number of cellular functions (chromosomal segregation, cellular cargo transport, etc.). Alteration of function of that leading towards cellular death. Hence, targeting tubule microtubule system; there would be a lesser chance to develop drug resistance. Our goal is to express P. falciparum tubulin for biophysical, biochemical characterization and drug targeting. P. falciparum have two alpha (1 & II) and one beta tubulin gene. We have created a codon preference table for Baculovirus expression system, depending upon host codon uses and designed as well as chemically synthesized our genes of interest. We have cloned and expressed the protein for drug targeting.

Email address for correspondence: choudhury_diptiman@rediffmail.com

156 Efficacy of anti-retroviral ‘HAART’ regimens on Plasmodium berghei in vivo in mice [MIM16697206]

O.O. Ebong, I.H Ogbuehi, E.K.I. Omogbai

Recent studies have indicated that anti-retroviral protease inhibitors may have anti-plasmodial effects. We investigated drugs in the ‘Highly Active Anti-Retroviral Therapy’ (HAART) group for anti-plasmodial effects in mice in vivo. Swiss
albino mice (20–24 g) in groups of five received intraperitoneally standard inoculums of $1 \times 10^{-7}$ chloroquine (CQ)-sensitive *Plasmodium berghei*-infected erythrocytes. Blood schizonticidal activities of drugs were estimated for repository, early and established infections. Mice received HAART in 2 regimens: Zidovudine + Lamivudine + Efavirenz combination (5–20 mg/kg) [Regimen 1] and Zidovudine + Lamivudine and Lopinavir-boosted Ritonavir combination (5–20 mg) [Regimen 2]. Control mice received dimethylsulfoxide (0.2 ml), CQ (5 mg/kg) and normal saline (0.2 ml). Mean percentage parasitemia reductions (MPPR) were estimated from daily thick and thin smears made from mice tail blood. Mean survival time (MST) for the animals was also determined. For the two regimens, MPPR was highest at 15 mg/kg dose. For Regimen 1, results for prophylactic, early and late schizonticidal tests showed an MPPR of 28.0%, 59.3% and 67.0% respectively; MST was 10 days. For Regimen 2, MPPR for prophylactic, early and late schizonticidal tests were 86.1%, 89.4%, and 92.6% respectively, with an MST of over 30 days. CQ cleared parasitaemia within 7 days. The results show that the HAART regimen with the protease inhibitor showed a higher anti-plasmodial efficacy, in line with other studies on the anti-plasmodial effects of protease inhibitors. In addition, the combined synergistic effect of drugs in HAART regimen enhances the host’s immune system and may contribute to the anti-plasmodial effects of the drugs.

Email address for correspondence: tayoebong@gmail.com

157 Discovery and development of new antimalarial drugs: A novel academic–industrial partnership [MIM16700872]

Roger C. Wiegand, Edmund Sybertz, Dyann F. Wirth, Jeffrey D. Klinger, Robert H. Barker Jr.

Three years ago Genzyme and the Broad Institute, with support from Malaria Medicines Ventures, initiated an academic–industrial collaboration to discover new antimalarial drugs. This ambitious and evolving partnership combines Genzyme’s drug development expertise with the genomics/proteomics and malaria biology expertise at Harvard and the Broad to create a portfolio of projects designed to maximize the probability of identifying new drug candidates. Three approaches are used to identify and advance individual projects within the portfolio: (1) high throughput screens (HTS) of small molecule libraries from multiple sources are used to identify novel chemotypes that kill the parasite in vitro (these are assembled into a “bioactives” collection that will be available to other researchers). (2) HTS enzyme assays based upon validated plasmodial biochemical targets are used to identify inhibitors. (3) Target identification (mechanism of action) studies are carried out on compounds with known antimalarial activity (yet which may have toxicologic liabilities) whose mechanism of action is unknown. These approaches include affinity purification, SILAC, resistance selection and other means to identify the plasmodial target. Once identified, such new targets can become the basis of further HTS assays to identify novel inhibitors. Hits from these three approaches then undergo iterative synthetic medicinal chemistry to optimize potency and drug-like properties. In vivo efficacy in *Plasmodium berghei* models is the primary end point for efficacy, but numerous other pharmacologic and parasite-directed assays are utilized to understand parasite stage specificity of drug action, drug liabilities, potential to treat P. vivax infections and propensity to select for drug-resistance. This academic–industrial partnership provides a new model for using effectively the broadly diverse capabilities of all the partners to efficiently identify new drug candidates.

Email address for correspondence: Rhbarkerjr@comcast.net

158 Pharmacokinetics of sulfadoxine and pyrimethamine in Intermittent Preventive Treatment of malaria in pregnancy (IPTp) [MIM16593630]

Myaing Nyunt, Ishag Adam, Kassoum Kayentao, Janneke van Dijk, Phil Thuma, Francesca Little, Yasin Cassam, Etienne Guirou, Craig Hendrix, Boubaca Traore, Ogobara Doumbo, David Sullivan, Peter Smith, Karen Barnes

Sulfadoxine–pyrimethamine (SP) is used as Intermittent Preventive Treatment of malaria in pregnancy (IPTp) in many African countries to minimize falciparum malaria–related adverse effects, but limited data are available on the pharmacokinetics of SP in pregnancy. We predicted that pregnancy-related physiologic changes, particularly in hepatic and renal function, influence SP disposition in pregnancy. A prospective, self-matched study was conducted in Mali, Mozambique, Sudan, and Zambia to assess SP pharmacokinetics during pregnancy and after the postpartum period. Women received sulfadoxine 1500 mg and pyrimethamine 75 mg at 15–34 weeks of gestation, and again 8–56 weeks after delivery. Blood was taken after each dosing for drug pharmacokinetics. The presence of parasites in the blood was also monitored throughout the study. In this study of 97 women acting as their own controls, pyrimethamine concentrations were significantly higher (all sites except one), and sulfadoxine lower (Mozambique and Sudan) during pregnancy than postpartum. SP pharmacokinetics, particularly for pyrimethamine, differed significantly from site to site, suggesting possible pharmacogenetic differences in the study populations. Multiple factors including pregnancy status, gestational age, postpartum time, and hemoglobin levels were considered as factors that may influence the pharmacokinetics of SP. Results of multivariate analyses will be presented. The study results highlight the importance of careful evaluation and understanding the pharmacokinetics of drugs in this vulnerable population. Given the inconsistency of the changes in PK parameters among study sites and between sulfadoxine and pyrimethamine, no SP dose adjustment for IPTp is indicated by our study findings.

Email address for correspondence: mnyunt@jhsphs.edu

159 Impact of the introduction of artemether–lumefantrine as first line treatment policy on underfive mortality in two rural districts of Tanzania [MIM16669785]


The rationale for deploying the highly effective artemisinin-based combinations (ACT) is to reduce severe malaria and deaths due to malaria. As part of the ALIVE project [Artemether–Lumefantrine (AL) In Vulnerable patients: Exploring health Impact], we aimed at assessing the impact of the introduction of AL as first line treatment for uncomplicated malaria on mortality in children <5 years. We made use of the mortality data collected through the Demography Surveillance System that covers about 85,000 individuals living in Kilombero and Ulanga districts. We compared mortality rates of children underfive during the sulfadoxine–pyrimethamine (SP) era (years 2005 & 2006) with that of 2007 and 2008, which corresponds to the first 24 months of ALu implementation. Underfive mortality rate per 1000 person–year was 126.5 in 2005, 106.2.1 in 2006 and 99.9 in 2007. The malaria cause specific mortality rates (per 1000) between these two period almost remained the same (7.6, 6.8 and 7.3 per 100 in 2005, 2006 and 2007 respectively). These data show a decline of underfive mortality from 2005 to 2007. Since the slope of the curve did not change before and after AL implementation, it
is unlikely that the mortality reduction between 2006 and 2007 is the result of the change in treatment policy. Also, the malaria cause specific almost remained the same.

Email address for correspondence: vasudevan.ajith@yahoo.com

160 Intermittent preventive treatment (IPT) in schoolchildren [MIM16558558]

Joaniter Nankabirwa, Sian Clarke, Narcis Kabatereine, Bonnie Cundill, Simon Brooker, Sarah G. Staedke

Intermittent preventive treatment (IPT) in pregnancy is an important component of malaria control, and may also benefit infants and children. A recent trial in Kenya found that provision of amodiaquine + sulfadoxine–pyrimethamine (AQ+SP) for IPT in schoolchildren reduced rates of malaria parasitemia and anaemia. However, the optimal antimalarial regimen for IPT in schoolchildren remain. We conducted a randomized, single-blinded, placebo-controlled trial to compare the efficacy, safety, and tolerability of sulfadoxine–pyrimethamine, AQ+SP, and dihydroartemisinin–piperine (DP) among primary schoolchildren in Uganda. Asymptomatic children aged 8–12 years (girls) and 8–14 years (boys) were randomized to receive one of the study regimens and were followed for 42 days. Participants were assessed for treatment outcomes over 42 days according to modified World Health Organization criteria. Of 780 participants enrolled in the study, 769 (99%) completed follow-up and were assigned a treatment outcome. The risk of parasitemia was lowest with DP (11%) followed by AQ+SP (44%); both were superior to SP alone (p < 0.001). There was no difference in the risk of parasitemia between SP and placebo (80% versus 85%, p = 0.22). Mean hemoglobin improved significantly in children receiving DP and SP+AQ (p < 0.001). Adverse events were reported in 477 (61%) of the children and all were mild or moderate in severity. No serious adverse events occurred. Our preliminary results suggest that AQ+SP and DP would be appropriate for IPT in schoolchildren. The role of SP for IPT in settings of high-level resistance may need to be re-examined.

Email address for correspondence: jnankabirwa@yahoo.co.uk

161 Intermittent preventive treatment of infants (IPTi) with amodiaquine/artesunate, SP/artesunate or chlorproguanil–dapsone in western Kenya: A randomized, double-blind placebo-controlled trial [MIM16689321]

Frank Odhiambo

Intermittent preventive treatment in infants (IPTi) with sulphadoxine–pyrimethamine (SP) to prevent malaria has shown encouraging results in six trials. Resistance to SP is rising and alternative drug combinations for IPTi need to be evaluated. Objectives: To evaluate the efficacy of short-acting and long-acting anti-malarial combinations used for IPTi, and to understand the role of treatment versus prophylactic effects. We conducted a randomized, double-blind placebo-controlled trial with SP plus 3 days of artesunate (SP–AS3), 3 days of amodiaquine–artesunate (AQ3–AS3), or 3 days of chlorproguanil–dapsone (CD3) administered at routine programme of immunization visits (10 weeks, 14 weeks and 9 months) in an area of western Kenya with perennial malaria transmission and high ITN coverage. 1365 children received ≥1 dose of study drug and were included in the analysis. The protective efficacy (PE) and 95% confidence intervals against the first or only episode of clinical malaria were: 25.7% (6.3, 41.1); 25.9% (6.8, 41.0); and 16.3% (−5.2, 33.5) in the SP–AS3, AQ3–AS3, and CD3 groups, respectively. The PEs for moderate-to-severe anaemia were 27.5% (−6.9, 50.8); 23.1% (−11.9, 47.2); and 11.4% (−28.6, 39.0). There was no evidence for a sustained beneficial or rebound effect in the second year. The results suggest that IPTi provides protection against clinical malaria even in the presence of high ITN coverage. The results also suggest that long-acting regimens are more suitable for IPTi than short-acting regimens in areas of perennial malaria transmission; the prophylactic rather than the treatment effect of IPTi appears central to its protective efficacy.

Email address for correspondence: fodhiambo@ke.cdc.gov

162 Phase II randomised trial of Dextran 70 and Hetastarch for correction of volume deficits in severe malaria [MIM15053308]

Samuel Akech, Molline Timbwa, Mwanavuva Boga, Charles Newton, Kathryn Maitland

Recent trials and a meta-analysis have shown that volume expansion with human albumin solution (HAS) results in a lower mortality in children with severe malaria acidosis (shock). As HAS is costly and not available in Africa in this study we examine whether two lower cost colloids, Dextran 70 and Hetastarch, have similar benefit. We conducted a phase II trial in children >6 months old with severe falciparum malaria (defined as impaired consciousness and/or deep breathing) complicated by metabolic acidosis (base deficit >8 mmol/l). Eligible children were randomised to receive either Dextran 70 or Hetastarch. Children received 20–40 ml/kg of resuscitation fluid over the first hour, volumes given dependent upon resolution of clinical features of shock. Efficacy was determined by the volume of colloid required for correction of the physiological parameters of shock. Safety was determined by incidence of adverse events/reactions to either colloid (allergic reactions, pulmonary oedema or raised intracranial pressure). Total of 80 children recruited; 40 to Dextran 70 and 40 to Hetastarch. No difference in volumes required to correct clinical features of shock. Three deaths (3.8%) occurred; one in Dextran 70 and two in Hetastarch groups. No adverse events (pulmonary oedema, raised intracranial pressure, allergic reaction, or bleeding episodes). Dextran 70 and Hetastarch safely correct hypovolaemia in non-intensive children with severe malarial acidosis. Non-inferiority trials in comparison to HAS should be considered if current phase III trials show that HAS is better than saline.

Email address for correspondence: sakech@kilifi.kemri-wellcome.org

163 A single blinded clinical trial comparing Arco a new antimalarial drug and Coartem in the treatment of uncomplicated malaria in adult patients in Uganda [MIM16494700]

Mworozi A. Edison, Rujumba Joseph, Kiguba Ronald, Maganda Albert, Nsobya Sam, Rwakimari Bosco

Malaria is a major cause of child morbidity and mortality in Uganda. The situation is worsened by the development of drug resistance and lengthy antimalarial drug treatment which pose problems of compliance to medication. We conducted a study to compare the efficacy and safety of Arco a single dose drug and Coartem in the treatment of uncomplicated malaria in children aged 4 months–16 years attending Mulago Hospital, Kampala, Uganda. A single blinded randomized clinical trial carried out between November 2007 and June 2008. We screened 3344 patients with
fever for malaria of whom 353 had positive blood smear, 225 fulfilled the inclusion criteria and were randomized into the two study arms. Study patients were followed up for 42 days. There was 100% parasite clearance by day 2 and 14 in both ARCO and Coartem arms of the study. Recrudescence occurred in 2 patients in the Arco arm of the study by day 42 and in two patients on Coartem by day 21 of follow up. There was no statistically significant difference in resolution of fever, vomiting, dizziness and back pain through out follow up. No difference was observed in the clinical presentation between the two treatment arms. No serious adverse events were reported/observed in any of the study arms. Arco and Coartem are equally effective and safe in the treatment of uncomplicated malaria in children aged 4 months to 16 years. Arco is a suitable ACT in the treatment of uncomplicated malaria in children.

Email address for correspondence: arwanire@yahoo.com

164
Severe sequelae and disability after cerebral malaria in a cohort of Ugandan children attending a specialist neurology clinic [MIM15066003]
Richard Idro

Cerebral malaria is a leading cause of disability in African children. The pathogenesis of sequelae is poorly understood and there are no guidelines for assessment, management or rehabilitation. Objectives: This study aimed to describe the forms of severe sequelae, pattern of brain injury on computerized tomography (CT) scans and encephalographic (EEG) characteristics (in those with epilepsy) in children who attended a specialist child neurology clinic. Children with severe cerebral malaria sequelae who attended the child neurology clinic in Mulago hospital, Kampala, Uganda, were assessed for neurological, cognitive, behavioral and psychiatric sequelae. Severe sequelae was defined as presence or a combination of generalized hypotonia, spastic hemiplegia or quadripareisis, blindness, loss of hearing or speech, epilepsy, behavioral abnormalities that impaired daily activities of the child or play with other children, regression in milestones, mental handicap or cognitive impairment requiring assistance with tasks of daily living. Children with focal neurological signs had CT imaging performed while those with epileptic seizures had EEG recordings. Specific treatment and rehabilitation was offered as appropriate and patients followed up to describe changes over time. In this paper, we describe these severe sequelae, patterns of brain injury, seizure characteristics and treatment/rehabilitation needs. Preliminary findings suggest that other the recognized neurologic and cognitive sequelae, behavior and psychiatric problems such as hyperactivity, inattention and aggressive behavior are common. The paper provides insight into the pathogenesis of severe sequelae after cerebral malaria and the areas for intervention. Guidelines are however urgently needed for their care and rehabilitation needs.
Email address for correspondence: ridro@kilifi.kemri.wellcome

165
Identification of P450s involved with insecticide metabolism in Anopheles gambiae [MIM16696609]
Bradley J. Stevenson, Patricia M. Pignatelli, Andrew Steven, and Mark J.I. Paine Vector Group

Anopheles gambiae is increasingly resistant to insecticides, in particular to pyrethroids which are used for treating bednets and indoor residual spraying. A multitude of genes are associated with insecticide detoxification, most notably cytochrome P450s. Functional validation of insecticide metabolism is essential to aid the identification of metabolic resistance markers for the development of new tools for monitoring resistance. Recombinant E. coli expression systems have been developed to enable mosquito P450s enzymes to be screened for the capacity to metabolise insecticides. Alongside, diagnostic antibodies and assays are being produced for the identification and characterisation of candidate metabolic resistance markers. Several resistance candidates have been examined for insecticide metabolism. Two P450s, CYP6P3 and CYP6M2, with consistently strong associations with permethrin resistance in field caught populations of An. gambiae have been shown to metabolise pyrethroids. Other metabolic resistance candidates are being investigated. We are starting to build a functional profile of the determinants of insecticide metabolism in mosquitoes. We describe how these may be translated into new tools to aid the implementation of malaria control interventions.
Email address for correspondence: m.j.paine@liverpool.ac.uk

166
Molecular investigations of the effect of a blood meal on insecticide resistance in Anopheles funestus [MIM16705948]
Belinda L. Spillings, Lizette L. Koekemoer, Basil D. Brooke, Maureen coetzee

The increasing prevalence of insecticide resistance in the southern African malaria vector Anopheles funestus raises concerns over the effectiveness of insecticide use for malaria vector control. In South Africa, vector control is based on indoor residual spraying (IRS) of DDT and pyrethroids. IRS specifically targets blood feeding, malaria transmitting females. It has previously been shown that the presence of a blood meal in insecticide resistant females confers increased levels of insecticide tolerance by an unknown mechanism that is distinct from vigour tolerance. We hypothesize that the process of blood digestion activates a suite of enzymes responsible for detoxifying xenobiotics and that this process inadvertently enhances their insecticide detoxifying capability. Cohorts of An. funestus females were divided into blood fed and unfed groups. Both were maintained under standard insectary conditions and RNA extractions were carried out 4 h post-blood feeding. A microarray, the An. gambiae detox chip, was used to compare gene expression levels between unfed and blood fed females. Quantitative PCR was then used to validate the gene expression levels. Anopheles funestus samples were hybridized onto the detox chip using low levels of stringency in order to maximize the number of signals obtained. This method proved to be inefficient and the experiment was repeated using An. gambiae samples. Anopheles funestus orthologues of the An. gambiae genes differentially expressed with respect to blood feeding were identified. The levels of overexpression of these candidate genes are currently being quantified using qPCR.
Email address for correspondence: lizettek@nicd.ac.za

167
A rapid colorimetric field test to determine levels of deltamethrin on PermaNet® surfaces: Association with mosquito bioactivity [MIM16808313]
Michael D. Green, Frances Atieli, Martin Akogbeto

Long-lasting insecticidal nets (LLINs) have made a significant impact in reducing malaria. Recent developments in insecticide application techniques have resulted in nets remaining effective (insecticidal) on an average of 3 years based on controlled laboratory washing experiments and a few field studies. However, factors
such as the washing manner/frequency and contact with other surfaces can cause significant deviation from the estimated 3-year life. Since mosquito bioactivity is a function of available insecticide on the net surface, we have developed a simple and inexpensive colorimetric test to measure the amount of cyanopyrethroid insecticide residue from filter paper exposed to the net surfaces. The net sampling protocol and colorimetric test (NetTest) was evaluated for deltamethrin-impregnated PermaNet® 2.0 by comparison with high-performance liquid chromatographic assays and mosquito mortality (WHO Cone Test). A good correlation (0.967 n = 5 assays) was observed between the amount of deltamethrin adsorbed onto the filter paper and the entire amount of deltamethrin per unit area of net material. The relationship between the surface levels of deltamethrin determined by the colorimetric test and the “gold standard” mosquito bioassay reveals a relatively accurate field test with a sensitivity of 91.4% and specificity if 85.4% (n = 76 samplings from n = 19 nets). An evaluation of nets collected after 11 months of use in Benin revealed a significant reduction (60% loss) of deltamethrin surface levels at the bottom of the net relative to the top.

Email address for correspondence: mdg4@cdc.gov

168 Molecular basis of pyrethroid resistance in Anopheles funestus, major malaria vector in Africa [MIM16735212]

Charles Wondji

Pyrethroid resistance in Anopheles funestus is a potential obstacle to malaria control in Africa. Tools are needed to detect resistance in field populations. We have been using a positional cloning approach to identify the major genes conferring pyrethroid resistance in this vector. A Quantitative Trait Locus (QTL) named rp1 explains 87% of the genetic variance in pyrethroid susceptibility in two families from reciprocal crosses between susceptible and resistant strains. Two additional QTLs of minor effect rp2 and rp3 were also detected. We sequenced a 120 kb BAC clone spanning the rp1 QTL and identified fourteen protein coding genes and one putative pseudogene. Ten of the fourteen genes encoded cytochrome P450s and expression analysis indicated that four of these P450s were differentially expressed between susceptible and resistant strains. Furthermore, two of these genes, CYP6P9 and CYP6P4, 25 and 51 times over expressed in resistant females, are tandemly duplicated in the BAC clone as well as in laboratory and field samples suggesting that P450 gene duplication could contribute to pyrethroid resistance in An. funestus. Single Nucleotide Polymorphisms (SNPs) were identified within CYP6P9 and CYP6P4 and genotyping of the progeny of the genetic crosses revealed a maximum penetrance value f2 = 1 confirming that these SNPs are valid resistance markers in the laboratory strains. This serves as proof of principle that a DNA-based diagnostic test could be designed to trace metabolic resistance in field populations. This will be a major advance for insecticide resistance management in malaria vectors which requires the early detection of resistance alleles.

Email address for correspondence: c.s.wondji@liverpool.ac.uk

170 Highly selective carbamates towards the malaria mosquito, Anopheles gambiae: Design, synthesis, potency and toxicity testing [MIM15694816]

James M. Mutunga, Troy D. Anderson, Bryan T. Jackson, Dawn M. Wong, Joshua A. Hartsel, Sally L. Paulson, Maxim Totrov, Paul R. Carlier and Jeffrey R. Bloomquist

Widespread resistance to pyrethroids and lack of alternative chemicals undermine the use of ITNs for mosquito control. The use of carbamates and organophosphates is limited due to their high mammalian toxicity. We report the re-engineering of carbamate insecticides to increase mosquito-selectivity and mitigate resistance development in An. gambiae. Based on mosquito acetylcholinesterase (AChE) protein homology modeling and in silico ligand docking, we have synthesized new carbamates that are highly selective to An. gambiae AChE. Using in vitro biochemical analysis, anticholinesterase activities of each carbamate were evaluated for both human and mosquito AChEs and compared to those of propoxur (WHO standard). We tested for topical and contact toxicity, and synergism assays using WHO methods. On-going studies will demonstrate possible roles of the mosquito blood–brain barrier (BBB) and cuticle in chemical pharmacokinetics. The novel carbamates have greater selectivity (ca. >1200-fold) towards An. gambiae AChE, compared to 5-fold selectivity with propoxur. The intrinsic and contact mosquito toxicity of these carbamates is comparable to that of propoxur. We obtained a 4-fold gain in toxicity with synergism studies meaning that redesigning of chemicals may further increase toxicity. We will discuss the role of the BBB and the insect cuticle in chemical penetration. With such high levels of selectivity and comparable toxicity to propoxur, these novel carbamates provide valuable leads to developing of alternative mosquitoicides for use in insecticide treated nets and indoor residual sprays. Our findings are important in the search for new mosquito-selective insecticides.

Email address for correspondence: jmutunga@vt.edu
171 The threat of pyrethroid resistance for control of malaria caused by *Anopheles gambiae* and the prospect for sustaining insecticidal control using chlorfenapyr and chlorpyrifos methyl [MIM16966777]

R. N’Guessan, A. Asidi, R. Oxborough, S. Magesa, M. Akogbeto, F. Mosha, M. Rowland

Pyrethroid resistance has become widespread in *Anopheles gambiae* in West Africa partly as a consequence of insecticide use in agriculture but increasingly as a result of scaling up of long lasting insecticidal nets (LLIN) and indoor residual spraying (IRS). Recent observations made in Southern Benin, Burkina Faso and Bioko indicate that vector control with ITNs and IRS is being undermined by pyrethroid resistance. There is an urgent need to identify new insecticides to control pyrethroid resistant mosquitoes and if possible reduce selection pressure for pyrethroid resistance. Two unrelated insecticides show no cross-resistance to pyrethroids: the pyrrole chlorfenapyr and the organophosphate chlorpyrifos methyl. These were evaluated through PAMVERC (www.pamverc.org), a consortium of African vector control research sites in Benin and Tanzania specialized in the development and evaluation of new vector control products. Small-scale trials of chlorpyrifos methyl and chlorfenapyr in experimental huts confirm that relative to pyrethroids IRS treatments with the new insecticides kill the majority of resistant and susceptible mosquitoes that enter huts. Recent advances in microencapsulation formulation technology show promise to greatly prolong the residual activity of insecticides that are otherwise short-lived. Both insecticides showed potential for deployment on ITNs, as single actives but more likely as combination products with pyrethroids. While both insecticides show potential to supplement the pyrethroids, far greater urgency, resources and public–private partnerships are required to make this a reality before pyrethroid resistance fatally undermines current malaria control successes.

Email address for correspondence: mark.rowland@lshtm.ac.uk

172 Durable Residual Wall Lining (DL): Acceptability, durability, and performance for malaria vector management in South Africa [MIM15032896]

Simone Nicolaisen, Christiaan E.G. Mulder

Indoor Residual Spraying (IRS) has the limitation of requiring repeated applications due to many factors that reduce residual effectiveness against malaria vectors. Durable Residual Wall Lining (DL) provides a controlled release of insecticide sustained over an extended period regardless of wall surface. The fabric is factory-treated so that the concentration of insecticide is uniform across the entire surface where the material is applied. The primary objective of this experiment is to evaluate the ability of DL to retain residual effectiveness for the duration of the trial and support its use as a replacement for IRS. The secondary objectives are to evaluate the acceptability of DL to homeowners and the durability and home improvement value of the fabric under real-use conditions. The trial was located in Mbombela Local Municipality, approximately 4 km from center of Nelspruit, South Africa. The trial period was from October, 2008 until July, 2009. Informed consent was obtained from participants prior to the start of the trial. Technicians were trained to follow best practices detailed in a comprehensive Installation Manual. Assessments in the form of surveys, inspections, and photographic documentation were conducted periodically during the trial. At 4 and 6 months post-install entomological assessments of residual efficacy were conducted using WHO/PES bioassay cone test methods. Results of these observations will be discussed in this presentation.

Email address for correspondence: sn@permanet.com

173 Insecticide susceptibility status of the malaria mosquito: *Anopheles gambiae* s.l. 17 years after The Gambian Nationwide Insecticide Bed Net Impregnated Program [MIM16259075]

Sam Awolola, Martha Betson, Musa Jawara

Following evidence of the impact of pyrethroids insecticide treated nets (ITNs) on reduction of malaria associated morbidity; The Gambian Government initiated a Nationwide Insecticide Impregnated Bednet Program (NIBP) in 1992. Since then, ITNs usage (>65%) has been impressive in The Gambia. Unfortunately, resistance of the malaria mosquito to pyrethroids has emerged in neighbouring countries and a threat facing malaria control. Resistance is mainly due to target site insensitivity often refers to as knock down resistance (kdr) that confers cross-resistance to pyrethroid and DDT. In spite of the history and high usage of ITNs in The Gambia, little is known about the current level of pyrethroids resistance. Here we report a country wide survey on *Anopheles* susceptibility level to insecticides 17 years after The Gambian NIBP. *Anopheles* larvae were collected from 12 sites in the 6 regions of The Gambia from June to November 2008. Six of these sites were established for malaria surveillance in The Gambia. Others were among those used for The Gambian NIBP. 2–3-days-old adult females were exposed to permethrin, deltamethrin and DDT in WHO bioassay test kits and identified by PCR. Further PCR analysis was carried out to detect the kdr alleles in the mosquitoes assayed. A total of 2650 *Anopheles* tested belongs to the *An. gambiae* complex. *Anopheles gambiae* s.s. (41.7%) and *An. arabienisis* (40.4%) were predominant at all sites. The rest were *An. melas* found at three sites. Mosquitoes from 11 sites were susceptible to the three insecticides. DDT resistance was found in *An. gambiae* s.s. from a site where the 24 h post-exposure mortality was <80%. There was no resistance to pyrethroids and the kdr mutations were not found in the mosquitoes assayed. The use of pyrethroids treated bednets has been sustained in The Gambia with no evidence of pyrethroids resistance. This is a welcome development. However continue monitoring is essential to provide early signs of resistance.

Email address for correspondence: awololas@hotmail.com

174 Insecticide resistance in African malaria vectors: Protocols and results from a TDR sponsored network [MIM16521720]

Hilary Ranson, Hiba Abdalla, Maureen Coetzee, Vincent Corbel, Filomeno Fortes, Clément Kerah Hinzoumbé, Sagnon NFalé, Frédéric Simard

Insecticide resistance is one of the biggest threats to sustainable malaria control in Africa. We have established a network of scientists who are actively engaged in insecticide resistance monitoring and research to assess the extent of current levels of insecticide resistance in malaria vectors, to identify the mechanisms responsible for this resistance and to use this information to provide guidance to malaria control programmes in the management of insecticide resistance. Four sentinel sites have been selected in each of five countries (Angola, Benin, Burkina Faso, Chad and Sudan) for biannual monitoring for insecticide resistance. Mosquito larvae are reared to adults and exposed to insecticide treated papers using standard WHO methods. Progeny from the bioassays are preserved for molecular analysis. Adult *Anopheles* are collected indoors
and out to test for parasite infection rates. Bioassay tests revealed high levels of pyrethroid and DDT resistance in Anopheles gambiae s.l. from multiple sentinel sites. With the exception of Burkina Faso and in a lesser extend Benin, the majority of the mosquitoes surveyed were susceptible to carbamates and bendiocarb. Marked variations in resistance levels between sites and collection seasons were noted. This longitudinal, multi-site study will provide valuable data on the status of insecticide resistance in malaria vectors in Africa. Molecular analysis will identify the major mechanisms responsible and improve the detection and management of insecticide resistance in malaria control programmes. 

Email address for correspondence: hanson@liv.ac.uk

175 Ecological divergence between the M and S forms of Anopheles gambiae and the role of chromosomal inversions in local adaptation [MIM16651887]

Frederic Simard, Diego Ayala, Marco Pombi, Colince Kamdem, Moussa Guelbeogo, N’Falé Sagnon, Nora J. Besansky, Carlo Costantini

Incipient speciation within Anopheles gambiae is thought to be promoted by disruptive selection and ecological divergence acting on sets of adaptation genes protected from recombination by polymorphic paracentric chromosomal inversions. However, shared chromosomal polymorphisms between the M and S molecular forms of An. gambiae and insufficient information about their relationship with ecological divergence challenge this view. We used Geographic Information Systems, Ecological Niche Factor Analysis, Correspondence Analysis and Bayesian Individual Multilocus Genetic Clustering to explore the nature and extent of ecological and chromosomal differentiation of M and S across Cameroon and Burkina Faso, and to question the role of chromosomal arrangements in ecological specialisation within and among molecular forms. More than 9000 An. gambiae s.s. specimens were collected from >300 villages in each country. Full karyotypes were obtained for 5364 An. gambiae s.s.s mosquitoes. Species’ distribution modelling revealed differences in the ecological niche of both molecular forms. Population structure analysis identified three chromosomal clusters in Cameroon and two in Burkina, each containing a mixture of M and S specimens. In both molecular forms, alternative karyotypes were segregating in contrasted environments, in agreement with a strong ecological adaptive value of chromosomal inversions. Our results confirmed the role played by polymorphic inversions on chromosome-2 in the adaptation to extensive eco-geographical gradients in both M and S. The exact role of each inversion, however, appears modulated by other regions of the genome of reduced recombination (other chromosomal inversions, and the pericentromeric regions known as “speciation islands”).

Email address for correspondence: simard@ird.fr

176 Population genetic structure of the malaria vector Anopheles funestus in the Senegal river basin, using microsatellite markers [MIM16674951]

Badara Samb, Ibrahima dia, Anna cohuet, Lassana konate, Didier fontenille

Anopheles funestus Giles is one of the major vectors of malaria in sub-Saharan Africa with members of the Anopheles gambiae complex. Following the recurrent droughts that have occurred during the seventies, this species seemed to have disappeared from many parts of Senegal including the Senegal River basin. However, its comeback was recently observed in this zone. The genetic structure of the newly established populations was studied using 11 microsatellite markers in Mbilor (low valley of Senegal River), Gan-kette Balla, Diaminar Keur Kane (Guiers Lake area) and Loboudou (north west part of low Ferlo) and compared to population of the village of Dielmo located in Sudanian zone. Each of these populations studied was in Hardy Weinberg equilibrium suggesting a situation of panmixia. The tests of genetic differentiation revealed that they could be subdivided into three distinct genetic entities: populations around Guiers lake area, population from the low valley of Senegal River, and population from Dielmo. Correlation between genetic and geographical distance has been observed. Gene flow exists between these subpopulations and differences observed would be mainly due to geographical distance. The existence of gene flow between the populations from Senegal River basin and Dielmo may indicate that the recolonization was probably carried out by dispersion starting from the neighboring areas where An. funestus had not disappeared.

Email address for correspondence: badarasambe@yahoo.fr

177 Increasing frequency of kdr alleles associated with increasing coverage of insecticide-treated bed nets [MIM16679622]

Damaris Matoke, Derrick Mathias, Nabie Bayoh, John Gimnig, Edward Walker, John Vulule, Luna Kamau

Ownership of insecticide-treated nets (ITN) has increased in western Kenya in the last 10 years with subsequent declines in malaria transmission and malaria-related mortality. However, a threat to this intervention is insecticide resistance within the major malaria vectors. In this study, we document trends in the kdr allele associated with insecticide resistance in An. gambiae as ITN ownership has increased. We evaluated insecticide resistance and associated genotypes in Asembo Bay and Seme, western Kenya from 1996 until 2008. Asembo Bay was the site of a large ITN trial and ITN ownership has been high from 1997 until the present. Ownership in Seme is lower and rose from <5% in 2000 to >75% by 2008. Adult mosquitoes were collected by indoors beginning in 1996. Larval collections were initiated in 2004. An. gambiae s.l. mosquitoes were identified to species by PCR. Mosquitoes were assayed by RT–PCR for the presence of the kdr mutations. In Asembo, the kdr allele (leucine–serine mutation) rose from <5% in 1996 to over 70% by 2008. In Seme, the frequency was 10% in 2004 but rose to >80% by 2008. We did not observe increasing phenotypic resistance. No evidence of phenotypic insecticide resistance was observed; however, the rapid rise in the frequency of the kdr allele is a cause for concern as it suggests selective pressure. Continued monitoring is needed to track the spread of this allele and to determine whether phenotypic resistance will arise in western Kenya due to widespread ITN use.

Email address for correspondence: dmatoke@kemri.org

178 Bionomic and population genetic structure of the malaria vector Anopheles moucetii in the equatorial forest region of Africa: An update [MIM16696680]

Christophe Antonio-Nkontjio, Cyrille Ndo, Pierre Kengne, Parfait Awono-Ambene, Didier Fontenille, Frédéric Simard

Anopheles moucetii is a major malaria vector in forested areas of Africa. However, despite its important epidemiological role, it remains poorly known and insufficiently studied. Here, we report data on its bionomics and population genetic structure using mosquito populations sampled throughout its distribution.
range in Central Africa. *An. moucheti* samples were collected from Cameroon, the Democratic Republic of Congo, Nigeria and Uganda. Microsatellite data and rDNA sequences were used to estimate genetic diversity within populations and their level of genetic differentiation. Bionomic studies consisted on the determination of mosquito epidemiological role, biting habit and feeding habits. All specimens collected in Tsakalakuku (Democratic Republic of Congo) were identified as A. m. Bervoetsi, those collected in Akaka Nigeria were An. m. nigeriensis while the rest consisted of A. m. moucheti. High levels of genetic differentiation were recorded between A. m. bervoetsi, An. m. nigeriensis and each A. m. moucheti sample using both microsatellite markers and rDNA sequences. Within An. m. moucheti samples a low to high genetic differentiation was detected. Using sequence variation of ITS1 rDNA a diagnostic polymerase chain reaction technique was set up for a reliable identification of members of this group. An. bervoetsi and An. moucheti were highly anthropophilic and were found infected by *Plasmodium falciparum*. High levels of genetic differentiation supports complete speciation of A. m. bervoetsi and An. m. nigeriensis. Much attention has to be given to members of this group which could support malaria endemicity in areas where An. gambiae is absent or scarce.

Email address for correspondence: antonionkondjio@yahoo.fr

179 Population genetic structure of Anopheles funestus group in Southern Africa [MIM16704888]
K.S. Choi, L.L. Koekemoer, M. Coetzee

*Anopheles funestus* is one of the major malaria vectors in sub-Saharan Africa. The population structure of *An. funestus* based on mitochondrial DNA (ND5) data led to the description of two cryptic subdivisions, common type—clade I and clade II only from Mozambique and Madagascar. We extended these studies to include other common members of the *Anopheles funestus* group in order to determine relationships within the group. The population genetic structure of *An. funestus*, *An. parensis*, *An. rivulorum* and *An. vanee- deni* using the ND5 mitochondrial gene was assessed. Samples were sourced from Malawi, Mozambique and South Africa. Preliminary results confirmed the two subdivisions in *An. funestus* from Mozambique. The new species from Malawi (unpublished data) presented as a distinct clade very distant from the other samples tested. Interestingly, both *An. rivulorum* and *An. parensis* exhibited two clades each but further investigations are needed to determine what these results mean.

Email address for correspondence: kwangshik@gmail.com

180 Ontologies and databases for the control of vectors and disease [MIM16700225]
Christos Louis

We are developing a set of IT-based tools that will hopefully help control arthropod vectors of disease in a more efficient way. The aim of the whole undertaking, which is worked out in the frame of the VectorBase and the international IDO (Infectious Disease Ontology) project, is to provide the vector control community with a set of ontologies that will be used both in order to drive specific databases and, most importantly, in the construction of decision support systems to control these diseases. This work, although mainly based in Crete, is carried out by an ad hoc, international network. Our ultimate plan is to develop these tools to cover most important vector-borne diseases and the vectors that transmit them; we initiated our project, though, focusing entirely on malaria and (anopheline) mosquitoes. For practical reasons (application priorities), we started with an ontology of insecticide resistance (MIRO), soon followed by a series of ontologies that describe malaria (IDO_Mal) as well as physiological processes of mosquitoes that are relevant to, and involved in, disease transmission. In addition, we have also designed and made available through VectorBase a dedicated database that is being populated by data on insecticide resistance, both from the literature and from recent field studies. This database now (February 2009) includes figures from more than 800 mosquito populations tested, and we hope that more data will keep flowing in from the international community.

Email address for correspondence: louis@immb.forth.gr

181 The glycosphingolipid pathway as target for new antimalarial drugs [MIM16694906]
Malena Landoni, Vilma G. Duschak, Alejandro M. Katzin, Alicia S. Couto

Although the biochemical pathways of glycosphingolipid biosynthesis are relatively well understood in mammalian cells, little is known about them in parasites. In a previous report, we have shown the presence of an active glucosyl ceramide synthase in *Plasmodium falciparum* with a particular substrate specificity. The fact that at variance with mammals, this key enzyme uses dihydroceramide as the lipidic substrate led us to perform a metabolic incorporation by using NBD–ceramide and NBD–DHceramide as substrates. Analysis of the labeled lipids showed that NBD–ceramide is very fast metabolized as well as a big amount of sphingomyelin was obtained. By contrast, when NBD–DHceramide was used as substrate, its levels remained almost unchanged and although practically no sphingomyelin was detected, glucosylceramide and lactosylceramide were shown. In addition, when NBD–sphingomyelin was metabolically incorporated, no glucosyl or lactosylceramides were detected. Altogether, the results obtained indicate that *P. falciparum* has two different sphingolipid pathways: one of them, the de novo biosynthetic pathway for the synthesis of GSLs and another one using ceramide from the host cell to synthesize the parasite sphingomy- line. At difference with the mammalian cells, these pathways seem not to be connected, highlighting the malarial enzyme as a putative new target for antimalarial drugs.

Email address for correspondence: acouto@qo.fcen.uba.ar

182 Signals for sequential release of apical organelles during erythrocyte invasion by malaria parasites [MIM16196093]
Shailja Singh, M. Mahmood Alam, Ipsita Pal-Bhowmick, Joseph A. Brzotowski, Chetan E. Chitnis

The clinical symptoms of malaria are attributed to the blood stage of the malaria parasite’s life cycle. The invasion of erythrocytes by malaria parasites is, thus, key to malaria pathogenesis. Invasion of erythrocytes by plasmodium merozoites is mediated by specific molecular interactions. A number of parasite proteins that mediate receptor engagement during erythrocyte invasion are secreted to the merozoite surface from apical organelles called micronemes and rhoptries. The sequence of these secretion events and the external signals that trigger protein translocation to the merozoite surface are not known. Live cell imaging and flow cytometric method have been used for measurement of calcium and translocation of micronemal and rhoptry proteins to the merozoite surface. Exposure of merozoites to low potassium ion
concentration as found in blood plasma leads to rise in free intracellular calcium through a phospholipase C mediated pathway and triggers translocation of microneme proteins such as the 175 kDa erythrocyte binding antigen (EBA175) to the merozoite surface. Subsequently, engagement of EBA175 with its receptor glycoporphin A, restores basal intracellular calcium levels and triggers release of rhoptry proteins to the merozoite surface. Our results identify for the first time the external signals responsible for the sequential release of microneme and rhoptry proteins during erythrocyte invasion and provide a starting point for dissection of signaling pathways involved in regulated exocytosis of these key apical organelles. Signaling pathway components involved in apical organelle discharge may serve as novel drug targets since inhibition of microneme and rhoptry secretion will block erythrocyte invasion by malaria parasites.

Email address for correspondence: shailja@icgeb.res.in

183 Apoptotic machinery in *Plasmodium falciparum* growing in a continuous culture [MIM16574236]

Beth Mutai, John Waitumbi (PhD)

Unicellular parasites seem to lack caspase protein that is involved in the “classical apoptosis” in multicellular organisms but have an equivalent proteolytic enzyme referred to as metacaspase that has caspase-like activity and is absent in mammals. Apoptosis and indeed metacaspase have not been sufficiently evaluated to show their role in *P. falciparum*. To study apoptosis, synchronized *P. falciparum* cultures were initiated at a parasitemia of 0.5% at 5% hematocrit and parasite density allowed to increase until the parasite culture crashed. To make sure that the crash was not due to limiting nutrients and/or red blood cells, growth medium was changed once every 24 h, while hematocrit was adjusted to 5% after each 48 h of schizogony cycle. Parasite growth was monitored by making Giemsa-stained thin smears that were examined microscopically and by flow cytometry following staining of cells with SYBR green. At every developmental cycle, equal number of infected red blood cells (iRBCs) were sampled and used for evaluation of apoptotic machinery. DNA fragmentation was evaluated by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (TUNEL) assay and analyzed by flow cytometry and fluorescent microscopy. Mitochondrial membrane potential was assessed by flow cytometry and fluorescent microscope after staining with tetra methyl rhodamine ester (TMRE) dye. Metacaspase gene expression was determined by quantitative real time reverse transcriptase polymerase chain reaction (qRT-PCR) using metacaspase gene specific primers. Housekeeping seryl tRNA transferase gene primers were used to control for gene expression. Gene expression was reported by comparing the Ct values of the two genes. Differential expression of metacaspase protein was assessed by probing a western blot loaded with equal amount of proteins with human polyclonal anti-caspase 3 and 7. Under the growth conditions described, *P. falciparum* initiated at 0.5% ring-stage parasitemia doubled every 48 h to 2% parasitemia, but the rate of growth declined in the third and fourth generations. Consistently, the culture then crashed at about 6% parasitemia when the growth requirements were not limiting. Up to a parasite density of 2%, the malaria parasites were morphologically healthy, but thereafter, the proportion of healthy parasites declined. Unhealthy parasites were smaller (pyknotic) and had condensed nucleus. DNA fragmentation as indicated by TUNEL positive staining increased from 0.2% at early parasitemia to 5% by the time the culture crashed. TMRE staining of the mitochondria showed collapse of mitochondrial membrane potential as the parasite density increased. By qRT-PCR, expression of metacaspase gene was not evident in the ring stages until the parasite density of approached 4%. The metacaspase gene was present in trophozoites at 0.5% and 4% parasitemia while in the schizonts it was present at all parasitemia levels. Because the expression pattern of seryl tRNA transferase gene was relatively similar in all the stages and at different parasite densities, we concluded that the observed differential expression of metacaspase gene as being real. Western blot analysis by anti-caspase 3 and 7 revealed presence of 45 and 28 kDa fragments as has been reported in other studies. Unlike the metacaspase gene, the protein expression was observed in all the stages. In the ring stages, the protein expression was highest at parasite density of 1.36% and lowest during the crash. The protein expression pattern was similar in the schizont stages, while at trophozoites stage protein abundance increased as the density increased. Taken together, these results indicate existence of apoptotic machinery in *P. falciparum* that seem to operate in tandem with parasite density. These findings offer important insights into *P. falciparum* survival strategies that could open new avenues for designing rational therapeutic interventions for malaria. HAD TO TRUNCATE. TOO BIG.

Email address for correspondence: bmutai@wrp-nbo.org

184 Fas-pathway modulation by *P. berghei* liver stage [MIM16699842]

Lígia A. Gonçalves, Mafalda Lopes-Silva, Carlos Penha-Gonçalves

The malaria liver stage is an obligatory step to *Plasmodium* development and is seen as a target to control malaria infection before any clinical symptoms. It is widely accepted that the success of malaria liver infection depends on parasite abilities to deter and counteract host mechanisms, namely the *Plasmodium* capability to confer an exquisite degree of apoptosis resistance in the infected hepatocyte. There is still a gap of knowledge on how the parasite manages the complex death-signaling pathway to its advantage. In connection with this theme we have developed a research line on the involvement of the Fas-pathway during hepatocyte infection by *P. berghei*, as Fas is constitutively expressed in hepatocytes. We used mouse primary hepatocyte cultures infected with GFP-*P. berghei*. Parasite expansion was accessed by qRT-PCR and fluorescent microscopy. Apoptosis was accessed microscopically by TUNEL staining. Fas-pathway was modulated by siRNA experiments and agonist antibodies. Our results indicate that parasite inhibits Fas-signaling in individual infected hepatocytes and on other hand, the neighboring non-infected cells show increased susceptibility to Fas-mediated death. Cells from Fas-deficient (lpr) mice revealed a *P. berghei* enhance expansion comparing to WT hepatocytes. Our data also shows that in the presence of siRNA for Cflar (a Fas-pathway inhibitor) parasite expansion was reduced and conversely, in the presence of a blocking antibody that inhibits Fas-signaling, the parasite burden is increased. The data highlight a dual role for Fas-signaling during hepatocyte infection, suggesting a path how *P. berghei* manage to block apoptosis.

Email address for correspondence: ligdeus@igc.gulbenkian.pt

185 Assessment of *Plasmodium falciparum* Tyrosine Kinase-like (TKL) protein kinases as potential anti-malarial drug targets [MIM16228941]

Abdirahman Abdi, Sylvain Eschenlauer, Christian Doerig

Protein kinases are targets for cancer chemotherapy, and we propose that enzymes of this class might also be considered as targets...
in the context of malaria. The vast phylogenetic distance between *Plasmodium* and human protein kinases may translate into selective inhibition of the former. In this study, we assessed the potential of a group of five parasite protein kinase that belong to tyrosine kinase-like kinases (TKLs) as anti-malarial drug target. We expressed five *Plasmodium* TKLs as recombinant proteins in *E. coli* to gather information about their biochemical properties, and used reverse genetics to assess their role in the parasite's life cycle. We succeeded in producing three PTKLs as active recombinant proteins, the activity of one of these (PTKL3) is dependent on a regulatory domain called sterile alpha motif (SAM domain) that mediates oligomerisation of the kinase. Disrupting SAM domain-dependent protein–protein interaction represents a possible strategy for chemotherapy, in addition to the classical approach consisting of targeting the catalytic domain. We obtained reverse genetics evidence that PTKL3 and PTKL1 are essential for completion of the erythrocytic asexual cycle, while the other three PTKLs are dispensable for this stage. Through biochemical and functional analysis, we validated two members of *P. falciparum* TKLs as potential schizonticidal targets, one of which is active as a recombinant enzyme that can be used for the screening of chemical libraries.

Email address for correspondence: aabdi001@udcf.gla.ac.uk

186 Malaria liver infection is decreased during blood-stage infection

**[MIM16299455]**

S. Portugal, L.A. Gonçalves, V. Zuzarte-Luis, S. Epiphonio, C. Carret, K. K. Hanson, F. Baptista, M.M. Mota

One-fifth of the world population lives in highly endemic malaria areas, where constant exposure to *Plasmodium*, and its high transmission rate most certainly promote re-infection of already infected individuals. In vertebrate hosts, *Plasmodium* life cycle comprises sequentially liver and blood stages of infection. Little is known about the temporal coincidence of these steps of malaria infection. Still, in highly endemic areas, the two stages must often occur simultaneously in a single host. To understand the dynamics of interaction between these two stages of infection, we simulated the re-infection situation in a mouse model, and determined whether an ongoing *Plasmodium* blood-stage infection interferes with the establishment of a secondary liver-stage infection. Our results show that blood-stage infection strongly decreases the ability of sporozoites to establish a secondary liver infection. Gene expression levels and histological inspection revealed that lower hepatocyte infection and decremented parasite development inside hepatocytes account for this decrease. Targeting the adaptive immune response using immunodeficient mice models, antibodies and inhibitors we could not observe any modulation of this impairment. We are now focusing on cellular alterations within hepatocytes that could be responsible for the observed phenotype and on innate immune mechanisms responding to the blood-stage infection. Our model ascertains that an ongoing *Plasmodium* blood-stage infection has a remarkable effect on the establishment of a secondary liver-stage allowing a better understanding on how *Plasmodium* blood and liver-stages interact. Importantly, this will give insights into why in endemic populations, high *Plasmodium* transmission rates associate with partial protection from severe disease.

Email address for correspondence: sportugal@fm.ul.pt

187 Reverse genetics of the *Plasmodium falciparum* Aurora-related protein kinase Pfark-1

**[MIM16696871]**

Luc Reininger, Christian Doerig

Aurora-related protein kinases play key roles in phosphorylation events that regulate the mitotic phase of the cell cycle, they are involved in the control of centrosome and nuclear cycles. We identified Pfark-1 (PlasmoDB ID PFF0260w) as an aurora-related protein kinase and here investigated its function. *P. falciparum* 3D7 parasites were transfected with Pfark-1 KO and GFP constructs and selected for resistance to blasticidin. Integration of plasmid constructs by homologous recombination was monitored by PCR. Pfark-1–GFP transgenic lines were cloned by limiting dilution. Phenotypic analysis of clones was performed by fluorescence microscopy. To test whether Pfark-1 is important for asexual multiplication, we attempted to disrupt the Pfark-1 gene by homologous recombination using a KO construct. Blasticidin-resistant parasites were obtained but no integration-specific PCR products were detected, suggesting that the gene is essential. In contrast the endogenous Pfark-1 gene could be modified by fusing the GFP sequence using targeted homologous recombination. The subcellular localization of Pfark-1 was then analyzed by fluorescence microscopy. The Ark-1–GFP fusion protein is observed from early to late schizont stages only and as rounded green dots associated with a small proportion of nuclei. The results of our study strongly suggest that Pfark-1 is an essential gene in *P. falciparum* parasite development and identify this protein kinase as a validated target for the development of new anti-malarials. The nuclear localization of Pfark-1 and its association within a limited number of nuclei during schizogy are consistent with a function in the regulation of nuclear division.

Email address for correspondence: lrein001@udcf.gla.ac.uk

188 Spatial distribution and dynamics of *Anopheles gambiae* s.l. larval habitats in the city of Yaoundé, central Cameroon

**[MIM16672309]**

Billy Tene fossog, Christophe Antonio-Nkondjo, Philippe Bousses, Collince Kamdem, Nora J. Besansky, Frederic Simard, Carlo Costantini

Increasing urbanization in Africa refocuses the attention of public health managers on urban malaria and begs the question of whether the vectors can adapt to the environmental conditions encountered in the most densely populated cities. A longitudinal survey was conducted in the town of Yaoundé, in the forest domain of central Cameroon, to assess the distribution of *An. gambiae* s.l. larval habitats and its relation with human activities. Larval habitats were examined monthly from May to December 2008 during the course of 1 week in 16 neighborhoods ranging from the centre to the rural outskirts of Yaoundé. Mosquito larvae were sampled by dipping. Larval habitats were characterized based on size, type of breeding site, water quality, location, and their relation with human activities. A total of 2449 potential mosquito breeding sites were examined: 482 (19.7%) contained *An. gambiae* s.l. larvae. Anopheline larval habitats were more abundant in urban than in rural or periurban areas. Large drains, swamps and gutters were associated with no or low larval densities. Human activities such as market gardening, house construction in swampy areas and road construction were associated with potential breeding sites for *An. gambiae*. Unexpectedly, anopheline larvae were collected in urban breeding sites highly polluted with organic matter. PCR identification revealed that only the M molecular form of *An.
Anopheles gambiae was present in the most urbanized areas, where the S molecular form was the most abundant in periurban and rural sites. These findings confirm that the malaria vector An. gambiae s.s. is adapting to the urban environment, and clearly partition the distribution of An. gambiae s.s. molecular forms M and S between urban and peripheral or rural areas.

Email address for correspondence: billytene@yahoo.fr

189 Larval development of the chromosomal forms of Anopheles funestus in different habitats in Western Burkina Faso: A transplantation experiment [MIM16689639]

Cytogenetic investigations within Anopheles funestus populations in Burkina Faso revealed the existence of two chromosomal incipient entities known as “Kiribina” and “Folonzo” forms genetically well structured in the field. Ecological and biological factors that exert the genetic barrier between these forms are poorly investigated. In this study we compared the development of both forms in different habitats in the West of Burkina Faso. First stage larvae (L1s) of wild-caught karyotyped-females were transferred into cages in natural habitats of the “Kiribina” (rice fields at Bama) or the “Folonzo” (puddles at Soumoussou). Each cage was covered with cloth permitting exchange of water, solutes and small particles and was seeded with 100 L1s of a single chromosomal form. The emergence was monitored during 35 days. The emergence success of both forms was higher in puddles than in rice fields. The emergence rate of “Kiribina” was higher (35% vs. 18%) than that of “Folonzo” in rice fields. Inversely it was lower (15% vs. 43%) than that of “Folonzo” in puddles. The emergence rate of “Folonzo” was higher than that of “Kiribina” in rainy season and higher for “Kiribina” during the dry season (68% vs. 15%). Overall larval development was always shorter for “Folonzo” than for “Kiribina”. The results suggest that the two chromosomal forms should differ in their habitat exploiting which should explain their naturally structured-populations reported by previous studies. Further studies are required to better explore the premating behaviour that can enhance the reproductive isolation between these chromosomal forms.

Email address for correspondence: toe_kob@yahoo.fr

191 Spatial and temporal distribution of the malaria mosquito Anopheles arabiensis in northern Sudan: Influence of environmental factors and implications for vector control [MIM15080823]
T.B. Ageep, J. Cox, M. Hassan, B. Knols, M. Benedict, C. Malcolm, A. Babiker, B.B. El Sayed

Malaria is an important public health problem in Northern State of Sudan. The only known vector species in this area is A. arabiensis. The State has been selected for a new vector control method based on the use of sterile insect technique (SIT) that requires comprehensive information about the vector breeding sites and factors that determine its abundance and distribution. This will help in achieving appropriate planning and implementation to avoid problems of previous trials such as the emergence of wild mosquitoes from unexpected breeding sites. Cross-sectional and longitudinal larval surveys were carried out every month between May 2005 and May 2007 in Dongola and Merowe localities. Specific sampling strategy was designed using Remote Sensing (RS), Geographical Information System (GIS) and Global Positioning System (GPS). Sixty blocks in each site were covered during the survey to collect intensive data about the breeding sites, and larval existence and abundance. A. arabiensis larval existence varied significantly between types of breeding sites. The most important breeding sites were flood pools, pipes leakage, irrigation channel seepages and brick works. The existence of A. arabiensis larvae was influenced by physical, chemical and biological characteristics of the sites. Habitats with warm water, low salinity, high pH number, small surface area, shallow depth, sunlight, algae, emergent vegetations, other mosquitoes and other invertebrates were more favorable for breeding of A. arabiensis. A significant association was found between A. arabiensis larval existence and classes of land-use and land-cover (LULC). The river sites had the highest proportion of positive sites followed by river settlements, mosaic trees, mosaic fields and inland settlements, respectively. An inverse relationship was found between A. arabiensis larval existences and the River Nile level, distance of breeding sites from the river and distance of breeding site from the nearest settlements. A. arabiensis immature stages were collected all the year round with seasonal fluctuations. The highest percentage of positive breeding sites was found during the post-flood season. The use of RS, GIS and GPS techniques has significant value in providing accurate spatial and temporal information about A. arabiensis breeding sites which is strongly required for the implementation of the SIT project. They will help in achieving a high coverage during the release phase of the SIT and support any future vector control strategies in Northern Sudan.

Email address for correspondence: tellalageep@yahoo.com
192 Development of vegetable farming: A cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin [MIM16649938]

Ainges W. Yadoulent, A. Asidi, Rousseau Djouaka, J. Braïma, C. Agossou, M.C. Akogbeto

A development of urban agriculture has recently taken place in many areas in Benin. This study aims to assess the rapid expansion of urban agriculture especially, its contribution to the emergence of insecticide resistance in *Anopheles gambiae*. The protocol was articulated on collecting sociological data by interviewing vegetable farmers on the types of pesticides used. Bioassay tests were performed to assess the susceptibility of malaria vectors and biochemical analysis was done to characterize molecular status of *Anopheles gambiae*. This research showed that: (i) the development of urban agriculture is related to unemployment observed in cities, rural exodus and the search for a balanced diet by urban populations; (ii) urban agriculture increases the farmers’ households incomes and their living standard; (iii) PCR analysis revealed three sub-species of *Anopheles gambiae*. The kdr west mutation recorded in samples from the three sites and more specifically on the M forms. Insecticide susceptibility tests revealed a clear pattern of resistance to permethrin (76% mortality rate at Parakou; 23.5% at Porto-Novo and 17% at Cotonou). This study confirmed an increase activity of the vegetable farming in urban areas of Benin. This has led to the use of insecticide in an improper manner to control vegetable pests, thus exerting a huge selection pressure on mosquito larval which resulted to the emergence of insecticide resistance in malaria vectors.

Email address for correspondence: anges33@yahoo.fr

193 Predisposed to adapt? Urbanization and diversification of *Anopheles gambiae* in the African equatorial forest [MIM16399191]

Colince Kamdem, Frédéric Simard, Joachim Etouna, François-Xavier Etoa, Didier Fontenille, Nora J. Besansky, Carlo Costantini

Humans represent the most disruptive biotic selective force on earth, by altering virtually every environment at unprecedented rates and extent. Our interest lies in understanding how such profound modifications of natural ecosystems contribute to the ecological, behavioural, and phylogenetic diversification of mosquitoes, and ultimately to the creation of new species, with particular reference to members of the *Anopheles gambiae* cryptic species complex, the most important vectors of human malaria in Africa. Such knowledge will be important for developing better strategies to control malaria transmission, and to predict the epidemiological impact of global changes in human-dominated ecosystems. We have used ecological niche modelling; combining geospatial species data with layers of remotely sensed environmental data and logistic regression in a Geographic Information System to investigate the causal link between divergence on low-dimensional niche axes and speciation. We demonstrate the existence of a link between ecological divergence and adaptive speciation within the nominal taxon of the complex, *Anopheles gambiae* Giles sensu stricto (Diptera: Culicidae), driven by the recent transformation of the African equatorial forest due to urbanization. Adaptation of incipient species to the novel urban ecosystem might progressively change the degree of exposure of the human population to malaria vectors, thereby affecting the epidemiology of malaria in this eco-geographical domain.

Email address for correspondence: kamdem_d@yahoo.com

194 Malaria vector species composition and their role in disease transmission in Mpongwe district of Zambia [MIM16197033]

Mbang Mbuleba

Malaria is the number one cause of illness in Zambia, affecting children and pregnant women. *Plasmodium falciparum* malaria is predominant in the country. *Anopheles gambiae* complex and *An. funestus* mosquitoes are supposed malaria vectors in Zambia. Mpongwe is a malaria endemic rural district of Zambia. Cases of malaria were 578 and 618/1000 in 2000 and 2001 respectively. Before this study, in 2005 and 2006, the African Malaria Network Trust supported studies carried out by the Tropical Diseases Research Centre to prepare the district for malaria vaccine trials. Entomological surveys were carried out during 2007 and 2008 transmission season to determine the composition of the malaria vector species by PCR and their contribution to transmission of *P. falciparum* malaria by ELISA. Mosquitoes were sampled from human dwellings by CDC light traps and pyrethrum spray catch methods. Malaria cases seen at Mpongwe Mission hospital laboratory were obtained. *Anopheles gambiae* s.s. and *An. funestus* were found but *An. gambiae* s.s. was most involved in malaria transmission. EIR was estimated at 32.5/ib/person/yr lower than an earlier estimation of 88/ib/person/yr in 2006. Malaria cases (38/1000) found were much lower than previous reports and this correlated well with the infection rates (1.7%) in the vectors. Malaria in recent years has progressively reduced probably due to the widespread use of insecticide treated nets. Future EIR estimation should be evaluated against a wide range of control measures including vaccines. Current malaria control measures should be continued and/or scaled-up.

Email address for correspondence: mulebem@yahoo.com

195 Distribution and larval habitat characterization of *Anopheles nili* and *An. moucheti* along river networks in southern Cameroon [MIM16689308]

Christophe Antonio-Nkondjo, Cyrielle Ndo, Carlo Costantini, Parfait Awono-Ambene, Didier Fontenille, Frédéric Simard

Despite their importance as malaria vectors in central Africa, little is known on larval ecology of *Anopheles nili* and *An. moucheti*. We explored the spatial distribution of their larval habitats and associated environmental parameters along river systems in Cameroon. Larvae were collected by dipping in 24 locations across the dense hydrographic network of southern Cameroon. Larval habitats were characterized visually and by the use of hand-held electronic probes for physical water parameters measurements. Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA) were used to determine key ecological factors associated with mosquito distributions. A total of 2269 anopheline mosquito larvae at the late instars were collected, including *An. nili* s.s. (47.4%), *An. moucheti* (22.6%), *An. carnevalei* (5.6%), *An. ovengensis* (2.9%) and *An. somaliensis* (0.1%). Five environmental variables were significantly associated with species’ distribution and abundance: river flow (lotic/lentic), sunlight exposure (sunny/shady), vegetation (presence/absence), temperature, and debris (presence/absence). Using CCA, it appeared that lotic rivers, exposed to sunlight, with vegetation or debris were the best predictors of *An.
**nili** larval abundance, whereas *An. moucheti* and *An. ovengensis* were associated with lentic river flows, lower temperature, and floating vegetation. The distribution of *An. nili* conforms to that of a generalist species that is able to exploit a variety of environmental conditions, whereas *An. moucheti*, *An. ovengensis* and *An. carnevalei* appear as more specialized forest mosquitoes.

**Email address for correspondence:** antonio_nk@yahoo.fr

**196 Ecology of malaria vectors in a highly populated semi-arid environment in north-western Kenya: Impact of local environmental modifications on disease outbreaks [MIM16679722]**

N. Bayoh, M. Hamel, David Sang, Willis Akwale, D. Koros, S. Chagal, H. Williams, E. Walker

Kakuma refugee camp is located in a semi-arid region of north-west Kenya, considered non-endemic for malaria transmission. Following a malaria outbreak in 2005, KEMRI/CDC and the Kenya Division of Malaria Control conducted entomologic surveys in the camp to investigate the source of the outbreak. Entomologic surveys were conducted in the dry season (February, 2006) and wet season (June, 2006). Using health facility-based disease surveillance records, we purposively selected areas of the camp most affected by the outbreak for our surveys. We mapped and surveyed all potential larval habitats using GPS units and dippers, and selected houses within those areas for adult mosquito surveys by pyrethrum spray collection. Tap stands for water supply were present throughout the camp and each had at least one dugout pit associated with it. These dugout pits accounted for the bulk of larval habitats: 74/91 (dry season) and 78/128 (wet season). Habitat occupancy was 23% (dry season) and 56% (wet season). Productive habitats were highly aggregated with more than 80% of total larvae collected from less than 10% of habitats. Indoor resting density (adult mosquitoes/house) for *Anopheles* was 270/142 during the dry season and 671/301 during the wet season. Constructed pits near tap stands were responsible for nearly all mosquitoes in the camp.

Because these pits are continuously refilled with water, there is potential for year round malaria transmission in an otherwise semi-arid environment. Local ecologies are important when investigating disease risk and should form the basis for any mosquito control measure.

**Email address for correspondence:** nbayoh@ke.cdc.gov

**197 Establishment of a Semi-Field System for the study of malaria vector ecology in Tanzania [MIM16699480]**


Medical entomologists increasingly recognize that the ability to make inferences between laboratory experiments of vector biology and epidemiological trends observed in the field is hindered by a conceptual and methodological gap occurring between these approaches which prevents hypothesis-driven empirical research from being conducted on relatively large and environmentally realistic scales. The development of Semi-Field Systems (SFS) has been proposed as the best mechanism for bridging this gap. We define SFS as enclosed environments, ideally situated within the natural ecosystem of a target disease vector and exposed to ambient environmental conditions, in which all features necessary for its life cycle completion are present. A 625 m² SFS for large-scale experimentation on malaria vector ecology and control was established at the Ifakara Health Institute in southern Tanzania, where malaria transmission is endemic. The interior of the SFS has been set up for a variety of research activities including mass-rearing for African malaria vectors under natural conditions, high throughput evaluation of novel mosquito control techniques, short-term assays of host-seeking behaviour and olfaction, and longer-term experimental investigation of anopheline population dynamics and gene flow. The SFS at Ifakara was completed and ready for use in under 2 years. Preliminary observations indicate that realistic and repeatable observations of anopheline behaviour are obtainable within the SFS, and that habitat and climatic features representative of field conditions can be simulated within it. The major opportunities and challenges to the successful establishment and application of SFS for malaria vector research and control are discussed.

**Email address for correspondence:** h.ferguson@bio.gla.ac.uk

**198 Transmission of Plasmodium falciparum dhfr haplotypes in the Gambia [MIM16316032]**

Amani Kheir, Davis Nwakanma, Yagut Akbarov, Salma Al-Saai, Aisha Al-Gazali, Göte Swedberg, Hamza A. Babiker

Sulfadoxine–pyrimethamine (SP) is a common partner of artemisinin-based combination therapy (ACT) in Africa. A high-level pyrimethamine resistance *P. falciparum* lineage with triple dhfr mutations (51I, 59R, 108N) prevails across Africa, however, additional minority lineages of this genotype were also seen. This genotype transmits readily to mosquito following SP treatment; however, it is not known whether different lineages of this genotype vary in their transmission capacity. Alleles of dhfr, microsatellites flanking dhfr and MSP-1 were typed among *P. falciparum* infected children prior to SP-treatment, and infected mosquitoes fed on blood collected post-treatment. Sixteen dhfr haplotypes existed among infected children, 2 carried double mutations (511,108N) while 14 harboured triple mutations. However, only 9 haplotypes with triple mutations transmitted to mosquitoes. A single triple mutant dhfr haplotype (haplotype-11) predominated among children (42%) and mosquitoes (68%). The major triple dhfr haplotypes in the Gambia, which exhibited substantial transmission advantage following SP-treatment, has great similarity to those in other African countries. This agrees with the hypothesis of migration of a high-level pyrimethamine resistance lineage across Africa. However, presence of multiple triple mutant haplotypes, and evidence of cross-mating between them, signifies the role of local evolution.

**Email address for correspondence:** amanikheir@hotmail.com

**199 Owards understanding the mechanisms of Lumefantrine resistance [MIM16371242]**

Leah Mwai, Steven Muriithi, Abdirahman Abdi, Victor Masseno, Steffen Bornmann, Alexis Nzila

Lumefantrine (LM)/artemether (Co-artem®) is now the first line treatment for uncomplicated malaria, and piperaquine (PQ)/dihydroartemisinin (DHA) [Artekin®] is being evaluated as antimalarial. Reports indicate that resistance to LM and PQ could arise quickly. We investigated the relationship between polymorphism at Pfcrt76 and Pfmdr186 and in vitro activity of chloroquine (CQ), Piperaquine (PQ), Lumefantrine (LM) and Dihydroartemisinin (DHA) in Kenyan *Plasmodium falciparum* isolates. We have adapted more than 100 *P. falciparum* isolates in vitro and assayed drug chemo-sensitivity profiles using the hypoxanthine assay. Results are presented as inhibitory concentrations that kill
50% of parasitemia (IC50). The median IC50 for CQ (n = 162), LM (n = 78), PQ (n = 78), and DHA (n = 78) were 41 (19–68), 48 (27–94), 27 (17–39), and 2 (2–4) nM respectively. As expected, DHA was the most active, followed by PQ, LM and CQ. However, about 20% of isolates had LM IC50 > 100 nM, an indication of decreased LM susceptibility. Interestingly, we have found an inverse relationship between CQ and LM activity (r² = −0.315, p < 0.01). The presence of wild-type codons at position Pfcr76 and Pfmdr186 was associated with decrease in LM activity and increase in CQ activity. PQ and DHA activity were not associated with Pfcr76 and Pfmdr186 genotypes. Surprisingly, the use of Artekin® tended to select parasites with higher LM activity. We conclude that Pfmdr1 and Pfcr7 could have a bearing on LM activity.

Email address for correspondence: lmwai@kilifi.kemri-wellcome.org

### 200 Prevalence of sulfadoxine–pyrimethamine-resistant alleles of *Plasmodium falciparum* isolates from pregnant women after introduction of IPTp-SP in Gabon [MIM16425141]


Intermittent preventive treatment with sulfadoxine–pyrimethamine (IPTp-SP) is adopted in Gabon since 3 years. Resistance to SP appears rapidly in areas where it has been largely used. In Gabon, significant rates of treatment failure with SP have been described. This study aims to assess the frequencies of molecular markers of SP resistance in plasmodial isolates from pregnant women living in Gabon. *P. falciparum* isolates from mothers and placenta were obtained at delivery. Molecular analysis of dhfr mutants (Asn-108, Arg-59, Ile-51 and Leu-164) and dhps mutants (A BA-436, Gly-437, Glu-540) was performed using nested PCR and allele-specific restriction enzyme digestion. Overall, 31 pairs (matched mother and placental isolates) and 51 single isolates were analyzed. *P. falciparum* dhfr triple mutants (Asn-108 + Arg-59 + Ile-51) were found in more than 80% of samples. Point mutations prevalence for dhps gene was of 43%, 65% and 4% respectively for Ala-436, Gly-437 and Glu-540. Analysis of the Leu-164 mutation is ongoing. The quintuple (dhfr triple + dhps double) mutant was observed in 22% of samples. The concordance of parasite resistance alleles in matched peripheral blood–placental samples was of 91–96%. Monitoring SP resistance is essential for estimating the effectiveness of IPTp, and molecular markers are increasingly applied for this purpose. These preliminary results suggest that prevalence of these markers associated with *P. falciparum* resistance to SP is high in maternal and placental samples. Complementary analysis including Leu-164 marker, data on IPTp-SP compliance during pregnancy and data on clinical malaria are needed.

Email address for correspondence: mariellebouyou@gmail.com

### 201 Intra-host model of malaria infection: Characterizing artemisinin resistance [MIM16558158]

Sompob Saralamba, Richard Maude, Arjen Dondorp, Joel Tarning, Niklas Lindegardh, Wiritchada Pongtavornpinyo, Lisa White

Increasing parasite clearance times in patients receiving artemunate monotherapy for *P. falciparum* malaria have been observed in western Cambodia. This is of major international concern since it signals the beginnings of artemisinin resistance. One hypothesis for this phenomenon is reduced drug efficacy against the asexual blood stages specifically the ring stage of the parasite life cycle. Plasma drug concentration–time data was collected from 42 patients during artemunate monotherapy. Parasite counts were measured every 6 h. A mathematical pharmacokinetic–pharmacodynamic (PKPD) model using difference equations was built to describe the effect of artesunate on the asexual blood stage parasites in age and time (PD), within an individual host, dynamically incorporating drug exposure over time (PK). To test the hypothesis, concentration–effect relationships for each of ring, trophozoite and schizont stages were modelled explicitly. The model outcomes, parasite clearance dynamics and recrudescence, were compared with the observed data. The model not only reproduced observed clearance times for sensitive parasites, but also the extended clearance times observed in western Cambodia that are thought to indicate resistance. The model provided a good fit to the data if the PKPD parameters for trophozoite and schizont stages were similar for individual patients, but those for rings were variable. We conclude that the most likely phenotypic characterization of artemisinin resistant malaria is a reduction of drug efficacy against ring stage parasites. We used the model to simulate potential alternative dosing regimens for improved efficacy of artemunate against parasites with this form of resistance.

Email address for correspondence: sompob@tropmedres.ac

### 202 Molecular monitoring of *Plasmodium falciparum* drug resistance [MIM16673396]


In Sudan, malaria is a leading cause of morbidity and mortality, resulting in an estimated 7.5 million cases and 35,000 deaths annually. In 2004, Sudan adopted the Artesunate + Sulfadoxine/Pyrimethamine (AS+SP) combination as first line drug, to face the high degree of chloroquine (CQ). In 2007, a molecular epidemiological study was conducted in two Sudanese regions, aimed to determine the genetic mutations linked to antimalarial resistance. A total of 198 *Plasmodium falciparum* isolates from Gezira State (Central Sudan) and from Gedarif State (Eastern Sudan) were examined by the Real-time PCR to assess the frequency of mutant alleles of the pfdhfr/pfdhps genes, associated with S/P resistance. The prevalence of PfATPas6 gene, the putative target for artemisinins, was also evaluated. The majority of the investigated parasite isolates harboured mutant alleles in genes associated with SP resistance, in particular a high frequency of point mutation pfdhfr 51I (75.2%) and 108N (72.7%) associated with pyrimethamine resistance was detected. We observed that 45.5% of isolates carried at least two point mutations linked to SP resistance, while quadruple (pfdhfr 51I/108N, pfdhps 437G/540E) and quintuple (pfdhfr 51I/59R/108N, pfdhps 437G/540E) mutant alleles were recorded in 11.7% and 2% of the samples, respectively. PfATPas6–431K was also detected in 17.7% of the isolates. This study represents the first molecular investigation on antimalarial resistance in these two Sudanese areas after the adoption of AS/SP in Sudan as a first line treatment. The results highlight the importance of the drug resistance molecular monitoring studies integrated in the malaria surveillance activities in endemic regions.

Email address for correspondence: bakrinour@hotmail.com
203
A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in falciparum malaria [MIM16673684]
Stephane Picot

An assessment of the correlation between anti-malarial treatment outcome and known molecular markers would improve the early detection and monitoring of drug resistance by Plasmodium falciparum. The purpose was to determine the risk of treatment failure associated with specific polymorphisms in the parasite genome or gene copy number. Clinical studies of non-severe malaria reporting on target genetic markers (SNPs for pfmdr1, pfcr, dhfr, dhps; gene copy number for pfmdr1) providing complete information on inclusion criteria, outcome, follow up and genotyping, were included. Three investigators independently extracted data from articles. Results were stratified by gene, codon, drug and duration of follow-up. For each study and aggregate data the random effect odds ratio (OR) with 95% CIs was estimated and presented as Forest plots. An OR with a lower 95th confidence interval >1 was considered consistent with a failure being associated to a given gene mutation. Funnel plots and Q-statistic were used to investigate heterogeneity. 91 studies were eligible among the selection from computerized search, with information on pfcr (27/159 studies), pfmdr1 (25/236 studies), dhfr (18/373 studies), dhps (21/195 studies). The risk of therapeutic failure after chloroquine was increased by the presence of pfcr K76T (Day 28, OR = 1.8 [95% CI: 1.3–2.4]) and amodiaquine failures (OR = 5.4 [95% CI: 2.6–11.3, p < 0.001]). For sulphadoxine-pyrimethamine the dhfr single (S108N) (OR = 2.2 [95% CI: 1.4–3.3]) and triple mutants (S108N, NS51, CS98) (OR = 4.3 [95% CI: 3.0–6.3]) and dhfr–dhps quintuple mutants (OR = 5.3) also increased the risk of treatment failure. Increased pfmdr1 copy number was related with treatment failure following mefloquine (OR = 8.6, [95% CI: 3.3–22.9, p < 0.001]) and artemether-lumefantrine (OR = 5.1 [95% CI: 1.4–20]; p = 0.012). When applying the selection procedure for comparative analysis, few studies fulfilled all inclusion criteria compared to the large number of papers identified. However there was limited heterogeneity. Genetic molecular markers were related to an increased risk of therapeutic failure. Guidelines are discussed and a check-list for further studies is proposed.
Email address for correspondence: picot@sante.univ-lyon1.fr

204
IL-22 SNP implications in clearance of drug resistant Plasmodium falciparum in Cameroon? [MIM16686273]
Wilfred F. Mbacham, Evehe Marie Solange, Honoré Ngora, Palmer M. Netongo, Ireene Domkam, Akindeh Nji, Diakite Mahamadou, Dominic Kwiatkowski, Lisa Ranford-Cartwright, Baldip Khan, Geoff Targett, Brian Greenwood

The ability of the body to clear parasites when given drugs is contingent on a functional immune system yet increasing reports demonstrate that certain children despite their less developed immunity still clear parasites with mutations mutations that confer resistance to anti-malaria drugs. We therefore set out to determine the relationship of SNPs on immune molecules among children in Cameroon being administered SP, AQ and SP/AQ. A randomised double-blind control study in the towns of Garoua, Yaounde and Mutengene in Cameroon amongst 750 children between the ages of 6 and 59 months was performed. Patients were followed up for 28 days and scored using the WHO 2003 protocol. Molecular markers were investigated by PCR-restriction or the dot blot for mutations on dhfr, dhps, pfcr and pfmdr. Allelle frequencies were calculated for 67 SNPs on 17 chromosomes for their possible implication to clear (ACPR) or not to clear resistant parasites with the triple mutations of pfcr76T, pfmdr686Y and pfmdr1042N (TYN). PCR corrected ACPR for D28 were AQ, 36.4%; SPAQ, 15.4%; SP, 18.1%. One SNP within IL-22 could influence the ability of children to clear resistant parasites, with a chi-squared p-value of 0.02 and an odds ratio of the C allele of 1.4 [95% CI (OR) of 1.06–1.95; p < 0.05]. IL-22 is a pro-inflammatory cytokine related to IL-10 that is produced by T cells. In West Africa, a large case-controlled study of two haplotypes of the IL-22 were shown to be associated with susceptibility and resistance to malaria.
Email address for correspondence: wfmbacham@yahoo.com

205
The search for molecular markers of artemisinin resistance: Implications for Africa [MIM16703548]
Shannon L. Takala, Arjen Dondorp, Francois Nosten, Harald Noedl, Dennis E. Kyle, Michael P. Cummings, Pardis Sabeti, Dominic Kwiatkowski, Xin-Zhuan Su, Peter Starzengruber, Frederic Ariley, Duong Sochet, Nicholas J. White, Pascal Ringwald, Christopher Targett, Brian Greenwood

Artemisinin-based combination therapies (ACTs) are the first line treatment for Plasmodium falciparum malaria in most endemic countries. The successful use of ACTs with other control tools has contributed to optimism about the possibility of malaria eradication. However, recent reports indicate a trend of prolonged parasite clearance time and increasing ACT treatment failure on the Thai-Cambodian border. If artemisinin resistance has emerged, it could spread globally at immense cost to human life, dashing eradication prospects. An international collaboration has formed to confirm clinical resistance in Southeast Asia, define in vitro resistance phenotypes, and identify molecular markers of resistance. To detect signatures of selection in drug resistant parasites, P. falciparum population genetics will be investigated at four sites in Southeast Asia by estimating the extent of diversity, linkage disequilibrium, and population structure in parasites collected in clinical trials of artemisinin and artesunate-mefloquine. Genome screens of resistant and sensitive isolates will be performed, using single nucleotide polymorphism markers and/or next generation sequencing technologies. Regions of the genome associated with the artemisinin resistance phenotype will be identified using statistical methods for detecting genotype–phenotype associations, such as random forests and long range haplotype tests. Estimates of population genetic parameters, as well as preliminary results from analyses of genotype–phenotype associations will be presented. If resistance markers are identified, rapid assays for detecting these markers in clinical samples can be developed, validated, and deployed in efforts to track and contain artemisinin resistance before it spreads to other malaria endemic areas, including Africa.
Email address for correspondence: stakala@medicine.umaryland.edu

206
Immunoepidemiology approach for the development and optimization of malaria vaccine candidates through a round trip strategy from the lab to the field or the necessary collaboration between basic research and field research [MIM16733976]
Aissatou Toure, Christian Roussillon, Georgette Aribot, Adama Tall, Cheikh Sokhna, Christophe Rogier, Jean-François Trape, Mouhamadou Ndiaye, Babacar Mbengue, Jean-Pierre Sauzet, Pierre Druiilhe

www.mimalaria.org
In the pathway of targeting and validation of malaria vaccine candidate the classical approach is the process of selecting and testing the potential of new antigens and constructs guided by the in-depth evaluation of the protection that could be induced in particular animal models. Another strategy is the selection and validation of antigens of interest on the basis of comparative studies of selected pairs of individuals differing in their protection status. It has been well established that the kinetics, magnitude, and the pattern type of immune response play a critical role in the balance between protection and disease in malaria infection. Such an approach has to be carried out in populations living in endemic areas of transmission that acquired natural partial protective immunity to acute infection by *Plasmodium falciparum*. It permits the analysis of the association of any immune response with specific steps along the cascade of events from infection to disease or parasite elimination. Previous experiences in the accurate definition of infection and morbidity phenotypes and the role of various cofactors to infection, led to integrated immuno-epidemiology approaches to demonstrate the association of particular antibody responses and/or functional potency with parameters of "protection" in prospective studies. The combination of these two approaches in repeated round trip processes has led to fruitful collaborative work that we describe taking the example of two antigens (MSP3, LSA3). This strategy illustrates the need of strong cooperation between basic research and translational research to guide the progressive evidence-based construction of a malaria vaccine candidate.

Email address for correspondence: paul.milligan@lshtm.ac.uk

207 Interpreting measures of vaccine efficacy from malaria vaccine trials [MIM16558590]
Paul Milligan

Vaccine efficacy is usually defined as the percentage reduction in disease incidence due to vaccination. Incidence can be thought of as the proportion of children who have had an episode of malaria after a given period of time, or the number of episodes per person over a given period. Such measures are useful to public health planners, but different statistical approaches can yield different estimates of vaccine efficacy and it is not always obvious how the results should be interpreted. The parent of a vaccinated child will be anxious to know whether his child is completely protected and how long the protection can be expected to last, but it is difficult to provide clear answers to these questions from trials of malaria vaccines. The purpose of this study was to review the methods used to estimate vaccine efficacy and the sources of bias. Probability models were used to calculate vaccine efficacy from time-to-first-event analysis, and from analysis of all events, in different scenarios: sustained efficacy; waning efficacy; heterogeneity in malaria risk; and when vaccine is assumed to interfere with acquisition of immunity. Vaccine efficacy estimates derived from time-to-first-episode can be biased. The reduction in the total number of malaria episodes is a more valid measure, and is relevant for public-health decision making. It applies only to the time-period of the trial. All episodes should be included when estimating efficacy. It may be impossible to infer the magnitude and duration of individual protection from trial data.

Email address for correspondence: paul.milligan@lshtm.ac.uk

208 MSP-1 block1/block2 hybrid: A vaccine candidate against *Plasmodium falciparum* [MIM16700249]
Graeme Cowan, Kelwalin Dhanasarnsombut, Roberta Spilotri, Alison Creasey, David Cavanagh

Two lines of evidence suggest that MSP-1 block2 may be promising vaccine candidate. Firstly, studies have shown that antibodies to MSP-1 block2 are strongly associated with a lower incidence of malaria in naturally exposed individuals, in two cohorts of differing endemicity in both Gambia and Ghana. Secondly, in an immunization and challenge trial, two of four Aotus monkeys were protected from parasite challenge after immunization with MSP-1 block2, and this protection was directly correlated with antibody titre (manuscript in preparation). However, unlike most other malaria vaccine candidates, MSP-1 block2 is based upon a polymorphic region of a parasite surface protein. The challenge of this polymorphism has been overcome by the design of a synthetic MSP-1 hybrid antigen which encompasses all of the sequence diversity present in natural parasite populations. This synthetic hybrid antigen construct was optimized for, and expressed in, three heterologous systems: *Escherichia coli*, Trypanosoma thermophila and Measles virus. Expression of the MSP-1 block2 hybrid antigen in all three expression systems has been successfully demonstrated. Preparations of block1/block2 hybrid antigen and recombinant MSP-1/measles virus have been subjected to biochemical characterization and immunogenicity testing in non-human primates and the results of these experiments will be presented. The results from these immunogenicity and antigenicity tests on preparations of block1/block2 hybrid antigen and recombinant MSP-1/measles virus show that MSP-1 block2 is a valid vaccine candidate against malaria.

Email address for correspondence: graeme.cowan@ed.ac.uk

209 *Plasmodium falciparum* Histidine-Rich Protein 2 ELISA for use in malaria intervention trials [MIM16595853]
Carol Kifude, Ann Stewart, Carter Diggs, Douglas Walsh, John Waitumbi

Microscopy is the gold standard for detection and quantification of malaria asexual parasitemia. Unfortunately, a number of factors mitigate utility of malaria microscopy. i.e its inability to detect the sequestered late stages parasites, the method is poorly reproducible and even then, requires considerable expertise for correct diagnosis and quantification. Due to these reasons, parasite biomarkers such as *Plasmodium falciparum* Histidine Rich Protein 2 (PFHRP2) are increasingly being used to resolve problems of malaria diagnosis. In a series of studies, we have sought to develop PFHRP2 ELISA as a quantitative assay that could ultimately benefit malaria intervention trials. The dynamic range of PFHRP2 ELISA has been determined as 3.91–250 ng/mL for rPFHRP2 (CV of 0.29–7.56%) and 11.7–750 infected RBC/μL (iRBC/μL) for spiked iRBC (CVs of 0.29–7.56%). The same spiked samples evaluated by microscopists had a similar sensitivity, but CVs were unacceptably high (20.7–161.6%). PFHRP2 is known to persist in circulation for up to 28 days even after a successful anti-malarial chemotherapy. We therefore designed experiments to determine blood compartment survival of PFHRP2. Compartment analysis by ELISA, flow cytometry and immuno-fluorescence indicate that the bulk of persistent PFHRP2 is inside the RBC and not in plasma. We conclude that PFHRP2 ELISA is a suitable adjunct to microscopy and could benefit malaria intervention trials. Finally, the compartment survival findings imply that PFHRP2
210

Randomized, controlled, phase 2b clinical trial to evaluate the safety, immunogenicity and efficacy of WRAIR’s AMA-1 malaria vaccine (FMP2.1) adjuvanted in GSK Biologicals’ AS02A vs rabies vaccine in 1–6 year old children in Bandiagara, Mali [MIM16681243]

Mahamadou A. Thera, Ogobara K. Doumbo, Drissa Coulibaly, Matthew B. Laurens, Abdoulaye Kone, Ando Guindo, Dapa A. Diallo, Karim Traore, Issa Diarra, Amadou Niangaly, Amagana Dolo, Modibo Daou, Mady Sissoko, Mahamadou S. Sissoko, Boureema Kouriba, Drissa Tr

The malaria vaccine candidate antigen FMP2.1 is a recombinant protein based on the 3D7 strain Plasmodium falciparum apical membrane antigen-1 (AMA-1). The purpose of this randomized, controlled Phase 2 clinical trial (NCT00460525) is to evaluate the safety, immunogenicity and efficacy of FMP2.1 formulated in GlaxoSmithKline’s Adjuvant System AS02A in children in Bandiagara, Mali, West Africa. Four hundred healthy children aged 1–6 were randomized 1:1 to receive three doses of 50 μg of FMP2.1 in 0.5 mL of AS02A or rabies vaccine, 30 days apart. The primary efficacy endpoint is time to first or only clinical malaria episode occurring between randomization and 6 months after the third immunization. Secondary endpoints include incidence density of clinical malaria episodes, time to first or only clinical malaria episode caused by parasites with AMA-1 genotypes identical to the 3D7 vaccine strain with respect to designated polymorphic codons, and asexual parasite density. Titters of anti-FMP2.1 antibody will be measured by ELISA. Data from the time of enrollment through 6 months after the third immunization will be unblinded and analyzed while the study continues in a single-blind fashion. In May–July of 2007 745 children were screened and 400 enrolled, of whom 377 (94%) received all the three immunizations. Safety, efficacy and immunogenicity results will be presented. If this trial demonstrates efficacy against genetically diverse parasites and acceptable safety and tolerability, further clinical development can be envisioned, either alone or as part of a multi-stage, multi-antigen malaria vaccine.

Email address for correspondence: mthera@mrtcbko.org

211

Simian adenoviral malaria vaccine [MIM16706095]


Protective immunity against liver-stage Plasmodium falciparum requires the induction of strong cellular immune responses. We have assessed adenoviruses as promising vectors circumventing problems with pre-existing immunity by using a simian adenovirus. We describe a phase I dose escalation clinical trial in healthy malaria naïve volunteers. In this trial groups of UK volunteers have been administered a single dose of AdCh63 ME-TRAP of 1 × 108, 1 × 109, 1 × 1010 or 5 × 1010 vp. In each group four of the volunteers received a further vaccination with MVA ME-TRAP at a dose of 2 × 108 pfu. We found AdCh63 ME-TRAP and MVA ME-TRAP as a heterologous prime boost regime showed a good safety profile and produced much stronger T cell responses than adenoviral vector alone, with evidence of higher T cell responses using higher doses. Responses using the 1 × 1010 priming dose followed by MVA exceeded the threshold level associated with protection in previous studies with vectored vaccines and this insert. These data identify a preferred heterologous prime-boost immunisation regime for pre-erythrocytic malaria and suggest that simian adenoviruses should provide safe and potent alternatives to adenovirus serotype 5 vectors for widespread use. We have initiated a phase 2a sporozoite challenge study to assess the protective efficacy of this regime.

Email address for correspondence: adrian.hill@ndm.ox.ac.uk

212

The antibody dependent respiratory burst (ADRB) assay is correlated with clinical protection from falciparum malaria, and validates the baculovirus MSP1p19 vaccine candidate [MIM155394909]

Charlotte Joos, Laurence Marrama, Hannah E.J. Polson, Aïssatou Toure, Ronald Perraut, Shirley Longacre

Effective vaccines to combat malaria are urgently needed, but have proved elusive without validated correlates of natural immunity. This study evaluated antibody dependent respiratory burst (ADRB) activity in polymorphonuclear neutrophils (PMN), induced by Plasmodium falciparum merozoites and serum antibodies from African endemic populations, and investigated associations with acquired clinical protection. The role of BvMSP1p19 specific antibodies in mediating ADRB activity was also determined. Respiratory bursts by freshly harvested PMN were quantified using chemiluminescence detected with isoluminol in a standardized protocol. Sera were analyzed from 230 individuals of all age groups living in Ndiop or Dielmo, and enrolled in a cross-sectional prospective follow-up study. Statistical significance was determined using non-parametric tests and Poisson regression models. Merozoites transgenic for MSP1p19 and sera depleted of specific anti-BvMSP1p19 were used to test the functional relevance of these antibodies in the ADRB assay. Most importantly, high PMN ADRB activity was correlated with a 17-fold lower risk factor (age-adjusted) for clinical malaria in a high transmission area (P=0.006), and a 12-fold lower risk factor for children in a low transmission area (P<0.001). Complementary results showed that ADRB activity was dependent on anti-merozoite opsonizing IgG1 and IgG3, and that anti-BvMSP1p19 antibodies mediated an average 30% of ADRB activity. This is the first demonstration of an in vitro functional correlate of immune protection against malaria. These results strongly support the use of the ADRB assay to guide pre-clinical and clinical development of MSP vaccine candidates, and confirm the relevance of the BvMSP1p19 vaccine candidate.

Email address for correspondence: cjoos@pasteur.sn

213

A phase 1 study to assess the safety and immunogenicity of the malaria vaccine candidate MSP31,LSP in children 12–24 months of age living in a stable malaria stable transmission area of Burkina Faso [MIM16690306]


The merozoite surface protein 3 (MSP3) is an antigen associated with the membrane of the free blood stage parasite. The aim of the study was to assess the safety and immunogenicity of this vaccine candidate in children aged 12–24 months. The study was a double-blind, randomized, controlled, dose escalation phase Ib trial. The three groups received three vaccine doses (days 0, 28 and
56) of either 15 µg of MSP3-LSP, 30 µg of MSP3-LSP or Engerix B. Children were visited to solicit symptoms. Antibody responses to MSP3-LSP were measured on days 0, 28, 56, 84, 168 and 365. All the 45 enrolled children received three vaccine doses. No serious adverse events were reported. The most common local reactions were pain (16), swelling (67) and induration (109). They were most frequent in MSP3-LSP groups. 15/35 swelling and 24/48 induration of grade 3 severity related to the vaccine were reported in the 30 µg group. Five cases of fever grade 3 no related to the vaccines were recorded as solicited general symptoms. Until day 84, both MSP3 doses regimens were able to elicit high levels of anti-MSP3 specific IgG1 and IgG3 antibodies in the volunteers with very little or no increase in IgG2, IgG4 and IgM classes. The assessment of immunology data from day 84 to day 365 is ongoing and will be presented during the conference. Our results support the promise of MSP3-LSP as a malaria vaccine candidate, in terms of tolerability and immunogenicity in our study population.

Email address for correspondence: alphonse.ouedraogo@yahoo.fr

214 Decreasing efficacy of antimalarial combination therapy in Uganda explained by decreasing host immunity rather than increasing drug resistance [MIM16627134]

Bryan Greenhouse

Improved control efforts are reducing the burden of malaria in Africa, but may result in decreased antimalarial immunity. A cohort of 129 children aged 1–10 years in Kampala, Uganda were treated with amodiaquine + sulfadoxine-pyrimethamine for 396 episodes of uncomplicated malaria over a 29-month period as part of a longitudinal clinical trial. The risk of treatment failure increased over the course of the study from 5% to 21% (HR = 2.4/year, 95% CI = 1.3–4.3). Parasite genetic polymorphisms were associated with an increased risk of failure, but their prevalence did not change over time. Three markers of antimalarial immunity were associated with a decreased risk of treatment failure: increased age (HR = 0.5/year, 95% CI = 0.2–1.2), living in an area of higher malaria incidence (HR = 0.26, 95% CI = 0.11–0.64), and recent asymptomatic parasitemia (HR = 0.06, 95% CI = 0.01–0.36). In multivariate analysis, adjustment for recent asymptomatic parasitemia, but not parasite polymorphisms, removed the association between calendar time and the risk of treatment failure (adjusted HR = 1.5/year, 95% CI = 0.7–3.4), suggesting that worsening treatment efficacy was best explained by decreasing host immunity. Declining immunity in our study population rather than increasing parasite resistance appeared to be the primary factor underlying decreased efficacy of amodiaquine + sulfadoxine-pyrimethamine. With improved malaria control efforts, decreasing immunity may unmask resistance to partially efficacious drugs.

Email address for correspondence: bgreenhouse@medsfgh.ucsf.edu

215 Evaluation of the public health impact of seasonal IPT in Senegal: Study design and description of the study site [MIM16645510]


Intermittent preventive treatment (IPT) with antimalarial drugs given to all children once a month during the transmission season can provide a high degree of protection against malaria. The purpose of this study is to determine to the feasibility of delivering this new strategy on a large scale to children in rural areas through routine health services, and to assess its safety, public health impact, and cost effectiveness. Following a 2-year pilot study, delivery of seasonal IPT is being introduced into 54 health posts in three rural districts (Fatick, Bamby and Mbour). Community health volunteers coordinated by the health post, visit homes once in September, October and November to deliver treatment with sulfadoxine-pyrimethamine and amodiaquine to all children aged 3–59 months. The intervention is being introduced in a staggered way over 3 years, allowing evaluation of its impact. The primary endpoint will be all causes mortality. Births, deaths and migrations, and ITN use, are recorded in 6-monthly demographic surveillance rounds. Passive surveillance for malaria and adverse events that may be drug-related is maintained in health facilities. The study started in January 2008 and the baseline census reported a study population of 602,000 residents (all-age). IPT was introduced to nine health posts, delivered to 16,200 children. No drug-related serious adverse events were reported. IPT remains safe after 1 year of delivery in nine health posts. Preliminary results on safety, acceptability and coverage will be presented.

Email address for correspondence: sokhna@ird.sn

216 Validity of verbal autopsy procedures [MIM16646346]

Arthur Mpimbaza, Agaba Katureebe, Linda Quick, Amy Ratcliffe, Scott Filler, Sarah G. Staedke

The goal of the US President’s Malaria Initiative is to reduce malaria deaths by 50% in 15 African countries, including Uganda. Verbal autopsy (VA) procedures can be used to estimate cause of death in settings with inadequate vital registries. However, the sensitivity and specificity of VA for determining malaria-specific mortality may be lower than for other diseases, and may vary with malaria transmission intensity. Misclassification errors can lead to large underestimates of the impact of interventions on malaria-specific mortality, even within a truly successful program. Objective: To assess the diagnostic accuracy of VA procedures as compared to hospital medical records for determining cause of death in children under five in three epidemiological settings in Uganda. Caretakers of children who died in hospital and who fulfilled entry criteria were interviewed using a standardized World Health Organization questionnaire. Medical records from the child’s hospitalization were also reviewed. Causes of death were assigned by two independent physicians. Preliminary results are available for 202 cases from a mesoendemic site. Causes of death as determined by the medical record included pneumonia (19%), diarrhea (15%), malnutrition (10%), malaria (9%), neonatal sepsis (9%), meningitis (8%), HIV (7%), and others (22%). VA procedures had a sensitivity of 63%, specificity of 91%, and positive predictive value of 41% for determining malaria deaths. Complete results from all sites will be presented. The performance of VA in different settings and the potential impact on the evaluation of control interventions and appropriate use of VA will be discussed.

Email address for correspondence: arthurwakg@yahoo.com

217 Malaria and tick-borne relapsing fever in Senegal: Two diseases usually confused [MIM16651523]

G. Diatta, A. Tall, E.H. Ba, H. Bouganali, N. Diagne, C. Sokhna, J.F. Trape

In Senegal, like anywhere in West Africa, malaria is the leading cause of mortality and morbidity by a vectorial disease. Tick-borne
relapsing fever (TBRF) due to Borrelia crocidurae is a bacterial disease widespread in the Sahel and Sudan savannah of West Africa. Daily medical and epidemiological monitoring of the population of Dielmo (Senegal) over 18 years (1990–2008) with collection of blood smears for all cases of fever. Of 59,545 thick blood smears examined, 34,476 presented malaria parasites and 1078 Borrelia spirochetes including 362 mixed infections. The average density of Borrelia was much lower than the density of malaria parasites. *Plasmodium falciparum* and *Borrelia crocidurae* infections were the two most frequent causes of fever both in children and in adults. The average incidence of TBRF was 12 per 100 person-years (range from 4 in 1990 to 25 in 2000). Contrary to malaria, the incidence of relapsing fever did not decrease with age and fever episodes lasting more than one day in adults were more likely to be caused by relapsing fever than by malaria. Symptoms of the two infections were very similar. TBRF is a very common cause of fever in all age groups with signs and symptoms similar to those of malaria. In many rural areas of West Africa, most long lasting fever cases attributed to malaria may be due to TBRF.

Email address for correspondence: diattag@ird.sn

218 Development and implementation of a cellular phone-based malaria early epidemic detection system for Zanzibar, 2008 [MIM16689034]

Abdul-wahid Al-mafazy

Household surveys in Zanzibar during 2007–08 confirm the population prevalence of *Plasmodium falciparum* infection is <1%—down from 15% in 2003. To ensure further reductions are achieved, the Zanzibar Malaria Control Programme (ZMCP) developed and implemented an early epidemic detection system for malaria. Fifty-two peripheral health facilities use a pre-printed, weekly form to record aggregate daily number of outpatient visits and malaria test results for outpatients with fever. Summarized data (stratified by <5 and ≥5 years) are entered into a customized cellular phone menu and transmitted weekly, via a toll-free number, to a remote server. Facility data are immediately available to ZMCP via a secure website displaying weekly trends in outpatient visits, malaria testing rates (patients tested for malaria/total outpatient visits), and malaria positivity rates (patients with confirmed malaria/total patients tested). During weeks 1–52 of 2008 and weeks 1–8 in 2009, the 52 health facilities submitted 2955 weekly reports (96.3% complete- ness) that captured 365,432 outpatient visits and 83,558 malaria diagnostic results. Outpatients <5 and ≥5 years of age had malaria testing rates of 30.1% and 13.1%, respectively (p < 0.001) and malaria positivity rates of 1.9% and 3.0%, respectively (p < 0.001). Overall positivity rate for weeks 1–8 in 2009 was 2.4%. This system successfully gathers weekly surveillance data from peripheral health facilities and with high completeness of reporting. Weekly testing rates indicate large proportions of outpatients <5 years are being tested for malaria, and positivity rates in early 2009 show no evidence of increase from 2008.

Email address for correspondence: mtash2@gmail.com

219 Impacts of nutritional status on malarial morbidity and mortality among children less than 5 years in Ibadan, South-West Nigeria [MIM16692647]

A.E. Orimadegun, O.K. Amodu, O.O. Omotade

The interactions of malaria and malnutrition remains a subject of controversy as findings from previous studies are inconsistent. This study describes the impact of malnutrition on malarial morbidity and mortality in children aged 6–59 months. a case control study; cases were malaria patients enrolled from three different children clinics in Ibadan and the control group were apparently healthy children with *Plasmodium falciparum* malaria parasitaemia from the same community. Parasitaemia was quantified by microscopy. The weight-for-height of subjects was compared with WHO standard using Anthro 2005 software. 1648 children (888 males, 760 females) aged 6–59 months; including 294 cases of cerebral malaria (CM), 285 severe malarial anaemia (SMA), 743 uncomplicated malaria (UM), and 326 with asymptomatic parasitaemia (AP) participated. The median (range) parasite counts per μl of blood for CM, SMA, UM and AP were 10,878/μl (4500–4,137,142), 8640/μl (2300–3,726,368), 2080/μl (1240–237,272) and 1057/μl (160–197,340), respectively (p < 0.05). The prevalence of malaria did not decrease with age and fever episodes lasting more than one day in adults were more likely to be caused by relapsing fever than by malaria. Symptoms of the two infections were very similar. TBRF is a very common cause of fever in all age groups with signs and symptoms similar to those of malaria. In many rural areas of West Africa, most long lasting fever cases attributed to malaria may be due to TBRF.

Email address for correspondence: beorimadegun@yahoo.com

220 Accounting for treatment in the analysis of longitudinal molecular data of Plasmodium falciparum infections [MIM16696848]

Michael Bretscher, Wilson Sama, Sonja Schöpflin, Ingrid Felger, Tom Smith

Planning and monitoring of elimination- and control efforts rely on good tools to measure the key parameters of malaria epidemiology. Among these are prevalence, multiplicity of infection, duration of infectiousness, force of infection, and seasonality of transmission. Immunity modulates the relationships of these measures in complicated ways, which need to be understood to predict the impact of interventions. Molecular data from cohort studies contain information on all these aspects. This is partly due to the ability of genotyping methods to distinguish individual infections by using highly variable loci, such as msp2. Unfortunately, asymptomatic infections are often not detected. This makes it necessary to use statistical models to interpret the data. We re-implemented, refined and extended existing immigration-death models for the analysis of molecular panel data of *Plasmodium falciparum* infections. By including a model for treatment, we were able to make use of an example dataset, where most participants received treatment at some point during the study. In addition, the proportion of cured infections could be estimated. Through the possibility to include treatment information in the analysis of molecular studies of malaria, immigration-death models can now be applied to a wider range of datasets. Re-implementation of the method in an extendable framework ensures continuity of future development and facilitates the use of the methods by other researchers.

Email address for correspondence: mthbretscher@gmail.com
Plasmodium falciparum gamocyte carriage is essential for mosquito infection and spread of the parasite. Human immune factors can reduce infectiousness and form the basis of transmission blocking vaccines. The occurrence and importance of this sexual stage immunity for low density infections has never been studied in natural settings. One hundred volunteers from an area of seasonal malaria transmission provided a total of 307 blood samples at the start, peak and end of the transmission season. Infectiousness was determined by membrane feeding assays and the same samples were used for gamocyte detection by PfS25-QT-NASBA and assessment of PfS45/45 and PfS230 antibody responses. At least one mosquito was infected in 32.6% (100/307) of the experiments. In total, 7.5% (916/12,079) of the mosquitoes were infected with 1–97 oocysts per midgut. Human individual infectiousness and the proportion of infected mosquitoes were negatively associated with age (p < 0.001 and p = 0.001 respectively) after adjustment for confounding factors. Individual infectiousness also declined over time with a significant change at the peak (OR = 0.56; p < 0.001) and end of the transmission season (OR = 0.20; p < 0.001) compared to the start. Submicroscopic gamocyte carriers, as detected by PfS25 QT-NASBA, were infectious to mosquitoes in 32.8% of the batches. PfS230 antibody prevalence increased with age and was negatively associated with human infectiousness. This study thus revealed age- and season-dependent patterns of gamocyte infectiousness in residents of an endemic area. These findings are relevant for characterizing the infectious reservoir and selecting seasons for interventions that aim at reducing malaria transmission.

Email address for correspondence: andrelin.cnrfp@fasonet.bf

### Using Bayesian methods to validate mathematical models of malaria transmission [MIM16698608]

J.T. Griffin, A.C. Ghani

Mathematical models were used in previous eradication campaigns to predict the expected rates of transmission decline. However, discrepancies between model predictions and outcomes seriously compromised the campaigns. We developed rigorous statistical methods to account for, and express, model uncertainties. We fitted models for malaria transmission dynamics to multiple datasets simultaneously, including age-stratified parasite prevalence measured by microscopy and in some cases PCR across a wide range of EIRs, and the age-stratified incidence of clinical disease from two EIR settings. We used Bayesian methods to incorporate additional information on parameters that cannot be readily identified from these data, such as the infectivity of humans to mosquitoes from feeding studies of mosquitoes on humans, and to formally compare different model structures. The best-fitting model included the development of immunity against acute clinical malaria and blood-stage parasites dependent on both age and force of infection, age-dependent heterogeneity in exposure and super-infection. The model reproduced well the patterns seen across different ages and transmission intensities. The ability of the methods to account for both model and parameter uncertainty by expressing predictions with uncertainty bounds will be demonstrated graphically. Bayesian methods allow us to incorporate prior knowledge of biological parameters when fitting models to epidemiological outcome data in a single coherent framework. They also provide a natural method to quantify the uncertainty in model predictions. Given the lessons from model use in the previous eradication campaigns, the latter is essential to avoid failure of the intervention program or the model.

Email address for correspondence: ccj@umn.edu

### Interruption of malaria transmission in two highland areas of Kenya [MIM16702471]

Chandy C. John, Melissa A. Riedesel, Gideon N. Magalk, Joseph Okweso, David M. Menge, Kim A. Lindblade, John M. Vulule, Willis Akhwale

Highland areas of unstable transmission are attractive targets for malaria elimination because malaria transmission decreases to very low levels during the dry season. Clinical visits for malaria at the local dispensaries in the highland areas of Kipsamiote and Kapisisiya, Kenya (population ~7400 individuals) were recorded from 2003 to 2008. The Ministry of Health performed household indoor residual spraying (IRS) with a synthetic pyrethroid annually in 2005, 2006 and 2007 (78% household coverage in 2007). Artemether-lumefantrine was implemented as first line malaria treatment in Kapisisiya in October 2006 and Kipsamiote in February 2007. Insecticide treated net ownership was estimated at 13.0% in 2007. From April 2003 to March 2007, average annual malaria incidence was 36.1/1000 persons across the two sites. From April 2007 to March 2008, no microscopy positive cases of malaria were seen in 416 symptomatic individuals (0%). Polymerase chain reaction (PCR) testing for Plasmodium falciparum was positive in 17 of 231 of these symptomatic individuals (7.3%). Among asymptomatic individuals assessed in cross-sectional surveys in May, August and November 2007 and April 2008, 0.1%, 0%, 0% and 0.2% of the population were positive for asexual P. falciparum by microscopy and 0.25%, 0.25%, 0% and 0% by PCR, respectively. Over the study period, there was no consistent pattern of changes in rainfall or temperature, but indoor resting vector density decreased significantly. In areas of unstable malaria transmission, interruption and eventual elimination of malaria may be achievable with widespread annual IRS treatment of households and the use of artesiminin combination therapy.
the previous night increased from 73.6% (95% CI: 68.2–79.1%) to 80.0% (75.9–84.1%) over the 3-year period. In unadjusted analyses of HH surveys, anemia (Hb < 8 g/dl) decreased from 18.4% (95% CI: 14.9–21.8%) to 15.4% (13.2–17.7%), while malaria, measured as positive-slide microscopy, decreased from 18.9% (14.7–23.2%) to 16.9% (13.8–20.0%), a relative reduction of 16% and 11%, respectively. In HF surveys, anemia decreased from 18.3% (95% CI: 14.9–21.7%) to 15.4% (12.7–18.2%), while malaria decreased from 30.6% (25.7–35.5%) to 13.2% (10.6–15.8%), a relative reduction of 15% and 57%, respectively. Increasing access to effective malaria prevention through ITNs appears to be associated with a reduced burden of malaria in young Malawian children. Anemia measured at the HF level at time of routine vaccination may be a good surrogate indicator for its measurement at the HH level in evaluating national malaria control programs.

Email address for correspondence: mud8@cdc.gov

### 225
Integration of insecticide-treated net distribution into routine immunization services in Malawi [MIM16697144]

Don P. Mathanga, MBBS, PhD, Elizabeth T. Luman, PhD, Carl H. Campbell, Jr., MPA, Chimwemwe Silwimba, Grace Malenga, MB, Chb, MMEd

To rapidly scale up ITN coverage, it is important that alternative distribution channels be explored. In this study we explored the feasibility of distributing insecticide-treated nets (ITNs) through routine immunization services. Free ITNs were distributed to infants attending immunization clinics in two districts of Malawi to increase ITN coverage and improve on timely immunization. Coverage of ITNs and timely immunization were evaluated through baseline and follow-up household surveys targeting children aged 12–23 months. ITN utilization among children aged 12–23 months roughly doubled in the two intervention districts and did not change in the control district. Timely vaccination coverage increased in all the three districts. The percentage of children aged 12–23 months who were both fully vaccinated by 12 months and slept under an ITN the night prior to the interview increased from 10–14% at baseline to 40–44% at follow-up in the intervention districts (p < .001), but did not change significantly in the control district. This study is the first to evaluate the provision of free ITNs at completion of a child's primary vaccination series, demonstrating that such a linkage is both feasible and can result in improved coverage with the combination of these two health services.

Email address for correspondence: dmmathang@mac.medcol.mw

### 226
A comparative, randomized clinical trial of artemisinin/naphtoquine twice daily one day versus artemether/lumefantrine six doses regimen in children and adults with uncomplicated falciparum malaria in Côte d'Ivoire [MIM16696593]

Offianan A. Toure, Louis K. Penali, Jean Didier Yapi, Aristide Berenger Ako, Walametchin Toure, Kali Djereea, Genevieve O. Gomez, Oyewole Makaila

Drug resistance in *Plasmodium falciparum* poses a major threat to malaria control. Combination anti-malarial therapy, including artemisinins, has been advocated to improve efficacy and limit the spread of resistance. The fixed combination of oral artemether-lumefantrine (AL) is highly effective and well-tolerated. Artemisinin/naphtoquine (AN) is a fixed-dose ACT that has recently become available in Africa. The objectives of the study were to compare the efficacy and safety of AN and AL for the treatment of uncomplicated falciparum malaria in a high transmission-intensity site in Ivory Coast. We enrolled 122 participants aged 6 months or more with uncomplicated falciparum malaria. Participants were randomized to receive either artemisinin/naphtoquine or artemether/lumefantrine with variable dose according to their weight. Primary endpoints were the risks of treatment failure within 28 days, either unadjusted or adjusted by genotyping to distinguish recrudescence from new infection. Among 125 participants enrolled, 123 (98.4%) completed follow-up. Clinical evaluation of the 123 participants showed that cumulative PCR-uncorrected cure rate on day 28 was 100% for artemisinin/naphtoquine and 98.4% for artemether/lumefantrine. Both artemisinin-based combinations effected rapid fever and parasite clearance. These data suggest that artemisinin/naphtoquine could prove to be suitable for use as combination antimalarial therapy. Meanwhile, pharmacokinetic studies and further efficacy assessment should be conducted before its widespread use can be supported.

Email address for correspondence: andre_offianan@yahoo.fr

### 227
A randomized open trial of amodiaquine + sulfalene-pyrimethamine vs artemether-lumefantrin for the treatment of uncomplicated malaria in Burkina Faso [MIM16694597]

A.H. Diallo, T.R. Guiguemde, O. Gaye, A. Wade

Malaria remains a major cause of child deaths in sub-Saharan Africa. Artemisinin-based combination therapy (ACT) has been recommended as malaria first line treatment by WHO. However the costs and the availability of these drugs make this policy not yet effective in several African countries. A randomized, open trial of AQ-SP versus A-L was conducted from August to December 2005 in Bobo-Dioulasso among patients aged from 2 years, and presenting uncomplicated falciparum malaria. The WHO standard 28-days follow-up was used to assess the efficacy and safety of both combinations. 239 patients were included in the study with 128 randomized to A-L arm and 111 to AQ-SP. At baseline, the use of antipyretics prior admission (74% for AQ-SP versus 50% for A-L, p < 0.001) and the mean temperature (38.6 °C for AQ-SP versus 38.4 °C, p = 0.04) differed. The overall lost-to-follow-up rate was 3.3%. In the intention to treat analysis, the efficacy risk (ACPR) at 28 days was respectively 90.7% [95 CI%: 85.3–96%] for AQ-SP, and 95.2% [95%CI: 91.1–99.4%] for A-L and p = 0.18. With the per protocol analysis and after PCR-correction, the efficacy risk was 96.5% for AQ-SP and 99%for A-L, p = 0.42. Given its high efficacy rate and its cheap cost, the association AQ-SP remains a competitive option for most African malaria endemic countries.

Email address for correspondence: hamadial@yahoo.fr

### 228
A trial of the combined effect of intermittent preventive treatment and insecticide treated bednets in reducing morbidity from malaria in African children [MIM16699756]

Daniel Chandramohan, Simon Cousens, Diadier Diallo, Alassane Dicko, Ogobara Doumbo, Amadou Konaté, Bocar Kouyaté, Paul Milligan, Brian Greenwood

A trial of intermittent preventive treatment of malaria in children (IPTc) and insecticide treated nets ITNs was implemented in 2008 in Burkina Faso and Mali to determine if IPT adds to the protection...
against malaria provided by ITNs without adversely affecting the development of naturally acquired immunity. The study was a randomised double blind placebo-controlled trial. Eligible children aged 3–59 months were given an ITN and then randomised to receive three rounds of treatment with amodiaquine (AQ) plus sulfadoxine pyrimethamine (SP) or placebos at monthly intervals during the high transmission season. Passive surveillance was set up to assess the efficacy of IPTc on malaria morbidity and determine whether IPTc is associated with increased morbidity after the intervention is stopped. Weekly visits to 150 children were carried out to monitor parasite prevalence. Cross-sectional surveys were conducted at baseline and at the end of the high malaria transmission season to monitor parasite prevalence, the prevalence of markers of resistance to SP and AQ and the prevalence of anaemia. 6025 children were enrolled in the trial. From August to November 2008, 10,035 clinic visits were recorded. During these, 5931 thick and thin blood films were prepared and 3017 (51%) showed malaria parasites. Eighty-six children were admitted to hospital and 14 deaths were recorded during this period. 2766 thick and thin blood films were prepared during weekly visits. The prevalence of malaria infection and anaemia were estimated in 701 at baseline and in 5736 children after the third round of IPTC administration. Slide reading and data entry are ongoing and data analysis for the assessment of the efficacy of IPTC in children using ITNs will be undertaken in May 2009. The trial results will guide decision making for future malaria control in areas with seasonal malaria transmission.

Email address for correspondence: diadier.diallo@lshtm.ac.uk

### 229

**An intervention to reduce case fatality from severe malaria [MIM16689560]**

M. Dicko, M. Willcox, B. Graz, J. Falquet, C. Diakite, S. Giani, D. Diallo

In the paediatric department of Sikasso Hospital, Mali, an audit in 2002 showed a case fatality rate of 24.3%, with 42% of deaths due to malaria. We intended to understand the reasons for this high case fatality and to test an intervention to reduce it. We conducted a retrospective study of patients’ notes for the years 2003–2005, and a case-control study to compare deaths and survivors. We then designed an intervention package including an emergency treatment kit on the ward (given free of charge), measurement of blood glucose in all patients with coma/convulsions, use of ceftriaxone for all comatose children, and treatment with intramuscular artemether. This was evaluated prospectively in a cohort of 437 patients admitted in 2006 with presumed severe malaria. Case fatality rates for presumed severe malaria were 8.2%, 17.5%, and 16.8% in 2003–2005 respectively. The case control study found that only the type of severe malaria was associated with outcome, whereas duration of illness and use of other treatments were not. When our intervention package was implemented, the prospective case fatality rate was 11.4%. In spite of higher admission rates than in previous years, an intervention package to improve quality of care and provide treatment free of charge was associated with a lower case fatality rate from severe malaria than in previous years, in spite of larger numbers of patients. This intervention must now be made sustainable and replicable in other areas.

Email address for correspondence: moussaidrissadicko@yahoo.fr

### 230

**Comparison of the safety, tolerability and efficacy of three potential drug combinations for intermittent preventive treatment of malaria in children—A randomised trial [MIM16313535]**

K. Bojang, F. Akor, O. Bittaye, D. Conway, C. Bottomley, P. Milligan, B. Greenwood

Sulphadoxine-pyrimethamine alone or in combination with other antimalarials has been used with good results in the intermittent preventive treatment of malaria in African children. Adverse events encountered include moderate vomiting and malaise. With increasing resistance of malaria parasite to sulphadoxine-pyrimethamine in many parts of Africa, it is important to investigate if other drug regimens might be equally effective in preventing malaria but less likely to cause adverse events. One thousand and eight (1008) children aged between 1 and 5 years were randomly assigned to receive either of three drug regimens-amodiaquine plus SP, piperaquine plus SP or dihydroartemisinin plus piperaquine at monthly intervals on three occasions (September, October and November) during the peak malaria transmission season in 2007. No severe adverse event related to the three IPT drugs was reported. Diarrhoea was present in 5.5%, 5.9%, 3.3% of study subjects who received SP + AQ, DA + PQ and PQ + SP respectively, compared to 9.8% in the control group. Loss of appetite was present in 4.6%, 2.5% and 4.2% of children in the SP + AQ, DA + PQ and PQ + SP groups respectively, compared to 5.6% in the control group. In general, adverse events were more common in the control group than in the three treatment groups. The incidence of malaria in the PQ + DHA, SP + AQ and SP + PQ groups were 0.10 (0.05, 0.22), 0.06 (0.02, 0.16) and 0.06 (0.02, 0.15) respectively. The incidence of malaria in the control group was 0.79 (0.58, 1.08). The three treatment regimens were safe, well tolerated and highly efficacious.

Email address for correspondence: fakor@mrc.gm

### 231

**Increased deposition of C3b on red cells with low CR1 and CD55 in a malaria-endemic region of western Kenya: Implications for the development of severe anemia [MIM16465311]**

Collins O. Odhiambo, Walter Otieno, Christine Adhiambo, Michael M. Odera, José A. Stouté

Severe anemia due to *Plasmodium falciparum* malaria is a major cause of mortality among young children. The factors leading to the age-specific incidence of this anemia are unknown. Previous studies have shown an age-related expression of erythrocyte complement regulatory proteins (CRPs), which protect erythrocytes from complement destruction. Objectives: To determine the factors contributing to age-related C3b deposition. 342 residents of a malaria holoendemic region of western Kenya were enrolled in a cross-sectional study and age-stratified. Parasitemic individuals were treated and blood collected when they were aparasitemic. We measured erythrocyte C3b, CR1, CD55, and immune complex binding capacity by flow cytometry. ANOVA was used to identify independent variables associated with the C3b-positive and hemoglobin level. Individuals of ages 6–36 months had the lowest CR1, highest C3b-positivity, highest parasite density and peak malaria prevalence. In children ≤24 months of age the C3b-positivity was higher in malaria treated than in untreated individuals with similarly low erythrocyte CR1 and CD55. C3b-positivity was strongly influenced by age, malaria status, and CD55 level. CR1 was more important than CD55 among malaria-treated individuals. Hemoglobin level was strongly influenced by
age, %C3b-positivity, CR1 and CD55. Conclusion: Increasing malaria prevalence among children <36 months and low CRP levels, results in increased C3b deposition on erythrocytes and low hemoglobin. The strong contribution of age to C3b deposition suggests additional age-related factors that increase erythrocyte susceptibility to C3b deposition and destruction.

Email address for correspondence: codhiamb@yahoo.com

232 Functional IL-10 promoter haplotypes (−1082A/G, −819T/C and −592A/C) condition susceptibility to severe malarial anemia in Kenyan children [MIM14921817]


Interleukin (IL)-10 plays an important role in determining malaria disease outcomes. Although individual polymorphisms in the IL-10 promoter (−1082A/G, −819T/C and −592A/C) are associated with malaria disease outcomes, the role of haplotypes constructed from these functional SNPs have not been reported. The association between IL-10 promoter haplotypes (−1082A/G, −819T/C and −592A/C) and susceptibility to SMA was investigated in a holoendemic Plasmodium falciparum transmission area. Children (n = 375) were enrolled at Siaya District Hospital, western Kenya, and complete hematological and clinical measures were determined. Parasitemic children were divided into two groups based on Hb status: non-SMA (Hb ≥ 6.0 g/dL) and SMA (Hb < 6.0 g/dL). IL-10 −592A/C was genotyped by PCR and RFLP (using Rsa I), while −819T/C and −1082A/G were genotyped by Taqman 5’ allelic discrimination assay. Circulating IL-10 was determined by a hu25-plex bead assay. Stratification into haplotypic groups yielded: 64.3% GCC; 35.3% ACC; 4.2% ATC; and 59.7% ATA in the non-SMA group and 55.5% GCC; 37.2% ACC; 5.8% ATC; and 59.1% ATA in the SMA group. Multivariate logistic regression analyses demonstrated that the GCC haplotype was associated with protection against SMA (OR; 0.68, 95% CI, 0.43–1.05; P = 0.044) and increased IL-10 production (P = 0.029). Although none of the other haplotypes were significantly associated with susceptibility to SMA, the ATA haplotype showed a tendency towards increased risk of SMA (OR; 1.92; 95% CI, 1.02–2.52; P = 0.042) and reduced circulating IL-10 levels (P = 0.042). Common IL-10 promoter haplotypes condition susceptibility to SMA and functional changes in circulating IL-10 levels in children with falciparum malaria.

Email address for correspondence: collinouma@yahoo.com

234 Parasite sequestration in murine model for pregnancy-associated malaria [MIM16698601]

Luciana Vieira de Moraes, Carlos Eduardo Tadokoro, Claudio R.F. Marinho, Rita Neres, Carlos Penha-Gonçalves

Pregnancy-associated malaria (PAM) is the major cause of maternal and fetal mortality in endemic areas. Recently, our group has described a mouse model for PAM with Plasmodium berghei that resembles severe malaria in pregnant women. To date, there has been no description regarding antigens involved in placental sequestration in Plasmodium berghei infection and if this parasite is submitted to antigenic variation as P. falciparum. We therefore sought to investigate if sequestration of infected erythrocytes occurs in our model in vivo and antigenic variation of sequestered parasites. Pregnant BALB/c mice were infected with 1 million P. berghei ANKA GFP-infected red blood cells (iRBC) at gestational day 13 (G13). At G18 placenta was exposed and images were acquired at different depths for 15 min using a multi-photon microscope. For investigation of putative antigen variation, iRBC extracts from placenta and from non-pregnant infected mice were loaded on a SDS-PAGE gel. Western Blott analysis was performed for serum reactivity from pregnant and non-pregnant infected mice. Our data showed that GFP-iRBC seems to interact with trophoblast cells in vivo in the placenta for an interval of time suggesting sequestration. Parasites maintained in pregnant mice exhibit different protein profile compared to iRBC from non pregnant animals. Western Blott analysis showed differential reactivity of protein bands when serum from pregnant and non-pregnant infected mice was collected. Collectively our results suggest parasite sequestration in the placenta and that binding of iRBC to trophoblast cells might involve upregulation or synthesis of new proteins.

Email address for correspondence: lmoraes@igc.gulbenkian.pt

235 Macrophage migration inhibitory factor (MIF) promoter polymorphisms and susceptibility to severe malarial anemia [MIM16592866]

Gordon A. Awandare, Jeremy J. Martinson, Tom Were, Collins Ouma, Gregory C. Davenport, John M. Ong’escha, Robert E. Ferrell, Richard Bucala, Douglas J. Perkins

Studies from our laboratory demonstrated that circulating levels of the innate immune mediator, macrophage migration inhibitory factor (MIF), are suppressed in children with severe malarial anemia (SMA). In addition, a functional polymorphism in the MIF promoter (−173G/C) was associated with increased susceptibility to high-density parasitemia but not SMA. To further investigate the influence of MIF genetic variation on susceptibility to SMA, haplotypes of MIF −173(G/C) and −794ATT5-8 polymorphisms

(Chern and Beutler, 1975). Recently, it has been proposed that this lowered erythrocytic activity of PdxK is a genetic trait which could affect the development of the disease.

Email address for correspondence: ifeyalio@yahoo.de
were examined in Kenyan children (aged 3–32 months; \(n=643\)) residing in a *Plasmodium falciparum* holoendemic transmission area. A statistically significant relationship between increasing frequencies of longer CATT repeats at \(−794\) and increasing disease severity was observed \((P=0.007)\). In addition, there was a strong association between lower MIF concentrations and longer CATT repeats \(\left(P<0.001\right)\). A likelihood ratio test showed significant evidence of linkage disequilibrium between the two polymorphic sites \(\chi^2=42.4, P<0.001\). Multivariate regression analyses, controlling for HIV-1 and sickle-cell status demonstrated that the MIF \(−794CATT6\)–\(−173G\) (6G) haplotype was associated with protection against SMA \([\text{OR }=0.63 (0.40–1.00), P=0.050]\), while carriers of 7C or 8C haplotypes had increased risk of developing SMA \([\text{OR }=1.71 (1.05–2.77), P=0.031]\). Furthermore, carriers of the 7C or 8C haplotypes had reduced plasma MIF levels during acute disease \((P=0.034)\). The findings demonstrate that variation in the MIF promoter influences susceptibility to SMA and peripheral MIF production. However, the MIF \(−173\) and \(−794\) polymorphisms appear to have both independent, as well as interactive effects on different measures of disease severity, suggesting a complex role for MIF in malarial pathogenesis.

Email address for correspondence: gawandare@hotmail.com

### 236

**Malaria vector mapping in East Africa [MIM15031435]**

Okara Robi

A detailed knowledge of the distribution of the main Anopheles malaria vectors of human malaria in East Africa may facilitate improved vector control. There are a lack of contemporary range maps of the locally dominant Anopheles vectors namely *Anopheles arabiensis*, *An. funestus*, *An. gambiae* s.s., *An. merus*, *An. nili* and *An. pharoensis* in the East Africa region. A comprehensive and systematic search of formal and informal literature was conducted and a database of the contemporary distribution of the main Anopheles vectors in East Africa was assembled. These records were read and entomological data abstracted for the period 1975–2008. Multi-temporal environmental data was obtained from Earth-observing satellite imagery. Various species mapping techniques including, boosted regression trees, generalized additive models and maximum entropy approaches were explored to relate species occurrence data to environmental variables and predict continuous maps of probability of presence of a species while taking into account prediction uncertainty. *An. arabiensis*, *An. funestus* and *An. gambiae* s.s. were found to be sympatric in many areas across the region. *An. arabiensis* and *An. pharoensis* were predicted to be present in the drier areas while *An. gambiae* s.s. was predicted to occur mostly in the wetter regions. *An. merus* was mainly predicted along the coastal region. Differences obtained using the different mapping techniques are explored. Contemporary range maps of the main Anopheles vectors, coupled with an understanding of their biomics will provide an evidence-based platform to guide targeted vector control choices for malaria control programmes in the region.

Email address for correspondence: bio_denloye@yahoo.com

### 237

**New developments in decision support systems [MIM16693724]**

M. Coleman, L. Eisen, S. Lozano-Fuentes, N. Morris, S. Coetzee, I. Seocharan, M. Coleman

The use of decision support systems for monitoring, evaluation and management of vector borne diseases is a relatively new concept. Through a generic framework and high end software development these systems can move beyond routine monitoring and surveillance approaches. Our malaria decision support system (MDSS) guides high quality decisions. A software package with database, application and presentation tiers was developed. System modules include entomology, case surveillance, intervention monitoring and indicator surveys. A GIS backbone allows spatial visualization from a dynamic query builder. The system allows integration of data from several sources while ensuring data quality. Maintenance users can set system parameters and module-specific variables, change alert thresholds and alter certain lookup tables. The MDSS is being tested in three southern African countries where we expect it to have a positive influence on the use of information for decision making and action. The intervention planning support allows operational managers to more accurately and timely plan for upcoming activities and allocate resources. The flexibility and adaptive characteristics of the system is useful for areas with different control strategies and transmission settings. The generation of data to support the monitoring and evaluation of country specific core process and impact indicators is demonstrated. Investment towards improved control and elimination of malaria prompted the enhancement of current monitoring and evaluation tools. This will improve allocation of resources and measurement of indicators. The flexible and adaptive MDSS allows integration of relevant data sets, with low maintenance and technical support making it a simple and effective decision support tool.

Email address for correspondence: dia@pasteur.sn

### 238

**The impact of indoor residual spraying in different epidemiological settings [MIM16689150]**

Rajendra Maharaj, Ishen Seocharan, Natasha Morris

Indoor residual spraying (IRS) forms the backbone of most malaria control programmes in southern Africa. In order to understand the impact that IRS will have on malaria transmission during the drive towards elimination, it is necessary to understand the impact that IRS will have in different epidemiological settings. Surveys were conducted in two provinces in Mozambique to understand the epidemiology of malaria transmission in these provinces. A review of historical literature suggested that the disease etiology was different in these provinces. Baseline surveys were conducted to establish baseline prevalence levels, anaemia levels, vector status and malaria knowledge. IRS was then introduced into these areas and follow up studies were conducted to measure the impact. Baseline studies indicated that the average prevalence of malaria in Maputo province was 76% and that in Gaza province was 54%. High levels of anaemia was measured in the populations of both provinces but it was higher in the Maputo province where the prevalence of the disease was higher. Two vectors with different behaviours were found in both these provinces, namely *Anopheles arabiensis* and *An. funestus*. *An. arabiensis*. Daily mosquito collections from window traps at sentinel sites showed that the mosquito abundance declined after the application of residual insecticides such as DDT and carbamates. Baseline prevalence decreased to 5% and 17% in Maputo and Gaza respectively following the introduction of an IRS programme. IRS does have an impact irrespective of the level of transmission. Sustained IRS is an effective means of controlling malaria in even low transmission settings.

Email address for correspondence: a_diabate@hotmail.com
Anopheles gambiae complex is represented by Anopheles arabiensis and A. gambiae composed of molecular S and M forms in Burkina Faso. Several field studies showed that An. gambiae is more anthropophilic and exhibit high Plasmodium infection comparatively to An. arabiensis. That would explain why many studies are focused on A. gambiae s.s. for mosquitoes control. This study aims to investigate the susceptibility of each species to Plasmodium infection in experimental condition. Mosquitoes larvae were sampled and growth to an insectary. Experimental infection by membrane feeding was done with 3–5 days old mosquitoes using blood from Plasmodium falciparum gametocytes carriers aged from 5 to 15 years. One week after infection experiments, midguts of surviving mosquitoes were examined for oocyst infection and carcasses typed for species and molecular form identification. The infection rate was similar between An. gambiae s.s. (23.8%) [n = 1901] and An. arabiensis (21.3%) [n = 955]. It was 24.9% [n = 1282] and 21.5% [n = 619] for the molecular form M and S respectively. The mean oocyst load was 7.2 ± 0.7 for An. gambiae s.s. and 3.6 ± 0.5 for An. arabiensis. It was 7.6 ± 0.9 for M and 6.5 ± 1.1 for S. No significant difference was observed either in the infection rate or in the mean oocyst load between species or molecular forms. This experimental study indicates that the susceptibility of the species and molecular forms of A. gambiae complex to natural P. falciparum parasites does not differ significantly. So An. arabiensis could be also an important vector.

Email address for correspondence: diattag@ird.sn

The Dieulmo project: A 18-year entomological study of malaria transmission and the bionomics of Anopheles gambiae and Anopheles funestus [MIM16599445]


The Dieulo project, initiated in 1990, consisted of long-term investigations on host-parasite relationships and the mechanisms of protective immunity in the 300 residents of a Senegalese village where malaria is holoendemic. Malaria vectors and transmission were continuously monitored during 18 years. Mosquitoes were collected weekly or monthly by human landing collection from April 1990 to June 2008. Sporozoites rates were investigated by dissection (1990–1993) and/or ELISA (1992–2008). Additional studies were conducted on the bionomics of Anopheles gambiae, An. arabiensis and An. funestus. A total of 41,351 An. gambiae s.l. and 42,903 An. funestus were collected during 2888 man-nights. The entomological inoculation rate varied from a maximum of 482 in 2000 to a minimum of 85 in 1994. Some years An. funestus was very abundant during the dry season and transmission was higher during the dry season than during the rainy season. Heterogeneity of transmission was found at three different levels: (1) the relative proportion of vectors according to month and year, (2) the infection rate of each vector, (3) and thus the number of infected bites for the whole vectors and for each species according to month and year. Our data show that even in areas of intense and perennial transmission, there are large longitudinal variations and strong heterogeneity in entomological parameters of malaria transmission. It is important to take these into account for the study of variations in clinical and biological parameters of human malaria.

Email address for correspondence: thdieng@refer.sn

Comparative susceptibility of the molecular forms M and S of Anopheles gambiae Theobald (Diptera: Culicidae) to Plasmodium falciparum [MIM15084348]

M.O. Dniath, A. Cohuet, C. Sokhna, L. Konaté, C. Boudin, D. Fontenille, J.F. Trape

The adaptation of Anopheles gambiae to its environment and humans involves an ongoing speciation process that can be best demonstrated by the existence of various chromosomal forms adapted to different environments and of two molecular forms known as incipient taxonomic units. Studies were conducted to determine the comparative susceptibility of Anopheles arabiensis (control group) and the molecular forms M and S of A. gambiae to Plasmodium falciparum. Mosquitoes were infected by direct membrane feeding assay then dissected on day 7–14 after feeding and examined for the presence of oocysts and sporozoite by ELISA. In feeding experiments involving 879 paired comparisons, the mean number of oocyst, oocyst rate and sporozoite rate were found to be significantly different. Of the test groups, molecular form S had the highest percentage of mosquitoes with oocyst (79.6%) and sporozoite (83.6%). There was no significant difference in oocyst (57.2% vs 55.3%) and sporozoite (50.9 % vs 50.8%) between the molecular form M and An. arabiensis. Infected in the same conditions the molecular form S seems to be more susceptible to infection by P. falciparum than the two other vectors. This susceptibility difference goes to further and suggests that these two forms are undergoing a
speciation process or support the hypothesis that these two forms are in fact two different species.

Email address for correspondence: bamideleedet@yahoo.com

243
Nationwide laboratory needs assessment to identify gaps in malaria laboratory diagnosis in Kenya [MIM16697363]

John Nyamuni

The Division of Malaria Control, Ministry of Medical Services, Kenya, supported by the USAID Improving Malaria Diagnostics Project under the President’s Malaria Initiative, conducted a nationwide malaria laboratory needs assessment to identify gaps in laboratory diagnostic services in government and faith-based health facilities. Two-day sensitization meetings were held with district and provincial laboratory supervisors to review a structured assessment tool. Information was captured on infrastructure, staffing, hours of operation, major equipment, supplies, reference materials, professional development, supervision, workload, malaria data, and external slide validation schemes. A team of national supervisors coordinated the exercise and provided support to provincial and district laboratory supervisors. In preliminary data from 56 representative facilities, 20% of laboratories lack functional microscopes and 11% had obsolete microscopes. Fifty-six percent of laboratory staff had not received training in malaria diagnosis since 2006, and 40% had not received supervision within the last year. Seventy-seven percent of laboratories experienced stock-outs of essential diagnostic supplies. A full analysis of approximately 1600 health facilities will be completed in March 2009. Final results will provide the Division of Malaria Control with health facility-specific information to guide decisions on human resource allocation, provision of supplies and equipment, training requirements, and quality assurance systems nationwide, and will provide a preliminary indication of the malaria case load by facility to identify priority intervention areas. These findings have relevance beyond malaria control, and will be shared with other health programs to strengthen laboratory services for improved health care delivery.

Email address for correspondence: mdmansu@yahoo.com

244
Systems effectiveness of a free distribution of long lasting insecticide treated nets in Zanzibar, Tanzania [MIM16698344]


Long lasting insecticide treated nets (LLINs) are prominent means of malaria prevention. Mass distribution of free LLINs to under-five children and pregnant women has been implemented in Zanzibar. We determined the outcomes of this distribution 4–9 months after implementation. A cross-sectional survey was performed in May 2006 in two districts of Zanzibar representing sub-optimal (Micheweni) and optimal (North A) distributions. Interviews with 509 caretakers of under-five children were conducted and inquired about socio-economic status, the distribution process, perceptions and use of bed nets. Degrees of success for different steps of the distribution process were assessed, and systems effectiveness and equity effectiveness were calculated. The proportion of children who slept under LLINs was 56.8% in Micheweni and 86.5% in North A. In Micheweni, the systems effectiveness was lower as compared with North A, and was higher in the least poor, whereas in North A it was higher in the poorest. Predicting factors for sleeping under an LLIN were residing in North A (OR = 3.5, p < 0.001), receiving an LLIN (OR = 3.5, p < 0.001), liking the LLIN size (OR = 0.5, p = 0.025), liking the LLIN mesh size (OR = 2.5, p < 0.001) and thinking that LLINs are better than conventional nets (OR = 2, p = 0.007). Although sustained use remains to be documented, we observed high LLIN coverage among under-five children. The different outcomes between the sub-optimal and optimal distributions point to the importance of a well planned distribution strategy which emphasizes on information, education and communication (IEC) and fair distribution to reach the poorest.

Email address for correspondence: eyobmk@yahoo.com

245
Development of a strategy to deliver a new malaria control tool: intermittent preventive treatment in Tanzanian infants [MIM16731256]

Fatuma Manzi, Joanna Schellenberg, Yuna Hamis, Mwifadhi Mrisho, Adiel K. Mushir, Kizito Shirima, Alex Mwita, Azma Simba, Neema Rusibamayila, Mary Kitambi, Marcel Tanner, Pedro Alonso, Hassan Mshinda, David Schellenberg

The translation of research findings into routine health care is challenging. Testing a new intervention under real life conditions should inform policy and practice. Efficacy information for Intermittent Preventive Treatment in infants (IPTi) was first available from southern Tanzania. Here we report development of a strategy for routine IPTi implementation in a total population of 1 million. A collaborative approach was employed to develop and implement IPTi under real life conditions. Researchers and policy makers at national and international levels worked with implementers at national, regional, district and facility level. The advice of key stakeholders on the development and fine tuning of the delivery system maintained awareness and knowledge sharing amongst the group. A gradual process was initiated to facilitate understanding of the local setting by collating evidence from monitoring and evaluation. The findings include contextualized evidence on how an intervention works in the local setting. Experience revealed the importance of institutionalizing the intervention for integrating the strategy into routine systems. The strategy was integrated into existing systems as far as possible and was well accepted by health staff. Thus, the collaborative approach effectively translated research findings into a strategy fit for public health implementation. We have showed how a promising health intervention can be prepared for deployment in real life to benefit those in need. This experience is relevant for other malaria control interventions.

Email address for correspondence: imayumana@yahoo.com

246
Pediatric diagnoses in a rural Kenyan hospital implementing the Kenyan inpatient pediatric guidelines [MIM16696658]


Due to lack of equipment and supplies, African rural hospitals commonly rely on signs and symptoms for some inpatient diagnoses. In preparation for a multi-site clinical trial, we implemented the Kenyan Inpatient Pediatric Guidelines (KIPG) and provided laboratory and radiology services at a rural district hospital. We describe admission signs, symptoms and diagnoses. Beginning August 2008, the first three acutely-ill children aged 2 months through 4 years admitted daily to the acute room in a rural hospital were enrolled. Study staff provided care following KIPG. Enrolled children provided a blood sample for malaria microscopy, complete
blood count, blood culture and sensitivity. Cerebral spinal fluid examination and culture were done when indicated. Children with respiratory symptoms had a chest X-ray. HIV-testing was offered. Thus far, 225 children, 105 males and 120 females, have been enrolled. Most children presented with fever (95%), cough (73%), difficulty in breathing (41%), diarrhea/nausea (35%) and/or seizures (24%). Overall, 63% were diagnosed with microscopy-proven malaria (35% severe malaria), 63% radiography-proven pneumonia, 22% meningitis, 10% critical anaemia (hemoglobin < 5 g/dL), and 5% severe diarrhea. In all, 19% had bacteraemia, with Salmonella most commonly isolated (9% of children). Chloramphenicol-resistance was common among salmonella isolates (90%, 18/20). HIV infection/exposure was common: 7% (11/164) of tested children were HIV antibody positive. Pneumonia, severe malaria, meningitis, and bacteraemia were major causes of hospitalization in this population. Further analysis and detail on specific etiologies and resistance patterns will help guide treatment and prevention efforts.

Email address for correspondence: akungu@gmail.com

247
Policy and implementation status of community case management of malaria, pneumonia, diarrhea, and neonatal sepsis in the countdown countries [MIM16703028]

Udita Patel Chek, David Marsh, Salim Sassrudin, Emmanuel D’Harcourt, Shamim Qazi, Stefan Petersen, Guelaye Sall, Franco Pagnoni, Karin Kallander, David Hamer, Mark Young, Alexandra de Sousa

Malaria, pneumonia, diarrhea and neonatal infections contribute to 76% of current mortality in children under five. Though there are effective interventions to prevent deaths related to these conditions they have failed to reach the children who need them most. Community Case Management (CCM) is a strategy that delivers antimalarials, antibiotics, and a combination of oral rehydration therapy and zinc at the community level by trained community health workers (CHWs) to the most vulnerable. At high coverage CCM can lead to significant mortality reductions to reach the fourth Millennium Development Goal. However, at present CCM is implemented at different levels in only few of the most needed countries. In order to build evidence for policy advocacy, a survey was conducted in the 68 countries were the world’s highest numbers of child mortality exist. UNICEF and MOH counterparts conducted a survey with 26 questions covering CCM policy and implementation status in the 68 countdown countries. The survey collected information on CCM of diarrhea, pneumonia, malaria and neonatal sepsis, and results were mapped in DevInfo. Of the 42 respondent countries, only 70% have a national program on CCM, whereas 26% have pilot programs conducted by NGOs and UN agencies. In only 71% countries more than one treatment is offered by CHWs. Our results show that great efforts are needed to scale effective interventions capable of reaching the most vulnerable. This survey reports countries profiles achieved on CCM as a strategy to increase worldwide accountability for progress in child survival.

Email address for correspondence: llaurent@ihi.or.tz

248
Pilot implementation of intermittent preventive treatment of malaria in infants (IPTI) [MIM16702418]

Alexandra de Sousa, Jean Louis Ndyae, Ebenizer Inkoom, Don Mathanga, Jacques Hassan, Alassane Dicko, Leon Paul Rabarijaona

IPTI is a new promising intervention that reduces an average of 30.1% malaria attacks during the first year of life in areas of moderate to high malaria transmission. IPTI consists of the delivery of the antimalarial drug sulfadoxine-pyrimethamine (SP) to infants alongside routine vaccinations of the Expanded Programme on Immunization (EPI). Evidence on programme effectiveness is critical to learn how to best reach universal and equitable coverage in a minimum amount of time. UNICEF conducted a pilot IPTI implementation in 20 districts in six African countries (Benin, Ghana, Mali, Senegal, Madagascar and Malawi) covering a population of 5 million people and 270,000 infants, with 0.5 million SP doses administered. Besides analysis of implementation bottlenecks and best practices, we evaluated the impact of IPTI on the EPI coverage and other malaria interventions, its cost, acceptability, drug resistance and pharmacovigilance safety profile. Maximum IPTI coverage was reached in 2 months, and the main bottleneck identified was the absence of a pediatric SP formulation for infants. IPTI significantly boosted the EPI coverage. Its delivery costs 15.48 cents per infant. IPTI was well accepted among health workers and communities, it did not increase the level of parasite molecular makers of resistance to SP after 1 year of implementation, and no Serious Adverse Events linked to IPTI were reported, neither by active follow up nor reported spontaneously. IPTI is a cheap and easy intervention to implement, able to boost the EPI program, and has an acceptable safety profile.

Email address for correspondence: adesousa@unicef.org

249
IFN-gamma and IL-4 responses induced by promiscuous T-cell epitopes of Plasmodium vivax Merozoite Surface Protein 9 (PvMSP9) in malaria naturally exposed individuals from Brazilian Amazon [MIM16143974]


PvMSP9 is a recently described vaccine candidate which stimulates both cellular and humoral immune responses in naturally exposed individuals. To identify possible promiscuous T-cell epitopes in PvMSP9, we used the MHC class-II binding peptide prediction software, ProPred algorithm, which contains 51 quantitative matrices for human MHC. Five peptide sequences [pE (147–159), pH (438–449), pJ (325–339), pK (434–448) and pL (443–456)] were predicted to bind to the largest number of HLA molecules and then tested in IFN-γ and IL-4 Elispot with PBMCs from 142 malaria-exposed individuals residents in the Brazilian Amazon, was then performed Multiplex PCR to HLA genotyping of this study cohort. The synthetic peptides elicited a robust IFN-γ and IL-4 recall responses. The overall frequencies of IFN-γ and IL-4 responders to at least one of the promiscuous peptides were 62% (88/142) and 46% (60/129), respectively. The frequencies of IFN-γ responders to each peptide were pE:50.7%, pH:36.6%, pJ:27.4%, pK:38.7% and pL:30.7% and the frequencies of IL-4 responders were pE:29.4%, pH:33.3%, pJ:25.4%, pK:26.3% and pL:30.4%. This cellular response was not associated to a particular HLA-DR and HLA-DQ allelic group, since most of the peptides induced a response in at least 12 HLA identified allelic groups. The experimental confirmation of promiscuous epitopes predicted using ProPred led to the identification of PvMSP9 immunodominant epitopes, recognized by PBMCs from a significant proportion of a genetically heterogeneous malaria-exposed population. The combination of such T cell epitopes in a vaccine may increase the frequency of responders and the overall effectiveness of immunizations in genetically distinct populations.

Email address for correspondence: josue@ioc.fiocruz.br
250

Analysis of cellular responses to recombinant PfEMP1 domain DBL1α tags dominantly expressed by the homologous parasite isolate [MIM15445979]


The immune response against the variant surface antigen Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) is a key component of clinical immunity against malaria. Studies investigating T-cell and antibody responses to PfEMP-1 have successfully used peptides based on the DBL1α domain of laboratory isolates suggesting that DBL1α-tags have the potential to be used as a model antigen in the analysis of PfEMP-1 variant specific immune responses. Parasites and PBMCs were isolated from 50 patients admitted to Kilifi District Hospital with an acute episode of malaria. DBLα-tags from PfEMP1 dominantly expressed by the homologous parasite isolate were cloned and expressed as recombinant proteins. The recombinant DBLα-tag was used to activate in PBMCs collected from the acute episode and from follow-up samples. CD4+ T cell responses were assessed by intracellular cytokine staining (ICS) for IFN-γ, IL-10, IL-2, IL-4, and CD 154. Cytokine expression profiles indicate that during acute infection, a proportion of T-cells in the peripheral circulation are activated and may secrete cytokines. During follow-up, CD4+ T-cells respond immediately to ex vivo stimulation using variant specific DBL1α-tags from PfEMP1. Recombinant DBLα-tag is a suitable antigen to study cellular responses to specific PfEMP-1 variants.

Email address for correspondence: egitau@kilifi.kemri-wellcome.org

251

IFN-γ responses to Plasmodium falciparum antigens in areas of unstable transmission decrease in periods of very low or absent transmission [MIM16696589]

Bilha Ogola, Gregory S. Noland, Ng’wena Gideon, Cyrus Ayieko, John M. Vulule, Chandy C. John

Immune responses to Plasmodium falciparum in areas of unstable malaria transmission, such as highland areas of Western Kenya, differ considerably from those in stable transmission areas. The longevity of cellular immunity in the absence of sustained transmission in these areas has not been described. Interferon gamma (IFN-γ) cytokine responses to pre-erythrocytic (CSP, LSA-1, and TRAP), blood-stage (MSP-1 and MB2), and pre-erythrocytic/blood stage (AMA-1) vaccine candidate antigens were measured by ELISA in PBMC culture supernatants from individuals residing in two unstable transmission areas of higher (n = 151) and lower (n = 141) baseline malaria incidence. Samples from the same individuals were collected in April and October 2008. During this period, cross sectional surveys showed an absence of asymptomatic parasitemia in the area and no microscopy positive cases of clinical malaria were seen in the area. Frequency of IFN-γ responses to all malaria antigens, except TRAP, declined by >2-fold between April (range 8.9–39.0%) and October 2008 (range 2.4–18.5%). Geometric mean levels of IFN-γ to CSP, LSA-1, MB2, and MSP-1 were also significantly reduced during this period. While there was no significant difference in frequency or levels of responses between sites in April, frequency of MB2 responses and geometric mean levels of MB2, LSA-1, and MSP-1 were significantly lower during October in the site with lower baseline transmission. IFN-γ responses to P. falciparum antigens in areas of unstable transmission wane during prolonged periods of low or absent transmission and wane more quickly in areas of historically less intense transmission.

Email address for correspondence: bilha2003@hotmail.com

252

Plasmodium falciparum GLURP and MSP3 induce higher Interleukin-21 in malaria-exposed compared to non-exposed malaria individuals [MIM16699089]

Ludovic Mewono, Selidji T. Agnandji, Davy W. Matondo Maya, Anne-Marie Nkoma, Berthe A. Iroungou, Saadou Issifou, Peter G. Kremsner

Interleukin-21 is type I cytokine which receptor belongs to the common gamma-chain receptor. IL-21 is produced by activated T-cells and may secrete cytokines. During follow-up, CD4+ T cell responses were assessed by intracellular cytokine staining (ICS) for IFN-γ, IL-10, IL-2, IL-4, and CD 154. Cytokine expression profiles indicate that during acute infection, a proportion of T-cells in the peripheral circulation are activated and may secrete cytokines. During follow-up, CD4+ T-cells respond immediately to ex vivo stimulation using variant specific DBL1α-tags from PfEMP1. Recombinant DBLα-tag is a suitable antigen to study cellular responses to specific PfEMP-1 variants.

Email address for correspondence: mewono2@yahoo.fr

253

Interferon-gamma (IFN-γ) promoter polymorphism (−1616A/G) conditions acquisition of parasitemia and malarial anemia in Kenyan children [MIM16614031]


The IFN-γ signaling pathway is important for mediating clinical outcomes in infectious and inflammatory diseases. Previous studies in malaria demonstrate that IFN-γ production is associated with both protection and pathogenesis. The role of IFN-γ −1616A/G promoter polymorphism in regulating malaria disease outcomes, however, remains largely unexplored, particularly in Plasmodium falciparum holoendemic transmission areas. As such, the impact of IFN-γ −1616A/G promoter variants on conditioning P. falciparum acquisition, severe malarial anemia (SMA; Hb < 6.0 g/dL), malarial anemia (MA; Hb < 8.0 g/dL) and high-density parasitemia (HDP ≥ 10,000 parasites/μL) were investigated. Children (aged 3–36 months; n = 623) were recruited at Siaya District Hospital, western Kenya, a P. falciparum holoendemic transmission area. Complete hematological, parasitical, and clinical indices were determined. Genotyping of IFN-γ −1616A/G was performed by Taqman 5’- allelic discrimination assay. Genotypic prevalence was AA (37.6%), AG (44.7%) and GG (17.7%), with allele frequencies of A = 0.60 and G = 0.40, respectively. Multivariate logistic regression analyses (controlling for age, gender, sickle-cell trait, HIV and bacteremia status) demonstrated that heterozygous individuals (AG) were 41% less likely of acquiring parasitemia (OR, 0.59; 95%
CI 0.39–0.90; P = 0.015) than wild-type (AA). Among children with acute malaria (n = 464), homozygous G individuals were protected against MA (OR, 0.49; 95% CI 0.24–0.99; P = 0.048), but not SMA of HDP. Variation in the IFN-γ promoter (−1616A/G) mediates acquisition of parasitemia and malarial anemia in children exposed to holoendemic \textit{P. falciparum} transmission.

Email address for correspondence: michaelongeha@yahoo.com

254
γδ T cells frequencies in sympatric ethnic groups with different susceptibility to malaria in Burkina Faso [MIM16697576]

W. Regis Tiendrebégou, Guillaume S. Sanou, André Lin Ouédraogo, Amidou Diarra, Alphonse Ouédraogo, Charlotte Behr, Marita Troye-Blomberg, David Modiano, Amagana Dolo, Maria G. Torcia, B. Sodimon Sirima, Issa Nébié

γδ T cells may associate with the malarial disease outcome through anti-parasitic responses or induction of immunomodulatory functions. To investigate these mechanisms involved in interethnic differences in cell- and cytokine-mediated responses to \textit{P. falciparum} infection, we carried out 2 case control studies comparing γδ T cells in two ethnic groups (Mossi and Fulani) living in sympatric with different susceptibility to malaria in Burkina Faso. A total of 60 Mossi and 72 Fulani adults were included in 2 cross-sectional studies high and low transmission season of 2007 and 2008 respectively. Collected peripheral blood mononuclear cells from the study subjects were stained for TCR-γδ and V62 phenotype assessment and intracellular IFNγ detection. There were no differences with season in the mean frequency of CD3 + V62+ subset cells either in Fulani or in Mossi (4.0% and 3.0% independently of season). Analyses in relation to ethnicity showed a higher frequency of CD3 + Pany6+ T cells in Fulani compared to Mossi during the high transmission season (8.2% and 6.3% respectively) but not at low transmission season (2.5% vs 2.7%). The mean frequency of cells producing interferon gamma (Panγ6+ IFNγ+) was higher in Fulani compared to Mossi (20.5% vs 12.4%). Our study shows that γδ T cells and interferon gamma production is associated with ethnicity. These data confirm the hypothesis that the resistance of Fulani ethnic group to malaria may be explained by the high rate of γδ T cells.

Email address for correspondence: tregis6@yahoo.fr

255
Regulatory T cell profiles in sympatric ethnic groups with different susceptibility to malaria in Burkina Faso [MIM16697466]

Guillaume S. Sanou, W. Regis Tiendrebégou, André Lin Ouédraogo, Amidou Diarra, Alphonse Ouédraogo, Charlotte Behr, Marita Troye-Blomberg, David Modiano, Amagana Dolo, Maria G. Torcia, B. Sodimon Sirima, Issa Nébié

Regulatory T cells have been reported to decrease human specific immune responses against \textit{P. falciparum}. To evaluate the role of these T cells, we carried out 2 case control studies comparing regulatory T cells in two ethnic groups (Mossi and Fulani) living in sympatric with different susceptibility to malaria in Burkina Faso. Two cross-sectional surveys were carried out at malaria high and low transmission season of 2007 and 2008 respectively in a hyperendemic transmission area. Peripheral blood mononuclear cells were isolated from 60 Mossi and 72 Fulani and stained for cell surface antigens expression and intracellular cytokine secretion profiles using flow cytometry. The prevalence of \textit{P. falciparum} was higher in Mossi compared to Fulani (27.1% and 8.8% at high transmission season) and (12.2% and 0% at low transmission season). The mean frequency of CD3 + CD4 + CD25+ T cells was lower in Fulani at high transmission season compared to Mossi (60.4% and 64.2% respectively) as well as at low transmission season (47.6% and 50.7% respectively). The peak frequency was observed during the high transmission season in both ethnic groups. Similarly, CD4 + CD25 + TGFB+ cells was found less frequent in Fulani compared to Mossi at high transmission (9.8% and 12.3% respectively) without an evident difference at low transmission season. In this study, regulatory T cells markers associate with ethnicity and season. These findings confirm the hypothesis that the resistance of Fulani to malaria might be linked to a deficit in regulatory T cells.

Email address for correspondence: sanougauillaume@gmail.com

256
Regulatory T cells in human geohelminth infection suppress immune responses to BCG and \textit{Plasmodium falciparum} [MIM16703891]


Chronic helminth infections induce T cell hyporesponsiveness, which may affect immune responses to other pathogens or to vaccine antigens. In experimental models of helminth infections T regulatory (Treg) cell numbers are increased, downregulating proliferation and cytokine responses of effector T cells. This study investigates the capacity of Treg cells to suppress immune responses to mycobacterial and malarial antigens during human geohelminth infection. CD4CD25hi T cells were magnetically depleted from freshly isolated PBMC of geohelminth infected and -uninfected Indonesian school children. Subsequently proliferation and cytokine production in response to BCG and \textit{P. falciparum} parasitized RBC (pRBC) were analyzed in total and depleted PBMC. Geohelminth-infected children’s in vitro T cell proliferation to either BCG or pRBC was reduced compared to that of uninfected children. Although the frequency of CD4 + CD25hiFOXP3+ T cells was similar regardless of infection status, the suppressive effect differed between geohelminth-infected and -uninfected groups: antigen-specific proliferative responses increased upon CD4 + CD25hi T cell depletion in geohelminth-infected subjects only. In addition IFN-γ production in response to both BCG and pRBC was increased after removal of CD4 + CD25hi T cells. In subjects free of geohelminths, CD4CD25hi T cells did not show such regulatory activity as depletion of these cells did not significantly enhance proliferation or cytokine production. These data demonstrate that geohelminth-associated human Treg cells influence immune responses to bystander antigens from mycobacteria and plasmodia. Geohelminth-induced immune modulation may thus have important consequences for vaccine efficacy assessments and immune responses to malaria coinfection. This work was supported by Royal Netherlands Academy of Arts and Sciences (KNAW 05-PP-35).

Email address for correspondence: l.j.wammes@lumc.nl

257
Measuring the impact of subsidized bednets in Tanzania [MIM16690024]

Chris Gingrich

Little information exists on the economics of insecticide treated mosquito nets (ITNs). In particular, no studies examine how consumer subsidies affect the ITN market and uptake in the community. This study analyzes Tanzania’s ITN market and measures the effect of the current national subsidy program on ITN uptake by both target and untargeted households. Data from a 2006 nationally representative survey reveal household demand preferences for...
258 Effectiveness and coverage of malaria social marketing communication mix [MIM16692648]

Christopher Mshana, Ahmed Makemba, Angel Dillip, Sandra Alba, Alexander Schulze, Christian Lengeler, Brigit Obrist Flora Kessy, Hassan Mshinda

The ACCESS Programme aims at understanding and improving access to prompt and effective malaria treatment and care in a rural Tanzanian district. A social marketing approach was adopted to increase awareness of malaria and to promote prompt and appropriate treatment seeking behaviour. Target audiences were segmented and different communication channels were used to convey key messages. Communication mix included road shows, posters, cinema, brochures, stickers, banners, billboards, a branded vehicle, community meetings, child and mother clinic campaign, T-shirts and caps. The paper assesses the coverage and effectiveness of the social marketing communication mix that we employed. A cross-sectional survey was conducted after 3 years of intervention (2004–2007) within the Demographic Surveillance System (DSS) which comprises a total population of 85,000. Our result revealed that people were 50–60 times more likely to recall the key malaria message if they noticed a T-shirt or caps compared to when they attended a video event (15–23 times). Having seen the branded vehicle and attending road shows also significantly increased the odds of recalling the key messages. In the intervention area, the intensive social marketing and health education campaign has resulted to increased preference for modern medicine by most patients; 87.5% of the fever cases in children and 80.7% in adults were treated with one of the recommended antimalarial. Social marketing with the selected channels was effective for communicating malaria messages. T-shirts and caps had the best impact, followed by seeing the branded vehicle and attending video shows. Email address for correspondence: cmshana2002@yahoo.co.uk

259 Community and health care providers’ perceptions of malaria prevention: ITNs and intermittent preventive treatment in children (IPTc) [MIM16698330]

Catherine Pitt, Amadou Konaté, Alassane Dicko, Diadier Diallo, Lesong Conteh

A placebo-controlled efficacy study in Burkina Faso and Mali was conducted in 2008–2009 to establish the degree to which intermittent preventive treatment of malaria in children under 5 (IPTc) can provide additional protection to children who sleep under an ITN. A qualitative follow-up study was conducted in the trial areas to examine the perceptions of health providers and families who would potentially be involved in implementing IPTc were it to become a recommended malaria prevention strategy. Semi-structured interviews and focus groups were conducted with approximately 100 participants. Participants included primary caregivers, community health workers, and health service staff members from the local to national levels. Analysis was conducted with NVivo software to identify and structure themes. The interviews and discussions explored perceptions of how IPTc delivery was associated with the use of ITNs and other malaria prevention strategies. Initial analysis suggests IPTc was seen as both a substitute and a complement to existing malaria prevention interventions. A number of important recommendations were made by health care personnel and caretakers about how to overcome the challenges of introducing and sustaining IPTc delivery. IPTc requires a delivery channel based in the community rather than health facilities. Successful widespread implementation of IPTc depends upon how providers and caretakers understand the intervention, whether they accept it, and the level of importance they place on it. Whether introduction of IPTc negatively influences attitudes towards ITNs and other health-promoting behaviours is important information when deciding the most appropriate and cost-effective malaria prevention strategies to adopt. Email address for correspondence: catherine.pitt@lshtm.ac.uk

260 Reaching the poor with health communication messages: Lessons learned from a National Voucher Scheme for treated nets in Tanzania [MIM16698995]

H. Mponda, J. Schellenberg, J. Bruce, Y. Sedekia, T. Marchant, C. Jones, K. Hanson

Health communication campaigns often targets to reach every one at risk, especially those from poor households. However, many of these campaigns have not yet been successfully reaching the target. We aimed to examine the effectiveness of the ongoing Tanzania National Voucher Scheme (TNVS) mass communication strategies reaching audiences and disaggregated findings by socio-economic status. 19 additional communication questions were added to TNVS household survey in 2006. The survey was a representative probability sample of 210 clusters, each had 30 households, selected from 21 districts across mainland Tanzania. The additional questions had two major topics on media reach and exposure of TNVS communication campaign. All household heads and women (15–49 years) were interviewed. We also involved stakeholders’ in-depth interviews exploring issues of comparative advantages and disadvantages of communication channels used. Further, operational barriers to media coverage, delivery and access of most popular media channels used for the campaign were explored through these interviews. Radio had the best media coverage and campaign exposure (58.7% households heads and 53.13% women). However, there were strong evidence on association of radio listening with SES, the poorest women had 32.6% while the better-off counterparts had 68.8%. Road shows reached poorest women slightly better than radio. Road shows if well organised could be better media for equitable communication coverage for health messages. However, a combination of the strategies is likely to be needed. Email address for correspondence: hadji.mponda@lshtm.ac.uk
261 Historical data from the US suggests affordable malaria treatment could have a significant impact on malaria transmission [MIM16739899]
Annemarie ter Veen, Menno Bouma, David Bradley, Anne Mills

US malaria incidence started declining after 1870, well before the advent of large-scale malaria control programmes in 1930. Until 1945 only cinchona bark derivatives and quinine sulphate (QS) were available for treatment. The onset of the decline in US malaria mortality coincided with a significant decrease in QS prices, from $4.50 per ounce in 1877 to a low of $0.17 in 1910. Import quantities increased over 70-fold during this period. In order to explore a possible association between malaria transmission and affordability of antimalarials, we used linear regression on time de-trended data from Mississippi. Here sharecroppers growing cotton represented the lowest income bracket, and malaria transmission exhibited strong epidemic cycles. Rates in the early 1900s were as high as in parts of Africa. Cotton prices represent the credit sharecroppers could obtain from landowners at the start of the growing season. 57% of inter-annual variability in malaria mortality rates in Mississippi between 1916 and 1945 could be explained using only two variables: QS price relative to cotton price (coef = 5.4, p < 0.001), and maximum May temperatures (coef = 4.18, p = 0.014), with QS/cotton ratios explaining most of the variability. Additional data suggests that relatively small improvements in per capita income/GDP over time correspond to significant improvements in malaria incidence in poorer US states and nations. Poverty reduction or the provision of free/reduced-cost antimalarials could independently contribute to the reduction of malaria transmission. A combination of the two strategies is expected to magnify the individual impacts and lead to an accelerated reduction in malaria transmission.

Email address for correspondence: annemarie.terveen@lshtm.ac.uk

263 Patterns of change in awareness, ownership, and use of ITNs from 2000 to 2008 [MIM16752935]
Carol Baume

In 2000, insecticide-treated nets (ITNs) to prevent malaria were virtually unknown in Africa, and major ITN programs began concerted efforts to create demand, reduce taxes and tariffs, spur the commercial market, and reach vulnerable populations with subsidized ITNs. The year 2005 marked a major shift in strategy when large infusions of donor money made ITNs available free of charge to households in order to make a rapid increase in ITN ownership. What was the impact of these strategies? Drawing on data from 15 standardized surveys from the USAID-sponsored AED/NetMark project, this study tracks awareness, ownership, and use of nets and ITNs from 2000 to 2008 in 5 countries in Africa. From 2000 to 2004 there were large increases in awareness and ownership of ITNs in all countries, usually with commensurate gains in the proportion of under-fives and pregnant women sleeping under ITNs. From 2005 to 2008, levels of ownership increased steeply following free net distribution. Levels of use increased, but did not keep pace with ownership. Purchased ITNs were more likely to be used than those obtained free. The “ITN picture” changed dramatically, both during the 2000–2004 period and the 2005–2008 period, but in different ways. The focus of ITN programs now should be on how to best sustain high levels of ownership and raise levels of use of ITNs owned.

Email address for correspondence: cbaume@aed.org

264 Characterization of antibody responses against adhesive domains of the malaria antigens Pf332 and PfEMP1 [MIM16669237]
Niloofar Rasti, Mats Wahlgren, Qijun Chen

Antibodies against the major surface antigen P. falciparum erythrocyte membrane protein 1 (PfEMP1) are believed to play an essential role in development of immunity to malaria. However other antigens expressed during the erythrocytic stage might be of equal importance. Recently, we identified an erythrocyte binding Duffy binding-like (DBL) domain of the surface associated antigen Pf332. We have analyzed naturally occurring antibodies towards the DBL-domain of Pf332 as well as the DBL-domain from a severe subtype of PfEMP1 in individuals residing in distinct malaria endemic regions. Further, human antibodies highly specific towards the DBL-domain of Pf332 were affinity purified and used to study invasion inhibition. Antibodies to both domains were induced after repeated exposure to P. falciparum. In general the IgG reactivity was significantly higher towards the conserved DBL-domain of Pf332 as compared to the more variable DBL-domain of PfEMP1. Children living in areas of intense malaria transmission had acquired antibodies against both antigens already at a very young age. Interestingly, the IgG levels to the DBL-domain of PfEMP1 were significantly higher in individuals in Mali known to be protected against malaria. The adhesive DBL-domain of Pf332 is very immunogenic and capable of eliciting a strong antibody response after infection with respectively, depending on the level of access to inpatient care. Pre-referral treatment with rectal artesunate is a cost-effective adjunct to standard parenteral treatment of severe malaria cases, judged by a standard of under US$ 50 per DALY averted for a very cost-effective intervention.

Email address for correspondence: tozan@bu.edu
**265**

Developmental shift in var gene transcription in *Plasmodium falciparum* patient isolates [MIM16672589]

Karin Blomqvist, Johan Normark, Daniel Nilsson, Ulf Ribacke, Judy Orikiriza, Petter Trillikott, Justus Byarugaba, Thomas G. Egwang, Fred Kironde, Björn Andersson, Mats Wahlgren

A major feature of *P. falciparum* parasitized red blood cells (pRBC) is their capacity to sequester in the microcirculation. The binding is mediated by PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), a variable protein encoded by the var gene family. *P. falciparum* avoids the host antibody response generated against previously used variants by switching the expression of PfEMP1, which also affects the disease outcome. We have here studied var gene transcription over time within the life cycle of the parasite by semi-quantitative PCR and sequencing by employing three sets of degenerate primers. To accurately determine transcript levels, subsequent in-depth analysis by quantitative PCR (Q-PCR) was made possible through the use of the gathered var sequences. Our data show that the intra-isolate var gene transcription dominance order often changes between different developmental stages. The maximum peak in var gene transcription varies in time among parasites, however in five out of seven isolates and strains, peak transcription occurs at 22–26 h post-invasion or is equal in ring and trophozoite stage. In addition to the unique var genes transcribed, var2CSA was found to be transcribed in all of the clinical isolates and varCOMMON/variCSA in six of the seven isolates and strains. The work presented here suggests that var gene transcription is more dynamic than previously thought, and that trophozoite pRBC is an optimal source of RNA for predicting the translated var gene species.

Email address for correspondence: karin.blomqvist@ki.se

**266**

Genetic diversity of expressed *Plasmodium falciparum* var genes from Tanzanian children with severe malaria [MIM16696510]

Joseph Paschal Mugasa, Wei Hong Qi, Sebastian Rusch, Matthias Rottman, Hans-Peter Beck

Severe malaria has been attributed to the expression of a restricted subset of the var multi-gene family, which encodes for *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 mediates cytoadherence to a variety of host cell receptors and causes a key element in the pathology of malaria, the sequestration of infected erythrocytes in post-capillary venules of the vital organs such as the brain or placenta. var genes are highly diverse, and can be classified in three major groups (ups A, B and C) and two intermediate groups (B/A and B/C) based on the genomic location, gene orientation and the upstream sequences. We have shown previously that var group A and B are up-regulated in clinical malaria compared to children with asymptomatic infections. We studied subsequently the genetic diversity of expressed var genes 2 associated with severe childhood malaria. By use of biotinylated magnetic beads tagged with the reverse complement of the conserved exon 2, full-length var mRNA was isolated and reverse transcribed into var cDNA. Different N-terminal domain tags were amplified by PCR, cloned and sequenced from isolates of children with severe (SM) and asymptomatic malaria (AM). Our analyses show high sequence diversity of the amplified var DBL-1α and upstream regions with minimal overlap among the isolates, providing strong evidence that var gene repertoire is immense and indefinite in high endemic areas. var DBL-1α sequences from AM isolates were more diverse with more singletons (p < 0.05) than those from SM cases.

Email address for correspondence: j.mugasa@bio.gla.ac.uk

**267**

Mechanisms regulating the surface expression of VAR2CSA and the importance for pregnancy associated malaria [MIM15030802]

Kim Brolin, Sandra Nilsson, Mats Wahlgren, Ulf Ribacke, Qijun Chen

VAR2CSA of the protein family PfEMP1 is the main adhesin involved in placental parasite sequestration of pregnant women with malaria. VAR2CSA is the main vaccine candidate for malaria in pregnancy, however, due to its polymorphism and size, more knowledge about this protein is needed. In this study, we have investigated gene structure, transcriptional and translational mechanisms as well as protein expression and function of VAR2CSA. Parasites were cultured according to standard procedures and repeatedly selected for a CSA-binding phenotype. Genomic DNA and RNA was extracted and analysed using an allelic discrimination and relative quantification approach with Q-RT-PCR. Protein translation and surface expression and reactivity were investigated using western blot, IFA and FACS. Findings demonstrate that VAR2CSA is up-regulated in CSA-selected parasites, that these translate VAR2CSA at various levels, and also show increased surface reactivity in a gender and parity specific pattern. VAR2CSA transcription, protein abundance, surface expression and reactivity varies between the different isolates, due to regulatory mechanisms in the parasite. By using a wide range of methods we have followed three parasite strains and their CSA-selected counterparts from gene structure to protein expression. This study, using an allelic discrimination approach to distinguish between sequence variations of VAR2CSA, is showing the many levels of differential regulatory mechanisms in these virulent parasites. Our results suggest that gene dosage as well as transcriptional and translational mechanisms partake in regulation of VAR2CSA surface expression. This has implications for the development of a vaccine protecting pregnant women against malaria.

Email address for correspondence: kim.brolin@smi.se
28-day in vivo test (WHO 2003). Blood samples for the molecular analysis were collected (day 0 before treatment and at the time of recurrent parasitaemia). DNA was extracted using the Chelex-100 method (Plowe et al., 1995). Detection of Pfct T76 and Pfmdr-1 Y86 mutations was done by using PCR followed by sequence-specific restriction enzyme digestion (Djimdé et al., 2001a). Nested PCR (Ranford-Cartwright et al., 1997) was adopted for the analysis of Msp1 and Msp2 to distinguish between recrudescence and new infection. Outcome at day 28 was known for most patients enrolled (90%, 195/217). The PCR-corrected total treatment failure (TTF) was 6% (12/195) with no difference between the two sites (P = 0.6) nor age groups (<5 years vs. >5 years (P = 0.11]). Before treatment, the prevalence of the Pfct T76, Pfmdr-1 Y86 or both mutations in the same infection was significantly higher among the TTF than among the adequate clinical and parasitological response (ACPR) samples (P = 0.03; P = 0.007; and P = 0.001, respectively). The prevalence of molecular markers did not differ between sites or age groups with the exception of Pfmdr-1 Y86, whose prevalence was significantly higher in children <5 years old (40%) than in the older group (21%) (P = 0.03). The Pfct T76 mutation is the main determinant for CQ resistance (Djimdé et al., 2001a; Dorsey et al., 2001; Tinto et al., 2003). In our study, the Pfct T76 mutation was strongly associated with AQ resistance, a result consistent with earlier studies in Burkina Faso (Dokomajilar et al., 2006) and in other African countries (Ochong et al., 2003; Holmgren et al., 2006), confirming its primary role in determining aminoquinolones resistance. The prevalence of Pfmdr-1 mutation was also significantly high in TTF than in ACPR samples, even if the relation was not as strong as for Pfct T76, a result consistent with earlier studies in Kenya and Nigeria, where AQ resistance is substantially higher than in Burkina Faso (Happi et al., 2006; Holmgren et al., 2006). In our study, the prevalence of both mutations Pfct T76 and Pfmdr-1 Y86 was higher in post-treatment than in pre-treatment samples, even if the difference was not statistically significant, possibly because of low AQ resistance. From the results obtained in this study, the definition of an AQ Genotype Failure Index, similar to that established for CQ (Djimdé et al., 2001b; Tinto et al., 2005) would be extremely useful in countries where AQ is used in combination with artesunate as the first or second-line treatment. However, we were unable to define that, possibly because of the limited number of failures we detected. Conclusion: In conclusion, the two molecular markers of CQ resistance seem to be linked to AQ resistance as well and could be used for surveillance purposes. However, larger studies in areas of higher AQ resistance should be conducted for this purpose.

Email address for correspondence: glougue5@yahoo.fr

Polymorphism of Merozoite surface protein (MSP)-1 and Merozoite surface protein (MSP)-2 of Plasmodium falciparum in Burkina Faso [MIM16716506]

Nikiéma Rosalie, Tinto Halidou, Valéa Innocent, Issaka Zongo, Ouédraogo Jean-Bosco, Umberto d’Alessandro, Guiguemdé T. Robert

Plasmodium falciparum antigenic diversity and polymorphism is an obstacle of antimalarial vaccine development. Merozoite surface protein (MSP)-1 and -2 are two highly polymorphic vaccine candidates. Characterization of their polymorphisms in endemic regions may facilitate the design of an effective vaccine. In the context carried out a study to investigate the polymorphism of Plasmodium falciparum two genes Merozoite surface protein (MSP)-1 and -2 in Burkina Faso. 165 children from 6 to 59 months of age consulting in Nanoro Medical center have been enrolled from September to December 2006. Children were treated with two combinations, Artéméther-Luméfantrine (AL) and Amodiaquine + Artésunate (AQ + AS). Nested-PCR was used to analyze the block 2 of MSP1 and block 3 of MSP2. The analysis of polymorphisms showed that K1 for MSP1 and 3D7 for MSP2 were the most circulating alleles in the parasites population. For MSP1, the number of alleles varied between 1 and 4, for MSP2, this number varied from 1 to 7. We also noted a high rate of monoclonal infections for FC27 (53.7%) before treatment, in opposite to 3D7 which showed as well monoclonal infections as 2, 3 or 4 clones. After treatment we observed a significant selection of 3D7 selection (p = 0.01). For FC27, we also observed a significant selection of infections (p = 0.02). Plasmodium falciparum polymorphism is extensive in Burkina Faso, and most of infections comprise multiple clones. The fluctuation of clones contributes to parasite diversity. Email address for correspondence: rosalienikiema@yahoo.fr

270 Relationship between the Plasmodium falciparum Pfmdr-1 gene mutations and the responses to the treatment with two artemisinin-based combinations in Burkina Faso [MIM16680387]

Da Dari, Tinto Halidou, Nikiéma Rosalie, Valéa Innocent, Issaka Zongo, Rouamba Noël, Ouédraogo Jean-Bosco, Umberto d’Alessandro, Guiguemdé T. Robert

Malaria treatment has been compromised by the Plasmodium falciparum resistance to commonly used antimalarial drugs. Therefore, many African countries have changed their drug policy with the adoption of ACTs. However, such choice has been done without knowing these drugs local effectiveness. We therefore propose to study the effectiveness of two combinations, artesunate + amodiaquine (AS + AQ) and artemether-lumefantrine (AL) in Burkina Faso, by investigating the relationship between the outcomes to treatments and the Pfmdr-1 gene mutations. The study was carried out from September to December 2006 in Nanoro, Burkina Faso. Patients were randomly assigned to receive standard doses of either AL or AS + AQ and followed up during 42 days. Primary endpoints were the treatment outcome PCR-adjusted. Afterwards, we evaluated polymorphisms in the Pfmdr-1 gene and their relationship with treatments outcomes. In total, 165 children with uncomplicated malaria were enrolled. Clinical evaluation of patients showed that cumulative PCR-corrected cure rates were 79.75% for AS + AQ and 78.05% for AL. For the pfmdr-1 gene, there was no mutation at position 1034. However, 34.36% and 6.25% of mutant alleles were found at position 86 and at position 1246 respectively. However, we found no relationship between the mutant genotypes and treatment failures for both AL and AS + AQ. We noted a high rate of treatment failures to both combinations and this is worrying as they are recommended for the treatment of uncomplicated malaria in Burkina Faso.

Email address for correspondence: dafrenick@yahoo.fr

271 Whether the number of concurrent clones in asymptomatic Plasmodium falciparum infections reflects the degree of host protection needs further elucidation: With the view to establishing the impact of transmission on diversity of P. falciparum infections an [MIM16699698]

Anna Färnert, Thomas N. Williams, Tabitha W. Mwangi, Anna Ehlin, Greg Fegan, Alex Macharia, Brett S. Lowe, Scott M. Montgomery, Kevin Marsh

The number of clones, determined by genotyping the highly polymorphic domain of the vaccine candidate antigen merozoite
272 Population replacement of An. gambiae by An. arabiensis mediated by the widespread use of insecticide-treated nets [MIM16678292]

Nabie Bayoh, Derrick Mathias, Luna Kamau, Maurice Odiere, John Gimnig, William Hawley, John Vulule, Edward Walker

Insecticide-treated bed nets (ITN) are increasingly being scaled up throughout Africa. Asembo Bay, Kenya was the site of a large community trial of ITNs. This trial revealed that widespread use of ITNs reduced the overall population of mosquitoes. In Asembo Bay, net ownership and use has been maintained at very high levels since the trial ended in 1999, and has increased in the surrounding areas. We report on our continuous monitoring of the mosquito population in the study and surrounding areas. Larval-stage mosquitoes were collected using area samplers and adults were collected by pyrethrum spray catches along a transect from 6 km within the original ITN study area to 6 km outside of the study area. The sampling was done in 2003, 2005, 2007 and 2008. In transects conducted in 2003 and 2005, there was a predominance of An. arabiensis in collections in the study area compared to the area outside of the study. The differences were largest in the larval samples. By 2007 and 2008, bednet ownership had increased throughout the entire region to >70% and An. gambiae became rare, with An. arabiensis representing >90% of both larval and adult samples in both the study and non-study areas. Overall, these results support the conclusion that high-coverage ITN programs have led to sustainable malaria control over many years, mediated through dramatic shifts to a vector which is less efficient in transmitting malaria but more difficult to control. Further transmission reduction interventions must adequately target An. arabiensis.

Email address for correspondence: jgimnig@cdc.gov

273 Estimation of infection rates and entomological inoculation rates (EIR) in the African malaria vector: When should CSP-ELISA be the ultimate? [MIM16733359]

J.D. Bigoga, L. Manga, R. Leke

Although CSP ELISA is considered as standard by most laboratories for infectivity and EIR determination, it is widely known to overestimate CSP rates. However, in many studies particularly in Africa the results are further confirmed using PCR, which is costly and expertise demanding. This study proposes a threshold cut-off value for positive CSP ELISA that should not require further confirmation by PCR. Indoor resting mosquitoes collected during three months in Douala, were comparatively assayed for P. falciparum infection using three methods (classical salivary gland dissections, CSA ELISA confirmed by PCR and the Vectest) and EIR determined. CSA ELISA specimens were assayed in three batches and the cut-off positive values for optical density (OD) readings taken at OD + 2SD, OD + 3SD, and at OD + 4SD respectively. Positive ELISA specimens were re-examined by PCR. The CSP rates by ELISA and Vect test were similar (5.8% and 7.5% respectively). Both methods were comparable in identifying P. falciparum CSA in mosquitoes. However, ELISA was more sensitive with regards to the number of infections detected. All positive CSP ELISAs at OD + 4SD were PCR positive while there was a 50% decrease in PCR compared to ELISA at OD + 2SD and 25% decrease at OD + 3SD. The findings suggest that cutoff values for CSP ELISA set at OD + 2SD are largely prone to producing false positive infections. However, cut-off values set at OD + 4SD is tantamount to obtaining PCR positive results on the mosquitoes and should therefore not warrant PCR confirmation.

Email address for correspondence: juldebigoga@yahoo.com

274 Comparison of PCR, ELISA-CSP and direct microscopic observation methods for the detection of Plasmodium falciparum sporozoites in Anopheles gambiae M. [MIM16705366]


The aim of the present study was to compare the enzyme-linked immuno sorbent assay for circumsporozoite antigen detection method (ELISA-CSP) and the polymerase chain reaction (PCR) technique used to identify Plasmodium falciparum genomic DNA markers, for the evaluation of both the sporozoïtic index (ICSP) and the entomological inoculation rate (EIR), two robust indicators of malaria transmission by mosquitoes. The study was conducted in laboratory on 86 specimens of Anopheles gambiae M infected after feeding with the blood of gametocytes-carrying individuals from Dielmo (Senegal). Salivary glands of 48 specimens randomly selected among the infected 86 were tested, after manual dissection and microscopically determination of sporozoïte positivity, using ELISA-CSP and PCR methods (test A). The head/thorax of the remaining 38 specimens was crushed, divided into two aliquots and used for both tests as previously (test B). The positive and negative results obtained were recorded and summed for each method. A pair-comparison of the results obtained with each method revealed in any case a good sensitivity (59–95%) and an excellent specificity (80–94%). The kappa coefficient (K) of test A indicated a “moderate” to “excellent” concordance between the three different methods performed. Using the crushed head/thorax sample, generally used to determine the transmission parameters (ICSP and EIR), the PCR/ELISA-CSP concordance was excellent (K = 0.84). More than an over-estimation of ICSP by ELISA-CSP, the use of DISS would occasion its under-estimation. Our results indicate that the PCR amplification on specific plasmodial DNA marker is a reliable method for the assessment of sporozoïtic index and the entomological inoculation rate.

Email address for correspondence: hubert.bassene@ird.sn

275 Biting and resting behaviour of Anopheles nili in North-Western Burkina Faso [MIM16677954]

Wamdaogo M. Guellbeogo, E.R. Saizonou/Poutya Marie-Rose, Ali Sié, Rainer Sauerborn, N’Fale Sagnon

The Anopheles nili group includes 4 species which are difficult to identify morphologically. Mosquitoes of this group are recognized as major human malaria vectors in tropical Africa, especially along
Anopheles mosquitoes: Not just flying malaria vectors...especially in the field [MIM16697167]

Christophe Boëte

The polymorphism of genes involved in the immunity of malaria vectors has been the subject of several recent studies with mosquitoes from natural populations. By looking at these recent studies, it appears that most of the genes examined are known for their role against Plasmodium berghei and not necessarily for their role against Plasmodium falciparum. In addition, it has recently been shown that the interactions of the mosquito larva with micro-organisms can have consequences on the future potential interactions of the adult mosquito with malaria parasites. It appears then highly important to be very careful when linking natural selection with malaria epidemiology. Moreover, we also need to consider the importance of other parasites and the environment on the mosquito genome. Developing field studies in ecological immunology and taking into account the potential effect of the environment and the other parasites on the mosquito genome seem to be major issues in better understanding the finely tuned host–pathogen relationships, particularly with regard to being relevant in terms of malaria control.

Email address for correspondence: cboete@gmail.com

Altitudinal and latitudinal variations in species composition of Anopheles gambiae complex (Diptera: Culicidae) along the volcanic Line of Cameroon [MIM16700479]

Timoléon Tchuinkam, Esperance Lélé-Défo, Billy Téné-Fossog, Samuel Wanji, Etienne Fondjo, Mpooame Mbida, Frédéric Simard, Didier Fontenille

Cameroon is considered as Africa in miniature and one main reason of this assertion is that it displays from the southern tropical humid forest to the northern arid savannah, a gradient of ecosystems and facies. The country therefore offers excellent opportunity to study variations in the sibling species within Anopheles gambiae complex. Mosquitoes were collected in three geographical regions along the Cameroon Volcanic Line, characterized by their topography: the Mount Cameroon region, the Bamilèke plateau and the Mandara-Kapsiki Mountains. In each of these zones, An. gambiae sl was sampled over an altitudinal transect by: dipping, human landing and pyrethrum spray catches. DNA of specimens were extracted from legs, amplified in a PCR-rDNA and identified to sibling species. A PCR-RFLP was performed to distinguish between molecular forms M/S of An. gambiae ss. Two members of An. gambiae complex were detected at Debundscha coast in Mount Cameroon region: 93.2% An. gambiae ss (100% of M molecular form), and 6.8% An. melas. Only An. gambiae ss was found at midway, both in Mutengene where M and S molecular forms were present at comparable rates (45.8% and 54.2% respectively), and in Meanja with a higher rate for S form (87.5%). Despite of the sympatry of the two molecular forms here, no hybrid was found. Uphill at Likoko and Ewonda, An. gambiae ss was still the single species, but with 100% of S molecular form. There was homogeneity in the composition of An. gambiae complex on the Bamilèke plateau, as all specimens were detected to be An. gambiae ss, with high predominance of about 98% of S molecular form in the plain of Santschou and on the plateau of Dschang, but 100% uphill at Djuttitsa. In the Mandara–Kapsiki Mountains there was again two sibling species An. gambiae ss and An. arabiensis, with a predominance of An. arabiensis both in lowland of Godola and at the elevated site of Moko. The S molecular form of An. gambiae ss was present in higher rate, 85.7% and 96% in Godola and Moko respectively. An. melas was found at the lowest altitude and latitude point in tropical humid forest, although at a low rate and in sympatry with An. gambiae ss, but disappeared relatively quick as we moved away from the sea coast, confirming thus that larvae stages of this species are definitely adapted to sea water. The M molecular form of An. gambiae ss was widely spread in this southern tropical forest and was progressively substituted by the S form as we moved farther both latitudinally within the continent and altitudinally at the same geographical region. The absence of hybrid of molecular forms at Mutengene indicates a completion of the speciation. The ecological conditions here seem to be appropriated for the two molecular forms. The S form was widely adapted to the Savannah region of the continent and progressively replaced by An. arabiensis upwards and northwards. An. gambiae sl exhibited an adaptive flexibility in response to environmental and climatic changes, in the form of genetic variations, both latitudinally and altitudinally, with a ratio of altitudinal changing distance 1000-fold the latitudinal one, and giving successively: An. melas, An. gambiae ss-M, An. gambiae ss–S and An. arabiensis. The nature of the selection pressures is still to be determined at the different changing points. Email address for correspondence: ddembele@mrtcbko.org

The Vector Population Monitoring Tool: DNA diagnostics for insecticide resistance monitoring in disease vectors [MIM16731635]

Martin J. Donnelly on behalf of the VPMT team

Pyrethroid-treated bednets and indoor residual spraying with insecticides are the mainstays of malaria vector control programmes in sub-Saharan Africa. Resistance to these insecticides threatens the sustainability of control. An understanding of the mechanisms conferring resistance facilitates the development of simple monitoring tools and may eventually lead to novel strategies to restore the efficacy of the insecticide. The Vector Population Monitoring Tool is an integrated series of assays that are intended to enable vector control programmes in Disease Endemic Countries (DECs) to rapidly identify and screen mosquito vector populations for Plasmodium infection and insecticide resistance. In addition
to designing assays for well established resistance markers we use whole genome microarray and candidate association mapping approaches to identify novel genes conferring metabolic resistance to insecticides in wild populations of *Anopheles gambiae*. This presentation will demonstrate how we have identified and validated some key metabolic resistance genes and are developing field appropriate technologies which can be used to screen for genetic markers for the early detection of insecticide resistance in field populations.

For improving malaria control, WHO recommend to develop new indicators evaluating anti-vector strategies. Previous studies had shown that the study of human Ab response to arthropod salivary proteins represent an epidemiological exposure marker to vector bites. This study aimed to validate this potential marker based on anti-saliva IgG Ab levels as a new indicator to evaluate the ITNs use efficacy in malaria control programs. A longitudinal study, concerning individuals (n = 108, children and adults) living in malaria endemic area in Angola, was performed from March 2005 to October 2006. The studied cohort was followed for parasitological, clinical, entomological and immunological data, each six weeks before and after the well-controlled use of Permanet® mosquito nets (installation in February 2006). Seasonal variations of IgG Ab levels to *An. gambiae* saliva were observed before and after the installation of ITNs, and appeared to be associated with the exposure level to *An. gambiae* and the prevalence/intensity of malaria infection. Moreover, a significant decrease of the anti-saliva IgG response was observed after the ITNs use which was correlated with the decrease of malaria parasitemia, the current and referent criteria showing the efficacy of ITNs. Results show here that anti-saliva IgG response in exposed individuals could not only be an immuno-epidemiological exposure marker to An. gambiae bites, but also a potential indicator for evaluating the ITNs efficacy. However, several future studies are needed to confirm this hypothesis in other transmission areas and using different anti-vector strategies.

For improving malaria control, WHO recommend to develop new indicators evaluating anti-vector strategies. Previous studies had shown that the study of human Ab response to arthropod salivary proteins represent an epidemiological exposure marker to vector bites. This study aimed to validate this potential marker based on anti-saliva IgG Ab levels as a new indicator to evaluate the ITNs use efficacy in malaria control programs. A longitudinal study, concerning individuals (n = 108, children and adults) living in malaria endemic area in Angola, was performed from March 2005 to October 2006. The studied cohort was followed for parasitological, clinical, entomological and immunological data, each six weeks before and after the well-controlled use of Permanet® mosquito nets (installation in February 2006). Seasonal variations of IgG Ab levels to *An. gambiae* saliva were observed before and after the installation of ITNs, and appeared to be associated with the exposure level to *An. gambiae* and the prevalence/intensity of malaria infection. Moreover, a significant decrease of the anti-saliva IgG response was observed after the ITNs use which was correlated with the decrease of malaria parasitemia, the current and referent criteria showing the efficacy of ITNs. Results show here that anti-saliva IgG response in exposed individuals could not only be an immuno-epidemiological exposure marker to An. gambiae bites, but also a potential indicator for evaluating the ITNs efficacy. However, several future studies are needed to confirm this hypothesis in other transmission areas and using different anti-vector strategies.

**279 Human antibody response to Anopheles gambiae saliva: A new immuno-epidemiological marker to evaluate the efficacy of insecticides treated nets (ITNs)?** [MIM15044378]

P.M. Drame, A. Poisignon, P. Besnard, S. Cornelia, V. Fournane, C. Sow, J. Le Mire, F. Fortes, D. Boulanger, F. Simondon, P. Carnevale, F. Remoué

For improving malaria control, WHO recommend to develop new indicators evaluating anti-vector strategies. Previous studies had shown that the study of human Ab response to arthropod salivary proteins represent an epidemiological exposure marker to vector bites. This study aimed to validate this potential marker based on anti-saliva IgG Ab levels as a new indicator to evaluate the ITNs use efficacy in malaria control programs. A longitudinal study, concerning individuals (n = 108, children and adults) living in malaria endemic area in Angola, was performed from March 2005 to October 2006. The studied cohort was followed for parasitological, clinical, entomological and immunological data, each six weeks before and after the well-controlled use of Permanet® mosquito nets (installation in February 2006). Seasonal variations of IgG Ab levels to *An. gambiae* saliva were observed before and after the installation of ITNs, and appeared to be associated with the exposure level to *An. gambiae* and the prevalence/intensity of malaria infection. Moreover, a significant decrease of the anti-saliva IgG response was observed after the ITNs use which was correlated with the decrease of malaria parasitemia, the current and referent criteria showing the efficacy of ITNs. Results show here that anti-saliva IgG response in exposed individuals could not only be an immuno-epidemiological exposure marker to An. gambiae bites, but also a potential indicator for evaluating the ITNs efficacy. However, several future studies are needed to confirm this hypothesis in other transmission areas and using different anti-vector strategies.

Email address for correspondence: dnairibodor@yahoo.co.uk

**280 Effectiveness of artemether-lumefantrine in underfive children with uncomplicated malaria at community level in rural Tanzania: Open label prospective study** [MIM14690090]

Billy Ngasala, Andreas Mårtensson, Anja M. Carlsson, Zul Premji, Costanzo, Aldo Morrone, Nathan Mulure, Luigi Toma

**281 Pharmacovigilance of antimalarial drugs: A prospective study on artesunate–amodiaquine treatment in Senegal** [MIM16197646]


The National Malaria Control Program in Senegal, under his new guidelines for malaria treatment, has introduced since 2006, Artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria cases. In this framework an antimalarial pharmacovigilance plan was developed and implemented in all health districts in Senegal. This study reports and analyzes adverse drug reactions (ADRs) collected in health facilities after antimalarial treatment. This was a prospective study based on spontaneous reports of ADRs after artesunate–amodiaquine combination treatment in health facilities and sent to NMCP between 1st January and 31st December 2007. Sixty-four (64) patients were enrolled during the study. There were more female (59%) than male patients (41%). The mean age of patients was 28.6 years. Most of cases (70%) were reported by nurses in heath posts. One hundred and thirty-eight (138) symptoms were identified in the population study. Gastrointestinal disorders (57%, 80/138) and neurological symptoms (24%, 34/138), were frequently observed. Imputability of spontaneous reports showed that 6% (8/138) of ADRs were linked to artesunate–amodiaquine. Imputability was likely in 25% of cases (36/138), possible in 33% of cases (47/138), unlikely in 28% of cases (39/138) and non-assessable in 8% of cases (8/138). No severe adverse events were reported. Artesunate–amodiaquine combination is well tolerated. However, it is necessary to expand the policy of collecting ADRs in all health facilities including hospitals and private structures and to strengthen the medical staff commitment to report all cases of ADRs.

Email address for correspondence: ssyllat@gmail.com

**282 Community-level deployment of artemether lumefantrine (AL) with rapid diagnostic testing: Effect on malaria mortality and health service utilization in a rural setting** [MIM16063302]

Hailemariam Lemma, Alem Desta, Edward Fottrell, Gehré Ab Barnabas Angela Bianchi, Andrea Bosman, Peter Byass, Gianfranco Costanzo, Aldo Morrone, Nathan Mulure, Luigi Toma

Prompt diagnosis of malaria and administration of artemether lumefantrine (AL) at community level may improve malaria...
control. A two-year observational study was undertaken (May 2005–June 2007) to evaluate the impact of deploying AL with rapid diagnostic testing through community health workers (CHWs). The study took place in the Alamata and Raya Azebo districts of Tigray, Northern Ethiopia. AL was first-line treatment for P. falciparum. In the intervention district (Alamata) AL was provided at village level through 33 trained CHWs and in health facilities, with phased introduction of rapid diagnostic tests (RDTs) by 50% of CHWs during year 2 of the project. In the control district, AL was provided only through health facilities without involvement of CHWs. In the intervention district, 75,654 patients with suspected malaria were managed by CHWs; only 54,774 patients were managed at health facilities compared to 101,535 patients in the control district. Over the two-year study, there was a significantly lower risk of malaria-specific death in the intervention district compared to the control district (adjusted incidence rate ratio 0.60, 95%CI 0.40–0.90, P = 0.013). Among 5123 patients assessed by CHWs using RDTs, only 526 (10.3%) were P. falciparum positive and received AL. In contrast, 38% patients examined with microscopy or RDTs at health facilities were diagnosed with P. falciparum and received AL. In this large-scale study in a resource-constrained rural setting, community-level rapid diagnosis and AL treatment was associated with a significantly lower risk of malaria-specific mortality and reduced transmission. RDT use facilitated targeted AL treatment.

Email address for correspondence: haile20022003@yahoo.com

283 Pharmacokinetics of artemisinin combination therapy in children in Kampala, Uganda [MIM16597761]

Julia Mwesigwa, Sunil Parikh, Bryan McGee, Polina German, Tamara D. Clark, Joan Nakayaga Grant Dorsey, Philip J. Rosenthal, Troy Drysdale, Niklas Lindegardh, Moses R. Kamya, Francesca Aweeka

WHO and Uganda Ministry of Health recommend the use of ACTs for the treatment of uncomplicated malaria. Pharmacokinetic (PK) data informing optimum dosing is limited in children who may exhibit enhanced metabolism and increased risk of treatment failure. An intensive PK study was completed in Ugandan children, aged 5–13 years, treated with artemether-lumefantrine (AL, n = 21) or artesunate/amodiaquine (AS/AQ, n = 20). For AL PK analysis was done for artemether, its active metabolite dihydroartemisinin (DHA) and lumefantrine. For AS/AQ PK analysis was done for AS, DHA and desethylamodiaquine (DEAQ) the active metabolite, rapidly formed from AQ. For AL, AUC/O–∞ for artemether, DHA, and lumefantrine were 193 h ng/mL, 404 h ng/mL and 248 h ng/mL respectively. Exposure to lumefantrine was 46% lower in children compared to a prior study in healthy adults. Exposure to artemether and DHA was approximately 3-fold and 2-fold higher, respectively compared to results from healthy adults. For AS/AQ, AUC/O–∞ for AS, DHA, and DEAQ were, 113 h ng/mL, 1509 h ng/mL, and 15,070 h ng/mL respectively. AS exposure was lower in children, DHA levels were roughly equivalent and DEAQ levels were higher in children as compared to data from two adult studies. These findings indicate lumefantrine AUC exposure is markedly lower in children treated with AL compared to healthy adults. This warrants caution for pediatric dosing of AL. For AS/AQ, higher DEAQ in children compared to adults suggests more rapid metabolic conversion to the active metabolite. Distinct PK characteristics of children must be considered for optimum dosing of antimalarial drugs.

Email address for correspondence: julimwesigwa@yahoo.com

284 Pharmacokinetics of dihydroartemisinin and piperazine in children with uncomplicated malaria in Burkina Faso [MIM16675320]

Halidou Tinto, Innocent Valéa, Silvia Pace, Marco Corsi, David Ubben, Umberto D’Alessandro, Allan Evans

Dihydroartemisinin-piperazine phosphate (DHA–PQP) is a promising fixed-dose combination treatment. During its clinical development, a pharmacokinetic (PK) study involving sparse sample analysis in children with uncomplicated malaria was carried out in Burkina Faso. Thirty children 6–59 months old with clinical malaria were treated with DHA–PQP (20 mg of DHA and 160 mg of PQP), daily for three consecutive days. Five blood samples were collected from each child (from 0 to 90 days) with no more than 3 samples in the first 24 h period. DHA–PQP efficacy (PCR-corrected) was 96.7% at day 28, 89.6% at day 42 and 86.2% at day 90. DHA exhibited multiple-peak phenomena due to three absorption processes with slow and fast absorption rate constants varying from 0.76 to 6.20 L/h. The total (apparent) volume of distribution of DHA was 0.705 L/kg and the apparent total clearance (CL/F) was approximately 1.45 L/(h kg). The mean of apparent terminal elimination half-life was around 1.45 h. For PQP, the plasma concentration versus time profiles were characterized by one slow absorption process that started within 1 h of dosing and one very quick input (almost bolus like) that arose about 3–4 h after dosing. The elimination half-life was very long with a mean value of about 23 days. The apparent total clearance (CL/F) was approximately 1.3 L/(h kg). The fixed DHA–PQP combination therapy was highly effective for the treatment of falciparum malaria and well tolerated. The PK profiles of both DHA and PQP in children are similar to those already observed in adults.

Email address for correspondence: tintohalidou@yahoo.fr

285 Cochrane review of artemisinin-based combination therapy [MIM15050408]

David Sinclair, Babalwa Zani, Hasifa Bukirwa, Sarah Donegan, Piero Olliaro, Paul Garner, Cochrane Infectious Diseases Group

The World Health Organization recommends Artemisinin Combination Therapy (ACT) for the treatment of uncomplicated malaria. As new combinations are developed careful evaluation of their safety and efficacy is required. We are currently preparing a Cochrane review of available ACTs, for use by policy makers, which provides a robust, scientifically defensible, global summary of their comparative effects. Objectives: To summarise the effects of ACTs in uncomplicated P. falciparum malaria. A secondary objective was to explore the effects of the combinations on P. vivax infection. A Cochrane systematic review of randomized controlled trials of adults and children with microscopically confirmed uncomplicated P. falciparum malaria. The primary outcome is treatment failure by days 28, 42 and 63 with adjustment for new infections. Secondary outcomes include gametocyte clearance, haemoglobin, effects on P. vivax and adverse events. The current draft version of this review includes 46 trials from a search in November 2008. The review provides substantive information on the relative and absolute effectiveness of (a) dihydroartemisinin-piperazine; (b) artesunate plus mefloquine; (c) artemether-lumefantrine; (d) artesunate plus amodiaquine; (e) artesunate plus sulfadoxine-pyrimethamine and (f) amodiaquine plus sulfadoxine-pyrimethamine. In September 2009 we will be updating the Cochrane review, and at the meeting we will present the Cochrane review and meta-analysis of head to head comparisons of ACTs.

Email address for correspondence: davesinkers@yahoo.com

www.mimalaria.org
286 Implications of price and availability of ACTs on malaria treatment in Uganda [MIM15247388]

Malaria ranks as the leading cause of morbidity and mortality in Uganda and is highly endemic throughout the country. In 2005, the Ugandan Ministry of Health changed the national policy on malaria treatment to artemisinin-based combination therapies (ACT) as first line drug. Despite this development, Uganda has lacked adequate data on levels and trends in price, availability and volumes of ACTs. In September 2008 a nationwide longitudinal panel study of outlets was conducted to provide benchmarks of trends and levels of ACTs and other anti-malarial medicines, followed by another round conducted in February 2009. A census of outlets was conducted in 38 sub-counties. Data was collected on availability of ACTs in public, NGO and mission health facilities, community based medicine distributors, pharmacies, drug shops, clinics and other informal outlets in both high and low malaria endemicity areas. Global Positioning System devices were used to record the coordinates of every outlet. Findings on coverage, access, availability and volume of ACTs throughout Uganda are presented. Findings will also highlight trends in providers’ perceptions and knowledge. Maps will be used to visually present and evaluate the spatial distribution of ACT availability. Availability of ACTs in private sector will inform design and review of ACT policy in Uganda leading to a possible nationwide change in the prescription status. Information on the pricing of ACTs will inform policies for the Affordable Medicines Facility for Malaria.

Email address for correspondence: ssensalire@psi.co.ug

287 Comparing different artemisinin-based combination therapies in Uganda: Implication for policy [MIM16644175]

Adoke Yeka, Hasifa Bukirwa, Sarah G. Staedke, Ambrose O. Talsiuna, Philip J. Rosenthal, Fred Wabwire-Mangen, Grant Dorsey, Moses R. Kamya

Most African countries have adopted artemisinin-based combination therapy (ACT) as first-line treatment for uncomplicated P. falciparum malaria. However, there is still some debate as to which ACT regimen is best. We summarize the treatment efficacy of 3 different ACT regimens from a series of randomized clinical trials conducted in different transmission settings in Uganda and discuss implications for policy. Data come from 10 randomized clinical trials conducted at 6 sites among children 6 months to 10 years of age with uncomplicated P. falciparum malaria treated with the following ACTs: amodiaquine + artesunate (7 studies, 1245 patients), artemether-lumefantrine (5 studies, 1109 patients) and dihydroartemisinin-piperaquine (3 studies, 777 patients).

288 Quality of artemesunate-containing antimalarials marketed in Nigeria [MIM16701029]

Oluseye O. Bolaji, Adeniyi E. Olagunuju

Artemisinin-based combination therapy has been adopted, in Nigeria and many African countries, as a first-line drug for malaria. However, as in many developing countries, the poor quality of antimalarials presents an obstacle to malaria control in Nigeria. Antimalarials of poor quality are known to contribute to the growing resistance of the major parasite, P. falciparum, to the medicines. In view of the potential danger that substandard antimalarial medicines are already posing in the fight against malaria in Nigeria we analysed the active ingredients or artemesunate-containing antimalarials marketed in the country. The contents of active ingredients of twelve (12) artemesunate-containing antimalarials were determined by HPLC-UV. All analyses were performed according to the specifications of the International Pharmacopoeia. All the samples contained the labelled active ingredients although with in amounts. Only nine (9) samples complied with the pharmacopeial requirements although two (2) of these samples also contained unidentified peaks thought to be those of other artemisinin derivatives. The presence of poor quality artemisinin-based antimalarials in Nigeria, as in other parts of Africa is real. This represents a potential route for resistance to these drugs in future. Every malaria patient treated with fake ACT is in danger of progressing to severe illness and in some cases of dying. The presence, on a wider scale, of fake or counterfeit artemisinins in the market may precipitate a collapse of confidence the artemisinin-based therapy. Appropriate steps must be taken to ensure a functioning drug control system in the country.

Email address for correspondence: obolaji@oauife.edu.ng

289 GSARIMA, a tool for malaria time series analysis during advanced phases of elimination campaigns with low case numbers [MIM4819027]

Olivier J.T. Briët, Penelope Vounatsou, Priyanie H. Amerasinghe

With the renewed drive towards malaria elimination, there is a need for improved surveillance tools. While time series analysis is an important tool for surveillance, prediction, and for measuring interventions’ impact, approximations by standard Gaussian methods are prone to inaccuracies when counts are low. Therefore, especially during “consolidation” and “pre-elimination” phases, statistical methods appropriate for count data are required. Generalized autoregressive moving average models (GSARIMA) were extended to generalized seasonal autoregressive integrated moving average (GSARIMA) models for modeling non-stationary and/or seasonal time series counts. The models were demonstrated using monthly malaria episode time series in a district in Sri Lanka, where malaria has decreased dramatically in recent years. The malaria series showed long-term changes in the mean, unstable variance, and seasonality. After fitting negative-binomial Bayesian models, both a GSARIMA and a GARIMA deterministic seasonality model were selected based on different criteria. The Bayesian modeling allowed for analysis of the posterior distributions of fitted observations. Those of negative-binomial models were more appropriate...
than those of Gaussian models. The G(S)ARIMA models satisfac-
torily accounted for the autocorrelation in the series, and produced
plausible prediction intervals GSARIMA models may be particularly
useful in the drive towards malaria elimination, since episode count
series are often seasonal and non-stationary, especially when con-
control is increased. Although building and fitting of GSARIMA models
is laborious, they provide more realistic prediction intervals than
Gaussian methods, and are more suitable for surveillance and the
evaluation of interventions when counts are low.

Email address for correspondence: o.briet@gmail.com

290
Developing maps of malaria risk and intervention coverage in
Kenya [MIM15000432]
Abdisalan M. Noor, Peter W. Gething, Anand P. Patil, Victor A.
Ale-gana, Simon I. Hay, Robert W. Snow

Examining changes in parasite prevalence against the evolution
of key malaria control strategies over a similar time and space
domain provides useful insight into changing malaria epidemi-
ology and resource needs. *Plasmodium falciparum* parasite prevalence
data for the period 1975–2008 were assembled from unpublished
random community and school surveys, national reports, and peer-
reviewed articles. Presence of parasites was examined using either
rapid diagnostic tests or microscopy. Data on insecticide treated
nets (ITN) coverage among children under the age of five years
were derived from three national cluster sample surveys conducted
between 2006 and 2007. Spatial-temporal Bayesian geostatistical
models were used with environmental and survey covariates to
predict continuous maps of malaria prevalence and ITN coverage in
Kenya for each year from 1975 to 2008. Changes in parasite preva-
ience were examined against changes in malaria control strategies.
Areas of highest malaria prevalence were along the Indian Ocean
coast and Lake Victoria. Although overall ITN coverage scaled well
with malaria risk, several high risk areas had low coverage. The
time-series plots showed peaks in parasite prevalence coinciding
failures. A significant drop of parasite prevalence was registered in
the periods following the national-scale of ITN (2005–2008). Com-
bining spatial-temporal maps of malaria prevalence and ITN coverage
provides useful tools to track the evolution of malaria epidemi-
ology. These maps should be used in determining target areas for
malaria control and forecasting changes in disease epidemiology.
Email address for correspondence: anoor@nairobi.kemri-wellcome.org

291
Malaria risk modeling in semi-arid areas of Africa: Somalia and
Djibouti [MIM15000338]
Abdisalan M. Noor, Peter W. Gething, Graine Moloney,
Mohammed Borle, Mouna A. Osman, Mauolid Mohamed, Simon I.
Hay, Robert W. Snow

Maps of malaria distribution are vital for optimal allocation of
resources for anti-malarial activities. There is a lack of reliable
contemporary malaria maps in semi-arid countries in sub-Saharan
Africa such as those located in the horn of Africa. Data were
assembled from a national malaria cluster sample survey in 2005
and routine cluster surveys in 2007 for Somalia and a malaria
indicator survey in 2008 for Djibouti. Rapid diagnostic tests were
used to examine the presence of *Plasmodium falciparum* parasites
in finger-prick blood samples obtained from individuals across all
age-groups. Bayesian geostatistical models, with environmental
and survey covariates, were used to predict continuous maps of
malaria prevalence across Somalia and Djibouti and to define
the uncertainty associated with the predictions. The inclusion of
covariates improved model fit for both countries. Model precision
was highest for Djibouti where data locations were evenly spread
across the country. The majority of areas in the two countries had
predicted prevalence of <5%. Areas with ≥5% prevalence were
predominantly in the south of Somalia near the Juba and Shabelle
rivers. The maps showed that malaria transmission in Somalia and
Djibouti varied from hypo- to meso-endemic. However, even after
including the selected covariates in the model, there still remained
a considerable amount of unexplained spatial variation in parasite
prevalence, indicating effects of other factors not captured in the
study. Nonetheless the maps presented here provide the best
contemporary information on malaria prevalence in Somalia and
Djibouti.

Email address for correspondence: anoor@nairobi.kemri-wellcome.org

292
An updated epidemiology of malaria in Kenyan school children
[MIM15050127]
Caroline Gitonga, Jimmy Kihara, George Okello, Hugh Sturrock,
Robert Snow, Simon Brooker

In Kenya, there is increasing evidence of the dramatic reduc-
tions in malaria mortality and morbidity in early childhood due
to recent expansion of malaria control efforts. These gains in
early childhood may, as a consequence of a slower acquisition of
exposure-dependent immunity, lead to an increased incidence of
malaria among older children, including school children. Malaria
among this age group has detrimental effects on haemoglobin lev-
els and learning and educational achievement. Despite this growing
awareness of the importance of malaria in schoolchildren there are
little contemporary and comprehensive data on malaria and the use
of bed nets among this age group. Therefore, we undertook a large-
scale survey which aimed to describe the epidemiology of malaria
in Kenyan schoolchildren and provide baseline information before
delivering a package of health interventions whose impact could
then be monitored over time. A cross-sectional survey was under-
taken in 65 schools in six districts along the Kenyan coast, from
the Tanzanian border to Malindi, between September and Octo-
ber 2008. In each school, 110 children were randomly selected and
tested for malaria using OptiMal® rapid test, haemoglobin, height
and weight, and helminth infections. Children were also asked
about their use of bed nets. A total of 7150 children in 65 schools
were selected for the study. In 13 schools, no children were found
were test positive for *Plasmodium* spp. The prevalence of infection
showed marked geographical variation, with prevalence reaching
35% in schools close to the Tanzanian border. Reported bednet
coverage also varied markedly between schools. Anaemia was com-
mon among the children. Information on the relationships between
malaria and bednet use and malaria and anaemia will also be pre-
presented. Comparison with earlier surveys will also be presented. It
is clear that levels of malaria parasitaemia among schoolchildren
living on the Kenya coast have been reduced in recent years, pre-
sumably the result of recent expansion of malaria control efforts.
However, there remains areas where infection remains prevalent.
This strongly supports the need for school health programmes
aimed at reducing the burden of malaria in school children, with
potentially beneficial effects on educational outcomes.

Email address for correspondence: cgitonga@nairobi.kemri-wellcome.org
Breastfeeding protects children against respiratory tract infection and diarrheal illness, but data on its effect on malaria are limited. We conducted a prospective cohort study of 348 Ugandan children, aged 1.5–9 months, living in an area of high malaria transmission. All children were given insecticide-treated bed nets and children born to HIV-infected mothers (n = 248) were given trimethoprim-sulfamethoxazole (TS) throughout follow-up. Malaria was diagnosed when a child presented with fever and a positive blood smear. Date of breastfeeding cessation was determined using monthly questionnaires. Generalized estimating equations were used to model the association between breastfeeding and the daily risk of malaria using multivariate analysis adjusting for repeated measures and age. 30% and 82% of children born to HIV-uninfected and HIV-infected mothers stopped breastfeeding during 15 months average observation, respectively. Adherence to TS was 99% among children born to HIV-infected mothers and 92% among HIV-infected mothers. Among children born to HIV-uninfected mothers, any breastfeeding was associated with an increased risk of malaria (RR = 1.80; p = 0.008). Among children born to HIV-infected mothers, any breastfeeding was associated with a decreased risk of malaria if mothers were taking TS (RR = 0.58; p = 0.001) but not if mothers were not taking TS (RR = 1.21, p = 0.44). Among children born to HIV-uninfected mothers breastfeeding was associated with an increased risk of malaria. We are exploring whether this finding may be explained by other confounding factors. Interestingly, among children born to HIV-infected mothers, breastfeeding was associated with a decrease risk of malaria but only if mothers were taking TS.

Email address for correspondence: abelkakuru@gmail.com

Regional influence of antimalarial drug pressure on epidemiology of malaria in Swaziland [MIM16645591]

Sabelo Dlamini, Colin Sutherland, Simon Kunene, Zuî’sile Zulu, Khalid Beshir

Despite widespread reports of chloroquine resistance (CQR) in Southern Africa, Swaziland still relies on chloroquine (CQ) for treating uncomplicated malaria. Influence of continued use of CQ on CQR markers, malaria incidence and death rates has never been investigated. We analysed DNA samples from Swaziland for markers of CQR between 1999 (73 thick blood smears) and 2007 (6 filter paper blood spots). Archive data on malaria incidence, case-fatality rates (CFRs), rainfall and quality of intervention programmes between 1997/98 and 2005/06 were also analysed to identify trends and correlations. Prevalence of 76T and 86Y mutations was high (71% and 83%, respectively) in Swaziland in 1999. 76T mutations remained high (OR = 0.4 95%CI 0.04, 24.0 p = 0.42) in 2007 but 86Y mutations decreased from 73% in 1999 to 33% in 2007 (OR = 0.14 95%CI 0.01–1.2, p = 0.04). A recovery of both 86N (OR = 6.5 95%CI 0.8, 77.0 p = 0.04) and 1246Y wild-type alleles was observed in 2007 following implementation of ACT policies in South Africa (KwaZulu-Natal) and Mozambique. A new mutation 86F, probably associated with amodiaquine uptake was observed in 4% (n = 51) of the 1999 samples. Increase of NFD (OR = 23 95%CI 2.2–294, p = 0.003) haplotypes and decrease of YYY haplotypes was also observed among 2007 samples. The CFR was found to increase against decreasing incidence rate between 1997/98 and 2005/06, and was finally interrupted in 2002/03 following AL implementation in KwaZulu-Natal (KZN). Use of ineffective CQ for case-management probably increased CFRs between 1997/98 and 2002/03 in Swaziland. Implementation of ACT policies in KZN could be associated with reversal of CQR and interruption of the increase of CFRs. A regional study of distribution and prevalence of CQR markers could add to our understanding of the impact of drug pressure on malaria epidemiology in Southern Africa.

Email address for correspondence: sabelo.dlamini@lshtm.ac.uk

The effect of malaria in pregnancy on neonatal and infant mortality in southern Mozambique [MIM16696437]

Azucena Bardaji, Betuel Siguavo, Maria Maixenchs, Sergi Sanz, Jaime Ordi, John Aponte, Pedro L. Alonso, Clara Menendez

There is some evidence on the effect of malaria in pregnancy on malaria morbidity in infancy. However, studies investigating the impact of malaria in pregnancy on neonatal and infant mortality have shown conflicting results. This study was conducted in the context of a randomised, placebo-controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) in pregnant women, in which live born babies were followed until 12 months of age. There were a total of 25 neonatal deaths and 58 infant deaths out of the 997 live born babies to 1030 enrolled Mozambican women in the IPTp trial. The risk of dying during the neonatal period was significantly higher among premature babies (RR, 6.24 [95% CI, 1.68–23.13]), live born to mothers with active placental infection (RR, 4.65 [95% CI, 1.40–15.46]), and in those with malaria parasitemia in cord blood (RR, 16.81 [95% CI, 2.45–115.25]).
Furthermore, the risk of dying within the first year of life was higher among low birth weight (RR, 2.82 [95% CI, 1.27–6.28]) and premature babies (RR, 3.19 [95% CI, 1.14–8.95]), in those whose mothers harboured acute placental infection (RR, 5.08 [95% CI, 1.77–14.53]), and among babies with parasitemia in cord blood (RR, 19.31 [95% CI, 4.44–84.02]). In this study it has been observed that malaria in pregnancy affects infant survival, and more importantly deaths occurring during the neonatal period. These findings highlight that promotion of malaria prevention strategies during pregnancy is critical in the fight against the burden that malaria represents in child health.

Email address for correspondence: abardaji@clinic.ub.es

297
Plasmodium falciparum genotype diversity in symptomatic malaria children under five years living in two different settings in Burkina Faso: Urban and rural area [MIM16696646]
Issiaka Soulama

The clinical presentation of malaria, resulting in a complex interaction between the parasite and the human genetic, is described to be different between rural and urban area. Analysis of the P. falciparum genetic diversity in uncomplicated malaria children living in these two different areas may have an interest to understand urbanization effect on P. falciparum genotypes distribution. Isolates collected in 75 and 89 uncomplicated malaria children living in rural and urban area of Burkina Faso respectively, were analyzed by a nested PCR amplification of msp1 and msp2 genes to compare P. falciparum diversity. The K1 allelic family (>90%) was widespread in the two sites compared to others msp1 allelic families. MAD 20 allelic family of msp1 was more prevalent (p = 0.0001) in urban (85.3%) vs. rural area (63.2%). In urban area, the 3D7 alleles were more prevalent compared to FC27 alleles with a high frequency for the 3D7300 bp allele (>30%). The multiplicity of infection was in the range of 1–6 in urban area and of 1–7 in rural area. There was no difference in the frequency of multiple infections (p = 0.6): 96.0% in urban versus 93.1% in rural area. The complexity of infection increased with age [p = 0.04 (rural area), p = 0.06 (urban area)]. Urban-rural area differences were observed in some allelic families (MAD20, FC27, 3D7), suggesting a probable impact of urbanization on genetic variability of P. falciparum. This should be taking into account in the implementation of malaria control measures.

Email address for correspondence: a.soulama.cnrfp@fasonet.bf

298
Using demographic surveillance system to record early inadvertent exposure of antimalarial during pregnancy [MIM16731756]
Abdunoor M. Kabanywanyi, Aggrey Malila, Mathew Alexander, Honesta Mzyangiziyangi, Honorati Masanja, Salim Abdulla

The risk of malaria infection and its severity tend to increase during pregnancy and for the same reason WHO recommends the use of antimalarial for case management and intermittent prevention of malaria during pregnancy (IPTp). In sub-Saharan Africa inadvertent exposure of antimalarial in early periods of pregnancy is common and there is no infrastructure to monitor drug safety during pregnancy. Random sampling of households with women of child bearing age was done during household survey in Ifakara demographic surveillance system (IDSS) in 2005. Women who were inadvertently exposed to antimalarial in the past two weeks were identified and offered a urinary pregnancy test. Pregnant women who tested positive were followed up to delivery and thereafter mother–child pair followed up quarterly for one year. Overall 268 women were identified and 253 reported use of antimalarial during the past two weeks preceding the interview. Nearly all, 94% agreed to undergo urine pregnancy tests. For those tested, 38 were pregnant and 10 (5%) of all who said are not pregnant before the test were found pregnant after test. There was no evidence of an adverse pregnancy outcome in any woman. Neonatal death was recorded for a birth that happened at home. This assessment has provided a tool to monitor early drug exposure in pregnancy. It has also revealed a gap that exists in morbidity and mortality information available between household and health facility that could be linked to allow proper post-birth safety ascertainment.

Email address for correspondence: omulokoz@gmail.com

299
Acquisition of antibody against VarO, a Plasmodium falciparum rosette-forming variant, in a Senegalese holoendemic rural setting [MIM16671019]
Mercereau-Puijalon Odile, Guillotte Micheline, Igonet Sébastien, Juillerat Alexandre, Petres Stéphane, Kouevidiin Ekoué, Baril Laurence, Bentley Graham, Vigan-Womas Inês

The capacity of Plasmodium falciparum infected erythrocytes to form rosettes and the absence of antibodies disrupting rosettes are associated with severe malaria in African children. However, the antibody response against rosetting variants remains poorly documented. Seroprevalence to VarO, a rosette-forming variant of the Palo Alto 89F5 clone was studied at the village level in a holoendemic rural setting (Dielmo, Senegal). Reactivity to the infected red blood cell surface was assessed by immuno-fluorescence (S-IFA). Antibodies to three soluble, correctly folded, recombinant PEMP1-varO domains were measured by ELISA. The kinetic of anti-varO antibody acquisition during the first six years of life was studied. Over 90% of villagers had VarO-surface reacting antibodies. All permanent residents had seroconverted by the age of five years. All individuals above 10 years had VarO rosette-disrupting antibodies. Seroprevalence to the three recombinant domains was high, namely 88% for NTS-DBL1a, 75% for CIDR1γ and 80% for DBL1α/C2. Interestingly, antibodies to CIDRγ were positively correlated with protection against clinical malaria, i.e. individuals with high levels of anti-CIDRγ antibodies experienced a lower number of clinical attacks in the next 12 months. VarO is a frequent serotype, against which antibodies are acquired early in life. Acquisition of surface-reacting antibodies and of antibodies to DBL1α (the rosette-mediating domain) precedes acquisition of antibodies functionally disrupting rosettes. Protection was associated with acquisition of antibodies to CIDRγ the least conserved domain in PEMP1 adhesins. All together, these data show that VarO represents a relevant in vitro model to develop vaccine strategies.

Email address for correspondence: odile.puijalon@pasteur.fr

300
Plasmodium falciparum var gene expression patterns in children with different levels of immunity to malaria [MIM16645474]
George Warimwe, Thomas Keane, Gregory Fegan, Jennifer N. Musyoki, Charles R.J.C. Newton, Arnab Pain, Matthew Berriman, Kevin Marsh, Peter C. Bull

The variant surface antigens (VSA) inserted on the surface of P. falciparum-infected erythrocytes appear to be important targets of immunity. As this immunity is acquired, changes in the
serological properties of VSA occur. VSA expressed by parasites from young children and those with severe malaria are more commonly recognized by plasma from malaria-exposed individuals as compared to VSA from older children or those with non-severe malaria. A major component of VSA is the cytoadhesive family of molecules called PfEMP1 which is encoded by ≈60 var genes that undergo clonal switching. Here we examine how the var expression patterns among parasites sampled from 217 Kenyan children are modified by host immunity. Parasites from children with different syndromes of malaria were compared with respect to (1) their expression of different subgroups of var sequence tags amplified from cDNA, and (2) their VSA’s serological properties as measured by flow cytometry and agglutination assays. Expression of var genes carrying sequence tags with two cysteine residues (cys2) was negatively associated with host age and positively associated with disease severity. Analysis of individual severe malaria syndromes showed that whereas parasites from patients with impaired consciousness and severe anaemia showed evidence for elevated expression of cys2 sequences, those from severe respiratory distress did not. These data support the idea that naturally acquired immunity to malaria acts by imposing a selection pressure on PfEMP1 variants expressed by the infecting parasite population.

Email address for correspondence: gwarimwe@kilifi.kemri-wellcome.org

301

Are antibody responses to PfEMP-1 reflected by antibody responses to DBLα-tags? [MIM15309131]

James Tuju, Peter Bull, George Warimwe, Eva Kimani, Kevin Marsh, Evelyn Gitau, Britta Urban

Natural immunity to malaria develops partly through acquisition of a wide pool of antibodies to variant surface antigens on Plasmodium falciparum infected erythrocytes. Recognition of antigens on the surface infected erythrocyte has previously been studied using methods that collectively measure responses to all variant antigens on the infected cell. We are now testing how immune responses to a small oligopeptide of P. falciparum erythrocyte membrane protein 1 (PfEMP1), the DBLα-tag, relate to age and exposure. We amplified by PCR 50 DBLα-tags from cDNA from field isolates and determined the dominant transcript. The dominant transcript was cloned into an expression vector and expressed in BL21(DE3)pLysS E. coli cells and then purified using Ni-chelate sepharose. The recombinant fragment was refolded by dialysis and endotoxin was removed using an endotoxin removal resin. The antibody reactivity to the recombinant proteins was analyzed by ELISA. We will present data on antibody recognition of recombinant DBLα-tags in sera from children living in Kilifi District in relation to age and exposure. So far, variant-specific antibody responses can only be measured using field isolates, a precious and limited resource. If antibody responses to the surface of infected erythrocyte are concordant with responses to the recombinant DBLα-tags derived from the dominant expressed PfEMP1 variant of the infecting field isolate, high throughput screening method using the Lumexin platform can be employed. This would allow precise, sensitive and reproducible profiling of antibody responses to a wide variety of variant DBLα-tags in very short time.

Email address for correspondence: jtuju@kilifi.kemri-wellcome.org

302

Humoral immunity in Mozambican children with severe and uncomplicated malaria [MIM16288101]

Eduard Rovira-Vallbona, Quique Bassat, Ruth Aguilar, Sonia Macheco, Laura Puyats, Inacio Mandomando, Chetan Chitnis, Pedro Alonso, Alfredo Mayor

Factors involved in the progression from Plasmodium falciparum infection to severe clinical disease are unclear and probably include parasite, host, geographic and socio-economic determinants. Among host parameters, deficient immunity might increase the risk of developing severe manifestations of malaria. A case–control study matched by sex and age was carried out in 142 Mozambican children presenting with severe (prostration, respiratory distress, anaemia, multiple seizures, cerebral malaria and hypoglycaemia) or uncomplicated malaria. IgGs against erythrocyte surface antigens from field isolates and laboratory lines, as well as IgGs and IgMs against merozoite antigens and a DBLα rosetting domain from P. falciparum erythrocyte membrane protein-1 (DBLαrostett), were measured by flow cytometry and ELISA. Association of antibody responses and malaria severity was determined by conditional logistic regression. Children were also stratified into two age groups (≤30 and >30 months) and analysed separately. For most of the parasites and recombinant proteins tested, IgG responses did not show significant differences between severe and uncomplicated malaria. However, IgG levels against the parasite line ITG-ICAM were significantly lower in children with complicated disease, and a borderline association was found for DBLαrostett. Risk of severe malaria was higher in children with low IgM antibodies against apical membrane antigen-1 (AMA-1), DBLαrostett and almost significant for erythrocyte binding antigen–175 (EBA-175), especially during early childhood. IgM antibody responses may play a role in protection from severe malaria. IgG antibodies to DBLα, a highly polymorphic domain linked to parasite virulence, may be involved in natural acquisition of immunity to severe disease.

Email address for correspondence: eduard.rovira@cresib.cat

303

Antibody responses to a panel of Plasmodium falciparum malaria blood-stage antigens in relation to clinical outcome in Sudan [MIM13188367]


Despite many intervention programmes aimed at curtailing the scourge, malaria remains a formidable problem of human health. Immunity to asexual blood-stage of Plasmodium falciparum malaria is thought to be associated with protective antibodies of certain immunoglobulin classes and subclasses. We have analysed immunoglobulin G profiles to six leading blood-stage antigens (AMA-1, MSP-2 3D7, FC27, MSP-3, GLURP-R0 and -R2) in relation to clinical malaria outcome in El-Obeid and Gedaref Hospitals, Sudan between 2004 and 2006. 545 individuals (176 severe, 230 uncomplicated malaria and 139 healthy controls) were consecutively recruited in the prospective study. The selection of antigens was based on their locations, expression and their association with protection in both preclinical and clinical studies. The measurement of the anti-malarial antibodies was performed by ELISA. Anti-malarial immunity were stratified into two sets of age (<5 and >5 years). To demonstrate the characteristics of the association we divided the distributions into groups. However, to maximise power we examined the linear association where it was appropriate. Our results revealed a linear association with anti-AMA-1-IgG1 antibodies in...
children <5 years and reduced risk of severe malaria as well as higher ratio of IgG1/IgG3, while the responses of the IgG3 antibodies against MSP-2, MSP-3, GLURP in individuals >5 years were bi-modal in a binary variable distribution analysis with multiple logistic regression. A dominance of IgG3 antibodies in >5 years was also observed with reduced ratio of IgG1/IgG3. In the final combined model, the highest levels of IgG1 antibodies to AMA-1, GLURP-R0, and the highest levels of IgG3 antibodies to 3D7 MSP-2 were independently associated with protection from clinical malaria. The study provides further support for the potential importance of the studied merozoite vaccine candidate antigens as targets for parasite neutralizing antibody responses of the IgG1 and IgG3 subclasses.

Email address for correspondence: kusi@bprc.nl

304
Humoral immune response to mixed AMA1 alleles; multivalent AMA1 vaccines induce broad specificity [MIM15066194]

Kwadwo A. Kusi, Bart W. Faber, Alan W. Thomas, Edmond J. Remarque

Apical membrane antigen 1 (AMA1) is a malaria merozoite membrane protein, essential for red cell invasion. Antibodies to AMA1 have anti-parasitic effects in experimental animal models and when isolated from AMA1-vaccinated or malaria-exposed humans. AMA1 is polymorphic, a property supposedly driven by host immune pressure, and thus of concern for its development as a vaccine. Indeed, antibodies to AMA1 less inhibit heterologous parasites in vitro. Here, the induction of broad strain antibody inhibitory antibodies with a multi-allele AMA1 vaccine, and the functional importance of cross-reactive and strain-specific IgG fractions elicited upon AMA1 immunization were assessed by competition ELISA and in vitro parasite growth inhibition assays. Immunization of rabbits with an AMA1 allele mixture yielded an increased proportion of antibodies to epitopes that are common to all vaccine alleles, compared to single allele immunization. Competition ELISA with affinity-purified cross-reactive antibodies between two different AMA1 alleles showed that over 80% of these common antibodies were shared with other AMA1 alleles. Furthermore, growth inhibition assays revealed that for any AMA1 allele, the cross-reactive fraction alone, on a per mg basis, had the same functional capacity on homologous parasites as the total affinity-purified IgGs. By contrast, the strain-specific fraction showed slightly less inhibition of red cell invasion by homologous strains. Thus multi-allele vaccination predominantly induces antibodies to common epitopes and reduces the proportion of antibodies to strain-specific epitopes. The focus on common epitopes explains the broadened cross-inhibition of diverse malaria parasites after immunization, and suggests multi-allele approaches warrant further clinical investigation. This work was funded by EMVI (grant number LSHP-CT-2007-037506).

Email address for correspondence: kusi@bprc.nl

305
Heterologous prime-boost immunizations with different alleles of MSP1 as strategy to overcome allele-specific immunity [MIM16682587]

Evelina Angov, Elizabeth H. Duncan, Kari M. Laquer, Elke S. Bergmann-Leitner

P. falciparum MSP1 is a leading erythrocytic-stage malaria vaccine candidate. The 195 kDa protein is processed to several fragments, and has been implicated in binding and/or invasion of erythrocytes by merozoites. At the time of erythrocyte invasion, the C-terminal fragment known as MSP142 undergoes secondary processing yielding a 33 and a 19 kDa fragment (MSP119). Previous results from pre-clinical and seroepidemiological studies have shown that antibody responses to fragments of MSP142, particularly to the MSP119, correlate with protection and/or reduced parasite densities, but these studies do not address the effect of pre-existing immunity or concurrent exposure and allelic heterogeneity. We have investigated this effect by using an experimental mouse model in which Balb/C mice were immunized with recombinant P. falciparum MSP142 antigens representing the three major alleles of MSP142, 3D7, FVO and Camp/FUP. Immunization strategies were designed to evaluate T cell and B cell recall responses induced by homologous or heterologous boosting (i.e. prime 3D7:3D7 or prime 3D7:FVO, etc.). We will report 1 allele specific antibody responses to p33, p19 (EGF-like domain 1 and 2) induced by homologous and heterologous immunization strategies measured by ELISA and bead-based luminox, (2) functional activity of MSP-1-specific antibodies against various alleles measured by pLDH-based GIA, and (3) dissecting allele-specific Th1 and Th2 cellular responses by IFNγ and IL-4 ELISpot assays. Since in malaria endemic areas, multiple strains P. falciparum circulate at any given instance, we set-out to address the potential for recombinant MSP142 subunit vaccines to prime and boost allele-cross-reactive recall responses.

Email address for correspondence: cmugyenyi@kilifi.kemri-wellcome.org

306
Antibodies to an invasion-inhibitory epitope on Plasmodium falciparum apical membrane antigen 1 (AMA1) are associated with protection from clinical disease [MIM16520741]

Cleopatra K. Mugyenyi, Fiona McCallum, Robin Anders, Kevin Marsh, James Beeson

Plasmodium falciparum AMA1 is a leading malaria vaccine candidate and is an important target of protective immunity. Antibodies to AMA1 may function by inhibition of invasion. We examined the prevalence and acquisition of antibodies to specific polymorphic and invasion-inhibitory AMA1 epitopes, and related these antibodies to protection from clinical malaria. Samples were obtained from individuals in a malaria-endemic area in Kenya who were monitored weekly by active surveillance over a period of 6 months for clinical malaria. Antibodies to recombinant AMA1 were measured by ELISA. Antibodies to an invasion-inhibitory epitope and a non-invasion inhibitory epitope were measured by competition ELISA using anti-AMA1 monoclonal antibodies 1F9 and 2CS. Protection from clinical malaria was investigated using Kaplan–Meier survival functions and Cox proportional hazard models. Prevalence of anti-AMA1 IgG (82.3%) and anti-2CS epitope IgG (52.0%) was high compared to those specific to the 1F9 invasion-inhibitory epitope (20.7%). Those with high antibody responses to AMA1 (HR = 0.36, p = 0.02), the 1F9 epitope (HR = 0.21, p = 0.01) and the 2CS epitope (HR = 0.38, p = 0.06) were less likely to experience a clinical malaria episode. Individuals with a high combined response to AMA1 and specific epitopes also showed significantly reduced risk of clinical disease (AMA1/1F9 HR = 0.15, p = 0.02; AMA1/2CS HR = 0.21, p = 0.02; 1F9/2CS HR = 0.23, p = 0.07). High levels of antibodies to the 1F9 epitope and AMA1 among individuals correlated with the growth-inhibitory activity of serum samples. Antibodies to polymorphic and invasion inhibitory epitopes were associated with reduced risk of malaria and could be involved in contributing to protective immunity to malaria.

Email address for correspondence: cmugyenyi@kilifi.kemri-wellcome.org
Impact of intermittent preventive treatment with sulfadoxine-pyrimethamine on immune responses to erythrocytic stage antigens in Mozambican children [MIM16818946]

Diana Quelhas, Laura Puyol, Llorenç Quintó, Tacilia Nhampossa, Eusebio Macete, Pedro Aide, Elisa Serra-Casas, Alfonso Jiménez, Pau Cisteró, Alfredo Mayor, Inacio Mandomando, Sergi Sanz, John J. Aponte, Virander S. Chauhan, Chetan

Intermittent preventive treatment in infants with sulfadoxine-pyrimethamine (IPTi-SP) along the EPI, has shown to be an efficacious and safe intervention against malaria in sub-Saharan Africa, and is currently being considered for adoption as policy. However, data on the effect of IPTi on the development of naturally acquired immunity is scarce. We evaluated the impact of IPTi-SP, given at 3, 4, and 9 months, on the development of antibody and cellular responses to Plasmodium falciparum in Mozambique. In a group of 302 infants at ages 5, 9, 12, and 24 months, we measured IgG to whole asexual parasites by IFAT and IgM and IgG subclass antibodies to recombinant merozoite antigens by ELISA. Using FACS, we measured IgG against VSA in the same children as well as growth inhibitory antibodies at ages 12 and 24 months. Intracellular and extracellular cytokines were measured by FACS and luminex, respectively. Antibody responses did not significantly differ between treatment groups at any time point measured, with the exception of IgG and IgG1 to AMA-1 and/or MSP-119, which were significantly higher in the control group at ages 5, 9, and/or 24 months. The effect of IPTi on the development of IgG to VSA and on growth inhibitory antibodies will also be presented. Preliminary data suggest that cytokine responses do not significantly vary in children receiving SP or placebo. IPTi-SP does not appear to negatively affect the development of immune responses to P. falciparum and, in some cases, it appears to be associated with higher antibody levels.

Email address for correspondence: diana.quelhas@manhica.net

Drug resistance characterization of pfdhfr, pfdhps and pfcrt haplotypes in P. falciparum parasites from Angolan patients [MIM15838929]

Bianca E. Gama, Guilhermina A.L. Pereira-Carvalho, Natalia K. Almeida, Filomena G. Silva, Filomeno Fortes, Cláudio T. Daniel-Ribeiro, Maria de Fátima Ferreira-da-Cruz

Malaria devastates million of lives, mainly in Africa. Although diagnosis and effective treatment are the mainstay of control strategy, this tactic is strongly compromised by the emergence of parasite resistance. In this way, the characterization of drug resistant parasites may contribute to morbidity reduction, as well as for monitoring the prevalence of chloroquine resistant parasites in population. Using a nested PCR associated to sequencing it was possible to genotype SNPs of pfdhfr and pfdhps as well as pfcrt genes, associated with sulfadoxine-pyrimethamine (SP) and chloroquine (CQ) resistance, in 84 Angolan samples. The pfdhfr analysis revealed the existence of 4 haplotypes (ACIRNV, ACICNV, ACNRNV, ARICNV) related to drug resistance profiles and the pfdhps typing showed 4 resistant haplotypes – SGKAA, AGKAA, SGEAA, SGECA – and the sensitive SAKAA. The pfcrt gene displayed a great number of single and mixed haplotypes: 5 single-type resistant haplotypes (SVMNT, CVIET, CVMNT, CVINT, CVMDT) and the sensitive CVNMK, besides 5 mixed-type resistant haplotypes. The high degree of SP resistance suggests the need of SP replacement by an alternative drug in the near future for the intermittent preventive treatment during pregnancy in Angola. Surprisingly, concerning the analysis of resistance to CQ, we detected the presence of wild P. falciparum parasites. Furthermore, SVMNT was the most prevalent haplotype, instead of the African typical CVIET. This finding could reflect that parasites carrying SVMNT haplotype of Asian or South American origin spread across Africa; an analysis of flanking microsatellites is being prepared to clarify this issue.

Email address for correspondence: bgama@ioc.fiocruz.br

Spread of Pfcrt, Pfmdr-1, dhfr and dhps genes in P. falciparum isolates treated with chloroquine and sulfadoxine-pyrimethaminem in Sudan [MIM16689499]


Malaria parasite resistant to chloroquine poses severe health problems in tropical countries. Molecular markers for monitoring the drugs resistance may be essential to overcome the problem. Forty patients out of 176 were completed in vivo sensitivity assay. The DNA was used to assess the prevalence of mutations of chloroquine resistance P. falciparum strains Pfcrt K76T, Pfmdr-1, N86Yand the prevalence of mutation of sulfadoxine-pyrimethaminem strains dhfr and dhps genes. Twenty two (54%) responded very well to chloroquine regimen with adequate clinical response (ACR), however, 18/40 (46%) were found to be chloroquine resistant. The in vitro assay showed that 32 (80%) of the isolates were resistant to Chloroquine. Thirteen (72%) isolates have mixed haplotypes. The high degree of SP resistance is related to drug resistance profiles and the pfdhps typing showed 4 resistant haplotypes – ACIRNVI, ACICNVI, ACNRNVI, ARICNVI. Using a nested PCR associated to sequencing it was possible to genotype SNPs of pfdhfr, pfdhps and pfcrt genes in 40 Angolan samples. The polymorphism...
of dhfr gene was in codons 108, 59 and 51. In codon 51 only 2 (4.5%) were mutant type and 28 (63.6%) were wild type, 3 (6.8%) were found as mixed infection (both mutant and wild types). One sample (2.2%) was dhfr 59 mutant and 31 (70.4%) were wild type, while 14 (31.8%) were dhfr 108 mutant and 24 (54.5%) were wild types, 3 (6.8%) were found as mixed infection. The screening of dhps 540 polymorphisms of the gene revealed that 2 (4.5%) were found as mixed infection, and 42 (95.5%) as wild type. The association between Pfcrt T76, and Pfmdr1 Y 86 for chloroquine resistant and dhfr, dhps polymorphisms from the current study, showed that 5/15 (33.3%) were found to be mutant at dhfr 108 and PfcrtT76 genes this means that there is a association between dhfr108 and Pfcrt76 genes. The high frequency of the mutant Pfcrt 76T gene among P. falciparum isolates in consistent with in vivo and in vitro study reveals that chloroquine is no longer suitable for treatment of malaria patients, However, the study concluded that the polymorphism in the dhfr and dhps genes are less abundant, but most likely increasing, and thus sulphadoxine-pyrimethamine (S/P) resistance may rapidly increasing.

Email address for correspondence: ahmedeltahirm@yahoo.com

311

Yronardin- artesunate: In vitro activity against clinical isolates of Plasmodium falciparum in Gabon [MIM16506517]

Florian Kurth, Peter G. Kremsner, Michael Ramharter

Pyronaridine is one of the most promising novel partner compounds for artemisinin combination therapy and is currently under consideration at international drug registration agencies for the treatment of uncomplicated Plasmodium falciparum and vivax malaria. Pyronaridine artesunate is likely to be used on a large scale in sub-Saharan Africa due to its low cost and based on data from malaria. Pyronaridine is likely to be used on a large scale treatment of uncomplicated malaria patients, However, the study concluded that the polymorphism in the dhfr and dhps genes are less abundant, but most likely increasing, and thus sulphadoxine-pyrimethamine (S/P) resistance may rapidly increasing.

Email address for correspondence: ahmedeltahirm@yahoo.com

312

Comparative study of the quality and efficiency of artemisinin drug based and Artemisia annua grown in Cameroon [MIM15225512]

R.D. Chougouo Kengne, J. Kouamouo, R. Moyou Somo, A. Penge On’Okoko

Malaria is the leading cause of death worldwide, especially in the Sub of Saharan Africa. This treatment has been a problem because of resistance. The Western part of Cameroon has 33% of counterfeit drugs in the market and Artemisia annua is a plant known for its antimalarial effect because containing artemisinin.

Email address for correspondence: ffefe@yahoo.com
314 Antiplasmodial activity of extracts from seven medicinal plants used in malaria treatment in Cameroon [MIM16790000]

Fabricc Fekam Boyom, Eugénie Madiisse Kemgne, Roselyne Tepongning, Wilfred Fon Mbacham, Etienne Tsamo, Paul Henri Amvam Zollo, Jiri Gut, Philip J. Rosenthal

Because the evolution of drug resistance is likely to compromise every drug in time, the demand for new antimalarial therapies is continuous. Accordingly, a vibrant drug discovery pipeline is needed to help ensure the availability of new products that will reduce mortality and morbidity resulting from malaria. To this end, we have carried out an ethnopharmacological study to evaluate the susceptibility of cultured *Plasmodium falciparum* to extracts and fractions from seven Cameroonian medicinal plants used in malaria treatment. We also explored inhibition of the *P. falciparum* cysteine protease falcipain-2. The majority of plant extracts were highly active against *P. falciparum in vitro*, with IC50 values lower than 5 μg/ml. Annonaceous extracts (acetogenins-rich fractions and interface precipitates) exhibited the highest potency. Some of these extracts exhibited modest inhibition of falcipain-2. These results support continued investigation of components of traditional medicines as potential new antimalarial agents.

Email address for correspondence: eugboyom@yahoo.fr

315 High prevalence of dhfr triple mutant and increased in vivo SP treatment failures in Gabonese children [MIM16790937]

Ghyslain Mombo-ngoma

Drug resistance contributes to the burden of malaria worldwide. Polymorphisms in *Plasmodium falciparum* dihydrofolate reductase (DHFR) cause resistance to the antifolate drugs, and polymorphisms in dihydropteroate synthase (DHPS) cause resistance to sulpha-
doxine. We determined the prevalence of DHFR and DHPS point mutations in falciparum-positive samples collected from 2005 to 2007. For assessment of the role of resistance-conferring parasite mutations on treatment responses to sulfadoxine-pyrimethamine (SP) and transmission potential, 29 children under five with uncomplicated falciparum-malaria from Lambarene, Gabon, were treated with SP and followed for 28 days between April and June 2007. DHFR and DHPS genes were typed using the sequence specific oligonucleotide probing method. Measurement of SP treatment efficacy in malaria-infected children was combined with MSP2 genotyping of parasites isolated before and after treatment. Most isolates (89%) presented with the triple mutant dhfr haplotype. Likewise, three point mutations were found in dhps, with five of the eight possible haplotypes being present. In the *in vivo* efficacy study, of all the subjects, only 60% cleared parasites by day 3 post-treatment. SP treatment failed to cure 62% children. On day 7, 50% of subjects carried gametocytes. These results show the high prevalence of dhfr triple mutant and dhps point mutations in Gabon, which might result in increased SP treatment failure rates and longer parasite clearance time. This in turn could explain the high rate of gametocyte carriers found after SP treatment.

Email address for correspondence: ghyslain.mombongoma@gmail.com

316 Toxicological and therapeutic efficacy assessment of *C. siamea* antimalarial Congolese traditional preparation and antiplasmodial in vitro activity of its isolated fractions and compounds [MIM15094649]

G.F. Nsonde Ntandou, M. Ndounga, J.T. Banzouzi, S.F. Mbatchi, T. Bansimba, B.J.-M. Ouamba, A. Berry, A.A. Abena, F. Benoit-Vical

The active antimalarial drugs nowadays certainly could become inactive in the future. It is necessary to develop new drugs, to associate to those already existing in order to minimize the speed of appearance of resistances, or to replace the drugs which are becoming ineffective against *Plasmodium falciparum*. Developing drugs from natural products may reduce the risk of toxicity and maintain its therapeutic effectiveness, when the drug is used clinically. To contribute to the safety of 80% of Africans without access to modern health care, we have studied the toxicity, established the antimalarial propriety of *Cassia siamea*, an African antimalarial traditional medicinal plant and determined their potential as sources of new antimalarial drugs. Water, ethanolic, dichloromethane and petroleum ether *C. siamea* stem bark extracts were assessed for acute and subchronic toxicities on Wistar rats (*n* = 5), and for cytotoxicity on KB (human epidermoid carcinoma) and Vero (African green monkey kidney) cells. Therapeutic efficacy of traditional extract was conducted in patients suffering from *P. falciparum* uncomplicated malaria, who normally and freely use this traditional treatment in south of Brazzaville-Congo. *In vitro* study was conducted on human erythrocytes infected by FcM29-Cameroon, a chloroquine-resistant strain of *P. falciparum*. The chemical following techniques were used: reaction tube for chemical screening, flash chromatography, preparative HPLC, CCM preparative, precipitation and crystallization for the fractionation and isolation. The structures of some compounds were assigned from spectroscopic evidence and comparison with published data. Petroleum ether and dichloromethane extracts LD50 were 1250 and 1300 mg/kg, respectively. These extracts are not cytotoxic particularly the water and ethanol extracts. There is no difference on the biochemical parameters between the traditional extract and control: glucose (87.2 ± 3.31–220 ± 4.99 mg/dl); AST (4.6 ± 0.67–9.6 ± 0.57 UI/dl); ALT (6.2 ± 0.42–12.6 ± 0.84 UI/dl); creatinin (3.16 ± 0.40–3.9 ± 0.38 UI/dl); Ht (31.2 ± 1.08–33.6 ± 0.48%). 85% treatment success with a significant reduction of parasitemia from the third day were observed with the extract traditional, which seems the standard drug (quinine). 38 fractions and 29 molecules *in vitro*, six fractions isolated IC50 (0.4–2 mg/ml) and two molecules not yet identified IC50 (0.3–1.2 μg/ml) exhibited most antiplasmodial activity. Following molecules: lupeol, betulina, betulina acid, stigmasterol, ursolic acid, oleanolic acid, emodin and barakol, already known about this plant have not shown a major antiplasmodial effect IC50 (>25 μg/ml). *C. siamea* is a medicinal plant that can provide new tolerated antimalarial molecules. It is essential to study the interaction of these pharmacological active compounds with chloroquine.

Email address for correspondence: nsonde.ntandou@yahoo.fr

317 Clinical trial of PR 259 CT1 vs artemunate-amodiaquine in uncomplicated malaria in Democratic Republic of Congo: A phase II clinical trial [MIM16670853]

Tona Lutete Gaston, Mesia Kahunu Gauthier, Cimanga Kanyakanga Richard, Mampunza Miezi, Muanda Tsobo, Ntamabyalirio Nsegni, Miatezilia Joe, Muyembe Tamfum Jean-Jacques, Totté Jozef, Pieters Luc, Vlietinck Arnold Jozef

Besides *Artemisia annua* extract, other plant extracts used for the treatment of malaria in traditional medicine are still candidate
for the development of active medicines from plant for the treat-
ment of uncomplicated malaria. The objective of this study was
to assess the efficacy of the herbal medicinal product PR259CT1, a
standardized extract formulated in capsules from a medical plant,
in human diagnosed with uncomplicated malaria. All patients were
selected according to the criteria defined by WHO 2003 guidelines.
Malaria was detected by medical examination using a thick blood
film stained with Giemsa. Only patients with axereal parasites/μL
>500 of parasitaemia were selected. A total of 32 patients/group
were treated with a drug regimen of two 500 mg capsules (3x/day,
each 8 h) for 3 days, followed by one 500 mg capsule (3x/day) the 4
next days during meals and, using the conventional As + Aq dosage,
under the supervision of a medical team. Patients were follow-up
until the 14th day (WHO, 2003). Results from the clinical investiga-
tions did not show a significant change in values of vital signs, ECG,
biochemical and haematological parameters. All values obtained
were in normal range. The study showed a clear significant decrease
of parasitaemia in patients treated with PC259CT1 (88% efficacy)
versus As + Aq (96.5% efficacy). PR259CT1 was better tolerated than
the As–Aq mixture since more side effects were observed for the
latter. The results pointed out that drug PR259CT1 can be consid-
ered as a promising candidate for the development of a medicine
for the treatment of uncomplicated malaria.

Email address for correspondence: mesia.kahunu@ua.ac.be

318
Antimalarial activity of 10 Gabonese medicinal plants
[MIM15055549]
Jean Bernard Lekana-Douki, Jean Bernard Bongui, Sandrine Lydie
Oyegue Liabagui, Sonya Estelle Dzang Edou, Ulrich Bivsgou,
Jacques Lebibi, Fousseyni Toure Ndouo, Maryvonne Kombila

Despite massive efforts to control P. falciparum malaria, it still
remains one of the most devastating tropical diseases, mainly
afflicting children from Sub-Saharan Africa. One of the causes of
malaria persistence is the increasing resistance of the para-
sites isolates to the commonly used drugs. So, antimalarial drug
research is essential. Plants represent a potential alternative.
Indeed, quinine and artemisinin, the best antimalarial drugs
currently used are plant derivatives. Gabonese populations use
some plants to treat malaria symptoms but their antiplasmodial
activities are not clearly known. In this study, we investigated
plants effects on P. falciparum growth. After ethnobotanic survey,
10 plants were collected from South-East of Gabon. Methanolic and
chloromethylenic extracts were prepared from trunk. Antiplas-
modial activities were evaluated in vitro against P. falciparum-
chloroquine-resistant-strains (FCR, W2) and field isolates by
DELI-test. Plant-extracts cytotoxicity was evaluated. Methanolic-
extracts of Stauditia gabonensis and chloromethylenic-extracts of
Adhatoda latibracteata showed high antiplasmodial activity
(IC50 < 1 μg/mL). Methanolic-extracts of Monodora myristica
and chloromethylenic-extracts of Afromomum giganteum and
Tetrapleura tetraptera showed good activity (1 < IC50 < 10 μg/mL).
Methanolic-extracts of Dorstenia klaineana and Tetrapleura
tetraptera, chloromethylenic-extracts of Copaifera religiosa, Mon-
odora myristica, and Leonotis africana showed moderate activity
of 10 < IC50 < 40 μg/mL. There were no differences between
extracts IC50 on reference strains and on field isolates. 90% of P.
falciparum field isolates collected were chloroquine-resistant.
Nine of plants tested shown antiplasmodial activity and weak
cytotoxicity. Investigations to identify active compounds in best
antiplasmodial-extracts are in progress.

Email address for correspondence: lekana jb@yahoo.fr

319
Effects of traditionally formulated trema orientalis on uncom-
pliated malaria [MIM16765621]
J.-V. Mombouli1, C. Nkoua Badzi1, A. Nzougani, M. Dounga,
D. Sianard, E. Mokondjimbe, A.A. Abuena, H.-J. Parra

Trema orientalis is used by healers throughout Congo to cure
malaria in adults. Our objective was to assess management of
uncomplicated malaria by a traditional healer using Trema orien-
talis. Participants had to provide informed consent before inclusion.
A blood smear was performed for confirmation of uncomplicated
malaria before enrollment. Clinical (age, gender, weight, body tem-
perature, blood pressure) and biochemical parameters (hematocrit,
glycemia, transaminases, creatinine) were recorded from each par-
ticipating subject. All were administered a solution prepared and
prescribed as instructed by the traditional healer. Thus, 200 g of
Trema orientalis leaves were boiled (in 5 L of water), filtered and
administered after cooling by 40 mL per os intake 3 times a day for 7
days. Group 1. These patients (n = 43) were recruited at the healer’s
cabin in Brazzaville. All had negative blood smears and could not
be included. Group 2. Patients (n = 7) were recruited at a primary
healthcare facility. The treatment had no effect on biochemical
parameters. The treatment cleared parasitemia on day 3. One
patient was removed at day 5 due to headaches. Group 3. 10 vol-
unteers were recruited and completed the regimen. 6 complained
of minor headaches. 3 reported relief from prior constipation. (1)
It is necessary to revisit systematically claims by traditional heal-
ers, when they do not have laboratory backing. (2) Trema orientalis
may be an antimalarial, provided that investigation on mechanism
demonstrates its mode(s) of action.

Email address for correspondence: jvmombouli@hotmail.com

320
Efficacy and safety of amodiaquine+artesunate (Arscum®)
and artemether-lumefantrine (Coartem®) against uncom-
plcipated malaria in the South West Province of Cameroon
[MIM16698494]
Mbacham F. Wilfred, N. Marcel Moyeh, Akindheh M. Nji, Netongo
M. Palmer, Brian Greenwood, Jeff Targett

The rapid onset and spread of parasites resistant to most of the easily
available and effective drugs has led to the implementation of
the combination therapy for treatment of uncomplicated malaria.
ACTs have been embraced by many countries without region
specific evidence based data to support such a move. This study
was thus carried out to determine the efficacy and safety of arte-
sunate plus amodiaquine (AS–AQ) and artemether lumefantrine
(AL) in the South west province. The study was carried out in the
Baptist Hospital in Mutengene. The study recruited children aged
6 months to 10 years with uncomplicated malaria, mono-infection
with P. falciparum with parasitaemia 1000–100,000 parasites per
microliter of blood, history of fever or axillary temperature above
37.50 °C and no severe malaria. Patients were randomised to take
AS–AQ or AL and followed up for 28 days. A total of 280 patients
took part in the study and randomised into one of the two arms. 212 children received the AS–AQ and 68 received the AL, both
combinations were well tolerated and effected rapid fever and
parasite clearance. The ACPR were 95.39% (n = 181) for AS–AQ and
92.86% (n = 52) for AL these findings provide enough evidence that
both drugs are very effective and well tolerated by the age group
under study.

Email address for correspondence: marcel7139@yahoo.com

Zul Premji, Rich E. Umeh, Seth Owusu-Agyei, Fabian Esamai, Emmanuel Ezedinachi, Stephen Oguche, Steffen Bornmann, Akinrunde Sovunni, Stephan Duparc, Paula L. Kirby, Allan Pamba, Lynda Kellam, Robert Guiguemde, Brian Greenwood, Stephan A. Ward, Peter A. Winstanley

The objective of this study was to compare chlorproguanil-dapsone-artesunate (CDA) and artemether-lumefantrine (AL) efficacy and safety in acute uncomplicated Plasmodium falciparum malaria. Haematological safety in glucose-6-phosphate dehydrogenase (G6PD)-deficient patients was studied carefully. Non-inferiority of CDA to AL for efficacy was tested in a randomised parallel-group, double-blind, double-dummy study conducted at 11 sites in 5 African countries. Patients (≥1–14 years) were randomised (2:1) to CDA od × 3 days or six-dose AL over 3 days. G6PD genotype and phenotype were determined. A haematological safety composite endpoint was defined as haemoglobin (Hb) drop of ≥40 g/L or ≥40% vs. baseline or Hb <50 g/L or blood transfusion. 1372 subjects were randomised; 914 to CDA, 458 to AL. Baseline demographic/clinical characteristics were similar between treatment groups. Parasitological cure rate (PCR-corrected) at D28 for the per-protocol population (primary efficacy comparison) was 94.1% (703/747) for CDA and 97.4% (369/379) for AL. CDA met the non-inferiority criterion of a lower 95% CI of >−7%; however, the upper 95% CI limit of <0 also indicated statistical superiority of AL. 282/1201 (23%) patients were G6PD A. For CDA, but not AL, occurrences of the composite haematological safety endpoint and blood transfusions were significantly more frequent in G6PD-deficient vs. normal patients. There were three deaths, unrelated to study medication (2 with CDA, 1 with AL). In conclusion, CDA was efficacious for the treatment of uncomplicated P. falciparum malaria, despite rapid elimination. However, the haematological safety profile of CDA in G6PD-deficient patients precludes its use in African countries.

Email address for correspondence: rossignolr@mmv.org

322 Minority-variant pfcr76T P. falciparum in Madagascar: Will they take more room? [MIM17256234]

Milijaona Randrianarivelosias, Benjamin Ramarosandratana, Frederic Ariey, Steven R. Meshnick, Jonathan J Juliano

Strains of Plasmodium falciparum genetically resistant to chloroquine (CQ) due to the presence of pfcr 76T appear to have been recently introduced to the island of Madagascar. Their prevalence is reported to be low (<3%) when evaluated by conventional PCR. However, these methods are insensitive to low levels of mutant parasites present in patients with polyclonal infections. Thus, the current estimates may be an under representation of the prevalence of the CQ-resistant P. falciparum isolates on the island. Previously we described an isotopic heteroduplex tracking assay (HTA) which can detect pfcr 76T-bearing P. falciparum minority variants that were undetectable by conventional PCR. However, as this assay required a radiolabeled probe, it could not be used in many resource-limited settings. Here we describe a digoxigenin (DIG)-labeled chemiluminescent heteroduplex tracking assay (DIG-HTA) to detect pfcr76T-bearing minority variant P. falciparum. This assay was compared to restriction fragment length polymorphism (RFLP) analysis and to the isotopic HTA for detection of genetically CQ-resistant parasites in clinical samples. Thirty-one clinical P. falciparum isolates (15 primary isolates and 16 recurrent isolates) from 17 Malagasy children treated with CQ for uncomplicated malaria were genotyped for the pfcr76T mutation. Two (11.7%) of 17 patients harbored genetically CQ-resistant P. falciparum strains after therapy as detected by HTA. RFLP analysis failed to detect any pfcr76T-bearing isolates. These findings indicate that HTAs for malaria drug resistance alleles are promising tools for the surveillance of antimalarial resistance. The use of a non-radioactive label allows for the use of HTAs in malaria endemic countries. Also, genetically CQ-resistant minority P. falciparum occur in Madagascar even though the fitness of the minority variant pfcr76T remains unclear. Taking into account the malaria treatment policy change with a shift to ACT, will these mutant parasites take more room?

Email address for correspondence: milijaon@pasteur.mg

323 Early development of the new artemether-lumefantrine dispersible tablet: Palatability and pharmacokinetics [MIM16667477]

S. Abdulla, G. LeFèvre, J. Lyimo, A. Agyemang, C. Reynolds, S. Pascoe, S. Fitoussi, C.M. Yeh, M. Nuortti, G.J. Rivière, R. Séchaud

An artemether-lumefantrine (A-L) dispersible tablet (DT) has been developed to allow convenient administration to malaria pediatric patients. During development of A-L DT, the palatability of 3 formulations with different flavors (study 1) and the relative bioavailability of DT compared to crushed commercial tablet (CT) (study 2) were assessed. In study 1 (single-blind, crossover), 48 Tanzanian healthy children (7–10 yrs) were randomized to receive A-L flavored with strawberry, orange or cherry (for 10 s without swallowing) followed by a rating of palatability using a visual analog scale (VAS) with a facial hedonic scale. VAS scores were analyzed using a SAS PROC MIXED procedure. In study 2 (open, randomized, crossover trial), 48 healthy adults of mainly Caucasian ethnicity (18–50 yrs) received in each of 2 consecutive periods a single dose of A-L (4 tablets, 80 mg artemether +480 mg lumefantrine of either dispersible or crushed tablets) under standard fed conditions. The pharmacokinetics of lumefantrine, artemether and dihydroartemisinin (DHA) between DT and CT were compared using standard bioequivalence tests. Study 1 showed no statistically significant difference in VAS scores between the 3 flavors; however, cherry was preferred overall. Study 2 demonstrated that DT delivered bioequivalent lumefantrine, artemether and DHA exposure (area under the curve) compared to CT. Bioequivalence criteria were also met for peak concentrations (Cmax) of lumefantrine and DHA. The cherry-flavored A-L dispersible tablet was selected for further clinical development in malaria patients.

Email address for correspondence: ajithkumar.vasudevan@novartis.com

324 Assessment of mutant genotypes in Plasmodium falciparum gametocytes following malaria treatment regimens in south eastern Tanzania [MIM15080168]

D. Sumari, K. Hosea, K. Mugittu, P. Kachur, S. Abdulla

This study was undertaken to assess the prevalence of mutant genotypes in gametocytes of P. falciparum following the treatment of malaria using SP, SP+artesunate and Coartem drugs in Kibiti-Rufiji district and Lupiro-Ulanga district. Children under fives with uncomplicated malaria were recruited in Kibiti and Lupiro villages in Tanzania and followed up for 28 days. The follow up
included any day after treatment that has shown the presence of parasitaemia and gametocytes microscopically. The analysis for the molecular markers was done by nested PCR followed by sequence specific oligonucleotide probing (SSOP). Results indicated that children who were treated with SP harbored significantly higher gametocyte numbers than those treated with ACT \((P = 0.0001)\). Furthermore, it was established that younger children (1–24 months) harbored higher gametocyte density than older ones (25–59 months). Drug resistant genotypes of PfDhfr and PfDhps genes analyzed by PCR-SSOP technique indicated that; frequency of PfDhfr and PfDhps haplotypes in gametocytes obtained after treatment with SP had significant \((P = 0.000001)\) higher prevalence of triple PfDhfr mutant genotypes (53.6%) than those in asexual stages. The prevalence of mutant genotypes on gametocytes determines the success of possessing selective advantages for the transmission dynamics and spread of drug resistance especially in endemic areas. Therefore, these findings confirm the fact that SP monotherapy is no longer the first line drug of choice as well as part of combination, hence support the adoption of ACT as the best treatment of uncomplicated malaria in Tanzania

Email address for correspondence: dsummy2000@yahoo.com

\[\text{325} \]
Antimalarial drug design by docking and 3D-QSAR: A case of *Plasmodium falciparum* protein kinase 7 (PFPK7) and dihydroorotate dehydrogenase (DOD) \([\text{MIM16669746}]\)

Maurice Hidebert

*Plasmodium falciparum* enzymes, PFK7 and DOD have been validated as targets since their inhibition leads to parasitic death and this study is aimed at developing their inhibitors. These two enzymes were obtained from Protein Databank and over 10,000 ligands were docked into each enzyme using ArgusLab programme. The enzyme–ligand complex with the lowest binding energy was further evaluated by using 3D-QSAR whereby pharmacophore was calculated and aligned by high throughput screening against a database Asinex using LigandScout programme. For DOD, chemical manipulation was carried out find out the best molecular interaction. For PFPK7, complexes that were considered to have the best scoring function were those whose binding free energy was less than \(-12.5 \text{ KCal/mol}\) and only 7 compounds attained the criteria. The best scoring ligand HM249 had their 3D-QSAR studied and the pharmacophore obtained was aligned to the database. Only compounds with the alignment scoring above 66 were selected and are being screened for their *in vitro* activities. For DOD, docking identified the ligand with lowest binding free energy \(-12.69 \text{ KCal/mol}\) and therefore, was taken to the 3D-QSAR and chemically manipulated. Ligand HM519 was identified. It was again docked and found to be a tight binder, aligned against a database and 30 analogues have been identified. Two enzymes have been studied and through docking and 3D-QSAR studies have identified compounds that are leading to the discovery of novel antimalarial compounds.

Email address for correspondence: hildebert1@yahoo.com

\[\text{326} \]
Prevalence of Pfcr t, Pfmdr1 resistance genes and efficacy of AS + SP three years after changing of first line treatments, in Sen nar city, Sudan \([\text{MIM15080077}]\)

Hanan A. Mohammed, Sahar A. Bashir, Tarig A. Mohamad, Suad M. Sulaiman

In 2004, the National Malaria Programme in Sudan changed the policy of treatment to AS + SP after levels of resistance to chloroquine were recorded from different areas. This study aimed to assess the efficacy of AS+SP three years after the change and to determine the prevalence of Pfcr t and Pfmdr1 as compared with a previous study in the same area. An *in vivo* study was conducted to assess the efficacy of AS + SP following WHO 2003 protocol. Molecular techniques were used to examine the prevalence of Pfcr t and Pfmdr1 from samples collected from malaria patients when enrolled in the study. A total of 100 patients who were positive with *P. falciparum* were followed for 28 days after treatment with AS + SP. Ninety-six patients were recorded as negative till day 28 giving 96% sensitivity to AS+SP. Four patients were classified as having resistance, and 3 of them had positive smears on day 14 and were classified as late treatment failure. One patient was detected positive on day 3 and recorded as early treatment failure. Molecular analysis for the Pfcr t and Pfmdr1 from 100 isolates revealed the mutation of Pfcr t 59% and 46%, respectively, while wild type was recorded as 41% and 54% for Pfcr t and Pfmdr1, respectively. AS + SP is highly effective for treatment of uncomplicated falciparum malaria. On the other hand there is a decrease in the level of the Pfcr t and Pfmdr1 after use of AS + SP. However, statistically there is no significant difference between the prevalence of Pfcr t and Pfmdr1 before and after shifting to AS + SP as first line treatment for malaria.

Email address for correspondence: hanan.mohamed@gmail.com

\[\text{327} \]
In vitro antimalarial drug sensitivities and associations with outcomes and genotypes of *Plasmodium falciparum* field isolates in Kampala, Uganda \([\text{MIM16559414}]\)

Samuel L. Nsobya, Moses Kiggundu, Sarah Nanyunja, Fatima Nawaz, Moses Joloba, Grant Dorsey, Philip J. Rosenthal

Evaluations of *in vitro* drug sensitivities of malaria parasites have been limited for components of artemisinin-based combination therapies, and associations between *in vitro* measures, clinical outcomes, and genotypes are uncertain. We evaluated sensitivities of *P. falciparum* parasites that caused symptomatic malaria in 2007–2008 in a longitudinal trial of 3 combination antimalarial regimens in Kampala. We cultured parasites, determined IC50s using an HRP-2-based ELISA, and evaluated 3 common pfmdr1 polymorphisms. Sensitivities (mean IC50 (nM), range) were assessed for: chloroquine (CQ, 101.1, 15.6–767, \(n = 181\)); monodesethylamodiaquine (MDAQ, 66.4, 6.5–312, \(n = 206\)); quinine (94.4, 15.4–761, \(n = 196\)); lumefantrine (LM, 0.51, 0.19–29.4, \(n = 200\)); piperaquine (PQ, 6.1, 5.5–6.8, \(n = 199\)); dihydroartemisinin (0.55, 0.13–4.8, \(n = 212\)). Sensitivities were positively correlated between CQ, MDAQ, and QN \((r = 0.4–0.6; p < 0.001\) by Pearson's correlation), but not for other comparisons. Sensitivities to CQ, MDAQ, and QN, but not to the other drugs, decreased over the course of the study. *In vitro* sensitivity did not appear to predict outcomes after treatment with combination therapies. Considering common Pfmdr1 polymorphisms, parasites most sensitive to MDAQ, QN, and LM, but not PQ, were more likely to have wild type sequence at allele N86Y; there were no clear associations at Y184F or D1246Y. Parasites had a wide range of sensitivities to other tested drugs were recorded showed some variation, but were generally very good.

Email address for correspondence: samnsobya@yahoo.co.uk
Malaria continues to be threatening more than 41% of the world's population and malaria parasite multi-drug resistance poses serious health problems in tropical countries. The aim of this study was to assess the sulfadoxine-pyrimethamine (Fansidar) resistance of Plasmodium falciparum parasite in central Sudan, using molecular markers. Forty-four patients with positive P. falciparum with no previous history of recent antimalarial medication were selected for this study. The genotyping of P. falciparum parasite showed that the polymorphism of dhfr gene was in codons 51, 59 and 108. In codon 51 only 2 (4.5%) patient samples were mutant type and 28 (63.6%) were wild type, 3 (6.8%) were found as mixed infection (both mutant and wild types). One sample (2.2%) was dhfr 59 mutant and 31 (70.4%) were wild type, while 14 (31.8%) were dhfr 108 mutant and 24 (54.5%) were wild type, 3 (6.8%) were found as mixed infection. The screening of dhps 540 polymorphisms of the gene revealed that 2 (4.5%) were found as mixed infection, and 42 (95.5%) as wild type. Fifteen samples were analyzed to find the association between Pfct76 and dhfr, dhps polymorphisms, the result showed that 5/15 (33.3%) were found to be mutant at dhfr 108 and Pfct76 genes indicating the association between dhfr108 and Pfct76 genes. Less incidence of polymorphism in the dhfr and dhps genes, indicate the effectiveness of sulfadoxine-pyrimethamine (S/P) as malaria chemotherapy.

Email address for correspondence: osamamama6@hotmail.com
complementation vectors. We demonstrated that the disruption of the PfHT gene does not occur in the absence of PfHT expression from the complementation episome. Our results provide evidence that PfHT is essential for the asexual stages of the *P. falciparum* and genetically validate PfHT as a novel drug target. Furthermore, we are currently assessing the effect of PfHT Q169N mutation (which mediates fructose/glucose discrimination) on the viability of the parasite supplied with either glucose or fructose as an energy source.

Email address for correspondence: p0606113@sgu.ac.uk

### 332

**Effects of internal deletions of hydroxymethylpteridine pyrophosphokinase-dihydropteroate synthase from *P. falciparum* [MIM16682936]**

Maria Jönsson, Worapol Ratanachuen, Worachart Sirawaraporn, Göte Swedberg

Folates are synthesised de novo in many eukaryotic and in plants. The bifunctional enzyme hydroxymethylpteridine pyrophosphokinase-dihydropteroate synthase (HPPK-DHPS) is a target for sulfadoxine, which is used in antimalarial chemotherapy. While both full length HPPK-DHPS and its separate HPPK part could complement an HPPK knock-out of *E. coli*, the full-length gene was required for complementation of a DHPS knock-out. Alignment of the HPPK domain of the plasmoidal bifunctional enzyme with HPPK sequences from other species revealed that *P. falciparum* HPPK contains extra amino acids within two extensive insertions. Insertion 1 encompassing amino acids 74–162 has sequences that are shared with rodent malaria and *P. vivax*. Even very small deletions within insertion 1 led to loss of HPPK activity, while DHPS activity remained. Larger deletions led to complete inactivation of the enzyme. Insertion 2 comprises amino acids 217–306, and most of it is unique for *P. falciparum*. The *P. falciparum*-specific part could be deleted without affecting the complementation of either HPPK or DHPS knockouts. However, a small decrease in enzyme activity was noticed by careful kinetic analysis. Expression studies showed that deletions in the parts common to all parasites led to diminished efficiency of expression. The conclusion is that apart from a *P. falciparum*-specific amino acid sequence, the extended HPPK is necessary for both activities of the bifunctional enzyme.

Email address for correspondence: gote.swedberg@imbim.uu.se

### 333

**Prevalence of Pfcr7 K76T and Pfmdr N86Y mutations in isolates [MIM16689380]**

Pembe Issamou Mayengue, Isabelle Morlais, Parfait Awono-Ambene, Emmanuel Bodzewan, Engelbert Manga, Françoise Benoit Vical, Antoine Berry

Several epidemiological studies have shown increase of gametocyte loads in children treated with chloroquine and carrying *P. falciparum* resistant parasites before treatment. We determine the prevalence of Pfcr7 K76T and Pfmdr1 N86Y mutations in isolates from asymptomatic *P. falciparum* infections and the relations between the prevalence of mutations and gametocyte carriage. Pfcr7 K76T and Pfmdr1 N86Y mutations were screened in falciparum isolates from asymptomatic children, 108 collected in November 2006 (long wet season) and 127 in April 2007 (short wet season). Collections were done in the out suburb of Yaoundé (Cameroon), a high *P. falciparum* transmission area. Samples were genotyped by real-time PCR. Cumulative analysis of the Pfcr7 K76T mutation indicated that 125 of the 235 (55%) samples had Pfcr7 76T mutant allele, while 176 (84%) had Pfmdr-1 86Y mutation. Separate analysis according to the season showed similar trend. No difference was found on the prevalence of Pfmdr-1 86Y mutation between gametocyte and non-gametocyte carriers. However, the prevalence of Pfcr7 76T mutation in November 2006 was statistically higher (*p* < 0.02) in gametocyte carriers compared with non carriers. Conversely, the mixed population (both K and T76 mutations) was higher (*p* < 0.01) in non gametocytes carriers. In April 2007 no difference was found. The higher prevalence of Pfcr7 76T mutation in gametocytes carriers during the long wet season may be due to intense transmission and consequently to a larger use of antimalarial drugs. Asymptomatic carriers could be implicated in the spread of resistant strains, particularly when the transmission level is high.

Email address for correspondence: pmayengue@yahoo.fr

### 334

**Community perceptions of first-line antimalarial treatments across three malaria treatment policy changes in Rufiji district, Tanzania [MIM16677324]**


Within a decade, Rufiji district in Tanzania has delivered three different first-line treatments for confirmed or suspected malaria. In 2001, sulfadoxine pyrimethamine (SP) replaced chloroquine (CQ) nationwide. In early-2003, SP + artesunate (AS) was introduced in Rufiji district alone to assess the feasibility and potential impact of artemisinin-containing combination treatment in real-life settings. In 2006, mainland Tanzania adopted artemether-lumefantrine (ALU) as the national treatment policy. From 2001 to 2005, we conducted 29 focus group discussions (FGD) and 47 illness narrative interviews with community members in 14 communities about their use and perceptions of SP and SP + AS. In 2007, we conducted seven FGDs in two communities to assess community perceptions of the government’s new ALU policy. Interview transcripts were imported into NVIVO 7 for content-analysis. Prior to the SP policy change, a variety of SP-branded products were recognized in the community as “strong” or “effective” against malaria. By the time the SP + AS policy was implemented in early-2003, many were indicating a preference for branded SP-containing products over the generic SP, which was perceived as potentially toxic. Perceived side-effects included skin disorders, nausea, and an initial increase in fever. After the introduction of SP + AS, most preferred it over SP alone, although concerns with SP side-effects continued and complaints about having to take “too many tablets” became common. Community perceptions of ALU were mixed. While most perceived it to be more efficacious than previous first-line treatments, many expressed dissatisfaction with the number of tablets prescribed and its complicated dosing schedule. Although both combination treatments were widely accepted shortly after their introduction, consumer dissatisfaction with the number of tablets and extended dosing schedule with ACTs was common. Having co-formulated products with fewer tablets may increase consumer acceptability, uptake and adherence.

Email address for correspondence: drothallen@cdc.gov

### 335

**Pattern of emergence of the dhps 540E mutation in African *P. falciparum* [MIM16465870]**

I. Naidoo, C. Roper

Sulphadoxine pyrimethamine (SP) treatment failures first occurred in eastern and southern Africa in the mid- to late-1990s. High grade
sulphadoxine resistance is caused by mutations at codons 437 and 540 of dhps. The 540E mutation commonly indicates the presence of a double mutant dhps allele containing both 437G and 540E. The double mutant allele was first recorded in east Africa in the mid-1990s but remains rare in west Africa. This work describes the spatio-temporal spread of 540E in Africa and contrasts it with the spread of chloroquine (CQ) resistance during 1978–1990. A literature survey of 540E was done between November 2006 and October 2007. Published studies of dhps sequence variation at codon 540 in P. falciparum isolates from African patients were collated and mapped. We compared these maps with others showing the emergence and spread of CQ resistance, also derived from published data. Data were available from 70 source documents covering 87 sites in Africa, between 1993 and 2005. The 540E mutation was reported first in east Africa, then in southern and western Africa and finally in central Africa. This is the first pan-African spatial study tracking the emergence of the dhps 540E mutation. Both CQ and SP resistance had an initial east African focus and gradually spread westwards across Africa. Monitoring by genotyping gives insight into the dynamics of SP resistance dispersal. It will be important to continue monitoring the progress of 540E in Africa while malaria control strategies deploy SP as intermittent preventive therapy.

Email address for correspondence: inaidoo@mrc.ac.za

336 Targeting polyamine metabolism of Plasmodium falciparum with polyamine analogues: Effect on biosynthesis, transport and cell growth [MIM16677175]

J. Niemand, A.I. Louw, K. Kirk, L. Birkholtz

Polyamines are polycations that play a central role in cell growth and replication. Polyamine metabolism is widely considered as a drug target in highly proliferative cells such as cancer cells and parasitic protozoa. Polyamine analogues are attractive therapeutic agents with the potential of having multiple activities. They may inhibit and/or be taken up by polyamine transport mechanisms, and once inside the cell they may inhibit polyamine biosynthetic enzymes as well as replace the functional intracellular polyamine pool. This study investigated the effect of polyamine analogues on the human malaria parasite Plasmodium falciparum. The effects of a variety of polyamine analogues on polyamine biosynthesis and transport and on parasite proliferation were investigated. Enzyme assays were performed by measuring the formation of radiolabeled products from the radiolabeled enzyme substrates, polyamine transport into isolated parasites were investigated using radioisotope flux techniques and parasite proliferation was monitored using in vitro [3H]hypoxanthine incorporation assays. The polyamine analogues inhibited in vitro P. falciparum parasite growth. Small aminooxy derivatives had the greatest inhibitory effect. These compounds reduced the uptake of radiolabeled putrescine into isolated trophozoite-stage parasites and affected the activities of the recombinantly expressed biosynthesis enzymes. The results of this study indicate that polyamine analogues may be more effective in targeting polyamine metabolism in the malaria parasite than the classical biosynthesis inhibitor Nα,Nα-difluoromethylornithine (DFMO). This therefore contributes to the development of polyamine analogues as lead molecules for the development of anti-malarial drugs.

Email address for correspondence: jandeli.niemand@googlemail.com

337 Efficacité thérapeutique de trois CTA administration répétée dans la prise en charge de paludisme non compliqué au Mali. [MIM16690710]

D. Dembele, B. Fofana, B. Sidibe, T. Toure, A. Togo, K. Sanogo, I. Sagara, S. Dama, A. Dicko, O.K. Doumbo, A.A. and Djimde

Les monothérapies ont été remplacées par les combinaisons thérapeutiques à base d’artémisinine (CTA) en Afrique. Les différents CTA testées sur 14 ou sur 28 jours ont montré une bonne efficacité thérapeutique mais leur efficacité en traitement répété est mal connue. Nous avons mené un essai clinique randomisé de phase IV à Bougoula Hameau au Mali. De 2005 à 2007 nous avons utilisé le protocole OMS 2003 pour comparer l’efficacité de l’artésunate-amodiaquione (AS/AQ), artésunate sulfadoxine-pyriméthamine (AS/SP) et artémétér-luméfantrine (AR-L) lors du traitement d’épisodes consécutifs de paludisme. Dès qu’un patient était randomisé à un bras de traitement, il recevait la même CTA durant toute l’étude. 780 participants ont été inclus dont 260 par bras de traitement. Au jour 28, les trois groupes étaient comparables après la correction moléculaire. De Janvier à Juin (RPCA = 100%), de Juillet à Septembre (RPCA = 98%; 99% et 100%) respectivement pour les bras AS/AQ, AR-L et AS/SP. D’octobre à Décembre (RPCA = 99%) pour l’ensemble des bras. Sans la correction moléculaire de Janvier à Mars (RPCA = 100% et 98%) respectivement dans les bras AS/AQ; AS/SP et AR-L d’Avril à Juin (RPCA = 79%; 91% et 75%), De Juillet à Septembre (RPCA = 75%; 85% et 57%). D’octobre à Décembre (RPCA = 80%; 94% et 58%), respectivement dans les bras AS/AQ, AS/SP et AR-L. Avec un (p < 0,001 (Chi-2)). Les trois CTA demeurent efficaces après correction moléculaire. Nous constatons des variations saisonnières de l’efficacité non corrigeée.

Email address for correspondence: ddembele@mrtcbko.org

338 Single nucleotide polymorphisms in the Plasmodium falciparum dhfr, dhps, pfcrt and pfmdr-1 genes and molecular indices of therapeutic failure to sulphadoxine-pyrimethamine and amodiaquine in Cameroon [MIM16701813]

Marie-Solange Evehe, Palmer Masumbe Netongo, Wilfred Fon Mbacham, Isabebe Akaragwe, Patrice Nsansou Mimche, Akindeh Nji, Domkam Irenee, Anthony Ajua, Jean Basile Echoufou, Bantar Tawe, Rachel Harlett, Cally Roper, Geoffrey Targett

Despite the change in policy from AQ and SP to ACTs, these drugs are still used in most parts of Africa. We propose equations and new molecular indices—specific genotype resistance index (SGRI) and specific genotype failure index (SGFI) that could be valuable for molecular monitoring of resistance to AQ and SP. 750 patients with uncomplicated Plasmodium falciparum malaria from towns within three distinct ecological regions of Cameroon were investigated for mutations on the pfcrt 76T and pfmdr-1 86Y, pfmdr1 genes following a double blind randomized trial. The occurrence of these mutations was used to establish equations and new molecular indices—SGRI and SGFI. Triple mutations on the dhfr (51I, 59R, 108N) coexisting with dhps 540E and pfmdr-1 86Y were detected invariably in ACPF and failure and were region-specific. The 437G or SGK haplotype increase in prevalence within three distinct ecological regions of Cameroon was correlated with SP failure across the three sites. There was the absence of 540E mutant that would make the quintuple mutation, seen in other African countries. Equations and indices were used to predict drug response in Ndu and other towns in Africa. These molecular indices—SGRI and SGFI could enhance the use of the GRI and GFI and usefulness of SNPs in predicting drug failure.

Email address for correspondence: seveheb@yahoo.com
Quinine is the drug recommended for severe malaria treatment. The mechanism of Plasmodium falciparum resistance to quinine is not known. Recently Ferdig et al. described the dissecting of low-level quinine resistance loci in parasites indicating that genetic loci on several chromosomes may be involved, including polymorphisms in a P. falciparum sodium–hydrogen exchanger (PfNHE) on chromosome 13. Prospective in vivo study was conducted in two hyperendemic villages in Mali to assess the quinine in efficacy: Kollé and Faladie. Consenting cases of non-per os malaria were included, treated with quinine standard doses of 5 days during 28 days follow up. Treatment outcome (ETF, LCF, LPF and ACPR) were classified using modifications of the WHO's 2003 protocol for the assessment of antimalarial efficacy. Molecular markers of polymorphisms msp1, msp2 and CA1 have been used to distinguish recrudescence from new infections. Overall 87 patients were included for Kolle with 0%, 12.8%, 46.8% and 40.4% of ETF, LCF, LPF and ACPR, 63 patients for Faladie with 15% LCF and 21.9% LPF. Molecular correction showed 100% of ACPR in two villages. The prevalence of microsatellite resistant form in post-treatment parasites increased for the two villages. Faladie's samples (n = 30) showed statistical difference between parasites collected before and after Quinine treatment (P = 0.0066) Mc Nemar. Kolle's samples (n = 49) also showed a significant difference (P = 0.0006). From patients presenting day 21 parasitemia and positive PCR by day 14, two had positive PCR at day 7 showing a selection by the drug of less sensitive parasites.

Email address for correspondence: amina@mrtcbko.org

340 Evaluation of the effect of red cell genetic factors on Pfmdr1 and chloroquine resistance [MIM16739388]

C.M. Nneji, C.O. Falade, O.G. Ademowo

Chloroquine which used to be the first drug of choice for malaria has been rendered impotent due to resistance. We investigated the effect of red cell genetic factors, sickle cell genetic trait, ABO blood group and glucose-6-phosphate (G6PD) deficiency on chloroquine resistance and its association with Pfmdr1 mutation. One hundred and twenty patients with acute uncomplicated falciparum malaria were recruited. They were administered 25 mg/kg body weight chloroquine over 3 days and followed up for 14 days for clinical and parasitological responses. Thick and thin blood smears were made for malaria parasite screening. Filter paper samples were collected for DNA analyses. One milliliter of blood was collected for determining of blood group, G6PD status, haemoglobin (Hb) genotype and G6PD status. Pfmdr1 gene was amplified by PCR to screen for Y86 mutation. MSP1 and MSP2 polymorphisms were used to differentiate between recrudescence and re-infection. Only 53% of the patients were cured with chloroquine. Pfmdr1 Y86 was more common among patients with clinical failure than among those cured. However, this was only significant among under 5 years old children. No significant association was found between Pfmdr1 alleles and ABO blood groups or G6PD status. Individuals with Hb AA were 2.3 times more prone to chloroquine resistance relative to Hb AS. Association between Pfmdr1 and chloroquine resistance was stronger among <5-year-old children. G6PD deficiency and blood group had no effect on chloroquine resistance. Hb AS individuals are less prone to chloroquine resistance.

Email address for correspondence: ogademowo@comui.edu.ng

342 Exposure to lumefantrine in infants and children receiving artemether-lumefantrine for uncomplicated malaria: Impact of African diet components [MIM15020198]

Abdoulaye Djimde PhD, William M. Sallas PhD, John Lyimo, Anne-Claire Marrast, Gilbert Lefèvre PhD, Steffen Borrmann MD

Artemether-lumefantrine (AL) shows high 28-day cure rates in children with uncomplicated P. falciparum malaria. Lumefantrine bioavailability is considerably enhanced by food, but data from the literature indicate that (a) only a small amount of dietary fat is necessary to ensure optimal efficacy with AL and (b) the fat content of standard meals or breast milk in sub-Saharan Africa is adequate. Methods: We evaluated lumefantrine exposure in children with P. falciparum malaria taking part in a randomized, multicenter study of two AL formulations (dispersible versus crushed tablet). Lumefantrine plasma concentrations were used to construct a two-compartment pharmacokinetic model. Results: The model incorporated data from 621 patients who received 3722 AL doses in total. Meals consumed at AL dosing were milk alone (57.4%), pancakes alone (27.8%), no meal (9.6%), and other meal (5.2%). For crushed tablet, relative bioavailability was 1.57 (90%CI: 1.29–1.96) for patients consuming milk and 2.74 (90%CI: 1.93–3.61) for patients eating pancakes versus patients who ate no meal. For the dispersible tablet, relative bioavailability was 1.65 (90%CI: 1.28–2.09) with milk and 1.83 (90%CI: 1.42–2.39) with pancakes versus no meal. Most patients (98.2%; 797/812) in

www.mimalaria.org
the primary analysis population had PCR-corrected parasitological cure at day 28. Patients who ate nothing with any AL dose all achieved parasitological cure. Conclusion: Consumption of milk or typical African food enhances lumefantrine bioavailability, providing adequate therapeutic exposure. Efficacy, as measured by the 28-day cure rate, was high (98%).

Email address for correspondence: adjimde@mrtcbko.org

343

PfNHE polymorphisms and Plasmodium falciparum sensitivity to quinine in Mali [MIM16694620]

Aaminatou Kone, Abdoul H. Beavogui, Omar B. Traore, Oumar Yattara, Antoine Dara, Souleymane Dama, Aly Kodio, Jianbing Mu, Ousmane Toure, Ogobara K. Doumbo, Thomas E. Wellems, Abdoulaye A. Djimde

Quinine is the drug recommended for severe malaria treatment. The mechanism of Plasmodium falciparum resistance to quinine is not known. Recently Ferdig et al. indicated that genetic loci on several chromosomes may be involved, including polymorphisms in a Pf. falciparum potassium–hydrogen exchanger (PfNHE) on chromosome 13. The microsatellite ms4760.1 was associated with decreased susceptibility of P. falciparum to quinine. Prospective in vivo studies were conducted in two hyperendemic villages (Faladje and Kollé) in Mali to assess quinine efficacy. Consenting cases of non-per os malaria were included, treated with quinine standard doses for 5 days and followed up for 28 days. Treatment outcome (ETF, LCF, LPF and ACPR) were classified using modifications of the WHO's 2003 protocol for the assessment of antimalarial molecules. Molecular markers of polymorphisms msp1, msp2 and CA1 were used to distinguish recrudescence from new infections. Overall 87 patients were included, treated with quinine standard doses for 5 days and followed up for 28 days. Treatment outcome (ETF, LCF, LPF and ACPR) were classified using modifications of the WHO's 2003 protocol for the assessment of antimalarial molecules. Overall 87 patients were included for Kolle with 0%, 12.8%, 46.8% and 40.4% of ETF, LCF, LPF and ACPR, 63 patients for Faladje with 15% LCF and 21.9% LPF before molecular correction. After molecular correction there was 100% of ACPR in the two villages. Compared to baseline the prevalence of ms4760.1 in post-treatment parasites significantly increased in both villages (n = 30, p = 0.006 for Faladje and n = 49, p = 0.0006 for Kolle; chi² McNemar) This data support a role for PfNHE in P. falciparum resistance to quinine.

Email address for correspondence: amina@mrtcbko.org

344

First line therapies of uncomplicated falciparum malaria in Burkina Faso: Efficacy and tolerance [MIM16748842]

Issaka Zongo, Noel Rouamba, AnyiréKun Fabrice Somé, Jean Eric Ouédraogo, Jean Bosco Ouédraogo

Burkina Faso switched to artemether-lumefantrine and amodiaquine-artesunate as first line therapy of uncomplicated falciparum malaria in 2005. We evaluated the efficacy and tolerance of these ACT in Bobo-Dioulasso. We enrolled 342 patients 6 months or older with uncomplicated falciparum malaria in Bobo-Dioulasso, Burkina Faso. Patients were randomly assigned to receive standard doses of either artemether-lumefantrine (175) or amodiaquine-artesunate (167) over 3 days and followed up for 28 days. The primary endpoint was the risk of treatment failure unadjusted or adjusted by genotyping to distinguish recrudescence and new infections. The compliance rate was 98.6% (331/342). We analyzed data using intention to treat and Per Protocol method and went to the same results. No treatment failure occurred up to 14 days of follow up. From that day, the risk of treatment failure was higher in artemether-lumefantrine group (29.2% versus 19.1%, risk difference 10.1%, p < 0.001). After msp2-PCR correction the treatment failure was respectively 13.42% and 10.78 for artemether-lumefantrine and amodiaquine-artesunate (risk difference 4.64%, p > 0.05). The genotyping work is ongoing for MSP1. All regimens were well tolerated. Three years after the new policy adoption, there are concerns about the decrease level of these ACTs efficacy. More studies need to be done countrywide to allow a better capture of the drug's efficacy and to give alert to the National Malaria Control Program. The evaluation of the impact of strategies (home-based managements, bed nets, IPT) is urgently required for malaria effective control.

Email address for correspondence: oyinoduola@yahoo.co.uk

345

Antiplasmodial activity and cytotoxicity of extracts from Terminalia catappa and Vitex doniana [MIM16705498]


Over the years ethnomedicine has been shown to be a potential source of antimalarial compounds or template for synthesis of novel antimalarial molecules. In continuation of our work on evaluation of the antimalarial activities of plants used in Nigerian ethnomedicine, the in vitro, in vivo and cytotoxicity of leaves and stem bark of Terminalia catappa and Vitex doniana were investigated. Plant parts were air-dried, powdered and extracted in methanol by maceration at room temperature for 72h or successively extracted with hexane, ethyl acetate and methanol using accelerated solvent extractor (ASE 200). In vitro antimalarial activity of plant extracts was determined against chloroquine/pyrimethamine resistant K1 and chloroquine sensitive NF54 strains of P. falciparum using [3H]-hypoxanthine based assay. Cytotoxicity was determined against mammalian L6 cells using Alamar blue assay. In vivo antimalarial activity of the plant extracts was evaluated using a mouse model of P. berghei ANKA transplanted with green fluorescence protein (GFP) in a 4-day suppressive test. Hexane and ethyl acetate extracts of leaves of V. doniana and ethyl acetate of leaves of T. catappa Linn (Combretaceae) displayed the highest in vitro antiplasmodial activity (IC50 < 5 μg/ml), elicited low cytotoxicity (selective index > 10) but lacked significant in vivo chemosuppression of parasite growth. However, the methanol extract of stem bark of T. catappa displayed moderate in vitro activity (IC50 = 9.5 μg/ml) and in vivo chemosuppression of parasite growth (45%). T. catappa from Nigerian ethnomedicine provides potentially valuable source of antimalarial agents that can be further developed.

Email address for correspondence: andleyfabricio1@yahoo.fr
weights were determined. The result indicated that at doses of 100 and 200 mg/kg of A. annua, there was no physically observable developmental toxicity in the fetuses. However, at 300 mg/kg dose, non-viable, abnormal and deformed fetuses were observed in some of the fetuses. One of the rats treated at this dose had the uterus and fetuses developed into a tumour-like mass. There was also a statistically significant decrease in the levels of estrogen at 100 mg/kg ($p < 0.001$) and 300 mg/kg ($p < 0.05$) compared with the control group. Percentage weight gains were significantly elevated at doses of 200 and 300 mg/kg extracts ($p < 0.05$) compared with the control group. The litter size showed no significant decrease at 100 mg/kg dose. The absolute weight of liver indicated a significant increase at 200 mg/kg ($p < 0.01$), the absolute and relative weights of the heart were significantly elevated at 200 and 300 mg/kg ($p < 0.01$ and $p < 0.05$, respectively) while the spleen absolute and relative weights were significantly reduced at 200 mg/kg ($p < 0.05$) and relative spleen weight showed a significant reduction ($p < 0.01$). We conclude that oral administration of Artemisia annua can adversely affect post-implantation development and pregnancy outcome in Wistar rats. Artemisia annua should therefore not be taken by pregnant women at any stage of pregnancy as it could lead to embryolethality and organs toxicity.

Email address for correspondence: adesokan_ayoade@yahoo.com

347

Antimalarial potentials of Enantia chlorantha stem bark in mice and its toxicological effect on rat liver [MIM15994931]

A.A. Adesokan, M.A. Akanji, O.G. Ademowo

Albino mice were infected by intraperitoneal injection of standard inoculum of chloroquine sensitive Plasmodium berghei (NK 65 strain). Two groups were treated with artesunate and chloroquine as standard antimalarial drugs. The other two groups received 100 and 400 mg/kg body weight of extract of Enantia chlorantha, respectively, for 4 consecutive days. Albino rats were also administered 100 and 400 mg/kg body weight of extract of E. chlorantha orally for 28 days. The results in mice showed 100% parasite clearance in the chloroquine, 45% in the artesunate, and 100% and 98.6% in the groups that received 400 and 100 mg/kg body weight of the extract, respectively, at the end of 28-day observational period. Resurgence of parasitaemia was recorded in the group that received artesunate. There was no survivor in the untreated mice but 60% survived in those that received artesunate, chloroquine and 100 mg/kg body weight of the extract. Forty percent (40%) survived in the group that received 400 mg/kg body weight of the extract. The mean survival time for the animals in the untreated group was 9 days, artesunate 22 days and chloroquine 19.8 days; while 19.6 and 17 days were recorded for the groups that received 100 and 400 mg/kg body weight of the extract, respectively. Rat liver aspartate and alanine transaminase activities showed varied responses but no significant changes in the serum levels. Histopathology of rat liver showed minimal but reversible changes only at the highest dose of 400 mg/kg body weight of the extract. These results showed that extract of E. chlorantha possess potent antimalarial activity with minimal but reversible toxic effect on the liver.

Email address for correspondence: bulusadzu@yahoo.com

348

Screening for antimalarial activity of Diospyros mespiliformis in mice [MIM14965940]

Bulus Adzuz, Karniyus Shingu Gamaniele

Diospyros mespiliformis is used in ethnomedical practice for treating symptoms of malaria attack. The freeze-dried aqueous extract of the plant's stem bark were investigated for in vivo antimalarial potency in mice. Curative effect against established infection, suppressive activity against earlier infection and prophylactic effect in residual infection were tested against Plasmodium berghei infected mice. Result shows that the extract (50–200 mg/kg, p.o.) has significant (p < 0.05) dose dependent activity against the parasite in the curative and suppressive test, and repository effect at high doses (100 and 200 mg/kg, p.o.). It also prolonged the survival time of the infected mice. Phytochemical test revealed the presence of saponins, alkaloids, tannins, steroids and terpenes, and the LD50 was established to be 1095.4 mg/kg, p. i. in mice. The result shows that the antimalarial activity of the extract is due to real antiplasmodial effect.

Email address for correspondence: bulusadzu@yahoo.com

349

Herbal tea for malaria? [MIM16694117]


Ten medicinal plant hot water extracts were evaluated for their suitability as herbal tea for malaria based on the results of the identification and evaluation of potential antimalarial components from the Nigeria phytomedicine and mimicking the ethnomedical extraction procedure. The in vitro antiplasmodial assay was done using the multi-resistant Plasmodium falciparum 3D7 strain in the parasite lactate dehydrogenase assay. Two aqueous extracts displayed good antiplasmodial activities with IC50 values of 1.56 and 4.87 μg/mL for Tithonia diversifolia leaf and Trichilin monaldelpha leaf, respectively. In the same assay, Azadirachta indica stem bark had IC50 value of 13.01 μg/mL, Phyllanthus amarus leaf; 16.30 μg/mL; Ocimum gratissimum leaf, 19.51 μg/mL; Khaya senegalensis stem bark; 21.34 μg/mL; Morinda lucida stem bark >25 μg/mL; Khaya ivorensis leaf >25 μg/mL. Chloroquin phosphate and artemisinin were included in the assay. The in vivo antimalaria study using Anka clone of P. falciparum in mice also showed that T. diversifolia had the highest in vivo antimalaria properties. The acute and sub-acute toxicological evaluation were also assessed in rats. There is need to have a closer look at the methods used traditionally in administering antimalaria herbal remedies and begin to look at the prospect of herbal tea for malaria.

Email address for correspondence: edaijaiye@yahoo.com

350

The suggestive potentiating effect of cod liver oil on the efficacy of artesunate in Plasmodium berghei infected mice [MIM16697872]

O. Awodele, M.O. Araoye, A.I. Oreagba, S.O. Kolawole, A. Akintonwa, D.F. Anisu

The effects of cod liver oil on the potency of artesunate was determined using plasmodium berghei infected mice. Fifty (50) adult albino mice weighing between 15 and 25 g were used for this experiment. There were five groups of ten animals each per group. Groups I–IV were infected with plasmodium berghei and also received 0.9% normal saline (Group I), artesunate (Group II), cod liver oil (Group III) and cod liver oil plus artesunate (Group IV). Group V was not infected and was not treated. The parasitaemia level was monitored for 8 days post-inoculation of the parasites to the animals. The Group IV animals that received the combination of both artesunate and cod liver oil demonstrated a better clearance of malaria parasite than artesunate monotherapy (Group II) with 48.7%, 90.3%, 98.9% and 99.2% suppression of parasitaemia from days 4 to 5, 5 to 6, 6 to 7 and 8.
7 and 7 to 8, respectively. These findings showed that the combination of artemunate and cod liver oil is more effective against Plasmodium berghei infection than artemunate alone. This combination may thus be considered as a suitable and cost effective combination therapy in malaria infection and should always be incorporated in artemisinin combination therapy used for malaria treatment.

Email address for correspondence: funmianisu@yahoo.com

351

In vitro susceptibility levels of Plasmodium falciparum isolates to antimalarial drugs in southern Benin [MIM16698919]

A. Wakpo, S. Ezinmegnon, S. Houze, A. Massougbodji, P. Deloron, J. Le Bras, A. Aubouy

Plasmodium falciparum resistance contributes to the increase of morbidity and mortality due to malaria in endemic areas. Within the context of the control of antimalarial drug efficacy in Benin, this study aimed to evaluate the in vitro efficacy of the following antimalarial drugs on P. falciparum isolates: monodeséthylamodi- aquine, quinine, mefloquine, dihydroartemisinine, lumefantrine and pyrimethamine. P. falciparum isolates were obtained from children in the area of Allada, where an in vivo study was carried out with artemether-lumefantrine and with artesunate-amodiaquine. Isolates were obtained at enrolment and in children presenting a treatment failure. Sensitivity levels of the isolates were obtained through the immunodetection of the P. lactate dehydrogenase (EMAT, Elisa malaria antigen test, Diamed). The tested isolates (n = 120 including the failures) showed no resistance to quinine, lumefantrine and dihydroartemisinine. However, 2.6%, 3.8%, and 61.8% of the isolates were resistant to mefloquine, monodesethylamodiaquine and pyrimethamine, respectively. In vitro results were consistent with in vivo results. The full data is still being analysed, particularly data obtained from in vivo failures. The resistance levels will be discussed in terms of antimalarial treatments use in Western Africa. The relevance of such method for the surveillance of drug susceptibility level will be considered.

Email address for correspondence: agnes.aubouy@ird.fr

352

Antagonistic effect of vitamin E on the efficacy of artemunate against Plasmodium berghei infection in mice [MIM16202147]

O. Awodele, P.M. Emeka, A. Akintonwa, O.O. Aina

The effect of antioxidant (vitamin E) on the efficacy of Artesunate was investigated using Plasmodium berghei infected mice. Fifty (50) adult albino mice weighing between 15 and 25 g were used for this study. There were five groups of ten animals each per group. Group I was the normal control group without the parasite and untreated, group II was infected with malaria parasite and 0.9% normal saline was administered, group III was infected with the parasite and treated with artesunate, group IV was infected with the parasite and vitamin E was administered and group V was infected with the parasite and combination of artesunate and vitamin E were administered. Parasitaemia level and haematocrit (PCV) were monitored upon the administration of antimalaria drug—artesunate, vitamin E, normal saline and combination therapy of both vitamin E and artesunate. The life span of the infected mice was found to be between the 7th and the 10th day post-inoculation, while the LD50 of the parasite was 156626.2 parasite/µl of blood. Artesunate was observed to rapidly clear the parasite with parasitaemia level of 21632.4 ± 513.3 parasite/µl on the 6th day, 11209.9 ± 363.7 parasite/µl on the 7th day, 1359.7 ± 14.3 parasite/µl on the 8th day, 7.8 ± 2.4 parasite/µl on the 9th day and zero parasitaemia on the 10th day. The effect of artemunate was significantly reduced when co-administered with vitamin E (p < 0.05) with parasitaemia 30542 ± 362.5 parasite/µl on the 6th day, 24705.2 ± 489.9 parasite/µl on the 7th day, 15485.0 ± 563.2 parasite/µl on the 8th day, 947.6 ± 37.8 parasite/µl on the 9th day and 8.0 ± 2.7 parasite/µl on the 10th day. The study suggests that co-administration of vitamin E with artesunate could reduce the efficacy of artemunate in malaria infection.

Email address for correspondence: awodeleo@yahoo.com

353

Ethnomedical survey of antimalarial herbs and antimalarial activity of Morinda charantia Linn. [MIM15887577]

C.M. Cyril-Olutayo, T.O. Elufioye, C.O. Adeloye

Malaria continues to cause morbidity and mortality on large scale in tropical countries. In an attempt to search for new antimalarial drugs, plants used by traditional healers to treat malaria were surveyed. Methanolic extract obtained from Morinda charantia L. was investigated at dose range 50 mg/kg, 100 mg/kg, 200 mg/kg, and 400 mg/kg per day, against early malarial infections in vivo, using Swiss Albino mice infected with malaria strain Plasmodium berghei var Anka I. Chloroquine at 5 mg/kg per day was used as positive control while distilled water was administered as negative control. The most effective dose of M. charantia L. extract was 50 mg/kg per day, i.e. 50 > 100 > 200 > 400 mg/kg. The suppression of parasitaemia by chloroquine at 5.0 mg/kg appeared lower than M. charantia at 50 mg/kg, 100 mg/kg and 200 mg/kg.

Email address for correspondence: mojioogunyemi@yahoo.com

354

In vitro Malian Plasmodium falciparum susceptibility to Artemisinin and prevalence of PfATP6 S769N mutation [MIM16690409]

Souleymane Dama, Bakary Fofana, Aly Kodio, Demba Dembele, Sekou Toure, Bakary Sidibe, Ogobara K. Doumbo, Abdoulaye A. Djimde

Artemisinin-based combination therapies are now first line malaria therapies in most malarious areas. Recent studies in French Gyanna showed that mutation was associated with raised IC50s of artemether. We investigated the in vitro susceptibility of Malian P. falciparum isolates to artemisinin and measured the prevalence of PfATP6 S769N. Fresh P. falciparum positive blood samples were collected in Bougoula-Hameau, Mali. In vitro 3H-hypoxanthine-based method was used to test IC50s for artemisinin. A nested PCR followed with restriction digestion was developed to analyze the mutation of PfATP6 S769N. Forty-five (45) P. falciparum field isolates were collected in 2004–2005 from Bougoula-Hameau. Two (4.3%) cases of PfATP6 S769N were detected in 41 samples successfully analyzed. Mean IC50 for artemisinin was 1.9±N. We show the presence of PfATP6 S769N mutation in Mali. All tested isolates were fully susceptible in vitro to artemisinin.

Email address for correspondence: dama@mrtcbko.org

355

Effect of Chloroquine and Arteether on malaria parasitaemia and gastric ulcer in mice [MIM16473557]

B.M. Duduyemi, S.B. Olaileye, O.G. Ademowo

Peptic ulcer disease is a ailment of the young adult to middle-age and is a common disease in our environment. Malaria has been found to have effects on the gastric mucosa, which include congestion with capillary stasis, necrosis, ulceration and haemorrhage.

www.mimalaria.org
We investigated the effect of Chloroquine and Artemether on parasite clearance and gastric ulcer in mice. Twenty mice were divided into four groups. One group constitutes the control with no parasite and no treatment, group 2 was parasitized but not treated, groups 3 and 4 were parasitized and were treated with Chloroquine and Artemether, respectively. The mice were passaged intraperitonially with Plasmodium berghei and gastric ulcer was induced with 70% alcohol and pylorus ligation after 24 h fast. The stomach of the mice were harvested and examined grossly and then fixed in 10% formalin for histological assessment. Blood films were made for assessment of parasite load for 7 days using standard techniques. The ulcer diameter in the group with ulcer and parasite (11 ± 1.72 mm) was significantly higher than the group with ulcer but no parasite (3.62 ± 1.6 mm) (p = 0.002). Chloroquine and artesether reduced the ulcer diameter remarkably. The ulcer diameters in chloroquine (4.83 ± 2.48 mm) and artesether (5.00 ± 0.89) groups were not significantly different (p > 0.889). Artemether restored PCV faster than chloroquine. Malaria contributes significantly to gastric ulceration. Administration of chloroquine and artesether brought about reduction in ulcer diameter, parasite load and subsequent restoration of PCV.

Email address for correspondence: babsdudu@yahoo.com

356 Effects of some selected ghanaian herbal preparations on the blood-stages of Plasmodium falciparum [MIM14505635]

D. Elewosi, S. Aduko, M.F. Ofori, R. Asmah, D. Edoh, K.A. Nyarko

Mortality and morbidity from malaria are high in Africa and may increase as drug-resistant malaria parasites spread. Malaria and fevers are treated using herbal preparations but the efficacy of these herbs is not scientifically established in Ghana. We therefore investigated anti-plasmodial activity of some of the plants being used traditionally to treat malaria. Chloroquine sensitive (3D7) and resistant (Dd2) strains of Plasmodium falciparum were cultured in vitro separately and in the presence of different concentrations of aqueous extracts of medicinal plants coded DE1, DE3, DE4, DE6 and DE7 and Artesunate for 48 h. Smears were prepared, Giemsa-stained, parasitaemia and growth inhibition determined. Destruction of parasite DNA by the extracts was assessed using comet assay. Concentrations of extracts used ranged from 833.3 µg/ml to 6.5 µg/ml. Highest concentration of DE1 inhibited growth of 3D7 by 71.67% while the maximum percentage growth inhibition of Dd2 parasite by the highest concentrations of DE3, DE4, DE6 and DE7 reached 79.11%, 61.94%, 62.19%, and 90.95%, respectively. Similarly, growth of Dd2 was inhibited up to 84.22% by DE7 with DE4 causing the least of 57.59%. The highest concentrations of DE1, DE3 and DE6 showed peak growth inhibition of 66.67%, 78.38%, and 61.20%, respectively. DE3 and DE7 extensively destroyed parasite’s DNA. All the extracts inhibited parasite growth in concentration dependent manner with DE7 exhibiting the highest anti-malarial activity. The anti-malaria activity of the plant may be due to destruction of the parasite’s DNA with DE7 and DE3 showing the highest destructive effect on the parasite’s DNA.

Email address for correspondence: doris.elewosi@gmail.com

357 Herbal combinatory drugs: A cheap, effective and wholesome remedy for combating malaria epidemic in third world countries [MIM16538841]

N.A.N. Emetu, H. Nuhu

In sub-saharan countries like Nigeria, more than 99% of citizens have the malaria parasite living in their liver. Pregnant women and children under 5 years are the worst hit. Conditions like Flu, HIV/AIDS, TB, Pneumonia, etc., exacerbates the incidence in other adult groups. Immune system’s suppression in host, among other reasons allows frequent and repeated attacks. Herbal remedies for the treatment of Malaria have been practiced for thousands of years with over 1277 plant species from 160 families being used. The most recent and effective Artemisinin and Quinine drugs like the others, lack the dual potency of immune system boosting and enhanced prophylactic remedial properties. This work presents the combinatory drug effect of using “ZOGLI” Moringa Oleifera and a new herbal antimalaria drug ‘HERB 25’ a product from not less than a decade of pharmacognostic research, recently approved by the Nigeria National Agency for Food and Drug Administration (NAFDAC), endorsed and accepted after trial by the world health organization (WHO). Zogali was analysed to contain % required dosage administration (ra) in a 25 g powder form: 42% protein, 310% vitamin A, 22% vitamin C, 71% iron, 41% Potassium, 125% calcium and 61% magnesium. This translates to 2 times the protein in milk, 4 times the calcium in milk, 4 times the vitamin A in carrots, 7 times the vitamin C in oranges and 3 times the potassium in bananas and 3 times the iron in spinach, gram for gram. This combinatory herbal remedy only not treats the malaria but also boost the immune system of patients thereby ensuring complete eradication, sustainance of body resistance and protection from subsequent attack by the parasite.

Email address for correspondence: emetun@yahoo.com

358 Effects of variation at the CYP2C19/CYP2C9 locus on pharmacokinetics of chlorocycloguanin in Gambians [MIM14517053]

Ramatoulie Janha

There is suggestive evidence that pharmacogenetic variation is important in determining response to treatment for common infections in Africa where the information on relevant drug metabolising alleles is limited. The antimalarial biguanides, proguanil and chlorproguanil, are activated by the cytochrome P450 enzyme CYP2C19 to their active metabolites. Polymorphisms in the CYP2C19 gene results in decreased or complete loss of enzyme activity that affect metabolism of other substances. However these alleles have not been shown to affect the pharmacokinetics of antimalarial biguanides and the frequencies of the alleles are not known in Africans. The range of genetic variation in CYP2C19 and CYP2C9 was profiled using a Bayesian algorithm for haplotype reconstruction from genotypic data (PHASE 2.2) and defined any haplotypes present in a Gambian population with malaria that was treated with and underwent detailed pharmacokinetic studies on chlorproguanil/dapsone. The effects of the defined haplotypes on chlorproguanil pharmacokinetics were examined. Participants with CYP2C19*17 had higher AUC0-24 for chlorocycloguanin than those without (geometric means 317 ng h/mL vs. 216 ng h/mL, ratio 1.46, 95%CI 1.03, 2.09, P = 0.0363) and higher Cmax (1.52, 95%CI 1.13, 2.05, P = 0.0071). Fast metabolisers had a higher AUC0-24 (335 ng h/mL) than intermediate (1.62, 95%CI 1.12–2.35, P = 0.0133), and slow (1.58, 95%CI 0.97–2.60 P = 0.0672) metabolisers CYP2C19*17 is the main determinant of antimalarial biguanide metabolic profile at the CYP2C19/C9 locus. Fast metabolisers had significantly increased AUC for chlorocycloguanil and approximately twice the maximum plasma concentration of slow metabolisers.

Email address for correspondence: rjanha@mrc.gm
359 Evolution of the prevalence of pfcrt K76T and pfmdr1 N86Y mutations in Pikine Senegal [MIM16737038]

Omar Ly

Senegal has changed in 2002 its first line treatment for uncomplicated malaria from CQ to an artemisinin combination therapy because of chloroquine resistance. The pfcrt K76T and pfmdr1 N86Y mutations have been associated with CQR. We have carried out a study to assess the prevalence of both mutations since the discontinuation of CQ use, because the re-introduction of CQ is discussed in areas where CQ is no longer used. Blood samples were collected from patients with uncomplicated malaria. Blood spots were dried on filter paper and parasite DNA was extracted and then amplified through nested PCR followed by RFLP. The prevalence of pfcrt 76T and pfmdr1 86Y point mutations was investigated. From 2002 to 2006, 444 samples were collected. In 2002, the prevalence of pfcrt 76T and pfmdr1 86Y was 68% and 38%, respectively. Our results show a significant decrease of both mutations over time (P = 0.001). Surprisingly, an increase of the prevalence of pfcrt 76T mutation was noticed from 2005 to 2006 going from 33% to 58%. However the prevalence of pfmdr1 86Y remained constant. Withdrawal of the use of CQ in Senegal appears to have reduced the prevalence of both mutant alleles suggesting that CQ pressure is needed to maintain mutations contributing to CQR. However the increase of the prevalence of pfcrt T76 from 2005 to 2006 remains unclear. It remains to be seen whether this pattern will continue to be observed before allowing the re-introduction of CQ.

Email address for correspondence: omarlyster@gmail.com

360 In vitro study and molecular analysis of Plasmodium falciparum resistance to antimalarial drugs in Bobo-Dioulasso, Burkina Faso [MIM16681142]


In regard to the quick spread of Plasmodium falciparum resistance to antimalarial drugs, we conducted a study to assess the in vitro susceptibility of P. falciparum to antimalarial drugs and its relationship with Pfmdr-1N86Y mutation. The study was carried out from September 2006 to May 2007 in Bobo-Dioulasso, Burkina Faso. The drugs tested were: dihydroartemesinin (DHA), Quinine (QN) and Chloroquine (CQ). In total, 87 patients with monospecific infection with parasitemiae above 4000 trophozoites/cL were enrolled. The presence of Pfmdr1-Y-86 mutation was assessed by Polymerase chain reaction (PCR). Out of 87 samples tested, 81 were interpretable. The in vitro susceptibility assessed after 42 h of drug exposure showed a geometric mean of IC50 of 1.5 nM for DHA. In vitro study and molecular analysis of Plasmodium falciparum have been performed since 2002 to 2006; 444 samples were collected. In 2002, the prevalence of pfcrt 76T and pfmdr1 86Y was 68% and 38%, respectively. Our results show a significant decrease of both mutations over time (P = 0.001). Surprisingly, an increase of the prevalence of pfcrt 76T mutation was noticed from 2005 to 2006 going from 33% to 58%. However the prevalence of pfmdr1 86Y remained constant. Withdrawal of the use of CQ in Senegal appears to have reduced the prevalence of both mutant alleles suggesting that CQ pressure is needed to maintain mutations contributing to CQR. However the increase of the prevalence of pfcrt T76 from 2005 to 2006 remains unclear. It remains to be seen whether this pattern will continue to be observed before allowing the re-introduction of CQ.

Email address for correspondence: leabonkian@yahoo.fr

361 Variable responses to antimalarials detected by ex vivo assay in Senegal: Increased sensitivity to chloroquine concomitant with increased tolerance to artemisinin [MIM16700038]

Daouda Ndiaye, Vishal Patel, Michelle LeRoux, Omar Ndir, Souleymane Mboup, Jon Clardy, Johanna P. Daily, Dyann F. Wirth

Drug resistance has eroded the efficacy of most antimalarials. Current assays for assessing drug responses in field isolates are not ideal—hypoxanthine assays require radioactive materials; DELI assays require unavailable antibodies; and microtests are incompatible with the high throughput necessary for multiple drug testing. We therefore developed a new DAPI assay (Baniecki, 2007) and adapted this for direct use on field samples (Ndiaye, 2007). IC50 values were determined using fresh patient isolates from Thiès Senegal. Briefly, 200 μL of erythrocytes (0.1–1% parasitemia; 2% hematocrit) were incubated with drug for 72 h. DAPI was added and RFU determined by plate reader to obtain IC50 values (Prism GraphPad). For 2007 and 2008, 44 and 110 samples were analyzed, respectively. Parasites were found resistant (data by year: 2007 and 2008) to chloroquine (36.4% and 32%); amodiaquine (9% and 8%); pyrimethamine (52.27% and 68%); and tolerant to artemisinin (19% and 18%). Quinine (2007 only: 27.7% resistance) and mefloquine (2008 only: 54% resistance) were only measured 1 year. Several parasites demonstrated multiple drug resistance, with no evidence of cross-resistance between chloroquine and amodiaquine. The DAPI assay is strongly adapted to field isolates and provides IC50 data in a valid and reproducible manner. Drug sensitivities were observed for all compound classes, with some parasites having significant levels of multiple drug resistance. This method is useful for testing new antimalarials and determining patterns of cross-resistance with known drugs, and for analyzing natural diversity with regard to parasite susceptibility to new chemical entities.

Email address for correspondence: dndiaye@hsph.harvard.edu

How can we implement and maintain effective anti-malarial pharmacovigilance system in remote rural areas: The Case of Saraya Health District, Senegal [MIM16700190]

Youssoupha Ndiaye, Jean Louis Ndiaye, Demetri Blanas, Jonas Bassene, Alexandra D. Sousa, Mouhamed Ndiaye, Doudou Sow, Babacar Faye, Badara Cissé, Ousmane Faye, Oumar Gaye

Artemisinin-Based Combination Therapy (ACT) was implemented in 2006 for the treatment of non-complicated malaria in Senegal. In addition, Sulfadoxin–Pyrimethamin (SP) has been adopted as intermittent preventive treatment (IPT) for pregnant women, and since November 2006 Intermittent Preventive treatment in infants (IPTi) in an operational research held in three districts. The objective was to assess the monitoring of pharmacovigilance and to ensure patient compliance in the use of ACT and SP. Saraya district is located in southeastern Senegal, a rural area where patients’ ability to have access to health structures is extremely limited. Health staff (14) and community health workers (30) have been trained in passive pharmacovigilance. Trainings were reinforced by formative supervisions and follow up meetings led by the district management team from July 2007 to December 2008. In a 35,159 population, 24 cases were notified, 3261 ACT treatment were administered, minor cases (8/24) were notified by health staff; some patients (4/24) were put under observation for 2 days. 2/24 cases were “possibly” due to SP or immunization; Ivermectin (9/24) and Cotrimoxazole (1/24); these latter were brought to the attention of the health team by the community; all cases were cured.
These results mainly include patients who are able to reach health units. To successfully implement a pharmacovigilance program, it is fundamental not only to reinforce health staff training but to involve communities by engaging leaders, families, schools, and traditional healers. It is also urgent to ensure the validity of information related to Pharmacovigilance.

Email address for correspondence: youlebou@yahoo.fr

363 Malarial suppressive activity of methanolic extracts from three Nigerian plants used alone and in combination against chloroquine sensitive Plasmodium in mice [MIM16974326]

Ifeoma Obidike

Malaria is one of the most serious health problems in malaria endemic developing countries, where herbal antimalarials and their combinations are still widely used in the treatment of malaria. It however unknown if some of these herbs afford a higher suppression of infection when used in combination. In this study, methanolic extracts of Nauclea latifolia (NL) and Senna occidentalis (SO) leaves and Cochlospermum tinctory (CT) rhizomes were screened for their plasmodial suppressive effects when employed alone or in combination in Plasmodium infected mice. Methanolic extracts of the three plants were prepared. Phytochemical screening of the extracts was performed, oral acute toxicity tests were carried out and LD50 was estimated. Evaluation malarial suppressive activity was done and the parameter used for the assessment was average % suppression. Different phytochemical constituents were found present in each extract. There were no obvious signs of clinical toxicity and mortality observed in mice that received the extracts at doses up to 5000 mg/kg. All the extracts showed a remarkable dose-dependent suppression of parasitaemia. CT extract possessed remarkable activity at a dose of 400 mg/kg (81.76%), compared to NL (78.41%) and SO (70.27%). However, at a dose of 400 mg/kg each, CT in combination with NS and CT in combination with SO showed 83.52% suppression of parasitaemia. The individual extracts suppressed parasitaemia significantly, and could be potential sources of new antimalarial drugs. However, the outcome of use of plant extracts in combination to suppress malaria is unpredictable, and may offer no significant advantage over a monothermal preparation.

Email address for correspondence: iphie_odike@yahoo.com

364 Effect of chloroquine, methylene blue and artemether on hepatic oxidative stress and antioxidant defence system of P. yoelii nigeriensis-infected mice [MIM16527332]

Mary Oguike

Most antimalarials are thought to be pro-oxidative in action, thus affecting the antioxidant defense system of both host and parasite. However, little is known of the effect of these drugs on the cellular antioxidant defense system and extent of lipid peroxidation in the hepatic tissues of the host during malaria chemotherapy. This study therefore aims at evaluating the antimalarial efficacy of chloroquine (CQ), methylene blue (MB) and artemether (ART) plus their effect on the malondialdehyde (MDA) level, glutathione (GSH) level and glutathione-S-transferase (GST) activity in hepatic tissues of the host during P. yoelii infection. One hundred and twenty mice were grouped into six treatment groups and CQ (10 mg/kg), MB (10 mg/kg) or ART (4 mg/kg) was administered to both the infected and uninfected mice for 3 consecutive days after established P. yoelii infection. Two groups of animals were used as positive (with malaria) and negative (without malaria) controls, respectively. Lipid peroxidation and antioxidant status were determined in liver samples using standard procedures. CQ, MB and ART caused significant increase (CQ → MB → ART) in MDA level in both infected and uninfected mice during therapy. Similarly, GSH level and GST activity were significantly increased during administration of the three drugs in both P. yoelii-infected and uninfected mice. In conclusion, malaria infection as well as CQ, MB and ART induce oxidative stress and disrupt the antioxidant defense system of the host.

Email address for correspondence: preciouskc23@yahoo.com

365 Comparative in vitro assessment of the antiplasmodial activity of quinine–zinc complex and quinine sulphate [MIM16748528]

O.O. Ogunlana, O.E. Ogunlana, O.G. Ademowo

Incipient malaria endemicity in the tropics and subtropical regions, recent work done on the synthesis of metal drug complexes of antimalarial drugs and the evaluation of their antimalarial activities in vitro; have led to the development of this study. Quinine–zinc complex (QZ) was synthesized using a modification of Singla and Wadhwa method. Melting point determination, TLC analysis, infra red, ultra violet, atomic absorption spectroscopy and mole ratio determination were all carried out on the complex synthesized. Direct measurement of the antimalarial activity of the potential new drug (QZ) against parasite growth in vitro was used to comparatively ascertain the antimalarial activity of QZ relative to Quinine sulphate (QS). Measurement of antimalarial activity was carried out based on the inhibition of parasite growth with respect to inhibition of schizont formation in freshly collected infected blood samples from patients. Results of melting point determination, TLC analysis and spectroscopy showed that QZ complex was formed. IC50 for QZ and QS were 3.98 and 14.13 pmol/200 μl of drugs respectively, indicating that antimalarial activity of QZ was three times that of QS alone. The difference in the antiplasmodial activities of QZ and QS was significant (p < 0.05). This study suggests that the quinine–zinc complex could have a better therapeutic activity than quinine.

Email address for correspondence: kellybee2001@yahoo.com

366 Evaluation of the antimalarial and antioxidant activities of methanolic extract of Nigella sativa in mice infected with Plasmodium yoelii nigeriensis [MIM16395386]

O.V. Okeola, O.G. Ademowo, C.M. Nneji, C.O. Falade, O.E. Farombi

Antimalarial activity and effect of methanolic extract of Nigella sativa (black seed) on oxidative stress and antioxidant defense system were investigated in mice infected with P. yoelii nigeriensis. Thirty adult albino mice were divided into five treatment groups. Three groups were inoculated by intraperitoneal injection with 1 × 10⁷ infected erythrocytes on day 0. After 72 h of inoculation, group 1 were administered 1.25 g/kg body weight N. sativa extract orally for 5 days, group 2 received chloroquine 10 mg/kg for 3 days and group 3 received normal saline. Groups 5 and 6 consisted uninfected mice but treated with extract alone and normal saline, respectively. The Rane test procedure was used to evaluate antimalarial activity. Oxidative status was evaluated by estimating malondialdehyde (MDA), reduced glutathione (GSH) and glutathione-S-transferase (GST) activity in the liver as well as catalase (CAT) and superoxide dismutase (SOD) in blood. The extract produced 99.2% and chloroquine 94.6% chemosuppression.
relative to untreated control. *P. yeolli* infection caused a significant (P < 0.05) elevation of MDA level and reduction in GST and GSH. *N. sativa* significantly decreased the elevation of MDA and also inhibited the depression in GST activity and GSH level in infected mice. The extract unlike chloroquine caused a significant increase in SOD and CAT activities in both infected and uninfected mice. In conclusion, *N. sativa* has appreciable antimalarial activity comparable to chloroquine in *P. yeolli* infected mice and caused an alteration in the antioxidant defense system.

Email address for correspondence: valeeokeola@yahoo.com

367
Preliminary formulation of a fixed-dose pediatric combination of artesunate and amodiaquine HCl [MIM16730134]
C.O. Okwelogu, M. De Matas, N.D. Ifudu, B.O. Silva, P. York

Combination therapies are becoming important options for the treatment of malaria. Since the introduction of artemisinin combination therapy (ACT), it has been recognised that challenges exist in presenting drugs as fixed dose combinations due to potential incompatibilities of the different chemical species. The aim of this study was therefore to develop a prototype, stable formulations combining Artesunate (AR) and Amodiaquine hydrochloride (AM). Two fast-disintegrating granular formulations, containing AR and AM, respectively, were produced by wet granulation. Samples were stored as single component formulations or blends in glass vials for periods up to 13 weeks at refrigerated storage conditions (10 °C), room temperature (20–25 °C) and ambient humidity (in the dark and light conditions), 25 °C/75% RH, and 50 °C/75% RH. The active agent content of the two drugs was determined using HPLC-UV at 1, 4 and 13 weeks from the start of the study. Statistical analyses of data were undertaken to determine the factors influencing the stability of the formulations, both alone and in combination. Interrogation of the data showed that the chemical stability of AR was markedly affected by relative humidity, with greatest levels of degradation occurring at 13 weeks after storage at 50 °C/75% RH. No significant loss of active agent content was observed for AM at any conditions over the duration of the study. The results indicated that stable fixed dose granular formulations of AR and AM can be produced which are stable under accelerated conditions. These formulations must however be protected from extremes of relative humidity using suitable packaging materials to avoid degradation of AR.

Email address for correspondence: cokwelogu@gmail.com

369
The use of Artemisinin-based Combination Therapies (ACTs) in public secondary health facilities in Lagos Nigeria—A follow up study [MIM16669534]
I.A. Oreagba, S.O. Olayemi, O. Awodele, A.T. Onajole, A.A. Akinyede

The objectives of this study was to assess the impact of an educational intervention program on the prescription pattern of Artemisinin Combination Therapies (ACTs) in public secondary health facilities in Lagos State Nigeria. Five out of the ten General Hospitals that were studied initially, were selected for the post-intervention study. A total of 1070 retrospective prescriptions of out-patients from March, 2008 were systematically sampled and assessed and the result compared with those of the baseline study. Intervention consisted of a national antimalarial policy sensitization programme for all stakeholders. The percentage of prescriptions containing ACTs had increased from 5.8% to 91.4%. The prescription of artemisinin derivatives as monotherapy had reduced from 18.2% to 4.57%. The prescription of chloroquine had decreased significantly from an average of 48.8% to 0.68% (p < 0.05). The percentage of prescriptions containing correct antimalarial doses had increased significantly from to 87.5% to 96.9%. Prescribers were more favourably disposed to the ACTs policy change, which tallied with prescribing pattern. Those who attended ACTs training seminar were more likely to prescribe correct doses than those who did not. A sustained educational intervention programme coupled with “Eko free” antimalaria programme have impacted positively in terms of rational use of ACTs and the overall adherence to the national antimalarial treatment policy.

Email address for correspondence: oreagbai@yahoo.com

368
In vitro bioequivalence study of nine brands of artesunate marketed in Nigeria [MIM16636427]

The availability of numerous brands of artesunate in our drug market today places clinicians and pharmacists in a difficult situation of choice of a suitable brand or the possibility of alternative use. The aim of the present study was to predict the bioequivalence of nine brands of artesunate tablets marketed in Nigeria using in vitro tests. The in vitro dissolution study was carried out on the nine brands of artesunate tablets using the basket method according to US Pharmacopoeia (USP) guidelines. Other general quality assessment tests like hardness and disintegration time were also determined. All the brands tested passed the British Pharmacopoeia (BP) standard for disintegration time. Only AT2, AT4, AT6 and AT9 passed the standard for hardness. There were significant differences in the dissolution profiles of the nine brands. All the brands except AT1, however, released >70% of artesunate within 30 min. Four of the brands AT5, AT6, AT7 and AT8 exhibited >90% dissolution in <10 min. The other brands AT1, AT2, AT3, AT4 and AT9 (innovator brand) have calculated similarity factors of 23.8, 59.8, 50, 54.8 and 100. Based on the in vitro tests, AT5, AT6, AT7 and AT8 are considered bioequivalent and interchangeable, while AT2, AT3 and AT4 are considered bioequivalent and interchangeable with the innovator brand (AT9). AT1 has very low dissolution rate, which will likely result in poor bioavailability. The results show the need for constant monitoring of new brands of artesunate introduced into the drug market to ascertain bioequivalence and conformity with pharmacopoeia standards.

Email address for correspondence: conocerhi@yahoo.com

370
Fever resolution time in children—0–5 years treated with artemisinin-based combination therapy at University of Calabar Teaching Hospital, Calabar, Nigeria [MIM16692738]
F.O. Siyanbade (RN, RM), I.I. Akpabio (Ph.D.)

This study aimed at evaluating fever resolution time in children with uncomplicated malaria, treated with artemisinin-based combination-therapy (ACT) at the Institute of Tropical Diseases, Research and Prevention, University Teaching Hospital, Calabar. The study focused on identifying the types of ACT in use, interval between the commencement of each type of ACT and the first recorded reduction in fever, the differences in fever resolution time with respect to gender, age and weight of the children. The study adopted descriptive design and a checklist to guide the review of records from hundred case-folders of children diagnosed with uncomplicated malaria and who were at the institute.
in August 2008, during the data collection period. Independent t-test was used to test four null hypotheses, at .05 levels of significance. Artemisinin-based combination-therapies in use during the period were Chlorproguanil-Dapsone-Artesunate (CDA); Arthometerum-Lumefantrine (Coartem); Artecom and Gsunate were all given orally. There were no significant differences in fever resolution time among children treated with the four different drugs (Cal.t = 0.95; 1.45 < crit.t = 2.01; p = .05) and between the males and females Cal.t = 1.30; .45; 1.15; .38 < crit.t = 2.01; p = .05). Conversely, fever resolution time was significantly different with respect to age and weight of children. With age, (CDA Cal.t = 8.51; Coartem = 10.4; Artecom = 8.9; Gsunate = 9.0 > crit.t = 2.07). Children aged 4–30 months had lower fever resolution time than those aged 31–60 months. With weight, (CDA Cal.t = 7.56; Coartem = 6.48; Artecom = 6.04; Gsunate = 7.2 > crit.t = 2.07; p = .05). Children who weighed less than 10 kg had lower fever resolution time than those who weighed above 10 kg. Age and weight of children significantly influenced efficacy of the drugs and should be highly considered when administering artemisinin-based combination-therapy.

Email address for correspondence: talk2funmilere@yahoo.com

371 Effects of amodiaquine and artesunate on sulfadoxine and pyrimethamine pharmacokinetics in children under five in Mali [MIM16694500]


Sulfadoxine-Pyrimethamine, in combination with artesunate or amodiaquine, is recommended in uncomplicated malaria treatment or used or evaluated for intermittent preventative treatment. Yet, the pharmacokinetic interactions of these drugs are poorly documented. In a randomized controlled trial, children aged 6–59 months with uncomplicated falciparum malaria, received either one dose of SP alone (SP), one dose of SP plus three daily doses of amodiaquine (SP + AQ) or one dose of SP plus 3 daily doses of artesunate (SP + AS). We collected exactly 100 µL of whole blood on a filter paper before drug administration at day 0 and at days 1, 3, 7, 14, 21 and 28 after drug administration for sulfadoxine and pyrimethamine pharmacokinetic parameter analysis. We analyzed 41, 39 and 33 samples in SP, SP + AQ and SP + AS arms, respectively. The mean concentrations on day 7: of pyrimethamine (in ng/mL) were [66.59 (6.48–162), 76.70 (24.20–222) and 68.23 (17.10–160)] and of Sulfadoxine (in mg/mL) were [33.79 (5.18–50.40), 35.11 (4.01–71.30) and 34.76 (11–60.50)] in SP, SP + AQ and SP + AS arms, respectively. We found a statistically significant difference between the Volumes of distribution, (in L/kg ± SD): [5.08 ± 1.39 versus 4.14 ± 1.57 versus 5.73 ± 1.97] and half life (in days ± SD): [3.47 ± 1.11 versus 2.98 ± 0.96 versus 4.09 ± 1.86] of pyrimethamine respectively in SP, SP + AQ and SP + AS arm. These data showed an increase of pyrimethamine concentration in the blood in SP + AQ arm.

Email address for correspondence: mtetete@mrtcbko.org

372 Tamarindus indica Linn. (Caesalpiniaeae) affects the Plasmodium infectivity in vivo [MIM16690912]


Burkina Faso is one of the tropical countries where people suffer from malaria besides having various kinds of plants for the treatment of the disease. New effective antimalarial drugs are urgently needed since Plasmodium falciparum is resistant to the available and safe drugs. The present work was undertaken to elucidate the antimalarial potential of the pulp extracts of the fruit of Tamarindus indica used in Burkina as a remedy for malaria [1]. TLC was applied for the preliminary phytochemical analysis of the plant components. The antimalarial activity was performed according to the 4-day suppressive test of Peters [2] using NMRI mice infected by the ANKA strain of Plasmodium berghei. Mice were divided into control and experimental groups which were treated with varying doses of the fruit extracts of Tamarindus indica. The toxicity was evaluated according to the modified method of Trevan [3]. The preliminary phytochemical screening revealed mainly terpenoids, anthracenosides and pectins. The extracts showed significant activity on mice (ED50 = 97.67 mg/kg with a therapeutic index (LD50/ED50) = 27.13) and exerted no toxicity in vivo in mice (LD50 = 2650 mg/kg). The extracts from the fruit of Tamarindus indica showed antimalarial effects in vivo in dose dependent manner. Awaiting complementary investigation in vitro on Plasmodium falciparum the activity displayed by the crude extracts may justifi the use of the plant in the treatment of malaria.

Email address for correspondence: tibienvenue3@yahoo.fr

373 Mild antiplasmodial constituents isolated from the roots of Canthium multiflorum (Thonn.) Hiern (Rubiacaeae) [MIM16606484]


In Burkina Faso, 17% of the population is reported to be infected with malaria each year and approximately 5000 die from the disease [2]. The tribal population still uses herbal medicine for treatment of malaria because of costs and availability. The increasing resistance of malaria parasites to available drugs also creates a growing interest in herbal remedies. Canthium multiflorum is used in Burkina as a remedy for malaria [1] and this study was undertaken to find lead compounds for antimalarial drug development. A bioactivity guided fractionation was carried out in order to characterize the antiplasmodial constituents. Column chromatography and HPLC were applied to isolate pure compounds which structures were elucidated using MS, 1H and 13C NMR spectroscopy. Assay for antiplasmodial activity was performed using chloroquine-sensitive P. falciparum strain 3D7 [3]. Chloroquine was used as a positive control. Pentacyclic triterpenes were tested for inducing change of the shape of membranes of erythrocytes according to a previous described method [4]. The fractionation of extracts from the roots (IC50 = 7 µg/ml) led to the isolation of four related coumarins, 6,7-dimethoxycoumarin, 6,7,8-trimethoxycoumarin, 7-hydroxy-6-methoxycoumarin, hymexelsin; one iridoid glucoside, 10-O-acetylgeniposidic acid and two new pentacyclic triterpenes (3-oxo-15,19,23-trihydroxyurs-1,12-dien-28-oic acid and 3-oxo-9β,13,15-trihydroxyurs-1,21-dien-28-oic acid). Coumarins and iridoids were found inactive (IC50 > 100 µg/ml) while penta cyclic triterpenes exhibited direct moderate antiplasmodial effects (12 < IC50 < 25 µg/ml). The antiplasmodial activity from the crude extracts to isolated compounds may justify the use of Canthium multiflorum as an antimalarial plant. However, further studies in vivo are needed to complete the in vitro findings.

Email address for correspondence: traore_maminata@yahoo.fr
374

Co-formulation of Artemether and Amodiaquine using a microemulsion system [MIM16828212]

O.J. Uwaezoke, N.D. Ifudu, N. Ngwuluka, O.A. Abioye

Malaria has remained a menace in children below 5 years of age, inspite of various co-formulated artemisinin-based drugs in use. Artemether-based drugs have other problems which includes having good absorption only in the presence of fats. This study sought to find solution to some of these problems by co-formulating Artemether and Amodiaquine in a microemulsion system. Tauroglycocholate, Ethanol and Oleic acid was the surfactant, co-surfactant and oil used, respectively. Various ratios of the oil to the surfactant/co-surfactant mixes was titrated with the aqueous phase for 10% (w/v), 20% (w/v), and 30% (w/v) mixes. Artemether was incorporated into the oil phase while Amodiaquine was incorporated into the aqueous phase. Phase behaviour, physicochemical characteristics and physical stability were studied. In vitro intestinal permeability was investigated in rat's ileum. A microemulsion system comprising 20% (w/v) Tauroglycocholate/ethanol mix:water:Oleic acid in the ratio 4:4:1 was selected for drug incorporation. Electroconductivity of the microemulsion was 1440 ± 3.7 Us/cm, the refractive index was 1.3760 ± 0.002 and the droplet size before and after drug incorporation was 34.86 ± 25.6 nm and 39.62 ± 21.1 nm, respectively. 32.64% and 25.80% of Artemether and Amodiaquine respectively diffused from the microemulsion after 1 h. Phase studies showed that different types of microemulsion were formed. Electroconductivity and refractive index measurement showed that the microemulsion was of the oil in water type. The volume of oil and the concentration of the surfactant/co-surfactant mix affected the viscosity. The droplets were monodispersed as shown by a polydispersity index of 0.091. There was a substantial release of the incorporated drugs after 1 h. This microemulsion system can be used to co-deliver Artemether and Amodiaquine in vivo thereby limiting the need for a fatty meal. Further work to determine dosage adjustment is recommended.

Email address for correspondence: onyinyemek@gmail.com

375

Malaria in Suriname: From control to elimination [MIM16691093]

L. Villegas, M. Eersel, G. Bretas

Malaria was an important public health problem in the interior of Suriname till 2007. Following a successful implementation of interventions, malaria was reduced dramatically. We describe the lessons learnt and the approaches for moving towards malaria elimination. Tailored-based malaria control interventions included: Insecticide Treated Nets (Long Lasting Nets-LLNs and non-LLNs), selective Indoor Residual Spraying (IRS), Active Case Detection (ACD), Artemisinin-Combination-Therapy (ACT) with Primaquine (P), Entomology surveillance (ES) and an intensive Behavioral Change Communication (BCC) campaign. The scaling up for impact was reached in 2007. The malaria information system is reporting cases in a weekly basis nationwide and epidemics are detected and contained in less than 2 weeks. Malaria diagnosis and treatment was increased to provide free services to mobile populations (gold miners). After scaling up malaria interventions (2006–2007), confirmed cases were reduced (>70% nationwide). Malaria cases have fallen from 9014 in 2005 to 2134 in 2008. 70–80% of all diagnosed cases in 2008 were reported among mobile populations (gold miners) and most of those infections (>50%) were imported from French Guyana. Malaria-related hospitalizations also showed a steep decline (2005–2008) and no malaria-related deaths have been reported (since 2006). Malaria remains a problem in mobile populations, especially along the border areas of Suriname with French Guyana. The achievements in malaria control have been maintained and a new strategic plan has been designed to address those mobile populations. The National Malaria Board has recently decided to move malaria control towards elimination (in a phased manner).

Email address for correspondence: leopoldovillegas2@gmail.com

376

Malaria in Pakistan [MIM15185155]

Mumtaz Khokhar

Management theories and practices have undergone radical transformation over last few years. The main driver for change is globalization and consequent congruence of many concepts and practices. The other issue has been emergence of some other centres of gravity of action like India and China in addition to traditional power centres of the West particularly USA and Europe. Free flow of information, improved and transparent systems of corporate governance in many countries, new tools and techniques of risk management, explosion of innovation in areas of technology, emergence of media and civil society as powerful social tools have added to the flavour of complexity. But the most important development in the world during last few years has been coupling between developed and developing countries and resultant growing complexity in all commercial and financial transactions. This has created serious increase in risk in balance sheets of many corporate, both domestic as well as global. The recent global sub-prime crisis is one indicator in this direction. All these, therefore, call for a fresh and innovative approach in management principles/theories and practices so that emerging and unfrequented challenges can be faced effectively. The management paradigms should also look into challenge of inclusive growth as poverty in many countries remains one of the biggest global challenges. The international conference on New Frontiers of Management is organized in the above backdrop to debate some of the issues related to new issues and challenges facing the new world and how the modern management theories and practices have effectively countered them. The conference thus focuses on innovation in management principles and practices. Areas in which papers and posters are sought. (1) Business strategy, (2) Finance and financial management, (3) Risk management principles and practices, (4) Internationalization of business, (5) Product development and related strategies, (6) Issues related to supply chain management, (7) Process management, (8) People management and all issues related thereto, (9) Corporate social responsibility, (10) Leadership, business ethics and corporate governance. Aims and objectives: (1) To discuss the emerging challenges in various domains of management (like marketing, finance, systems, human resources, corporate social responsibility, ethics, among others) and the suitability of existing theories and/or practices to face them and (2) To bring out the innovative practices or development of new theories to effectively face these emerging challenges. The word limit for the abstracts of the paper presentation is 300 words and for posters is 100 words. Mail your abstracts to Email: innovation.siescoms@gmail.com.

Email address for correspondence: mumtaaz005@yahoo.com
377 Dynamics of *Plasmodium falciparum* and *P. vivax* infection in highly endemic region of southwestern India [MIM16698638]

Rajeshwara N. Achur, Ravindra Puttaswamy, Srinivas Kakkilaya, D.C. Gowda

Like in many other parts of the world, *P. vivax* malaria infection preponderates in India. Malaria persists throughout the year in the southwestern region of India which receives high rainfall. Despite enormous health burden associated with malaria, there is no systematic study aimed at understanding the pathophysiology and immunology of infection in this region. Although *P. vivax* infection has been considered to be benign, in recent years, it has been associated with severe malaria with significant fatalities. Thus, we have undertaken a detailed epidemiological survey, drug resistance and clinical presentation of *P. falciparum* and *P. vivax* malaria. Two districts (Dakshina Kannada and Udupi) in this highly endemic region and the data were collected from 1998 onwards. The prevalence and distribution and *P. falciparum* and *P. vivax* infection in the study area suggests that *P. vivax* predominates to about 70–90% which varies to a certain degree among several locations. Notably, the data obtained between 1991 and 1994 for Mangalore, a major city in the study area, suggests that the infection was almost exclusively due to *P. vivax* (99.8–100%). However, during 1997–2001, *P. falciparum* infection was 5–10% and currently in the range of 10–30%. The month-wise incidence of infection during 2003–2008 also indicates that there is an increased infection rate during monsoon rainy season and reaching peak level in July and August. The collection of data on drug resistance and clinical presentations including pregnancy malaria are under way.

Email address for correspondence: rajachur@gmail.com

378 Sleeping arrangements under long lasting impregnated mosquito nets: Differences during low and high malaria transmission seasons [MIM13390117]

S.D. Fernando, R.R. Abeyasinghe, G.N.L. Galappaththy, L.C. Rajapakse

The sleeping arrangements under long lasting impregnated bed nets (LLINs) was recorded in 2467 households during the low malaria transmission season (May/June 2007) and the same families followed up during the high malaria transmission season (December/January 2008), in two malaria endemic areas of Sri Lanka. A community-based cross-sectional survey carried out in approximately 800 households from each of the three main ethnic groups to whom LLINs had been previously distributed. The number of families lost to follow up was 68. A significant rise was seen in the households that had used LLINs the previous night during the high transmission season as compared to the low transmission season ($p = 0.001$ for all three ethnic groups). When sleeping arrangements in the entire population was considered, priority to sleep under the LLIN was given to children under the age of 5 years during both seasons. Among the households with children under five, the percentage of children sleeping under a LLIN increased in all three ethnic groups during the high transmission season, the difference being statistically significant only among Tamils ($p = 0.018$). Utilization of LLINs by pregnant women was low as only approximately 45% of pregnant women slept under a LLIN during both seasons The study suggests the possible need for re-focusing of health education messages regarding importance of LLIN use among pregnant women. The prevalence of LLIN use for children under five in this study group is above the target set by the Roll Back Malaria guidelines.

Email address for correspondence: deepfern@slt.lk

379 Is Sri Lanka ready to move towards malaria elimination? [MIM15655047]

S.D. Fernando, R.R. Abeyasinghe, G.N.L. Galappaththy, L.C. Rajapakse

The number of malaria cases have steadily declined over the past 8 years and the goal of elimination of malaria from Sri Lanka was announced in April 2008. The presence of asymptomatic carriers and loss of natural immunity increases the risk of epidemic spread in the pre-elimination phase. This study was planned to determine the prevalence of asymptomatic malaria infections. Due to the very low rates of malaria infection, four MOH areas in the Trincomalee district and Polpitigama MOH area in Kurunegala which have reported high prevalence in past years were purposively selected. In each MOH area, GN divisions having high malaria risk were identified. From the identified GN divisions, 20% of the population was randomly selected for blood smear examination and in a 50% sub-sample blood was also collected for PCR assay. A population of 1825 from 8 GN divisions in the Kurunegala district and 1905 individuals from 5 GN divisions in the Trincomalee districts were sampled. Thick and thin Giemsa stained blood smears were negative for malaria parasites. PCR carried out in 50% of the study sample was also negative for malaria parasites. The study demonstrates that there were no asymptomatic carriers in the two selected high transmission areas in the country. An extensive and larger sample needs to be studied to confirm the low prevalence of asymptomatic carriers. The current national objectives of achieving malaria elimination in non-conflict and transitional zones by 2012 appears feasible.

Email address for correspondence: hapugalle@yahoo.co.uk

380 Acute neuropsychiatric profile of patients with [MIM15051406]


Cerebral malaria is a well known entity as one of the manifestation of severe falciparum malaria. Other miscellaneous neurological manifestations in cases of severe falciparum malaria have been reported from time to time. This spectrum ranges from acute to chronic complications includes seizures through cranial nerve palsies, hemiplegia, myelitis, polynuereitis to cerebellar disorders. Psychiatric disturbances like neuroaesthetic syndrome and malarial psychosis have also been identified. Aims and objectives: To observe the neuropsychiatric profile of both complicated and uncomplicated *P. falciparum* infected patients. We screened 200 patients presenting with fever to the Department of Medicine for malaria through peripheral blood smear examination and ‘Optimal/Falcivax’ (RDT-Rapid diagnostic test) during the epidemic of falciparum malaria from September to November 2006. Out of these we included 103 cases that were smear and/or RDT positive. Every case underwent a complete neurological examination including fundus and CSF examination as per need and a neuropsychiatric evaluation with mini mental state examination (MMSE), brief psychiatric rating scale (BPRS). Cases were also screened for haematological and metabolic derangements including hypoglycaemia, uraemia, and hepatic involvement. All patients received treatment as per the WHO guidelines. The total study population was 103 Plasmodium falciparum infected patients with M:F ratio of 7:58. The mean age of the study population was 32.27 ± 14.03 (males: 32.91 ± 13.82; females: 27.42 ± 15.28). Neuropsychiatric manifestations were seen in 21 patients out of total 103 cases as follows: altered consciousness 20 (19.41%), headache 17 (16.50%),
Le paludisme de l'enfant congolais de moins de 15 ans [MIM16754894]


Haemoglobin was performed according to the method of spectrophotometer. Blood thick and blood thin for malaria parasite were performed using Giensa stain and the activity of G6PD was evaluated for some children. We compare the 2 periods, before and after the policy change of malaria treatment in Democratic Republic of Congo. We assessed our results by the calculation of Relative Risk and Odd ratio, \( p < 0.05 \) was considered as statistically significant. During the period before the change of policy (7 years), 16 children with black water fever were admitted at Kinshasa University Hospital and 41 after the period of policy change (7 years). The majority of children with black water fever during the 2 periods were more than 5 years old (73.6\%), 82.4\% of children for both periods received quinine before to develop black water fever. Fourteen children performed the Coombs antiglobulin test; nine received quinine and 5 received other drugs. Three children have positive coomb's antiglobulin test among the nine who received quinine. The odds ratio is 5.9\%(IC: 0.017–2002, 40) Fisher exact test with \( p = 0.23 \). The average haemoglobin rate is very low among positive Coombs test children (3.5 g: dl) compared to those with negative Coombs test patients (6.2\%). 64.9\% of the children developed renal failure. The Risk to develop black water fever increases with the policy change when quinine was used at the first line in the treatment of malaria because of resistance of plasmodium to chloroquine. The majority of cases are more than 5 years old and received quinine before to develop black water fever. Association between the ingestion of quinine in the treatment of malaria and the occurrence of black water fever exit. More investigations such as the measure of quinine-dependent antibodies, G6PD activity must be performed to get best evidence. Increasing sample size of children tested for Coombs test will be helpful. Management of renal failure must be improved to take care of all renal failure cases due to black water fever.

Email address for correspondence: mireillensangun@yahoo.fr

---

**Co-infections of malaria and intestinal helminths in Ekona and Great Soppo: Two areas with contrasting levels of urbanisation in the Mount Cameroon Region [MIM16693195]**

Lum Emmaculate, Muh Bernice Fien, Mbu V. Judith., Samuel Wanji, Helen K. Kimbi

Malaria infections do co-exist with helminth infections and it has been speculated that urbanization alters the frequency and transmission dynamics of malaria as well as helminth infections. The overall objective of this study was to assess the impact of urbanization on co-infections of malaria and intestinal helminths and to establish if any relationship exist between these co-infections in school children in the Mount Cameroon Region. A total of 235 and 208 children from Ekona and Great Soppo respectively of both sexes aged 4–14 years were enrolled into a cross-sectional study between January and June 2007. Capillary blood samples were collected for detection and determination of malaria parasitaemia as well as PCV. Stool samples were also collected and examined by Kato-Katz technique for the presence and intensity of intestinal helminths (Ascaris lumbricoides, Trichuris trichuria and hookworm). Helminth infections were more prevalent in Ekona than Great Soppo. The most prevalent helminth infection in Ekona was Ascaris, followed by Trichuris and then hookworm. In Great Soppo, Trichuris was the most prevalent helminth species followed by Ascaris, then hookworm. More children had co-infections of malaria and helminths in Ekona than in Great Soppo. The most prevalent co-infecting species were Ascaris/P. falciparum and Trichuris/P. falciparum in Ekona while in Great Soppo Trichuris/P. falciparum infections were most prevalent. More children were infected with malaria and intestinal helminths as well as these co-infections in Ekona probably due to increased urbanization in Great Soppo than Ekona.

Email address for correspondence: ngongpanemma@yahoo.co.uk

---

**Genetic variability of Plasmodium falciparum during sporogonic development [MIM16807979]**

H.P. Awono-Ambene, W. Toussile, S. Nsango, R. Tabué, A. Berry, I. Morlais

The sporogonic development of Plasmodium falciparum parasites was monitored in local malaria vector mosquitoes to assess genetic diversity and parasite-vector compatibility in Cameroon. Feeding experiments of our local mosquito strain were performed using gametocyte containing blood from children recruited in Mfou, an area of stable malaria transmission. Plasmodium falciparum samples were collected from gametocyte isolates, oocytes and salivary glands. Gametocytes were isolated at the feeding day, and oocytes and sporozoites were obtained following mosquito dissection at day 9 and day 14 post-infection, respectively. DNAs were extracted and submitted to P. falciparum microsatellite amplification at 6 loci. PCR products were genotyped using GeneMapper software and analyses were done using FSTAT and MixMoGenD. Experimental infections were carried on in 2008. Infection rates in the Ngoussou strain ranged from 0 to 92\%, depending on the gametocyte densities, sex-ratio and gamete maturity. Preliminary results indicate 25\% of single gametocyte infections in the studied area. We observed high genetic polymorphism with an average of 10 allele per locus. Our data will help for a better knowledge of P. falciparum genetic differentiation through the sporogonic development, which is crucial to understand the spread of parasite resistances to drugs and vaccines.

Email address for correspondence: hpaawono@yahoo.fr
Knowledge of climatological and physical factors, housing type and level of urbanization are essential to the study of insect-borne diseases such as malaria. This study was designed to assess factors contributing to malaria heterogeneity in the Mount Cameroon region. A geographical positioning system (GPS) unit was used at each locality, to collect altitude, latitude and longitude data. A sketch map of the area was generated. Blood samples were collected from participants in each locality using sterile blood lancets and blood films produced. Slides were stained with 5% Giemsa and read for the presence of Plasmodium species. Data generated were analyzed using SPSS. Chi square test of heterogeneity was used to assess the differences of malaria prevalence in the study area. A logistic regression analysis was used to determine the significance of factors contributing to malaria in the region. One thousand three hundred and nineteen samples were included. Seven hundred and eighty-six samples were positive for malaria yielding a prevalence of 39.59%. Ekona, a low altitude locality, recorded the highest (92.34%) prevalence of malaria while Bonakanda, at the highest altitude, recorded the lowest (12.33) malaria prevalence, (p = 0.001). Logistic regression analysis suggested that altitude, and relative humidity were the factors contributing to malaria heterogeneity in this region. These results suggest that malaria prevalence in the Mount Cameroon region is heterogeneous. Malarial infection is significantly associated to altitude and relative humidity. The analyses in this work can be used by public health workers in allocating resources for malaria control.

Email address for correspondence: jeebanga@yahoo.com

388 Malaria and HIV/AIDS co-infection in a rural setting of Cameroon: Assessment of some parasitological and clinical parameters [MIM16685621]

Theresa Nkuo-Akenji, Frankline Nzang Ajoeh, Etienne Emgilbert Tevoufouet, Isaac Ngide Ebong

Co-infection with malaria and HIV/AIDS in a rural plantation setting such as Muyuka is expected. This study investigated the effect of co-infection on parasitological and clinical parameters over a 1 year period. 867 adults attending the Muyuka hospital comprised the study population. Parasitaemia was detected by microscopy. HIV infection was diagnosed using test kits. Prevalence of malaria, HIV-1 and co-infection was 90.7%, 27.0% and 25.6%, respectively. GMPD was higher in co-infected (3103.4 ± 773.3) than in malaria patients (2140.2 ± 291.9) (P < 0.004). Mean illness duration (days) was longer (25.8 ± 3.5) in co-infected patients followed by those with malaria (12.4 ± 1.0) and HIV/AIDS monoinfections (4.0 ± 0.7) (P < 0.001). Mean Hb concentration (g/dl) in co-infected patients was 11.4 ± 0.3 compared with 12.1 ± 0.2 for malaria patients (P < 0.05). Fever was higher in co-infected (73.0%) than in malaria patients (60.6%) (P < 0.05). Mean CD4+ count in co-infection was lower (384.5 ± 25.9) than that for monoinfection with HIV/AIDS (467.8 ± 84.8). While none of those solely infected with HIV/AIDS was in the advanced stage, 13.5% of co-infected patients fell in this category. CD4+ counts in febrile co-infected patients (346.6 ± 20.8) were lower than for those afebrile (475 ± 47.3) (P < 0.005). The GMPD in co-infected patients with CD4+ counts < 200 was 5539.9 ± 235.1 when compared with 2987.1 ± 1118.7 and 2015.7 ± 530.0 for those with counts in the range of 200–499 and >500 respectively (P < 0.062). Co-infection was more associated with lower CD4+ counts, high parasitaemia, high fever frequency, longer illness duration and low Hb concentration.

Email address for correspondence: wifon@yahoo.com

389 Using the Rapid Urban Malaria Appraisal (RUMA) method to elucidate the epidemiology of malaria in the city of Douala, Cameroon [MIM14344918]

Dickson Nsagha

Malaria used to be a disease of rural areas but urban malaria is an emerging disease in Africa. e used the Rapid Urban Malaria Appraisal (RUMA) method: literature review, health facility survey and an observational checklist to identify malaria high risk area for the implementation of home-based management of malaria with ACTs among the under-fives in the city of Douala. From chart review in 2005, the highest number of malaria cases was in the Bonassama health district (14,588) and the highest number of malaria deaths in the Log Babah health district (26). The corresponding figures for the under fives in these areas were 5444 (2.83%) and 5 (0.25), respectively. From the health facility survey, the highest percentage of feverish children with positive malaria parasite among all feverish children who attended health facilities were as follows: Deido (56.32%), Bonassama (45.01%) and Cite des Palmiers (42.58%). Literature review and health facility survey gave conflicting results; hence we designed an observational checklist of malaria risk factors. The results of the health facility survey, literature review and environmental malaria risk factors identified the Nkomba health area in the Bonassama health district as the malaria high risk area in Douala city for the implementation of home-based management of malaria with ACT among the under-fives. A clear picture of the malaria burden in the city of Douala is difficult because of under-reporting but using RUMA, the Nkomba health area is the highest malaria risk area in the city of Douala.

Email address for correspondence: dsnsagha@yahoo.com

390 Infection à Plasmodium falciparum chez les élèves camerounais [MIM13578445]

Ponka Roger, Fokou Elie

Le paludisme reste un problème de santé publique au Cameroun car il est la première cause de mortalité et de morbidité. Ainsi, cette étude recherche les facteurs nutritionnels en relation avec l’infection à Plasmodium falciparum, chez les élèves camerounais de Ngali Il. 211 et 200 élèves âgés de 5–18 ans ont été recrutés respectivement en saison des pluies et en saison sèche après accord de leurs parents. Leurs apports alimentaires ont été déterminés. Le sang prélevé sur ces sujets a permis de déterminer la parasitémie, de leurs parents. Leurs apports alimentaires ont été déterminés. Le sang prélevé sur ces sujets a permis de déterminer la parasitémie, l’hématocrite la zincémie et la cuprémie de 70%. Les corrélations négatives et significatives sont observées entre les apports en énergie, zinc, cuivre, vitamine A, ainsi que la parasitémie, l’hématocrite la zincémie et la cuprémie. Les infections sont dues à Plasmodium falciparum à plus de 70%. Les corrélations négatives et significatives sont observées entre les apports en énergie, zinc, cuivre, vitamine A, ainsi que la parasitémie, l’hématocrite la zincémie et la cuprémie. Les infections sont dues à Plasmodium falciparum à plus de 70%. Les corrélations négatives et significatives sont observées entre les apports en énergie, zinc, cuivre, vitamine A, ainsi que la parasitémie, l’hématocrite la zincémie et la cuprémie. Les infections sont dues à Plasmodium falciparum à plus de 70%. Les corrélations négatives et significatives sont observées entre les apports en énergie, zinc, cuivre, vitamine A, ainsi que la parasitémie, l’hématocrite la zincémie et la cuprémie.
En effet la vitamine A, le zinc et le cuivre sont impliqués dans le fonctionnement du système immunitaire. Une carence en fer se traduit par la réduction de la parasitémie.

Email address for correspondence: rponka@yahoo.fr

391
Using National Health Information System to monitor malaria morbidity [MIM16698991]
E. Edu, V. Sima, G. Nseng, J.L. Segura

With participation of WHO, International NGOs and MOHSW, Equatorial Guinea designed and launched a unique set of health records in 2006. Initially, under the support of Bioko Initiative Malaria Control Project (BIMCP) the registers were distributed in Bioko Island (Insular Region), and since 2008 these were introduced in all health facilities of Continental Region. This report is limited to Bioko Island, where 2 complete years of registers collected and processed are available. The National Health Information System monthly collects registers in all health facilities, and these are entered in one MS-Access-based ad hoc data entry application developed by MCDI. In 2008 a set of improved registers were introduced, to accurately track each record. These registers were distributed with books and pages pre-coded, to compare distributed and collected registers. During 2007 a total of 32,971 outpatients were recorded, and 18,111 (55%) of them were diagnosed as malaria cases. In 2008, only 36,943 total of outpatients 12,237 (33%) were diagnosed as malaria. During first quarter 2008 there was 32% less malaria patients compared to the same period in 2007. For last quarter the decrease reached a 53%. In 2008 a peak of cases was observed for April and May, reaching 50% of excess for all age groups, but in less than 1 year group it reached more than 300% (May 08). Apparently improvements in NHIS registers increased its sensitivity to epidemiologic events induced by rain seasonality and/or bednet distribution campaigns, increasing its value to support resources distribution.

Email address for correspondence: lsegura1212@gmail.com

392
Heterogeneous malaria incidence in children in an area of very high transmission [MIM16204074]

Malaria incidence is known to be affected by local environment in areas of low transmission, but is often assumed to be homogeneous in areas of very high transmission. Tororo, Uganda, is a rural district where malaria is holoendemic and the entomological inoculation rate is estimated at 562 bites per person-year (PPY). Infants aged 6 weeks to 12 months living within 30 km of our health facilities of Continental Region. This report is limited to Bioko Island, where 2 complete years of registers collected and processed are available. The National Health Information System monthly collects registers in all health facilities, and these are entered in one MS-Access-based ad hoc data entry application developed by MCDI. In 2008 a set of improved registers were introduced, to accurately track each record. These registers were distributed with books and pages pre-coded, to compare distributed and collected registers. During 2007 a total of 32,971 outpatients were recorded, and 18,111 (55%) of them were diagnosed as malaria cases. In 2008, only 36,943 total of outpatients 12,237 (33%) were diagnosed as malaria. During first quarter 2008 there was 32% less malaria patients compared to the same period in 2007. For last quarter the decrease reached a 53%. In 2008 a peak of cases was observed for April and May, reaching 50% of excess for all age groups, but in less than 1 year group it reached more than 300% (May 08). Apparently improvements in NHIS registers increased its sensitivity to epidemiologic events induced by rain seasonality and/or bednet distribution campaigns, increasing its value to support resources distribution.

Email address for correspondence: lsegura1212@gmail.com

393
Microscopy versus home-based presumptive diagnosis of malaria in a rural community in Western Kenya [MIM]
Rose Kakai, Josephine Nasimiyu, Wilson Odero

Malaria can be a life-threatening disease, especially in children, when left untreated. It is therefore important to have a quick and accurate diagnosis. Home-based management of malaria is promoted as a major strategy to improve prompt delivery of effective malaria treatment in Africa. To prevent unnecessary anti-malarial treatment, it is important to confirm clinical suspicions with a good laboratory test. The purpose of this study was to compare the results of routine malaria microscopy and caretakers’ presumptive diagnosis of malaria in a rural community in Western Kenya. Cross-sectional study done in November/December 2007 at Bokoli location, Webuye division of Bungoma East District. At the households, consenting caretakers of children with malaria (according to the caretakers’ diagnosis) were interviewed using a semi-structured questionnaire. Finger prick blood smears were collected from 96 children aged 6–36 months, stained by Field stain A and B then examined for malaria parasites by light microscopy. Data was analyzed by descriptive statistics. Only 31.3% (30/96) specimens were positive for Plasmodium falciparum. Although only 17.5% (10/57) patients had received anti-malarial treatment, 60% (6/10) of those were smear negative, and 81% (17/21) of those who had smear positive microscopy had not received any treatment. There was no relationship between caretakers’ age or level of education and presumptive diagnosis. The difficulty in making presumptive diagnosis of malaria necessitates urgent need for improved diagnostic tools that can be used at community level in poor populations. Those untreated may serve as reservoirs for malaria parasite transmission.

Email address for correspondence: kakairm@yahoo.com

394
The changing patterns of malaria admissions since 1999 at 18 hospitals across Kenya [MIM15064898]
Emelda A Okiro, Juliette Mutheu, Pete W. Gething, Elizabeth Juma, Robert W. Snow

The last few years have witnessed a rapid scaling up of key malaria interventions in a number of African countries, most notably insecticide treated nets (ITN) and improved therapeutics such as Artemisinin-based combination therapy (ACT). However, there is only limited information on the health impact of expanded coverage of these interventions in Kenya. Paediatric admission data were assembled over 9 years from 18 district hospital settings in Kenya. Study hospitals were selected to reflect the diverse malaria ecologies typical of Kenya. Trends in monthly malaria admissions between January 1999 and July 2008 were analysed using several time-series models controlling for covariates related to climate and service use to establish whether changes in admissions can be attributed to expanded coverage of ITNs and ACT. There was evidence that the hospital burden for children with a primary admission diagnosis of malaria has declined over the interval at several sites; notably in areas where starting endemicity was low,
Malaria-related mortality in hospitalized children in an area of high malaria transmission [MIM16671767]

Robert Opika Opoka, Teekam, Paul Bangirana, Charles Engoru, Justus Byarugaba, Chandy C. John

In areas of seasonal malaria transmission in sub-Saharan Africa, the presumptive treatment of febrile illnesses as malaria without laboratory confirmation of the diagnosis is associated with high impatient mortality. However this presumptive diagnosis and treatment of malaria and the related impatient mortality in high malaria endemic areas is not well characterized. A retrospective chart review was conducted of all children up to 15 years of age admitted to Soroti Hospital, Uganda from January 2002 to December 2004, with a diagnosis of malaria and clinical outcome analyzed according to microscopic confirmation of diagnosis. A total of 10,387 children were admitted with a diagnosis of malaria during the study period, 746 (7.2%) of whom died. Severe malarial anemia 3794 (36.5%) and malaria with convulsions 1764 (17.0%) were the two common causes of malaria related admissions. Children who did not receive microscopy testing had a higher case fatality rate than those with a positive blood smear (9.0% vs. 6.5%, P < 0.001). After adjustment for age, malaria complications and co-morbid conditions, children who did not have a smear done had a higher risk of death than those with a positive blood smear, Odds Ratio (OR) 2.08, 95% Confidence Interval (CI) 1.71, 2.53, P < 0.001. In high malaria endemic areas, diagnosis of malaria in the absence of microscopic confirmation is associated with significantly increased mortality in hospitalized Ugandan children. In patient diagnoses of malaria should be supported by laboratory confirmation of diagnosis.

Email address for correspondence: opokabob@yahoo.com

The current status of malaria infection risk, disease burden and intervention coverage in Africa in 2008: The Malaria Atlas Project in Afric [MIM15000361]

Robert W. Snow

Mapping the distribution of malaria risk is central to all effective planning of global initiatives for malaria control and elimination. The Malaria Atlas Project (MAP, http://www.map.ox.ac.uk) was founded to fill this niche and has to-date provided the most empirically defined maps of the global limits and transmission intensity distribution since 1968. Across several parts of Africa malaria transmission is clearly in transition and time-series maps of malaria risk have been used to provide a more evidence-based and dynamic picture of how this changing risk has impacted on disease burdens since 1985. All however is not equal and work undertaken by MAP has shown that intervention coverage and donor support varies enormously between countries on the continent and these inequities need to be addressed. Time-space modeled maps of parasite prevalence provide a powerful tool to track progress in malaria control nationally and regionally. The plenary session will summarize the collective work of MAP in Africa to the scientific and control communities to show-case how future national malaria indicator surveys, MAP models and disease burden estimations will serve to monitor future progress toward malaria control through to 2015.

Email address for correspondence: rsnov@nairobi.kemri-welcome.org

Prevalence of P. falciparum and P. vivax and its associated risk factors in Harar, Eastern Ethiopia [MIM15082324]

Balew Arega, Brhane G/mariam, Nigussie Mersha, Rigat Solomone, Tibebo Yisigat, Sisay Fesseha, Tamirat Gebru

Malaria is a communicable parasitic disease caused by a protozoan parasite and is transmitted by the bite of female anopheles mosquitoes. Globally about 300–500 million people suffer from malaria each year. Nearly 250 million clinical cases and over one million deaths were recorded annually. In sub-Saharan Africa where 74% of the population live in highly endemic area and a further of 18% live in malaria epidemic areas. In Ethiopia 3/4 of the country are malariaous and 40 million people live in this area being at risk of malaria attack. A cross-sectional descriptive, quantitative type of study was conducted to assess the prevalence and associated risk factors of malaria infection in Harar malaria center, Harar, Ethiopia, from March 13 to April 3, 2008. Once the data was collected and processed it was analyzed by calculating chi-square (x²) for each category of study groups and then the percentage and p value was measured. In this study, out of a total 234 sample-population diagnosed, 45(19.23%) individuals were positive for malaria, 17(40%) for P. falciparum and 28(60%) for P. vivax. 17(37.18%) males and 28(62.20%) female were positive for either of the two species of malaria. In this study, there was no significant association observed between travel to malaria endemic area, using of personal repellent, use of bed-net, sleeping out door, presence of stagnant water around their house and previous malaria attack (p < 0.05).

Email address for correspondence: tamiratgw2002@yahoo.com

Estimation of the actual malaria transmission levels in central-east of Sudan [MIM14954821]

Y.S. Nimr, M.A. Ata almannan

Malaria is a very significant health problem in Sudan, although the severity of the annual outbreaks is influenced by rainfall and possibly other factors. The spread of the disease in the Sennar state (Central-east of Sudan) depends mainly on rain. A. arabiensis is a major vector in this state. This study evaluated the seasonality of the actual malaria transmission by Anopheles arabiensis characterized the blood feeding behavior of An. arabiensis in two villages (Al camp 33 and Emsail village). The state is situated in the rich savanna environment. It has an area of 37,844 km² and an estimated population of approximately 143.059, it lies between latitude 12.5–14.7 N and longitude 32.9–35.4 O S. Summer starts in March and ends in May, with an average daily temperature 32–40 °C and relative humidity 25%. The rainy season starts earlier in June and ends in September. The temperature raging between 20 and 25 °C during winter, which starts in October. Two methods were used to collect mosquito’s monthly and them, human/landing catches and The
Knock-down method. PCR method was used to identify the a total of 400 members of An. gambiae complex from Sennar state in central Sudan, and then sandwich Elisa (Csp-Elisa) was used to determine the percentage of infective mosquitoes’. The majority, 72.5% (\(n = 290\)) were successfully identified as An. arabiensis, while the remaining 27.5% (\(n = 110\)) did not amplify even after two attempts. Transmission during March 2006–February 2007 dry season was zero because of widespread drought. In rainy season sporozoite rate of \(P. falciparum\) estimated 1.0% and 2.0% in \(P. vivax\). In post-rainy season sporozoite rate was increased up to 1.3% and 2.5% in \(P. falciparum\) and \(P. vivax\), respectively. Finally the estimated Entomological Inoculation Rate (EIR) values were 0.01 and 0.02 infective bite per person per transmission season of \(P. falciparum\) and \(P. vivax\) respectively in rainy season. Also EIR was increased up to 0.05 and 0.10 for two species respectively in post-rainy season.

Email address for correspondence: elyazidnimr@yahoo.com

399 Spatio-temporal distribution of clinical malaria cases in a highland site in western Kenya [MIM16669989]

Yaw Afrane, Andrew Githeko, Guiyun Yan

One important issue for evaluating the efficiency of new malaria control measures on febrile malaria is the accuracy of hospital-based malaria case data, including underreporting, misdiagnosis or over-reporting. Further, the distribution pattern of febrile malaria may be used to infer environmental risk factors. This study investigated the spatial distribution and temporal dynamics of clinical malaria cases through active and passive case surveillance in a western Kenya highland site. Active case surveillance was done with a cohort of over 1800 participants selected randomly from 400 houses in Kakamega District. These houses were stratified by topography. Participants were visited every 2 weeks and screened for clinical malaria whilst passive case surveillance was done from the local health facility in the study area. A clinical malaria case is defined as an individual with malaria-related symptoms (fever [axillary temperature \(\geq 37.5\) °C], chills, severe malaise, headache or vomiting) at the time of examination or 1–2 days prior to the examination and a presence of a \(P. falciparum\) positive blood smear. Topography was associated with increased malaria risk. Clinical malaria cases from active case surveillance were clustered along valley bottoms with a 2–2.5-fold increase during the rainy season. Children between the ages of 6–10 years had 45% of clinical malaria cases followed by children under the age of 5 years (40%), then people older than 10 years had the least (15%). Bednet coverage was over 60% among households. However, households with bednets still had clinical malaria cases. There was no significant correlation between case numbers obtained from active clinical cases and passive hospital hospital-based case surveillance, suggesting some level of unreliability of hospital-based clinical case data. Hospital cases seem to be the same throughout the year with no pattern of seasonality compared to the active clinical cases. We are investigating whether misdiagnosis and over treatment from the hospitals account for the ambiguity in the passive cases.

Email address for correspondence: yaw_afrane@yahoo.com

400 HIV, malnutrition and invasive bacterial infection among children with severe malaria [MIM16669311]

Jay Berkley

HIV infection, malnutrition and invasive bacterial infections (IBI) are reported among children with severe malaria. However, it is unclear whether their co-occurrence with falciparum parasitization and severe disease is by chance, or by association among children in malaria endemic areas We examined 3068 consecutive paediatric admissions to a Kenyan district hospital with clinical features of severe malaria, and 592 community controls. We performed multivariable regression analysis with each case weighted for their probability of being due to falciparum malaria using estimates of the fraction of severe disease attributable to malaria at different parasite densities derived from cross-sectional parasitological surveys from well children in the same community. HIV infection was present in 133/1071 (12%, 95%CI 11–15%) consecutive parasitic admissions. Parasite densities were higher in HIV infected children. The odds of admission associated with HIV infection for admission with true severe falciparum malaria were 9.6 (95%CI 4.9–19), however this effect was restricted to children age \(\geq 1\) year. Malnutrition was present in 307/2048 (25%, 95%CI 23–27%) consecutive parasitic admissions. The odds associated with malnutrition for admission with true severe falciparum malaria were 4.0 (95%CI 2.9–5.5). IBI was detected in 127/2048 (6.2%, 95%CI 5.2–7.3%) of consecutive parasitic admissions. All three comorbidities were associated with increased case fatality. HIV, malnutrition and IBI are biologically associated with severe disease due to falciparum malaria rather than being simply alternative diagnoses in co-incidentally parasitized children in an endemic area.

Email address for correspondence: jberkley@kilifi.kemri-welcome.org

401 Genetic diversity of Plasmodium falciparum and acquisition of strain specific immunity in individuals of north-eastern Tanzania [MIM16763859]


Malaria is the leading cause of morbidity and mortality in sub-Saharan Africa and polymorphism is common among malaria parasites. This represents major challenge in the implementation of different interventions such as vaccine trials. The study was conducted in 0.6–44 years individuals in Muheza district, Tanga region, northeastern-Tanzania. Axillary temperature, finger prick blood, thick and thin smears, Blood spotted 3 mm Whatman filter paper for genotyping Plasmodium falciparum merozoite surface protein 2 (Pfmsp-2) gene by PCR was done. Malaria prevalence was 31.4% and 34.6% during short-rains and long-rains, respectively. Genotyping results showed that 55.6% of samples had 3D7 and FC27 strains during short-rains and in long-rains had 3D7 (42.0%) (\(\chi^2 = 13.80, p = 0.001\)). We then found that Multiplicity of Infection (MOI) was 2.25, i.e. 2.74 during short-rains and in long-rains was 2.01 (\(p < 0.001\)). More fever cases were observed during short-rains than in long-rains (23/256, 9.0% vs. 29/604, 4.8%; \(\chi^2 = 5.5379, p = 0.019\)) which was associated with the increase in mean 3D7 clones. Malaria prevalence was higher during long rains indicating direct association between malaria transmissions and rainy seasons. The MOI showed persistence of 3D7 clone during long rains indicating acquisition of strain specific immunity in some individuals after short rains. The association of increased febrile cases during short rains with the increase in mean 3D7 clones indicate that FC27 clone is responsible for the strain specific clinical protection. These findings are critical while designing an effective antimalarial vaccine based on Pfmsp-2.

Email address for correspondence: mwanaidiktz@yahoo.co.uk
402 Estimates of malaria at community level through CORPs strategy by early diagnosis and treatment of febrile illnesses in North-Eastern Tanzania [MIM16671207]


Early diagnosis and prompt treatment has been advocated as best strategy for malaria case management. Community owned resource persons (CORPs) can provide such services at community level. The main aim of the study was to use CORPs strategy to estimate the burden of malaria in preparation for malaria vaccine trial. The study was conducted from February 2006 to December 2008 in Korogwe, Tanzania. Passive case detection (PCD) of fever was through CORPs. Malaria diagnosis was by both Paracheck Pf® and blood smears. Cases consulting CORPs had personal information recorded. Antimalarial given was sulfadoxine/pyrimethamine (SP) up to January 2007, thereafter artemether/lumefantrine. A total of 11,038 cases were attended during 35 months period. Around 30.8% of cases had measured fever (≥37.5 °C) in 2006, 39.1% in 2007, and 33.6% in 2008; being significantly higher in 2007. Overall, 41.5% of fever cases were positive for malaria parasites. Feverish under-fives positive for malaria parasites were 36.2%. Logistic regression, adjusting for strata showed malaria infection in 2007 was 2.26-fold compared with 2006 (OR = 2.262, 95%CI = 2.032, 2.52) and for 2008 it was 13% higher than 2006 (OR = 1.13, 95%CI = 1.01, 1.27). Clinical malaria (fever ≥37.5 plus Plasmodium falciparum ≥2500 rings/μl) was 9.5% across three years (2006: 6.4%, 2007: 13.1%, 2008: 8.6%).

Email address for correspondence: malinzi55@yahoo.com

403 Estimates of malaria at community level through CORPs strategy by early diagnosis and treatment of febrile illnesses in North-Eastern Tanzania [MIM16728593]


Early diagnosis and prompt treatment has been advocated as best strategy for malaria case management. Community owned resource persons (CORPs) can provide such services at community level. The main aim of the study was to use CORPs strategy to estimate the burden of malaria in preparation for malaria vaccine trial. The study was conducted from February 2006 to December 2008 in Korogwe, Tanzania. Passive case detection (PCD) of fever was through CORPs. Malaria diagnosis was by both Paracheck Pf® and blood smears. Cases consulting CORPs had personal information recorded. Antimalarial given was sulfadoxine/pyrimethamine (SP) up to January 2007, thereafter artemether/lumefantrine. A total of 11,038 cases were attended during 35 months period. Around 30.8% of cases had measured fever (≥37.5 °C) in 2006, 39.1% in 2007, and 33.6% in 2008; being significantly higher in 2007. Overall, 41.5% of fever cases were positive for malaria parasites. Feverish under-fives positive for malaria parasites were 36.2%. Logistic regression, adjusting for strata showed malaria infection in 2007 was 2.26-fold compared with 2006 (OR = 2.262, 95%CI = 2.032, 2.52) and for 2008 it was 13% higher than 2006 (OR = 1.13, 95%CI = 1.01, 1.27). Clinical malaria (fever ≥37.5 plus Plasmodium falciparum ≥2500 rings/μl) was 9.5% across three years (2006: 6.4%, 2007: 13.1%, 2008: 8.6%).

Email address for correspondence: malinzi55@yahoo.com

404 Malaria parasitaemia among farming communities in Mvomero District, Tanzania [MIM16824694]

Kesheni Senkoro

Objective: To determine malaria parasitaemia in Mvomero farming community. Four cross-sectional malariometric surveys were carried from August 2004 to May 2005 in villages with different agricultural practices in Mvomero District, Tanzania. Thick and thin blood smears were taken from individuals of all age-group at a community setting. A total of 7856 individuals were screened for malaria parasites. Of these, 4.7%, 68.2%, 11.4% and 15.6% were <5, 5–9, 10–14 and ≥15 years old, respectively. The overall prevalence of malaria parasitaemia was 34.5% (2708/7856). Plasmodium falciparum was the most dominant species (98.3%) of malaria parasites. Other malaria infections were due to Plasmodium malariae (0.013%) and P. falciparum + P. malariae (0.37%) and P. falciparum + Plasmodium ovale (0.07%). Malaria parasite prevalence varied between villages and agricultural practice. Highest prevalence rate was observed in community living in rice irrigation farming system at Komtonga (66.3%). The lowest prevalence was among community living in sugarcane plantation at Mtibwa (7.2%). The prevalence was highest (43.6%) and lowest (15.34%) among 10–4 and ≥15 year olds, respectively. Malaria prevalence was slightly higher (36.8%) in males than females (32.3%). Overall the prevalence of spleen enlargement was 5.6%, with children 10–14 years old being the highly affected group (20.0%). These findings show that there are significant variations in malaria parasitaemia and associated morbidity among individuals living in different agroecological settings within short distances.

Email address for correspondence: ksenkoro@nimr.or.tz

405 A community-based surveillance system to assess the effects of malaria interventions [MIM16571834]


The ACCESS Programme aims at understanding and improving access to prompt and effective malaria treatment and care in a rural Tanzanian setting with a set of integrated interventions. To evaluate the programme’s impact on reported incidence of fever and severe malaria disease at both the community and health facility levels, and to investigate the value of community-based reporting for routine malaria control programme monitoring. This work was implemented within the Ifakara Demographic Surveillance System (DSS) which comprises a total population of 80,000 in southern Tanzania. Besides data on mortality, the DSS staff routinely collected data on reported incidence of fever (2-week recall) and severe malaria disease in the community. In parallel we collected fever data from the 15 health facilities in the area. Reported fever rates in the community decreased from 47.2 to 41.4/1000 person weeks (IRR = 0.94, p < 0.001) between 2005 and 2007. The fever rates in the health facilities decreased slightly from 19.5 to 18.8/1000 person weeks between 2005 and 2007 (IRR = 0.96, p < 0.001). A good temporal and quantitative relationship was found between community-reported fever and health facility malaria diagnoses, suggesting that the former could be used for routine monitoring. Moreover, good internal and external consistency was found with a separate treatment seeking survey in the same community and with national data. The trends of fever cases indicated a reduction in malaria risk. This conclusion is strengthened by...
the great consistency of the data collected from four independent sources.
Email address for correspondence: sandra.alba@unibas.ch

406 Evidence for hotspots of Anopheles exposure that partially explain heterogeneity in malaria morbidity in northeastern Tanzania [MIM16669136]

Teun Bousema, Samwel Gesase, Ramadhan Hashim, Stephen Magesa, Frank Mosha, Silas Otieno, Magreth Mosha, Caroline Maxwell, Chris Drakeley, Roly Gosling

Variation in malaria transmission at micro-epidemiological levels has long been acknowledged but never utilized for malaria control. Detecting hotspots of malaria transmission will become increasingly important with malaria eradication efforts. We determined variation in exposure to (malaria-infected) mosquitoes and developed a predictive model for detecting mosquito hotspots. Mosquito exposure was assessed at three time-points in the dry and wet season in Korogwe, Tanzania. Hotspots of mosquito exposure were defined as households with the highest quintile of female Anopheles lines in the wet season. Consistency in hotspots over time was determined and related to distance to breeding sites, household characteristics and passive case-detection data over a three-year period. Mosquitoes were sampled in households in the wet season (n = 500), beginning (n = 506) and end of dry season (n = 450). 52,856 female Culicines and 8276 female Anopheles were caught with considerable variation between households. Twenty percent of the households experienced 75.1% of the exposure to female Anopheles lines and infected mosquitoes clustered in households (p < 0.001). Hotspots of female Anopheles exposure were consistent throughout seasons (p < 0.001). This was specific for Anopheles with Culicines not showing the same pattern (p = 0.93). There was a strong relation between Anopheles hotspots and the number of experienced malaria episodes (p < 0.001). Household factors predicting the presence of hotspots included distance to a breeding site, roofing material and number of individuals sleeping in a household. Our data show that hotspots of exposure to malaria transmitting mosquitoes are consistent over time. Our predictive model for hotspots can facilitate targeted control efforts.
Email address for correspondence: teun.bousema@lshtm.ac.uk

407 MARA: An open access malariometric database in Africa [MIM16541804]

Konstantina Boutsika, Tanja Jaeggi, Musa Mabaso, Colleen Fraser, Don de Savigny, Christian Lengeler, Penelope Vounatsou

The Mapping Malaria Risk in Africa (MARA) initiative is a comprehensive compilation of malaria prevalence data for Africa since 1900. It was started in 1995 as a Pan-African initiative. Currently, MARA is being maintained by the Swiss Tropical Institute (STI) in Basel, Switzerland, in the collaboration of Medical Research Council (MRC) in Durban, South Africa. Malariometric data from peer-reviewed publications as well as ‘grey literature’ are continuously identified and extracted. Direct communication with authors and researchers help to clarify issues as required. The database consists so far of more than 13,000 surveys collected over 12,000 locations, distributed in the continent as follow 25% in East Africa, 37% in Southern Africa, 5% in Central Africa and 33% in West Africa. The MARA database constitutes a precious global resource, especially in view of the rapid expansion of malaria control activities. The database is currently being converted into an open access, user-friendly database. Our goal is to develop an internet-based platform to enable users to extract and display updated raw data and disease burden estimates, as well as to enter new data and hence contribute to the expansion of the database.
Email address for correspondence: konstantina.boutsika@unibas.ch

408 Comparing the effectiveness of malaria vector control interventions through a mathematical model [MIM16672703]

Nakul Chitnis, Allan Schapira, Richard Steketee, Thomas Smith

Pregnancy-associated malaria is of major concern in sub-Saharan Africa causing maternal anemia, low birth weight and still births due to the accumulation of infected erythrocytes in the placenta. This accumulation is due to the interaction between the VAR2CSA protein on the surface of the infected erythrocytes (IE) and chondroitin sulfate A (CSA) in the placenta. Pregnant women gradually develop protective anti-VAR2CSA IgG over successive pregnancies. However, infected multigravid women seem to harbour parasite expressing distinct variants of VAR2CSA as compared to infected primigravidae. The aim of this study was to determine the dynamics of Plasmodium falciparum genotypes during pregnancy in relation to acquisition of anti-VAR2CSA immune response. To study the dynamics of P. falciparum population during pregnancy,msp2 genotyping and analysis of the var2csa DBL5e sequence were performed. ELISA was used to measure the level of VAR2CSA DBL5e specific IgG and their levels were analyzed in relation to infection. The results available now highlight the selection of some parasite genotypes able to persist over several weeks and, still present in the placenta at delivery. Anti-VAR2CSA antibody response is associated to clearance of some but not all genotypes. We will continue exploiting these results to determine (i) the possible existence of variant specific immune response against VAR2CSA. (ii) Whether parasites infecting primigravidae and multigravidae express different VAR2CSA DBL5 variants. (iii) Whether placental tropism is restricted to particular VAR2CSA variants.
Email address for correspondence: nakul.chitnis@unibas.ch

409 The MalariaGEN serum repository [MIM16758570]

Patrick Corran, Nilupa Silva, Paul Risley

One of the constituent Consortial Projects of the Grand Challenges in Global Health-funded MalariaGEN collaboration (CP2) was the establishment of a repository of serum samples from a subset of collaborating sites, each matched to a corresponding DNA sample. This repository was established and maintained at the National Institute for Biological Standards and Control Serum and plasma samples were logged and stored at −80 °C as two aliquots in individually bar-coded tubes in bar-coded racks. A portion (5 μl) was diluted and used to estimate total IgG to four Plasmodium falciparum antigens (MSP-1,AMA-1, MSP-2 and NANP) and total IgE as part of a standard characterisation schedule. Each sample corresponded to a matching DNA sample provided to the MalariaGEN DNA repository. Approximately 15,000 sample were received from 7 sites in 6 countries (5 in Africa and one Asian). Summary serological data has been generated for each site and merged into the central database containing results from corresponding genomic investigations together with clinical and epidemiological data. This has provided the basis of analyses in some of the accompanying presentations. We believe that this is the first
occasion in which a uniform set of immunological and genomic information has been obtained over such a wide range of samples. The samples lodged in the repository, together with the accompanying genomic information, provide an opportunity for generating a uniquely rich set of information about the genetic basis of different aspects of the development of humoral immunity to malaria.

Email address for correspondence: pcorran@nibsc.ac.uk

410 Treatment seeking patterns for fever and convulsion in rural Tanzania [MIM16520938]

Convulsions are manifestations of severe malaria among under five children leading to serious complications and death. Previous studies have provided contradictory evidence with regard to treatment of convulsions (traditional healers versus modern treatment). To clarify this, a study was carried out within the frame of the ACCESS Programme, an intervention study aiming at understanding and improving access to prompt and effective malaria treatment in rural Tanzania. The study used a locally constructed cultural-epidemiological approach. A baseline study was carried out in 2004/2005 and repeated in 2006/2008. Results from 2004/2005 showed that about 71.1% of all convulsion cases were timely brought to health facility, compared to 45.6% for mild fever cases. The patterns of distress associated with less timely health facility use and receipt of antimalarial among convulsion children were generalized symptoms, rather than the typical symptoms of convulsions. Caretakers who administered antimalarials to children without attending a health facility believed either the facilities were out of stock or there is no proper diagnosis there. Analysis of the 2006/2008 is in progress. Our findings contradict previous studies which have shown that children with convulsion are less likely to use health facilities. This may result from a weakening of traditional ideas. We expect more children with convulsion to timely attend health facility in the repeated studies as the result of ongoing interventions. However, the next aim is to improve the quality of care at health facilities by making drugs and diagnosis available and free for communities to access.

Email address for correspondence: adillip@ihi.or.tz

411 Estimating the rate of acquiring immunity to severe disease due to Plasmodium falciparum with age and exposure [MIM16694456]

Immunity to severe disease is known to develop with repeat infections of Plasmodium falciparum. Previous analysis suggested that immunity to non-cerebral severe malaria is acquired after only a couple of infections. However, evidence from longitudinal studies shows that some young children experience multiple episodes of severe disease, suggesting that immunity may not be acquired so quickly. We developed a mathematical model for infection and episodes of severe disease by age and fitted it to the age-distribution of severe malaria cases in children, stratified by cerebral malaria, severe malarial anaemia and respiratory distress. Models were fitted to data across a range of transmission settings. Mechanisms for the development of immunity to severe disease were investigated by evaluating the fit of different model structures to the data. The force of infection correlated well with the measured parasite rates in children. Maternal immunity was estimated to last up to a year. The best fitting models suggest that immunity to severe disease develops gradually, increasing with repeat infections. This analysis of data from a range of settings gives plausible estimates both for the force of infection in each setting and for the duration of maternal immunity to disease. Our results suggest that immunity to severe disease increases with each exposure, but more gradually than previously suggested. Further longitudinal studies would be required to test these estimates and to investigate the effect of heterogeneities in exposure.

Email address for correspondence: d.hollingsworth@imperial.ac.uk

412 Marked increase in child survival after four years of intensive malaria control on Bioko Island, Equatorial Guinea [MIM16669390]
Immo Kleinschmidt, Christopher Schwabe, Luis Benavente, Miguel Torres, Frances Ridi, Jose Luis Segura, Paul Ehmer, Gloria Nchama

In malaria endemic countries in Africa a large proportion of child deaths are attributable to infection with Plasmodium falciparum. In Bioko, Equatorial Guinea, comprehensive interventions consisting of vector control, case management and other measures were introduced in 2004. In annual household surveys, infection with parasites, anaemia and fever history in children, coverage of indoor residual spraying (IRS) and use of insecticide treated nets (ITN) were monitored. Changes in all cause under-five mortality were assessed through women's birth history questionnaires at baseline and after four years. All cause under-five mortality fell from 152 per 1000 births (95% CI 122–186) to 55 per 1000 (95% CI 38–77) during the intervention period, hazard ratio 0.34 (95% CI 0.23–0.49, p < 0.001). Simultaneously there were reductions in prevalence of infection (Odds ratio (OR) = 0.31, 95% CI 0.2–0.46, p < 0.001), anaemia (OR = 0.11, 95% CI 0.07–0.18, p < 0.001) and reported fevers (OR = 0.41, 95% CI 0.22–0.76, p = 0.008) in children. Vector control (IRS or ITN or both) reached 95% (95% CI 92–96) of children in 2008. Infectiveness in malaria vectors fell to near undetectable levels. In settings of high transmission intensity, effective malaria control measures which achieve a high degree of coverage, can result in major health improvements, and can play a key role in achieving millennium development goals.

Email address for correspondence: immo.kleinschmidt@lshtm.ac.uk

413 Influence of host genetic factors of the chromosomal region 5q31–33 on first Plasmodium falciparum infections in young children from a holoendemic area [MIM16663849]

Time and course of the first infections with Plasmodium falciparum in the life of a child determine the risk of severe malaria complications. The dynamics of malarial infections are dependent on environmental as well as on parasite and host genetic determinants. The chromosomal region 5q31–33 is considered a major candidate gene area for malaria risk, since high lod scores for high parasitaemia and increased numbers of re-infections were observed within this gene region in segregation analyses. Thousand seventy children from a holoendemic area in the Ashanti Region, Ghana,
were enrolled at the age of 3 months and followed by monthly active visits and passive case detection. The phenotypes that were considered comprise the time of first infection and first malaria episode, their frequencies, levels of parasitaemia, and anaemia. Two hundred thirty-eight single nucleotide polymorphisms (SNPs) from 37 genes within the multigene cluster were selected for typing of SNPs and subsequent association analyses. The malaria incidence was in average 1.2 episodes per person years. The analysis showed linkage disequilibria, haplotype blocks and haplotype frequencies which differed markedly from those of Caucasians but also from the few data known from other African populations. Data showed several haplotype blocks of loci with high linkage disequilibrium, an age-dependent occurrence of parasitaemia associated with distinct gene variants, and strong associations between the time and number of malaria episodes and some of the SNPs on 5q31–33. Host gene variants define the capability of the innate and acquired immune response and may point to potential pathophysiological mechanisms for the development of malaria.

Email address for correspondence: may@bni-hamburg.de

414 Evaluation of the risk of malaria re-emergence in Romania [MIM16693599]


Romania experienced about 300,000 malaria cases yearly, the last one being recorded in 1962. The permanent risk of malaria re-emergence because of the presence of the Anopheles maculipennis group species and imported malaria cases increased in the present conditions of the climatic and other environmental changes in Romania. The analysis of the evolution of this risk to anticipate, prevent and control the malaria re-emergence is performed. Analysis of both the current and historical data regarding the presence, dynamics and vectorial capacity of the anopheline vector populations and the presence of the imported Plasmodium strains in correlation with the evolution and present status of the environmental, social and economical conditions in the areas with human populations at risk is performed. Three periods of evolution of the risk of malaria re-emergence after the malaria eradication are characterized in Romania. The risk of malaria re-emergence in every period resulted from the balance of the factors increasing or decreasing this risk. These factors led to the low risk linked to low densities of the vector populations (1962–1985); the start of the time of first infection and first malaria episode by the dominance of the main vector, Anopheles atroparvus, and the gradual transition to the resistance to insecticides (1985–1993); the high risk linked to the increase of both the density and resistance to insecticides of vector populations dominated by An. atroparvus and the imported malaria cases (1993–to the day). The balance of all the factors leading to different risk level in every period is recorded and evaluated.

Email address for correspondence: gabrielamarianicolescu@yahoo.co.uk

415 Development of a new biomarker of exposure to Anopheles bites based on human antibody responses to salivary proteins: From the concept to the applications [MIM16525169]


Human antibody (Ab) IgG response to whole saliva of Anopheles gambiae could be an epidemiological biomarker of exposure to An. gambiae bites. In the objective to increase the specificity to Anopheles exposure, the second step is to identify the salivary proteins (i) specific to Anopheles genus and (ii) antigenic in children exposed to malaria. First, the identification of immunogenic salivary proteins of An. gambiae by an immunoproteomic approach was assessed. The second step was to design peptide sequences, from the selected An. gambiae gSG6 antigen using a bioinformatic approach, taking into consideration (i) their potential antigenic properties and (ii) the absence of cross-reactivity with other arthropods/organisms. The specific IgG Ab levels were then evaluated in Senegalese children in different context of malaria. From five gSG6 peptides, one gSG6-P1 peptide presented all criteria to be an optimal candidate biomarker for evaluating exposure to An. gambiae bites. Indeed, in addition to high specificity to Anopheles genus, the anti-gSG6-P1 IgG level was associated with the intensity of exposure to An. gambiae bites. In addition, complementary studies indicated that gSG6-P1 could be a specific biomarker for low exposure to An. gambiae and also to Anopheles funestus bites. This new “salivary” biomarker could be used as a geographic indicator for mapping the risk of malaria transmission and especially in low Anopheles density conditions, where entomological methods are limited in sensitivity (dry season, altitude or urban malaria). It could also represent a direct criterion of efficacy in the evaluation of anti-vector strategies.

Email address for correspondence: franck.remoue@ird.fr

416 Consequences of pregnancy-associated malaria on fetal growth and development of preeclampsia and gestational hypertension [MIM16690749]

Christentze Schmiegelow, John Lusingu, Martha Lemnge, Birgitte Bruun Nielsen, Vibeke Rasch, Mayke Oesterholt, Daniel Minja, Charles Tunuka, Nadine Fievey, Achille Massougbdji, Philippe Deloron, Thor Theander

Pregnancy-associated malaria (PAM) has detrimental effects on mother and fetus. PAM causes low birth weight and recent studies have shown a relation between PAM and development of gestational hypertension and preeclampsia which can itself affect the fetus. The interrelation of PAM, preeclampsia and gestational hypertension and the effect on the fetal growth is still not thoroughly investigated. We are conducting a longitudinal prospective study of 1000 pregnant mothers in Korogwe, North-eastern Tanzania. Using ultrasound investigation the gestational age is estimated before pregnancy week 24. The fetal growth is assessed on three consecutive ultrasound investigations, enabling us to diagnose Small for Gestational Age (SGA) fetuses and intrauterine growth retardation (IUGR). In parallel, screening for malaria, gestational hypertension and preeclampsia using clinical scoring and parametrical tests is performed during pregnancy and at delivery. Using the data collected on fetuses/newborns of mothers not diagnosed with PAM, gestational hypertension and preeclampsia we will describe a normal Tanzanian cohort. The prevalence of SGA and IUGR among fetuses carried by mothers suffering from one or more of the mentioned diseases is investigated and compared with this normal cohort. The synergistic effect of the three diseases and their interrelation will furthermore be described. Inclusion and follow-up is ongoing. The results will be based on women having completed their follow-up before the end of September 2009. We will compare our findings with recent studies on IUGR in malaria endemic areas as well as a parallel study in Benin, where malaria transmission is more intense.

Email address for correspondence: christentzes@yahoo.dk
417 The impact of host genes coding for candidate liver stage ligands on the susceptibility to P. falciparum malaria [MIM16670604]

A. Schulz, S. Borrmann, A. Macharia, S. Uyoga, K. Marsh, K. Matuschewski, T.N. Williams

Plasmodium parasites escape immune responses by alternating between different developmental stages within the human host and between the human and mosquito hosts. Genetic variability within the human host influences the susceptibility to malaria. Known genetic factors (e.g., hemoglobin S) can only explain a fraction of disease outcomes and are thought to be mainly mediated by interactions at the erythrocytic stage of the parasite. Factors with a potential impact on the development of pre-erythrocytic stages have so far not been explored. In this study we investigated single nucleotide polymorphisms (SNPs) in candidate genes coding for potential host-cell ligands implicated in the development of liver stage parasites. We identified SNPs by re-sequencing and by screening of genomic databases. We established a mini-sequencing assay for high-throughput genotyping of individuals in an established cohort of children living in a malaria endemic area at the Kenyan coast. We determined the relationship between genotypes/haplotypes and susceptibility to P. falciparum malaria using multiple failure-time survival analysis. Additionally, we used computational algorithms to explore haplotype structures in different populations (HapMap) to identify genetic signatures of recent selection. We found an association between candidate locus L3104 and the risk of P. falciparum malaria episodes and time to infection. Furthermore, no epistatic effect between sickle cell and L3104 was observed. Our results suggest an effect of L3104 on pre-erythrocytic parasite stages presumably influencing parasite development in the liver. This study highlights the epidemiological relevance of host–parasite interactions at the hepatocyte–liver stage interface.

Email address for correspondence: andris.schulz@med.uni-heidelberg.de

418 Association of malaria and salmonellosis in hospitalised Ghanaian children in an area highly endemic for both diseases [MIM16697551]


Individuals in areas endemic for falciparum malaria and salmonellosis are at substantial risk of contracting both diseases. It is under discussion whether one disease has an impact on the risk or the course of the other disease, either concurrently or an acute infection superimposing on a chronic one. The objective of this study is to analyse if there is a mutual influence of both diseases in African children. Children under 15 years of age who were admitted to the paediatric ward of a rural hospital in the Ashanti Region, Ghana, between September 07 and November 08 were included into a study on infectious diseases. Blood cultures were done using the Bactec® system with subtyping of Salmonella enterica group B, D, E and G and S. typhi. Malaria parasites were detected through thick smears. Of 402 children recruited until November 2008, 31% had falciparum parasitaemia and fever. Blood cultures were true positive in 77 patients (19%). Contaminations were found in 10%. The majority of these samples (71%) were positive for S. enterica spp. (17% serovar S. typhi). The occurrence of malaria and salmonellosis was spatially and seasonally correlated. Malaria and salmonellosis are dependent on spatio-temporal factors and are associated with each other. Control measures against one of the diseases may influence the epidemiology of the other.

Email address for correspondence: schwarznorbert@web.de

419 Treatment seeking patterns for fever and convulsion in rural Tanzania [MIM15817911]


Convulsions are manifestations of severe malaria among under five children leading to serious complications and death. Previous studies have provided contradictory evidence with regard to treatment of convulsions (traditional healers versus modern treatment). To clarify this, a study was carried out within the frame of the ACCESS Programme, an intervention study aiming at understanding and improving access to prompt and effective malaria treatment in rural Tanzania. The study used a locally constructed cultural-epidemiological approach. A baseline study was carried out in 2004/2005 and repeated in 2006/2008 Results from 2004/2005 showed that about 71.1% of all convulsion cases were timely brought to health facility, compared to 45.6% for mild fever cases. The patterns of distress associated with less timely health facility use and receipt of antimalarial among convulsion children were generalized symptoms, rather than the typical symptoms of convulsions. Caretakers who administered antimalarials to children without attending a health facility believed either the facilities were out of stock or there is no proper diagnosis there. Analysis of the 2006/2008 is in progress. Our findings contradict previous studies which have shown that children with convulsion are less likely to use health facilities. This may result from a weakening of traditional ideas. We expect more children with convulsion to timely attend health facility in the repeated studies as the result of on going interventions. However, the next aim is to improve the quality of care at health facilities by making drugs and diagnosis available and free for communities to access.

Email address for correspondence: adillip@ihi.or.tz

420 Relationship between persistence of subpatent asexual Plasmodium falciparum infections and subsequent recrudescence after antimalarial treatment [MIM16671023]


Malaria control relies mainly on efficacious treatment of uncomplicated disease episodes. The rate of inadequately treated primary infections is determined by the in vivo test. This test aims to detect persistent sub-microscopic blood stage infections by capturing subsequent recrudescences up to 8 weeks after treatment. In high transmission areas PCR-based molecular techniques are required to distinguish recrudescence from re-infections. These techniques are error-prone leading to unreliable treatment outcome estimates. We hypothesized that persistent, subpatent asexual blood stage infections can be detected on day 7 and that this could be used to predict subsequent patency. We analyzed venous blood samples on day 7 from 34 children who were treated with a supervised 3-day course of amodiaquine for uncomplicated P. falciparum malaria. The pre-determined detection threshold was ≥10 parasites/mL. In 16/51 (31%) samples we detected metabolically active persistent asexual parasites 7 days after start of treatment. A similar
421
Determining the association between lifetime migration and malaria transmission intensity in highland areas, southwest Uganda [MIM16696398]


Introduction: Travel is repeatedly implicated as a key factor in the recent increase in malaria transmission in highland areas. However, until now, research has been limited to estimating the effect of recent travel on parasite prevalence. This study uses newly developed serological tools to estimate the association between an individuals’ life time travel and their exposure to malaria. We conducted an altitudinally stratified cross-sectional survey in Kabale and Rukungiri highland districts of southwest Uganda (altitude range 1350–2400 m). Parasite prevalence was measured using Paracheck-Pf and seroprevalence of MSP1-19 was determined using indirect ELISA. Subjects lifetime migration was estimated using an adapted Life History Calendar methodology which focussed on main reasons for travel outside of subjects areas of residence. Force of infection (λ) and Entomological Inoculation Rates were estimated for ‘well-travelled’ and ‘settled’ subjects and results indicate that the force of infection of malaria is higher in well travelled subjects compared to those who have not travelled much throughout their life. However, the importance of age and household geographical location in relation to other risk factors are also significant. Understanding the association of human travel on malaria transmission in relation to other risk factors in highland areas can help national control programs prioritise malaria prevention strategies. This is especially important in light of renewed efforts to eliminate malaria.

Email address for correspondence: caroline.lynch@lshtm.ac.uk

422
Sporozoite rate and malaria mortality: Seasonality, spatial and temporal patterns within the Rufiji DSS, Tanzania [MIM16677555]

S.F. Rumisha, P. Vounatsou, S. Abdulla, T. Smith

The effect of transmission reduction on malaria morbidity and mortality is uncertain as the precise nature of the relationship between malaria mortality and levels of transmission is unclear. The Malaria Transmission Intensity and Mortality Burden across Africa (MTIMBA) project was initiated to collect entomological data within a number of DSS in sub-Saharan Africa to study the malaria-mortality relation. Bayesian geo-statistical logistic regression models were fitted on sporozoite rates (SR) in the Rufiji DSS to predict transmission at household locations with child mortality data. The models were adjusted for seasonality, environment, climatic factors, and temporal correlations. Predicted SR for Anopheles gambiae and funestus species were then used as covariates in a survival model on all-cause child mortality adjusted for age, socio-economic status and interventions. An. gambiae remains the predominant vector species accounting 60% (n = 1098) of all infections while An. funestus comprising the remaining 40%. SR was 4.3% and 2.4% for gambiae and funestus, respectively. SR was higher in wet seasons than in dry seasons. For gambiae, the highest SR was observed in the month June with no clear pattern presented for funestus. Less spatial variation was observed within the DSS area, however, temporal variations were significant with months after rain season showing higher SR. Preliminary analysis indicates lack of relationship between mortality and SR. Results show variation of transmission by month with weak relation with mortality outcomes. Despite stable pattern of transmission, significant reduction on the mortality was observed. This could imply success of interventions implemented.

Email address for correspondence: susan.rumisha@uniba.ch

423
ENDRA and NOS2A are malaria candidate genes in the Príncipe population [MIM16698441]

Maria de Jesus Trovoada, Lígia A. Gonçalves, Paulo Almeida, Roni Moya, Rita Neres, Rute Vieira, João Costa, Isabel Marques, Artur Borja, António Coutinho, Carlos Penha-Gonçalves.

We performed a whole population-based study to identify genetic factors that are associated to resistance/susceptibility to malaria in Príncipe Island. Príncipe is a 115 km² island with about 6000 inhabitants and belongs to the São Tomé and Príncipe archipelago located in the Gulf of Guinea. Here, we report on SNPs and haplotypes of malaria candidate genes that are associated to the carrier status. A cross-sectional study in May 2005 involved in 1390 volunteer donors. Parasite DNA was detected by nested PCR in peripheral blood and individuals with a positive PCR for Plasmodium were defined as carriers. 146 SNPs in 39 candidate genes were selected among high frequency polymorphisms that were identified in the HapMap African panel. The genotyping was carried out in the MassARRAY platform. Genotype quality control excluded SNPs with inconsistencies in HapMap control samples and SNPs failing Hardy–Weinberg equilibrium test. Allele frequencies were estimated by gene counting and genetic association was tested using allelic χ² tests. Parasitemia prevalence was 26.2% among asymptomatic individuals. Allelic association in tag SNPs by case–control analysis revealed that one SNP of the Endothelin receptor A (EDNRA) gene was significantly associated to a decrease risk in carrier status. Conversely, minor alleles in SNPs of the Nitric oxide Synthase (NOS2) gene we were found to confer, susceptibility to carrier status. These results suggest that NOS2A and EDNRA are candidate genes in establishing Plasmodium unapparent infection in the Príncipe population. This study highlights the relevance of host genetic susceptibility in asymptomatic malaria infection.

Email address for correspondence: mjesus@isc.gulbenkian.pt

424
Evaluation of two definitions of placental malaria as predictors of low birth weight in HIV—Infected and uninfected pregnant women in Tororo, Uganda [MIM16689028]

Patrick M. Newman, Humphrey Wanzira, Jane Achan, Moses R. Kamya, Diane Havlir, Philip J. Rosenthal, Sarah Waldman, Grant Dorsey, Deborah Cohan, Tamara D. Clark

For the prevention of placental malaria it is recommended that HIV-infected women receive daily trimethoprim-sulfamethoxazole (TS)
while HIV-uninfected women receive intermittent preventive therapy with sulfadoxine–pyrimethamine (IPT–SP). However, there are limited data on the efficacy of TS prophylaxis in HIV infected women and the associations between different definitions of placental malaria and low birth weight (LBW). Cross-sectional study of HIV-infected women taking TS prophylaxis and HIV-uninfected women taking IPT–SP (1:3 ratio) delivering at Tororo District Hospital. We measured the associations between placental malaria, defined as microscopic infection (positive placental blood smear) vs. submicroscopic infection (negative placental blood smear, positive PCR of placental blood), and the risk of LBW (<2500g). We enrolled 517 women. The prevalence of placental malaria defined as microscopic infection vs. submicroscopic infection was 6% and 16%, respectively among HIV-infected women and 9% and 19%, respectively among HIV-uninfected women. HIV status was not associated with either definition of placental malaria after controlling for gravidity. Microscopic infection was associated with LBW among HIV-infected (RR 3.04, 95% CI 2.31–10.97) and HIV-uninfected (RR 2.35, 95% CI 1.11–4.98) women. Submicroscopic infection did not predict LBW in HIV-infected (RR 1.30, 95% CI 0.40–4.16) women, but did predict LBW in HIV-uninfected (RR 1.98, 95% CI 1.05–3.74) women. We found similar prevalence of placental malaria among HIV-infected and HIV-uninfected women in the setting of daily TS prophylaxis in HIV-infected women. Microscopic infection was associated with LBW for all women, but submicroscopic infection was associated with LBW only among HIV-uninfected women.

Email address for correspondence: patrick.newman@ucsf.edu

425 Reduced malaria incidence found in a longitudinal cohort of infants living in a holoendemic region of Western Kenya between May 2006–June 2008 [MIM16668257]

E. Piriou, A. Asito, N.C. Fiore, J. Opondo, A.M. Moormann, P. Sumba Odada, R. Rochford

In a longitudinal study of infants from Chulaimbo District in Nyanza Province, Western Kenya, the frequency of malaria positive blood smears (BS) was much lower than anticipated based on historical data. The aim of this study was to determine if other methods to detect malaria infections (e.g. Plasmodium falciparum DNA and serology) confirmed the lower than expected incidence rate of malaria. 108 children born in May/June 2006 were enrolled, and followed until June 2008. Clinical data, blood smear and plasma were available from 15 to 20 time points for 69 children, in addition to data and BS from 725 acute illness visits. P. falciparum-specific IgG was measured by multiplexed serology for AMA1, MSP1kd42, LSA1 and CSP1; P. falciparum DNA by real-time PC After 24 months 69/108 children remained in study, with 1 death attributable to P. falciparum. While 71.0% of children had one or more malaria infections based on symptoms, in only 17/139 cases (12.2%) was the BS positive. Only 13/69 children (18.8%) had one or more positive BS, which was always confirmed by serology. In contrast, 94.3% of children had been exposed to P. falciparum 1–4 times based on serology, which was often but not always paralleled by detection of P. falciparum DNA. These data emphasize the need of longitudinal studies, making use of serological and molecular methods in addition to BS, in order to understand the changes in malaria exposure in relation to an increased frequency of both home-based and prescribed antimalarial treatment.

Email address for correspondence: pirioue@gmail.com

426 A multiplexed real-time PCR assay for malaria speciation with improved sensitivity for mixed infections [MIM15061188]

Sandra E. Shokoples, Momar Ndao, Kinga Kowalewska-Grzechowska, Stephanie K. Yanow

The implementation of real-time PCR for the diagnosis of malaria has been hampered by technical limitations affecting the speciation of Plasmodium and/or mixed infections. We have optimized a method which enhances the sensitivity for detecting minor species in mixed infections within a single, multiplex reaction. Patient samples were from regional laboratories in Alberta and the malaria repository at the National Reference Center for Parasitology (Canada). After DNA extraction, real-time PCR was performed under universal conditions using the ABI Taqman 7500. Genus specific detection of malaria was followed by a multiplex reaction using species-specific forward primers and probes and a conserved reverse primer. To quantify the DNA in the samples, a standard curve was derived using a plasmid containing the P. vivax 18S gene. With a blind panel of clinical samples, we successfully speciated 19/22 mixed infections, and demonstrated a specificity and sensitivity for single infections of 100% compared with nested PCR as the gold standard. This test has been implemented for routine species confirmation at the Provincial Laboratory for Public Health in Alberta, Canada. In comparison with speciation by front-line microscopy, the real-time PCR test demonstrated greater sensitivity for the speciation of low level infections, mixed infections, and discrimination of “non-falciparum” species. An initial assessment of parasite load by real-time PCR suggests a positive correlation between gene copy number and parasitemia. Our experience supports a role for real-time PCR in the speciation of malaria parasites in conjunction with front-line microscopy.

Email address for correspondence: sshokoples@gmail.com

427 Marked decline in hospital paediatric blood transfusions after introduction of artesinin combination therapy in Macha, Zambia [MIM16691532]

P. Thuma, S. Mharakurwa, J. van Dijk

Subsequent to the introduction of artemisinin combination therapy (ACT) in 2003 in Zambia, there has been a marked decrease in malaria case load in the Macha community, as evidenced by data from Macha Hospital. We examined hospital data to see if the decrease in malaria cases was associated with changes in the number of blood transfusions given to children at this district-level hospital. Data was gleaned from the hospital lab’s blood transfusion record ledger, and verified with review of pediatric inpatient records and patient files after discharge, for the ten-year period 1999–2009. Analysis of the data shows that over 50% of all blood transfusions given were to children under the age of six years. The number of paediatric blood transfusions for severe anaemia (defined as Hgb < 5 mg/dl), declined from an average of 457 transfusions per year in the five-year period after ACT introduction. The data demonstrates that blood transfusion services in rural Africa, where there has been a history of endemic malaria, can most likely be dramatically decreased by bringing malaria under control. Taking this into account, we believe that malaria control measures, including ACT, are an effective means to decrease the need for expensive and labor intensive blood transfusions services.

Email address for correspondence: phil.thuma@miam.org.zm
428
Detection of latent antifolate resistance mutants in sub-microscopic Plasmodium falciparum infections of Southern Zambia [MIM16672067]

Sungano Mharakurwa, Mwiche Siame, Jodi Chondoka, David Sullivan, Philip Thuma

The emergence of Plasmodium falciparum drug resistance remains a threat to successful malaria intervention, necessitating epidemiological surveillance. The present study examines antifolate resistance mutants in sub-microscopic asymptomatic P. falciparum infections of Southern Zambia. From a 2000 km² location, willing individuals of all ages were screened for malaria by microscopy. P. falciparum DNA samples were collected by finger-prick dry blood spots on filter paper. Chelex DNA extraction was performed on a random sub-set of 91 samples. Extracts were subjected to nested PCR using regular published P. falciparum DHFR primers or sensitive short-amplicon primers, and an alternative tertiary nested PCR strategy utilizing regular published primers. P. falciparum DHFR mutations were detected by allele-specific restriction enzyme digestion. Of the representative 91 sample sub-set, only 1 (1.1%) was thick-film positive, while 4 (4.4%) were positive by nested PCR. In contrast, up to 79 (86.8%) were positive by short amplicon primers and tertiary nested PCR. All infections positive by the thick-film or standard nested PCR bored either the DHFR Asn-108 (92%) or Ser-108 (8%) residues, as expected from drug selection due to S/P use over the years. However, short-amplicon and tertiary amplified sub-microscopic infections exhibited both pyrimethamine resistance and cycloguani resistance-conferring variants, such as Thr-108 (16.5%), so far considered rare or absent in Africa. This study documents reservoirs of latent antifolate resistance mutants among asymptomatic P. falciparum infections in an area of apparently decreasing malaria prevalence. The data may impact surveillance and containment strategies for P. falciparum malaria resurgence and drug resistance.

Email address for correspondence: sungano.mharakurwa@miam.org.zm

429
Prospects and challenges for malaria elimination in Southern Africa [MIM16669722]


The Southern African Development Community (SADC) has committed to eliminating malaria from six low transmission countries including Botswana; Namibia; South Africa and Swaziland, by 2015. In this review, we consider the prospects for successful elimination in these countries through existing evidence. A robust literature review was conducted on malaria elimination and malaria control for relevance to socio-economic and epidemiological conditions in the selected countries and compared against current global guidelines on malaria elimination. Reported malaria incidence ranges from 0.016 to 17.0 per 1000 population at risk in the selected countries, with Namibia reporting the highest case estimate. All countries except South Africa currently report incidence based on clinical rather than confirmed diagnosis. All countries except Swaziland currently implement ACTs for malaria treatment. IRS population coverage in 2007 ranges from 57% to 89% and ITN coverage ranges from 10% to 28%. Surveillance systems are being developed, but do not yet meet the standards necessary for an elimination program. Frequent migration from other malaria endemic countries poses a huge challenge to achievement and maintenance of elimination. Available data suggest that all targeted countries except Namibia have low malaria transmission. Substantial improvement in diagnosis and active community based surveillance of new malaria infections will be required for all countries to achieve and maintain elimination. Due to close proximity to highly endemic areas, elimination will also require sustained reductions of malaria transmission in neighboring countries through effective sustained cross-border programs.

Email address for correspondence: dmoonasar@clintonfoundation.org

430
Detection of asymptomatic Plasmodium falciparum infection reservoirs by tertiary nested PCR during scaled up malaria intervention in Southern Zambia [MIM16672246]

Mwiche Siame, Jodi Chondoka, Sandra Chishimba, Aniset Kamanga, Phil Thuma, Sungano Mharakurwa

As endemic countries scale up effective malaria intervention, the monitoring of asymptomatic infection reservoirs becomes increasingly paramount, to avoid potential resurgences. Using a tertiary amplification strategy, the present study reports the detection of widespread sub-microscopic asymptomatic Plasmodium falciparum infections during scaled up malaria intervention. From a 2000 km² area around Macha willing 1500 residents of all ages were screened for malaria by microscopy, with simultaneous collection of dried finger-prick blood spots on Whatman 3MM filter paper. Chelex extracts from the dried blood spots were subjected to regular nested PCR using primers targeting P. falciparum 18S small subunit ribosomal and DHFR genes. PfDHFR was amplified using regular nested PCR with published primers and a new tertiary PCR strategy using three sets of regular published primers. While the malarial parasite rate was 1.1% by microscopy, nested PCR showed 46.2% parasite rate with 18S ribosomal primers, 54.9% with regular published primers and 84.6% with short amplicon primers. Tertiary nested PCR enhanced the detectable parasite rate to 70.3% parasite rate (p = 0.04, n = 91) compared with two rounds of amplification. Using short amplicon primers or tertiary nested amplification with regular primers, this study documents the existence of ultra-low asymptomatic P. falciparum parasitaemia below detection limit of microscopy and standard nested PCR in an area of apparently reduced malaria prevalence. These methods can be used to significantly enhance detection of latent reservoirs of infection to minimize risk of resurgences.

Email address for correspondence: mwiche.siame@miam.org.zm

431
Comparison of dried blood sample-based RT-PCR against microscopy for the detection of Plasmodium falciparum gametocytaemia in Southern Zambia [MIM16696661]

Tamaki Kobayashi, Jodi Chondoka, Sandra Chishimba, Mwiche Siame, Jay Sikalima, Phil Thuma, Sungano Mharakurwa, Godfree Mlambo, William Moss

Detection of Plasmodium falciparum gametocytaemia is important for identifying foci of malaria transmission, especially as endemic countries intensify interventions against the disease. However, the current mainstay method, based on microscopy, has limited sensitivity and capacity for large numbers. The present study compares a dry blood spot-based RT-PCR assay against microscopy for gametocyte detection. A cross-sectional malaria survey was carried in randomly selected households in 2008. Finger prick blood samples
were collected from each member of a household on glass slides and Whatman 903 filter paper. The prevalence of \textit{P. falciparum} gametocytes was determined by light microscopy and RT-PCR analysis. From a total of 121 individuals screened by microscopy and RT-PCR, 0% were positive for \textit{P. falciparum} by microscopy, of which 0% were found carrying gametocytes. By contrast, RT-PCR detected gametocytaemia in 4% of the individuals. RT-PCR on dry blood spots provides a sensitive means of detecting gamocyte carriage in an area of declining malarial prevalence.

Email address for correspondence: jodi.chondoka@miam.org.zm

**432**

The prevalence of malaria in Mefloquine Hydrochloride—Mefliam® users during the deployment of military forces in Burundi, East Africa [MIM13238901]

Capt E. Basson (Epidemiologist)

Malaria represents one of the most important infectious disease threats to deployed military forces. Malaria in soldiers has a serious economic impact in terms of both lost productivity and treatment cost for the state. No information is available regarding the prevalence of malaria among military personnel during deployments in Burundi. Mefliam® has been used for the treatment of malaria infections amongst military forces deployed in Burundi and East Africa. The chemoprophylactic efficacy and usability of Mefloquine Hydrochloride—Mefliam® has never been determined in Burundi. Multidrug resistance necessitates the use of alternative drugs that may be expensive and difficult to administrate, and often have side-effects. The aim of the study was to investigate the prevalence of malaria in the users of Mefliam® when administrated to soldiers stationed in East Africa and specifically Burundi. If Mefloquine is the drug of choice and prove to be effective during military deployments to East Africa and Burundi, the presumption can be made that the Plasmodium organism in that areas are not resistant to the Mefloquine. Mefloquine will thus be an effective malaria management tool as a therapeutic and preventative drug. The target population was SANDF soldiers deployed in Burundi for more than three months. The best chance to successfully combat the disease will require collaboration between those who control and the researchers. With comparable attention to tailoring Mefloquine use, as that paid to appropriate uniform fit or weapon allocation, most service personnel will be well protected with Mefloquine during military operations in malarious areas.

Email address for correspondence: eldrian@telkomsa.net

**433**

Civic educating rural pregnant mothers and children under five to sleep under ITNs a tool to reducing malaria in Malawi [MIM16563927]

Kamoza Chimungu

It is estimated that 600,000 women get pregnant every year in Malawi. Primary health care plays a vital role. Malawi, one of Africa’s poorest nations, launched a nationwide anti-malaria campaign in 2008 by distributing one million free nets to children aged under five and pregnant mothers. Including the new nets, about 6.3 million dollars (4000 euros) worth of nets have been handed out for free in Malawi since 2003. Accessibility, availability and affordability of essential ITNs to women and children fewer than five, has proved not to be effective due to lack of knowledge on the end user. Global Hope Mobilization is providing education and counselling to pregnant women in rural setting to access and sleep under ITNs in Lilongwe district rural setting in the context of the project we are implementing. The project designed civic and education materials, including leaflets; local meetings with women, counselling sessions, home visitation one by one, questionnaires to assess knowledge on the use of ITNs were also administered. It is estimated that 1000 representing 80% of the community women population the project was targeting pregnant were sensitized on correctly use of ITNs and Malaria cases were dropped by 85% in the area. To date 92% of women and children under five are sleeping under ITNs using information given to them. The proportion of pregnant mothers and children under five living in rural areas is high. The main risk factor for lack of not sleeping and correct use of ITNs is lack of knowledge. Acceptance of civic education on the correct use of ITNs is good in this rural population setting.

Email address for correspondence: kchimungu@globemw.net

**434**

Empirical modelling and mapping of seasonality of malaria transmission by \textit{Anopheles gambiae} sensu lato in Africa [MIM16756469]

Musawenkosi Mabaso

Seasonality in climate drives the dynamic relationships between the entomological inoculation rate, parasite prevalence and disease outcome and this leads to strong seasonal forcing of malaria transmission. Seasonality is thus critically important for understanding disease risk and planning the timing of interventions. The only available maps of malaria seasonality in Africa are based on a simple dichotomous classification of months using climate variables and have ignored the available entomological field data. We use an approximation of the discrete Fourier transform for the \textit{Anopheles gambiae} sensu lato entomologic inoculation rate (EIR) and environmental covariates to fit a model for seasonality to the data from 54 locations across sub-Saharan Africa where these data have been collected for an entire year. The magnitude and timing of the annual and biannual seasonal cycles of the logarithm of the EIR was modelled as a function of the amplitude and phase of meteorological covariates by relating the Fourier coefficients using multiple linear regression. We apply this empirical model to generate maps of the degree and timing of malaria seasonality and of the duration of transmission. The degree of seasonality is predicted to be highest in northern and southern parts of the continent with a peak in August/September and April/May, respectively. The duration of the transmission season is longest in the central equatorial rainforest zone and in parts the savannah region. The seasonality maps produced are of value for determining the most effective moment and geographical position for control efforts to be applied throughout the continent. This analysis presents the first step towards the development of improved models of malaria seasonality, and can be refined as more malaria and high resolution environmental data becomes available.

Email address for correspondence: mmabaso@mrc.ac.za

**435**

Cerebral malaria genetic risk factors in angolan children [MIM16691882]

Maria Sambo

The challenge of distinguishing the genetic variants that are associated to malaria severity from those that specifically favor cerebral malaria syndrome persists. We aimed to identify specific genetic risk factors to cerebral malaria. A genetic association study was conducted in Angolan children comparing 130 patients with cerebral malaria with three control groups: 158 patients having severe malaria in the absence of cerebral malaria, 142 patients with
uncomplicated malaria and 319 uninfected controls. Transmission disequilibrium tests were performed using mothers. Two distinct haplotypes in the TGFB2 gene revealed opposite effects on the specific risk of cerebral malaria (corrected $P$-value = 0.03 and 0.001) as compared to other forms of severe malaria. One haplotype in the HMOX1 gene increased the risk of cerebral malaria (corrected $P$-value = 0.002) as compared to uncomplicated malaria. Analysis by transmission disequilibrium test excluded stratification as a confounder evidencing the undertransmission of the TGFB2 haplotype CACCACA (a CM protective factor) to cerebral malaria affected children ($P$ = 0.045). Our results underline the value of using a study design centered in a particular malaria clinical manifestation and comparing it with a variety of other malaria clinical status. This enabled us to identify novel CM genes, as is the case of the TGFB2 that would not be revealed in typical case-control studies. Also, this approach allowed to discern that genes as HMOX1 and CD36 although associated with CM, may be more generally implicated in disease severity and resistance to infection and are not specific CM syndrome susceptibility factors.

Email address for correspondence: rosariosambo@hotmail.com

436 Proximate determinants of usage of insecticide treated nets (ITN) among pregnant women and children under five in Namibia [MIM16729122]
Liezal Wolmarans, Elize Biermann, Uchenna Nwokenna

Malaria is the leading cause of illness and death among children under five and the third leading cause among adults. Approximately 65% of Namibia’s populations live in malaria endemic areas, with an average of 400,000 outpatient, 30,000 inpatients and 1000 malaria related deaths reported annually. Susceptibility/risk perception for pregnant women and children under five were significant determinants of behavior between ITN users and non-users during 2006. In 2005 and 2009, two rounds of malaria surveys were conducted in households with pregnant women and children under five, involving 480 and 720 households, respectively. Data was collected on demographic characteristics, exposure to program activities and selected determinants of net usage. Levels of usage were determined using descriptive statistics and comparisons were made using logistic regression to determine the association between usage of bed nets and risk perception. Results of DHS 2006 showed that bed nets use was 9% and 11% among pregnant women and under-5 children, respectively. Data was collected on demographic characteristics, exposure to program activities and selected determinants of net usage. Levels of usage were determined using descriptive statistics and comparisons were made using logistic regression to determine the association between usage of bed nets and risk perception. Results of DHS 2006 showed that bed nets use was 9% and 11% among pregnant women and under-5 children, respectively. Anticipated findings include an increase in ITN ownership and usage and decrease in the average number of people sleeping under a net. Also, malaria prevention and transmission knowledge is expected to improve significantly. The second round of data will be analysed to track whether susceptibility differences still exist between ITN users and non-users and will be also evaluated over time. This program is expected to demonstrate the efficacy of a family values based campaign in promoting net ownership.

Email address for correspondence: liezel.wolmarans@sma.org.na

437 Changes in antioxidant status in malaria patients treated with artemisinin combination therapy [MIM16475365]
O.G. Ademowo, C.M. Nneji, C.O. Falade

Reactive oxygen species (ROS) are mediators of tissue injury and are believed to be involved in pathophysiology of malaria. We studied the effect of malaria on the antioxidant defense system in patients treated with artemisinin combination therapy. Twenty-seven patients with falciparum malaria were recruited into the study. They were administered standard doses of dihydroartemisinin–sulphadoxine/pyrimethamine (DHA-SP) or artesunate–amodiaquine–chlorpheniramime (AQC) combinations and followed up on days 7 and 28. 2 ml of blood was withdrawn from each patient for the determination of PCV and levels of malondialdehyde MDA (an indicator of lipid peroxidation) and glutathione (GSH). Activities of glutathione-S-transferase, superoxide dismutase (SOD) and catalase (CAT) were also measured. Thick and thin blood films were made for malaria parasite screening. Parasitaemia cleared in all patients by day 7 except in two patients on DHA-SP group in whom parasite reappeared by day 28. There was a progressive reduction in MDA and SOD while GSH, GST and CAT significantly increased by day 28. There was no significant difference in changes of MDA, GSH, GST, SOD and CAT between baseline and days 7 or day 28 in the DHA-SP group. However, in the AQC group, the increase in PCV, GSH, GST and CAT and reduction in MDA level and SOD activity by day 28 were significant. Malaria infection induced oxidative stress in the host. Treatment with DHA-SP and AQC cleared parasitaemia, alleviated the oxidative stress and caused considerable alteration in the antioxidant defense system in the host.

Email address for correspondence: ademowo_g@yahoo.com

438 Mapping of malaria cases using Geographical Information systems (GIS) in The Gambia [MIM14940885]
Ebako Takem, Kebba Touray, Judith Satoguina, David Conway, Michael Walther

Over the last two years, we conducted a case–control study comparing cases of severe and uncomplicated malaria in an urban area of the Gambia, where malaria transmission is low. A recently performed malaria prevalence survey carried out in three rural sites in the Gambia indicates that malaria prevalence varies considerably among villages in the same sites. This prompted us to investigate the origin of the cases enrolled into the case–control study. We seek to map the residential distribution of the patients recruited into our case–control study, to explore if malaria cases do cluster in certain regions within the recruitment area; analyse the distribution according to (i) severity, (ii) year, and (iii) time of the season the patient was recruited. Study will include children with malaria recruited into our case–control study both in the 2007/2008 and the 2008/2009 malaria seasons. A GPS device will be used to collect the geographical location of the patient’s residence with the study ID as the waypoint number. Detailed maps by settlement will be used to document where cases occurred. Spatial analysis and space–time analyses will be performed. Results will be presented for a total of 307 cases; 124 enrolled during the 2007/2008 malaria season, and 183 during the 2008/2009 malaria season. This study will reveal whether in an area of low transmission malaria cases do fall into clusters which could then be targeted for focused interventions.

Email address for correspondence: etakem@mrc.gm

439 Estimation of the human to mosquito transmission (HMT) and transmission reducing immunity (TRI) by Direct Membrane Feeding Assay (DMFA), in a hypoendemic zone in Senegal [MIM16606014]
A. Gaye, M.O. Ndiath, L. Gadiaga, L. Konaté, C. Boudin, C. Sokhna, J.F. Molez, J.F. Trape

Malaria transmission is usually measured using entomological data, i.e. the mosquito to human transmission. The objective of this study is to measure the human to mosquito transmission.
Estimation of the Human to Mosquito Transmission (HMT) and Transmission Reducing Immunity (TRI) by Direct Membrane Feeding Assay (DMFA), in a hypoendemic area in Senegal [MIM16646851]

A. Gaye, M.O. Ndiath, L. Gadiaga, L. Konaté, C. Boudin, C. Sokhna, J.F. Molez, J.F. Trape

Malaria transmission is usually measured using entomological data, i.e. the mosquito to human transmission. The objective of this study is to measure the human to mosquito transmission (HMT) of malaria and the human transmission reducing immunity (TRI) in order to evaluate the global transmission of malaria in an hypoendemic area of Senegal. In order to study the HMT and TRI, we artificially infected mosquitoes and performed Direct Membrane Feeding Assay on gametocytes carriers from Thiès area with or without the replacement of autologue serum by non-immune serum in rural area. These infections were experimentally carried out or directly on the skin. The results of the HMT showed that 9% of the individuals infected mosquitoes although 13% were gametocytes carriers. The proportion of Plasmodium falciparum positive mosquitoes was 20%. 43% of tested plasma samples were able to inhibit significantly the development of P. falciparum in mosquitoes. Among the inhibiting subjects, the intensity of the TRI was 57%. Compared to similar studies in Cameroun where malaria transmission was much higher, there were no important differences both in the proportion of infected mosquitoes with a similar assay, the proportion of samples able to inhibit the development of P. falciparum and the intensity of TRI.

Email address for correspondence: layegaye@gmail.com

Epidemiology of malaria in the forest-savannah transitional zone of Ghana [MIM16697880]

Kwaku Poku Asante, Daniel Chandramohan, Martin Adjuk, George Adjei, Elizabeth Awini, Mohammed Adams, Sam Newton, David Dosoo, Dominique Dery, Akua Agyeman-Budu, John Gyapong, Brian Greenwood Seth Owusu-Agyei

Information on the epidemiology of malaria is essential for designing and interpreting results of clinical trials of drugs, vaccines and other interventions. As a background to the establishment of a site for antimalarial drugs and vaccine trials, we have investigated the epidemiology of malaria in a rural site in Ghana. Active surveillance of clinical malaria was carried among 335 children <5 years. The prevalence of malaria among subjects of all ages (n = 1484) was determined in two monthly surveys over a 12-month period. Participants were sampled from clusters drawn around sixteen index houses randomly selected from a total of about 22,000 houses within the study area. Estimation of entomological inoculation rate (EIR) was carried out. The average parasite prevalence in the all age cohort was 58% (95% CI 56.9, 59.4). More than 50% of all children less than 10 years of age were anaemic. Children less than 5 years of age had as many as seven malaria attacks per child per year. The attack rates decreased significantly with increasing cut-offs of parasite density. The average Multiplicity of Infection (MOI) was 6.1. All three pyrimethamine resistance mutant alleles of the Pfmdfr gene were prevalent in this population and 25% of infections had a fourth mutant of pfmdf-p-A437G. EIR was 269 infective bites/year. The transmission of malaria in the forest-savanna region of Ghana is high and perennial. Clinical trials of antimalarial drugs and vaccines can be appropriately carried out in this area.

Email address for correspondence: kwakupoku.asante@ghana-khrc.org

Level of trace elements and total antioxidants in gravid Balb/c mice infected with Plasmodium yoelii malaria parasites at different gestational periods [MIM16706533]

Sabur Badmos

No Abstract

Email address for correspondence: solargoodmus@yahoo.com
444 Using GIS technologies in the relationship between Malaria morbidity and environment, a study case in the suburb of Dakar Senegal [MIM16689502]

Birane Cisse, Jean Louis Ndiaye, Vincent Turmine, Adrien Coly, Yéré-makhan Keita, Aminata Niang Diene, Alioune Kane, Roger Tine, Babacar Faye, Oumar Gaye

The suburb of Dakar, which houses the Niayes region and the down town, constitutes a favorable environment for the development of vectors disease including malaria. The objective of this study was to determine environmental and social factors that promote the development of malaria in cities of Guediawaye and Pikine. We collected health data in the various health facilities from 2000 to 2008. Climate data that allowed us to make the relationship between malaria and rainfall were recorded at the National Meteorology Direction. In addition, a KPC (Knowledge Practices and Coverage) household survey determined knowledge of populations in relation to the degradation of their environment. Remote sensing and GIS technologies enabled us to identify areas at risk of malaria and to correlate morbidity and environment. Study area is located in several geomorphologic depressions. The rural exodus due to the drought of 1970 has facilitated the occupation of natural waterways and low areas. In 2000, the return of rainfall led to flooding and the establishment of breeding in this locality. This was followed by an increase in malaria morbidity from 2001 to 2005 from 26 to 32%. This increase in malaria morbidity was noted more in irregular areas without sewerage system. This study showed the diversity of factors influencing the malaria morbidity. The control of this scourge requires an integrated pest management involving all development sectors, particularly the communities. The study puts into perspective a determination of areas where efforts should be concentrated in the fight against malaria.

Email address for correspondence: diattag@ird.sn

445 Congenital and neonatal malaria in a tertiary reference hospital in Mali [MIM16689967]


Malaria contribution to mortality or morbidity in neonates in Mali is not known. We conducted a cross-sectional study in infants aged 0–28 days that were admitted for inpatient care to the Unit of Reanimation and Neonatology of a tertiary reference hospital in Mali. 300 mother–infant pairs were recruited. After informed parental consent was obtained venous blood was collected for malaria diagnosis by OptiMAL® IT, microscopy and PCR. 300 infants and 146 mothers were included between October 2006 and February 2008. The mean age of infants was 3.4 days but 44.4% of infants were included on their first day of life. The mean weight was 2922 g but 23.5% of the infant were low birth weight infants. In all infants both PCR and microscopy for malaria were negative. However, three infants were positive for Plasmodium falciparum malaria by the OptiMAL® IT test. The mean age of mothers was 25.2 years. No malaria prophylaxis was used by 5.3% of them during the pregnancy. Of the remaining women that used chemoprophylaxis, 58.7% used chloroquine while 36% used IPTp with sulfadoxine–pyrimethamine, the national policy for preventing malaria during pregnancy. All mothers were parasite negative by microscopy, the OptiMAL® IT was positive for Plasmodium falciparum in six cases while PCR was positive in 11 women. These data suggest that malaria is not a significant contributor to neonatal morbidity and mortality in this setting.

Email address for correspondence: tonydara@mrtchko.org

446 Placental malaria among pregnant women and low birthweight among newborn babies in six communities in Anambra State, southeast Nigeria [MIM14905165]

D. Aribodor, O. Nwaorgu, C. Eneanya

Placental malaria is known to have adverse outcomes including maternal death, low birthweight babies and neonatal death. Low birthweight defined as birthweight <2500 g has been reported to be a leading cause of poor infant survival and development in Africa. The cost implications of placental malaria in the family and the society are enormous thus the need to halt malaria during pregnancy. Thick blood films were prepared using blood from placenta of 500 women delivered of newborns after delivery. This was stained with Giemsa stain and examined for malaria parasites. Every newborn was weighed immediately after delivery and the birthweight matched with the malaria status of the mother. Of the 500 placentas of pregnant women examined for malaria parasites, 64.4% were positive for Plasmodium spp. There was no significant difference in the prevalence of placental malaria among the communities which included urban (64%) and rural areas (64.9%). Also of the 322 newborns whose mothers were positive for placental malaria, 45% were of low birthweight with a mean of 2140 g. The outcome of this study may not be justified by the holoendemicity of malaria within the study area. Such factors as quality of antenatal care services, nutritional status of pregnant mothers, and drug resistance may be responsible. The findings of the study are an indication that the burden of malaria in pregnancy is yet to be reduced. Practical, efficient and effective malaria-transmission preventive strategies are recommended for pregnant women.

Email address for correspondence: dnaribodor@yahoo.co.uk

447 Malaria and tick-borne relapsing fever in Senegal: Two diseases usually confused [MIM16653588]

G. Diatta, A. Tall, E.H. Ba, H. Bouganali, N. Diagne, C. Sokhna, J.F. Trape

In Senegal, like anywhere in West Africa, malaria is the leading cause of mortality and morbidity by a vectorial disease. Tick-borne relapsing fever (TBRF) due to Borrelia crocidurae is a bacterial disease widespread in the Sahel and Sudan savannah of West Africa. Daily medical and epidemiological monitoring of the population of Dielmo (Senegal) over 18 years (1990–2008) with collection of blood smears for all cases of fever. Of 59,545 thick blood smears examined, 34,476 presented malaria parasites and 1078 Borrelia spirochetes including 362 mixed infections. The average density of Borrelia was much lower than the density of malaria parasites. Plasmodium falciparum and Borrelia crocidurae infections were the two most frequent causes of fever both in children and in adults. The average incidence of TBRF was 12 per 100 person-years (range from 4 from 1990 to 25 in 2000). Contrary to malaria, the incidence of relapsing fever did not decrease with age and fever episodes lasting more than one day in adults were more likely to be caused by relapsing fever than by malaria. Symptoms of the two infections were very similar. TBRF is a very common cause of fever in all age groups with signs and symptoms similar to those of malaria. In many rural areas of West Africa, most long lasting fever cases attributed to malaria may be due to TBRF.

Email address for correspondence: diattag@ird.sn
448 Aspects épidémiologiques, parasitologiques et cliniques du paludisme à Louga (Sénégal) [MIM16760984]

Au Sénégal, le paludisme est la première cause de morbidité notamment dans la région de Louga où le taux de morbidité du paludisme était de 38.4% en 2005. Cette étude a pour but de déterminer les aspects épidémiologiques, parasitologiques et cliniques du paludisme simple vus du laboratoire de l'hôpital régional de Louga. Cette étude prospective s'est déroulée de septembre 2007 à juillet 2008 et a concerné les patients suspects d'accès palustres simples venus au laboratoire pour confirmation parasitologique. Une goutte épaissie et un frottis sanguins ont été réalisés et la parasitémie a été déterminée à partir de la goutte épaissie. Parmi les 2138 patients 20,4% hébergeaient Plasmodium falciparum. L’indice plasmodique était significativement plus élevé en octobre (30,1%) en début de saison sèche (26,8%), chez les enfants de 6 à 14 ans (30,4%). L’indice parasitaire était significativement plus élevé chez les patients qui n’utilisaient pas de moustiquaires imprégnées (21,3%) que chez ceux qui les utilisèrent (6%). Dans 53,7% des cas, les parasitémies se situait entre 500 et 4999/µL. Les principaux signes cliniques motivant la demande en goutte épaissie étaient la fièvre observée chez 80,1% des patients, les céphalées (46%) et les vomissements (40,9%). Ces signes étaient associés à la présence d’hématozoaires respectivement dans 15,9%, 32,5%, 6,4% des cas. Le paludisme est endémique à Louga avec une recrudescence saisonnière. L’utilisation des moustiquaires imprégnées efficaces pour prévenir le paludisme doit être renforcée au sein de la population.

Email address for correspondence: thdieng@refer.sn

449 Malaria infection among blood donors in Onitsha urban, southeast Nigeria [MIM14974783]
C.A. Ekwunife, O.C. Nwaorgu, C.I. Eneanya

Blood safety is an issue of major concern in transfusion medicine in developing countries where national blood transfusion services, policies guiding transfusion and financial resources are lacking. Transfusion transmitted malaria is a potential health hazard but is often neglected in many malaria endemic areas. Malaria infection among blood donors in Onitsha urban, South East Nigeria was studied between August and October 2008 Venous blood from 410 blood donors were collected in EDTA bottles and screened for malaria parasites using Giemsa-stained thick and thin blood films. Also ABO blood grouping and rhesus factor tests were performed and demographic data of donors documented Of the 410 films. Also ABO blood grouping and rhesus factor tests were performed and demographic data of donors documented Of the 410 blood donors were collected in EDTA bottles and screened for malaria parasites using Giemsa-stained thick and thin blood films. Density was estimated using 8000 white blood cells/µL of blood. Pyrethrum sprays and human bait collections of anopheline species were carried out weekly between 1800 and 0600 h. Eighty-three individuals (15.0%) were parasitaemic. There was no sex difference in infection rate but infection rate varied slightly with age (P<0.05). P. falciparum (13.8%) and P. malariae (1.3%) trphozoites were encountered. The difference was significant (P<0.05). An. funestus was the most abundant (36.7%) species followed by An. gambiae s.s (34.7%) and An. arabiensis (28.6%). This was not significant (P>0.05). Sporozote rate differed significantly, An. gambiae s.s having the highest rate (2.1%), followed by An. arabiensis (1.4%) and An. funestus (0.3%) (P<0.05). An. gambiae s.s also had P. malariae sporozoites. Results show urban malaria as on going in Enugu. Anophelines may be adapting to more polluted larval habitats and more exophilic. Control efforts targeted towards larval control, impregnated curtains more than bed nets may prove more effective in the control of urban malaria.

Email address for correspondence: ceneanya@yahoo.com

450 The epidemiology of urban malaria in a cosmopolitan city, southeast Nigeria [MIM14971336]
C.I. Eneanya, C.A. Ekwunife, F. Aneke

The implications of rapid urban growth in increased poverty, environmental degradation and increased rate of infectious diseases has been well documented. Urban malaria is emerging as a potential crisis in Nigeria, where urbanization is still a recent phenomenon. A study to define urban malaria was therefore made in an urban city, southeast Nigeria. A total of 552 persons were screened for malaria parasites between May and July 2007. The study site Uwani represented the high density area. Giemsa-stained thick and thin blood films were screened for malaria parasites. Density was estimated using 8000 white blood cells/µL of blood. Pyrethrum sprays and human bait collections of anopheline species were carried out weekly between 1800 and 0600 h. Eighty-three individuals (15.0%) were parasitaemic. There was no sex difference in infection rate but infection rate varied slightly with age (P<0.05). P. falciparum (13.8%) and P. malariae (1.3%) trphozoites were encountered. The difference was significant (P<0.05). An. funestus was the most abundant (36.7%) species followed by An. gambiae s.s (34.7%) and An. arabiensis (28.6%). This was not significant (P>0.05). Sporozote rate differed significantly, An. gambiae s.s having the highest rate (2.1%), followed by An. arabiensis (1.4%) and An. funestus (0.3%) (P<0.05). An. gambiae s.s also had P. malariae sporozoites. Results show urban malaria as on going in Enugu. Anophelines may be adapting to more polluted larval habitats and more exophilic. Control efforts targeted towards larval control, impregnated curtains more than bed nets may prove more effective in the control of urban malaria.

Email address for correspondence: ceneanya@yahoo.com

451 Plasmodium falciparum malaria and cotrimoxazole resistant Streptococcus pneumoniae infections among children under 5 years of age in Lagos, SouthWestern Nigeria due to Drug Misuse [MIM16757931]

Objectives: To examine of Plasmodium falciparum malaria and Streptococcus pneumoniae co-infection among children under 5 years of age. To determine the antibiocorial of susceptibility pattern of the Streptococcus pneumoniae isolates. To assess the Knowledge, Attitude and Practice of mothers/care-givers with positive children under 5 years of age for Plasmodium falciparum and Streptococcus pneumoniae infections. Approvals were obtained from the Ethics Committee of Lagos state Hospital Management Board and Institutional Review Board of Nigerain Institute of Medical Research, Yaba, Lagos. Informed written consent was voluntarily submitted by each mother/care-giver of each patient after being enrolled for the study. A total of 202 febrile children with difficult breathing and 202 mothers/care-givers were studied between April 2005 to June 2006. Only 5 ml of blood specimen was aseptically collected from each febrile with difficult breathing child by the attending clinician for blood culture, thick and thin Giemsa stained films. Data from the Laboratory tests and questionnaire were analyzed using EPISSTAT and EPI INFO statistical packages. More than 52.0% of the patients had positive culture for Streptococcus pneumoniae and 84% of the isolates were resistant to cotrimoxazol. It also showed that 70.6% of the care-givers misused cotrimoxazole. There is urgent need to control cotrimoxazole resistant by adequate supply of drugs for
free treatments for both acute respiratory and malaria infections in children under 5 years of age and training of mothers/care-givers to fill the gap in their knowledge of correct use of cotrimoxazole.

Email address for correspondence: veramails1@yahoo.com

452 Plasmodium falciparum genotypes during successive clinical episodes of malaria in Ghanian children [MIM16697102]


In endemic areas, persons infected with Plasmodium falciparum may harbour multiple strains. These multiple parasite sub-populations, which have a strong geographical correlate, may change in an asymptomatic individual as clinical disease develops. The polymorphic regions of the merozoite surface proteins 1 and 2 were used as genetic markers to analyse P. falciparum genotypes in successive malaria episodes in Ghanian children who participated in a longitudinal clinical trial of two artemisinin combination therapy regimens. Out of 247 children treated for uncomplicated malaria and followed up 1 year, 17 had recurrent episodes of P. falciparum malaria. The K1 and FC27 strains were most dominant, constituting 64% of all new infections. Multiple genotypes were detected in all children, and in 8(47%) subjects, the same genotypic patterns were observed in two consecutive clinical malaria episodes. In seven others (41%), genotype patterns were different from one episode to another. In this hyperendemic area, children were exposed to many different P. falciparum strains and in a large proportion of cases, the same parasite strains were observed in two discrete clinical attacks. This may indicate recrudescence after a long enough follow-up period, or circulation of genetically similar strains. The K1 and FC27 strains were the dominant genotypes present in new infections in the study area. The high multiplicity confirms the intense malaria transmission in this area, suggesting malaria control efforts in the area should be based on multiple approaches.

Email address for correspondence: shaggy1129@yahoo.co.uk

453 Heritability of Plasmodium falciparum malaria in two villages of rural Senegal (West Africa) in the ACT era [MIM16698024]

Cheikh Loucoubar, Julie Coutheret, Joseph Faye, Ekoue Kouev-idjin, Adama Tall, Cheikh Sokhna, Alioune Badara Ly, Hubert Bassene, Jean-François Trape, Anavaj Sakuntabhai, Aliou Diop, Odile Mercereau-Puijalon, Richard Paul, Laurence Baril

Since the introduction of the artemisinin-based combination therapy (ACT) there has been a rapid reduction in the burden of malaria. We used data from a community-based longitudinal survey performed in two rural villages of Senegal with holoendemic (V1) and mesoendemic (V2) transmission to assess the impact of human factors on parasite-based treatment strategies after the implementation of ACT. We calculated the incidence of Plasmodium falciparum malaria attacks in 2001 and 2007, and the adjusted odds-ratio (OR) of ACT from 2006 to 2008 according to several human factors (age, symptoms, parasite density, etc.). We estimated the human genetic contribution (heritability) to malaria attacks using pedigree-based variance components models. There was a decline of malaria attacks from 2001 to 2007: 103 and 143% increase of individuals without malaria infection and 68 and 80% decrease of malaria attacks in V1 and V2, respectively. Children under 7 years still represent the “at-risk” group for malaria attacks: OR = 9.09 (95% CI=[5.27–15.10]) and OR = 2.84 (95% CI=[1.66–4.92]) for the 0–7 age group compared to subjects ≥15 in V1 and V2, respectively. 34.4% of the unexplained variance of parasite-based ACT initiation was due to heritability in holoendemic V1, but none in mesoendemic V2 where environmental factors are probably the major contributors. Despite the radical decline in the incidence of malaria in the ACT era, human genetics still play a major role in the outcome of infection in high transmission intensity areas prior to the development of immunity. The causative genes remain to be identified.

Email address for correspondence: cloucobar@pasteur.sn

454 Placental malaria and low birth weight among neonates born at a tertiary centre in Awka, Anambra State, Nigeria [MIM14986031]

C.I. Eneanya, E.C. Mbanefo, J.M. Umeh, M.O. Obiukwu, M.O. Otiji

Placental malaria is a leading cause of low birth weight, a major factor in low infant survival and suboptimal growth. A pre-intervention survey of placental malaria parasitaemia and low birth weight among neonates was carried out in Awka, Anambra State, Nigeria. Giemsa stained smears of placental blood were examined using the WHO accepted standard procedure. Data on birth weights were promptly collected from birth attendants. The generated data was subjected to statistical analysis using Chi-square. The findings show that placental malaria parasitaemia and intensity are dependent on pregnancy status and parity of pregnancy (p < 0.05). It demonstrated that a baby is more likely to be born of low birth weight (<2500 g) if the mother’s placenta was infected at the time of delivery, especially during the first pregnancy (Primiparae: 79.49% and Multiparae: 47.37%). The mean birth weight recorded in the study for both parity groups show that placental malaria infection greatly lowers the birth weight of babies, thus indirectly affects their survival, optimal growth and development (Primiparae: 2826 g vs. 2231 g and Multiparae: 2848 g vs. 2553 g). The observation could be attributed to the immunosuppressive physiological changes associated with pregnancy, intensified by the development of the new immunologically naive utero-placental vasculature during the first pregnancy. The baseline data presented in this study show that placental malaria especially with Plasmodium falciparum has a more significant role in infant survival in Africa than previously assumed. The value of this study as a public health tool for planning, delivery and monitoring of intervention is enormous.

Email address for correspondence: evari4u@yahoo.com

455 Malaria morbidity in children living in Lagoon area on the Coast of Benin, West Africa: Determining factors and implications for malaria control [MIM16692495]

Alain Nahum, Annette Erhart, Ambroise Mayé, Daniel Ahounou, Chantal van Overmeir, Joris Menten, Harry van Loen, Martin Akogbeto, Marc Coosemans, Achille Massougbdji, Umberto D’Alessandro

The peri-urban lagoon sector of southern Benin is densely populated and subject to periodic flood, even in the dry season because of the overflowing of Lake Nokoué. Between July 2003 and December 2004, malaria episodes were identified by active case detection on a cohort of 553 children aged 6–59 months. Genotyping was used to distinguish between recrudescence and new infection. In addition, three cross-sectional surveys were carried out for determining the seasonal variation of malarialometric indices and related risk factors. 503 clinical episodes (438 due to new infections) of Plasmodium falciparum malaria were identified among 271
children of the cohort. The overall incidence rate was estimated at 1003/1000 person-years. The highest risk occurred either during the short rainy season or the first part of the long dry season. Poor housing conditions were identified as an important risk factor. At survey, the prevalence of *P. falciparum* infection was between 40 and 60%. The highest risk for malaria infection occurred after the annual flooding period. Malaria morbidity was strongly associated with the calendar months during which important environmental changes occurred following the flooding from Lake Nokoué. In this situation, environmental management may be able to reduce the risk of malaria. This should be implemented in parallel to vector control interventions and the provision of adequate treatment with artemisinin-based combination therapies.

Email address for correspondence: nahum_alain@yahoo.fr

---

456
Mosquito control strategies and prevalence of malaria in children (0–15 years) in Amucha Community, Abia State, Nigeria [MIM16399314]

F.O. Nduka, N.P. Gbajie

Malaria still remains a leading parasitic disease of global concern. It is ever present in the tropics with sub-Saharan Africa accounting for 90% of the disease. Various control measures have been introduced and employed by different individuals and households in the control of malaria infections. The effectiveness of some measures in the reduction of Malaria prevalence in children aged 0–15 years in Amucha rural community of Abia State was measured in this study. A total of 200 children (0–15 years) were randomly chosen from different households in Amucha Community, situated in the Suburb of Aba (a major town) in Abia State, Nigeria. Blood samples using venous puncture was collected from the selected children and screened for malaria parasites using thick and thin blood film method. Information on sex, age, type of mosquito control strategy used was collected from each child or parent by oral questioning. The result showed that 160 (80%) of the 200 children sampled were infected with *Plasmodium falciparum*. Age was statistically significant with children 6–10 years old having the highest infection of 89.2%. Sex was not significant though females had 81.6% of the infection against 78.4% in males. Children who used window net screening had 82.1% infection, those who used insecticide spray had 75% while those who slept under treated bed nets had 0% infection. The difference between the prevalence of different control measures was statistically significant. Amucha community is hyperendemic may be because of adequate breeding sites for the mosquitoes such as stagnant pools due to very poor drainage system, high population density, suitable temperature and nearby bushes. Treated bed nets proved effective but still poorly used as only 24 respondents slept under treated nets. The failure of window nets is instructive as the doors may have been kept open to allow in more air and of course mosquitoes. Mosquito resistance may have accounted for the high infection observed among insecticide spraying households. Efforts should be made to enlighten the communities and the distribution of the ITNs made more effective.

Email address for correspondence: floxai@yahoo.com

---

457
Identifying immunologic markers that protect against malaria infection in an endemic area in Mali [MIM16690051]

Niangaly Hamidou, Sangaré Cheick Papa Oumar, Diallo Nouhoum, Dembele Demba, Maiga Oumou, Doumbo Ogobara, Djimde Abdoulaye

The epidemiology of malaria in the low lands of the Dogon Country of Mali is poorly documented. We conducted four cross-sectional parasitological and clinical studies in children between 1 and 16 years of age and two cross-sectional entomological studies on anopheline population and malaria transmission from October 2005 to July 2007. Pongnon, a Malian village located in the plain of the Dogon Country was our study site. Between October 2006 and December 2007, we also conducted a longitudinal in vivo study of *Plasmodium falciparum* sensitivity to chloroquine in patients 6 months and older. Chloroquine was administered per os at the dose of 10 mg/kg during the two first days and 5 mg/kg on the third day. The plasmodic, gametocytic and spleen indices were 52.1, 7.4 and 11.2%, respectively. Two species were found (*Anopheles gambiae* s.l. and *Anopheles funestus*) but only *An. gambiae* s.l. was infected with 8.6% IAS and an entomology inoculation rate of one infected bite per person per month in October. After molecular correction, ACPR were 72.6 and 67.8%, respectively, in 2006 and 2007. The prevalence of *pfcrt* 76T was 32.4 and 32% in 2006 and 2007, respectively. Genotype resistance indices were 1.6 and 1.7, respectively, in 2006 and 2007. Our results suggest that malaria in the plain may be different from malaria on the Dogon escarpment. We confirm the validity of the current GRI model and propose a novel GRI that is more accurate and better in line with current WHO norms.

Email address for correspondence: hniangaly@mrctbko.org

---

458
Prevalence of malaria among children 1–10 years old in Awka North Local government Area, Anambra State, SE Nigeria [MIM15081185]

O.C. Nwaorgu, B. Orajiaka

Malaria is a major cause of illness and death especially among children under 5 years old and pregnant women. It is estimated that more than one million children living in Africa especially in remote areas with poor access to health services die annually from direct and indirect effects of malaria (Fawale and Onadeko, 2001). Fatally affected children often die within less than 72 h after developing the symptoms. In Nigeria, malaria consistently ranks among the five most common causes of death in children. As a result of increased mortality and morbidity there is need for proper understanding of the epidemiology of the disease among the most at risk groups. In the study of 1000 children, 1–10 years old were randomly selected from 20 primary and 31 nursery schools in the four randomly selected communities in Awka North LGA. Two milliliters venous blood was collected from each of the 1000 pupil (600 primary and 400 nursery) and stored in an anticoagulant specimen bottle. Thick and thin films were prepared, stained and examined for malaria parasite under the microscope using the oil immersion objective. Also both 12 h human bait collection and pyrethrum knocked down methods were used for identification of types of mosquitoes found in the study communities. Malaria infection is most prevalent among 1–4 years old, highest being among 3 years old (76.4%), followed by 1 and 4 years old with 71.3 and 71.2%, respectively, and 62.04% for 2 years old. This decreased as the children get older. There was no significant difference in prevalence among the male and female pupils, with 59.2 and 57.2%, respectively. The most prominent sex in the community is *Plasmodium falciparum* (51.8%). Forty-three percent of the pupil positive for malaria had low parasitic diversity below 1000, 12.4% between 1000 and 10,000, 2.3% between 10,000 and 100,000 and 0.2% above 100,000. Malaria is a problem among pupil 1–10 years old especially from age 2 years when their immunity from mothers start reducing. There is need to ensure that mothers protect
Malaria is a major cause of morbidity and mortality in young children in sub-Saharan Africa. Transmission in Nigeria occurs all year round with a major peak during the rainy season. Malaria is frequently overdiagnosed and results in failure to treat other life-threatening conditions. Anaemia is a very common presentation of malaria in children. Blood samples were examined for malaria parasite and packed cell volume (PCV) using standard methods and procedures in febrile children age 0–12 years attending, St. Kizito primary health centre, Lekki Lagos, Nigeria between July 2006 and March 2008. The PCV was used as an index of anaemia. Of the 1210 children screened, 20.7% (251/1210) of them were positive for malaria parasites. Of the positive children 11% of them were <1 year and 25.8% were >1 year. Children >5 years has highest parasite density compared to children <5 years. 74.4% of children ≤1 year and 58.4% of children ≤5 years had fever (temperature >37.5) at enrolment, out of which 11.9 and 16.5% were positive for malaria parasites, respectively. Children >1 year and >5 years in the parasitaemia range between 10,001 and 250,000 p/μl. Plasmodium falciparum (96.5%) was more frequent in the study. There was an association between malaria parasite density and PCV in parasitaemia range between 1–500 and 10,001–250,000 p/μl. Severe (PCV <15) to mild anaemia (PCV ≤30) were seen in parasite density groups; 501–1000, 1001–10,000, and 10,001–250,000 p/μl. There should be investment on accurate malaria diagnosis to reduce overdiagnosis, resulting in unnecessary treatment due to fever in children, malaria-related anaemia in children should be diagnosed and treated promptly.

Email address for correspondence: oladosu_dipo@yahoo.com

Malaria remains one of the world’s greatest childhood killers and is a substantial obstacle to social and economic development in the tropics. Plasmodium falciparum infection is the major cause of morbidity and mortality especially among the vulnerable groups to which children, especially aged less than 5 years belong. Studies were carried out to determine the prevalence of malaria parasite infection among infants and children (0–12 years) in Ota, South Western Nigeria between April and December 2008. The two hospitals used were Ota General Hospital and Covenant University Health Centre, Canaanland, Ota. Thick and thin films were made and stained using standard parasitological procedures. Structured questionnaires were distributed to ascertain the age, sex, drugs/insecticides used and state of health of the subjects before recruiting them into the study. Overall 215 (80.5%) of the 267 children investigated were found to have malaria infection. Age group 0–5 years had the highest frequency rate of 84.7% with mean parasite density of 900 and the difference between the age groups were statistically significant (p < 0.05). Children from suburb villages had the highest mean parasite density of 850 with 78.1% prevalence rate. Twenty percent were given local herbs and 22% used orthodox medicine as prophylaxis. Only 18% used insecticide treated mosquito nets while 24% of the parents spray insecticides against mosquito. There is therefore need for more awareness on effective use of drugs and insecticide treated bednets in malaria hyperendemic regions.

Email address for correspondence: golasehinde@yahoo.com

Pregnant women and children are at high risk of developing malaria because of a decrease of the immune response during pregnancy and the lack of immunity against malaria in infancy. Other factors have an influence on the occurrence of malaria: vector transmission and sensitization of the fetus to malaria antigens caused by the presence of parasites in the placenta. Our objective is to determine the relationship between placental malaria at delivery and the risk of developing parasitemia during the first year of life. A prospective cohort study was set up in Tori-Bossito from June 2007 to December 2008 to follow new born children (either or not born from mothers having a placental malaria) from their birth till the age of 12 months. Anthropometric, socio-demographic, obstetrical history and placental malaria status data were collected at inclusion. A thick blood smear was done monthly to check for parasitemia. We built a time-dependent variable by allocating to each child, the number of Anopheles gambiae captured monthly at four points in each village. A Cox model was used to characterize the time to first malarial parasitemia. Six hundred and twelve mother–infant pairs were included. The prevalence of placental malaria was 10.91%. Primigravid women presented more infected placenta than multigravid. The prevalence of premature babies was higher when the placenta was infected. A first analysis suggested that the influence of placental malaria on the date of first parasitemia might not be confirmed in this study, but final results will be presented at the congress.

Email address for correspondence: smaila11@yahoo.fr

The incidence of clinical malaria is one of the key endpoints measures for malaria vaccine candidates’ trials. These measures might be done either by active or by passive case detection (ACD and PCD) methods. This study aims to identify the most suitable method to be use in a site being prepared for future malaria vaccine candidates’ trials. Two cohort studies were conducted within the same study area of Saponé. The first cohort consisted (ACD cohort) of a group of 554 children aged 0–5 years followed up by biweekly home visit. The second cohort (PCD) included 927 children, whose parents were encouraged to report to the nearest community clinic should their child feel sick. Treatment was provided free of charge in both cohorts. At each visit, a malaria smear was obtained if fever. Study duration was 1 year. A malaria episode was defined
as positive *Plasmodium falciparum*-parasites density in presence of fever. In the PCD cohort, 3479 clinic visits were recorded with 1076 malaria episodes diagnosed. The incidence of clinical malaria was 1.17 episodes/child-year at risk (CI 95% [0.48–1.86]). In the ACD cohort, total 56716 home visits were performed. The children were seen during 49,062 visits. A total of 381 malaria episodes were diagnosed. The overall incidence of clinical malaria was 0.78 episode/child-year at risk (95% CI [0.70–0.86]). These finding suggest that in our setting with a treatment provided free of charge PCD will be the efficient method to assess malaria morbidity in under five children.

Email address for correspondence: aouedraogo.cnrfp@fasonet.bf

463

Epidemiology of malaria and insecticide resistance burden in Nigeria [MIM14982573]


The use of insecticide treated mosquito nets (ITNs) or indoor residual spraying (IRS) is an important component of this strategy. However, the reports on emergence of pyrethroid resistance mosquitoes in Africa is a cause for concern in lieu of the Nigerian government call for practical implementation of ITNs and IRS programmes throughout the country. Larvae of *Anopheles* from the breeding sites in five ecological zones in Nigeria were reared to adulthood in a standard insectary. Susceptibility tests were conducted on nonbloodfed, 2–3-day-old emerged adult female mosquitoes using standard WHO procedures, diagnostic kits and test papers (WHO, 1998). PCR assays were used for the identification of the species and characterization of the kdr allele. The mosquito samples were susceptible to the diagnostic doses of insecticides tested. Pyrethroid resistance mosquitoes were recorded in the forest-mosaic and Guinea savanna zones. The kdr frequency and mortality rates were lower in permethrin, indicating a level of resistance to this insecticide. Overall, kdr frequency was low in all the zones ranging between 37 and 53%. This study forms a baseline data for pyrethroid resistance status of the local anopheline mosquitoes in Nigeria and the need for continuous monitoring in order to guarantee the success of ITNs and IRS as malaria control measures.

Email address for correspondence: oyewoleoi@gmail.com

464

Effect of low protein diet and pregnancy on malaria in *Plasmodium berghei* infected mice [MIM16762570]


Malaria and malnutrition are major causes of morbidity and mortality. The relationship between malaria and malnutrition is not well understood. We hereby investigate the effect of low protein diet and pregnancy on malaria in mice. Fifteen BALB/c mice were divided into three groups. Mice in group 1 were fed on normal diet, group 2 were fed on low protein diet for 1 week and group 3 were pregnant mice fed on low protein diet for 1 week before the start of the experiment. The diet was continued till the end of the experiment. All the animals were then infected with 0.2 ml inoculum size *Plasmodium berghei*. Parasitaemia was monitored through blood smears which were made at intervals. Packed cell volume (PCV) and nitric oxide (NO) concentration were also monitored for 21 days post-inoculation. Low protein diet delayed the onset of parasitaemia in both pregnant and non-pregnant mice. Parasite count was significantly (p < 0.05) higher in normal mice relative to low protein fed mice. There was a progressive drop in PCV as parasitaemia increases. This was not significantly different between normal and malnourished mice. The mean survival time was higher in low protein than in mice fed on normal diet. NO concentration is markedly higher in normal than low protein fed mice. Malnourished mice had some degree of resistance to parasite growth due to the poor nutrient content. The high NO in normal mice may be due to the relatively high parasitaemia.

Email address for correspondence: wummiy@yahoo.com

465

Trends in malaria and all-causes mortality in Niakhar DSS, Senegal, 1962–2008 [MIM16600203]


In African rural settings, information on death rates and causes of deaths is largely lacking. However, valuable information can be obtained from the monitoring of small populations. The Niakhar Demographic Surveillance System, initiated in 1962, is the oldest DSS in Africa. The study population comprises 34 000 inhabitants in 2008. After an initial census in 1962, births, deaths and migrations were documented at least yearly. Since 1984, postmortem interviews were conducted for each death, using a standardized questionnaire. During the period 1962–2008, the probability of dying before the age of 5 years declined from 485 to 68 deaths per thousand live birth. However, this decrease has not been constant. After a progressive decline from 1962 to 1989, a plateau at around 180 was observed from 1990 to 1997. Mortality peaked again up to 263 during the period 1998–1999 before returning to 180 in 2000, then rapidly decreasing since 2004. Analysis of causes of deaths indicates that a doubling of malaria mortality in the 1990s, and an epidemic of meningitis in 1998–1999 were responsible for the plateau and the peak of mortality, respectively. The recent decline in malaria and all-cause mortality appears related to the introduction of malaria combination therapy and seasonal preventive treatment. Annual malaria mortality in children 0–4 years was 5.2 per thousand in 2007 vs. a maximum of 16.2 in 1995. New antimalarial drug policies and improvement of immunization and other health programmes have dramatically reduced malaria and all-causes mortality in Niakhar.

Email address for correspondence: sauvage@ird.sn
bite preventive measures, poverty, low maternal education, low-ranking paternal occupation, rural domicile and low social class were each associated with severe malaria (p < 0.05). Multivariate analyses show that only parasite density and poverty were the significant predictors of severe malaria. Progress in stemming the burden of malaria depends on accurate knowledge and understanding of the epidemiology and control of the disease in the affected populations. The non-use of mosquito bite preventive measures might be as a result of ignorance and poverty. These factors should be considered in the design of sustainable and effective locally relevant strategies for the prevention of malaria.

Email address for correspondence: senbanjo001@yahoo.com

467 Malaria prevalence in Tsunami-affected districts of Aceh, Indonesia [MIM16263150]
David Muiruki, Sigrid Hahn, Abiola Fasina, Richard Allan

Malaria is endemic to Indonesia. There is little prevalence data available from Aceh Province, however, due to the long-standing separatist conflict in the province and because surveillance systems were compromised by decentralization of the public health system. The MENTOR Initiative, which specializes in malaria control in humanitarian emergencies, was one of the non-governmental organizations to respond to the 2004 Indian Ocean Tsunami in Aceh. Data on malaria prevalence were gathered to guide and evaluate programmatic efforts. The MENTOR Initiative conducted community-based malaria prevalence surveys in 2005 and 2006 in five districts along the Tsunami-affected western coastline. Individuals in randomly selected households were consented for testing using blood smears. Positive cases were treated according to guidelines. 11,763 individuals in 3,771 households were tested. The overall slide positivity rate for Plasmodium spp. was 2.2%. Slide positivity rates ranged from 0 to 55% among villages. Overall, 57% of the 262 cases were infected with Plasmodium falciparum, while 43% were infected with Plasmodium vivax. A majority of affected patients were male, and over the age of 10 years. Local prevalence data is needed to design effective community-based malaria control programs, as endemicity varies greatly within districts. Certain villages were found to be hyperendemic, with slide positivity rates far higher than average in Indonesia, and similar to sub-Saharan Africa. There is a need for ongoing malaria surveillance in Aceh Province to monitor prevention and treatment efforts.

Email address for correspondence: sigridahahn@gmail.com

468 Assessing the relationship between mortality and malaria transmission: An overview of the MTIMBA project [MIM16699081]

MTIMBA (Malaria Transmission Intensity and Mortality Burden across Africa) is a multi-centre study of malaria-endemic sites in sub-Saharan Africa. The study aimed at estimating entomological inoculation rate (EIR), all-cause and malaria specific mortality plus malaria contextual factors that might influence the relationship between malaria transmission and mortality. MTIMBA entomological and mortality data were collected repeatedly from large number of household locations (up to 20,000) over time. Such data are correlated in terms of space and time. Bayesian geostatistical models are the most appropriate for such kind of data, however; due to many locations model fit is not practical. MTIMBA study team has developed Bayesian geostatistical methodology to solve large data problems. The methodology is currently being applied to assess the relation between mortality and malaria transmission in six DSS sites. The distribution in space and time of malaria transmission measures will be presented for the MTIMBA sites and their relation to mortality will be reported based on results available up to the point of presentation. The development of statistical methodological work enables us to explore the MTIMBA data fully. Results will contribute to further understanding of the relationships between mortality rates and malaria endemicity in Africa. Estimates of the relation between age specific mortality and malaria endemicity will make it possible to predict the consequences of malaria transmission reduction of control initiatives. Findings will be of greater value in planning malaria intervention especially in endemic area.

Email address for correspondence: simon.kasasa@unibas.ch

469 Genetic identification of Plasmodium falciparum parasite virulence markers according to local populations [MIM16259531]
Carole Eboumbou, Amadou Niangaly, Ghyslain Mombo-Ngoma, Eric Achidi, Albert Same Ekofo, Peter Kremsner, Saadou Issifou, Amed Ouattara, Ogobara Doumbo, Christophe Rogier

The mechanisms of the heterogeneous courses of severe malaria (SM) are not clearly understood but are thought to involve a complex combination of human host factors under the influence of his genetic background and parasite-specific factors. Their incidence vary greatly among people and epidemiological conditions. Our aim was to identify Plasmodium falciparum genetic factors associated with pathogenicity in several local populations. We are performing combined epidemiological, clinical and genetic analysis of more than 60 falciparum isolates from uncomplicated malaria (UM) and SM cases from three countries (Mali, Cameroon and Gabon). Parasite loci associated with pathogenicity will be identified using a genome wide gene mapping approach. The P. falciparum genotypes associated with severity and clinical presentation will be presented. Linkage disequilibrium blocks and susceptibility haplotypes should characterize parasite genetic factors that are involved in the pathogenesis of SM. These findings could benefit clinical decision-making process in allowing early identification of mild clinical cases that are more susceptible to become severe. They could also generate new hypothesis about the pathogenesis of SM and suggest new therapeutic approaches.

Email address for correspondence: elsecarole@yahoo.fr

470 Classification of the hypervariable regions of the rif and stever variant gene families sampled from Plasmodium falciparum [MIM16645983]
Vandana Thathy, Caroline Buckee, Peter C. Bull, Chris Newbold, Kevin Marsh

The Plasmodium falciparum genome contains diverse multigene families (var, rif and stever) encoding hypervariable antigens that are exported to the surface of the infected host erythrocyte and represent targets of naturally acquired immunity to malaria. With the exception of var-encoded P. falciparum erythrocyte membrane protein (PfEMP1), the functional significance of other VSA remains unknown. We aim to determine whether RIFIN and STEVOR are targets of natural immunity and whether the hypervariable regions of these structurally related proteins are exposed to antibody-mediated immune selection. We present a preliminary classification of rif and stever hypervariable domains that will be used to characterize the diversity of gene repertoires expressed in wild isolates from coastal Kenya. Standard phylogenetic approaches as well as novel approaches were applied to rif and stever sequences mined from coastal Kenya. Standard phylogenetic approaches as well as novel approaches were applied to rif and stever sequences mined from coastal Kenya.
from multiple \textit{P. falciparum} isolates in order to identify genetically distinct sets of genes. In addition, we adapted sequence networking approaches developed for the classification of \textit{var} sequence tags, to visualize relationships between the hypervariable regions of members of each gene family. Groupings derived using the various approaches were compared with each other and to previously defined groupings obtained using full-length sequences. We show that rif and stevor genes cluster into multiple highly divergent groups comprised of members that are conserved across multiple isolates. The networks obtained using the hypervariable regions revealed evidence of strong clustering of putative recombining sequences. We have derived distinct rif and stevor sequence groups present across multiple \textit{P. falciparum} isolates. We present evidence that these multigene families appear to undergo recombination-mediated variation between paralogs similar to the \textit{var} genes. Future work is required to determine whether the sequence groups we have identified are biologically meaningful.

Email address for correspondence: vthathy@kilifi.kemri-wellcome.org

471

\textbf{User impressions from long-term evaluation of durable residual wall lining (DL) as a replacement of IRS for malaria vector control in Nigeria [MIM15066128]}

John H. Thomas

Various published research projects demonstrated that covering walls of houses with insecticide impregnated durable residual wall lining (DL) holds promise as a means to overcome the limitations of indoor residual spraying (IRS). That is, the material is not complicated to install vs. the logistics of spraying; treatment is uniform across the entire surface due to factory-treatment vs. uneven spray coverage; and effectiveness is long-lasting due to controlled release of active ingredient vs. spray formulations which are rapidly degraded by different wall surfaces (mud, clay and wood). DL was installed in 180 houses near Lagos, Enugu, and Kano, Nigeria in November 2006. Three types of materials were used: shade cloth, netting, and plastic sheeting. A follow-up survey, including photographic documentation, was conducted in July, 2008 in which 50% of the original participants were available. Of this group 40% were urban and 60% were rural. After 2 years 67% of the houses still had wall-lining products hanging on the walls. For the rural participants in that group 78% of the DL was shade cloth. Key benefits derived from having DL on the walls included: killing/driving mosquitoes and flies away (79%); killing crawling insects/bedbugs (51%); and beautifying the home (28%). Reasons given for removal included: renovation of room/house (37%); and non-effectiveness (23%). An overwhelming majority of the occupants where DL remained installed (90%) and those where DL had been removed (60%) wanted to have it re-installed because of its effectiveness in eliminating vectors.

Email address for correspondence: john.thomas@phnxord.com

472

\textbf{Seasonal variation in species composition and frequency of insecticide resistance alleles (kdr and ace-1R) in the Anopheles gambiae complex from an irrigated rice fields area in Western Burkina Faso [MIM16689682]}


Monitoring of the spread of insecticide resistance in field vector populations is a prerequisite for the implementation of efficient and sustainable vector control strategies based on the use of insecticides. Screening for resistance alleles in \textit{Anopheles gambiae} populations is facilitated by the availability of molecular diagnostics to detect major target-site mutations, such as knock-down resistance (kdr) and insensitive acetylcholinesterase (ace-1R). \textit{An. gambiae} mosquitoes were collected resting indoors in two villages within a rice cultivation area in western Burkina Faso, from January to December 2007. Specimens were identified to species and molecular form and their genotype at the kdr and ace-1 locus was determined using PCR and RFLP protocols. The M form was largely predominant in our samples and was present all year round in both villages. S-form mosquitoes gradually appeared during the rainy season in the village at the margins of the rice fields (VK7) whereas it was very rare in the center of the rice cultivation area (VK5) throughout the survey. The frequency of both kdr and ace-1R mutations was higher in the S than in the M form at any time. In the M form, frequency of the kdr mutation was higher during the rainy season in both villages ($P<0.005$). We report occurrence of the ace-1R mutation in the M form, albeit at a low frequency ($<1\%$). Our results highlight the preoccupying status of insecticide resistance in \textit{An. gambiae} populations from Burkina Faso, and suggest that comprehensive monitoring strategies need to consider population dynamics.

Email address for correspondence: namountougou.d@yahoo.fr

473

\textbf{Exploring the role of Faith-Based Organizations for engaging communities towards malaria control: Evidence from Mozambique [MIM16772733]}

Anant Bhan, Sunita Sheel Bandewar, Linda Rozmovits, Mark Webster, Jean Duff, James V. Lavery

With the growing recognition that communities can suffer research-related harms and exploitation, community engagement (CE) has become an important ethical requirement for research. Despite this recognition, there is little guidance on what makes CE effective in biomedical research and public health practice. In Mozambique, a collaboration among the country’s major Faith-Based Organizations (FBOs)—Together Against Malaria (TAM)—is mobilizing communities to take action to control malaria. We conducted a case study of TAM, as one of a series of 10 case studies, to examine what makes CE effective, from a range of stakeholder perspectives. The study combined case study and grounded theory research methods. We examined TAMs CE process, with particular emphasis on perceptions of effectiveness among a sample of 61 key TAM stakeholders, including faith leaders, NGO staff, government health officials, funders and community members. The extensive reach of FBOs throughout the region (including their presence in remote areas), their unique convening power and ability to reliably deliver messages to regularly assembled congregations, and their structured hierarchical nature and leadership facilitate community engagement for public health purposes, including malaria prevention. Faith-based initiatives can play a crucial role in CE for malaria control. We present and discuss features of TAM that we believe may be replicable in other parts of Africa, and elsewhere, to provide policy makers, funders and researchers with insights about the unique potential of FBOs for community and public mobilization for public health purposes.

Email address for correspondence: bhan.anant@gmail.com
474
The scope of misconduct in scientific research [MIM16228882]
C. Chi Primus

In the past two decades, the volume of scientific research carried out in Africa has been on an increasing scale. In designing and implementing these researches, the inclusion of fundamental ethical principles remains an essential ingredient. However, the vast majority of scientists doing research have had no formal training in research ethics. This growing volume of research carried out by scientists with little or no formal training in research ethics has engendered a serious and widely acknowledged ethical issue of scientific misconduct; the violation of the standard codes of scholarly conduct and ethical behaviour in professional scientific research. The ethical issue of scientific misconduct cuts across all the categories of scientific research; be it physical, chemical, biological or otherwise. Although scientists generally acknowledge that scientific misconduct is unethical, experience has, however, shown that there is some disagreement on their appreciation of the scope of scientific misconduct. The paper will diligently address the conditions favouring the practice of scientific misconduct as well as the consequences, detection, management and prevention of this unethical practice. Furthermore, online resources offering free training courses on research ethics will equally be provided. Materials for the paper will be obtained from the review of training curricula on research ethics, scientific publications, codes of ethics of professional scientific organizations and other online resources. It is hoped that the paper will sensitizes young scientists on the scope of scientific misconduct and nurture within them a culture of integrity in scientific research.

Email address for correspondence: chi.primus@yahoo.com

475
Community engagement in biomedical research in a low-income setting: Preliminary evaluation of a new network of community representatives [MIM16204952]
D.M. Kamuya, V. Marsh, P.W. Geissler, S. Molyneux

Recent debates on ethical practice in international research emphasize collaborative partnerships with communities where research is conducted. However, there is little documented experience on developing such partnerships. KEMRI-CGMRC in Kilifi, facilitated setting up a network of 140 volunteer community representatives from 240,000 residents where research is done. This study was a preliminary evaluation of this network, focusing on perceptions of roles, challenges and benefits. Qualitative analysis of meeting minutes (n = 64), in-depth interviews (n = 3) and focus group discussions (n = 3) with selected community representatives. Quantitative analysis of self-administered questionnaires (n = 140) and comparison of key attributes with population data. The network has been well sustained over 20 months, with high meeting attendance (70% over seven meetings) and low turnover rates. Issues raised by KCR members contributed to institutional policy changes and closer relationships with community to mediate several complex situations. KCR members reported good understanding of their roles, faced significant challenges undertaking these roles, including explaining how research differs from standard health care. These challenges are understandable in this setting given the range of research activities, frequent overlaps between health research and treatment, and livelihood struggles for many community members. Together, these factors make achieving equal partnerships difficult. Our experiences support importance of involving communities in international research and highlight challenges to identifying appropriate representatives and achieving equal partnerships. In settings similar to ours, it maybe realistic to aim for community participation (i.e. increased interactions, co-learning and mutual consultation) than partnership.

Email address for correspondence: dkamuya@kilifi.kemri-wellcome.org

476
“We knew that children were being treated...” Therapeutic misconceptions in a clinical trial done in a low resource setting [MIM14852100]
Rose Mwangi

Therapeutic misconception, when a research subject mistakenly understands the main purpose of a clinical trial to be therapeutic, is a major concern in many developing countries, and may affect the informed consent and informed decision-making processes. Objective: To explore potential therapeutic misconceptions and or confusion among research participants and their decision-making strategies, to ascertain whether research recruits understand the non-therapeutic nature of the research. The study was done in north east Tanzania, with a group of individuals who had been asked to participate in a non-therapeutic randomized double blinded placebo controlled clinical trial of a malaria drug. In-depth interviews were conducted, using the local language (Kiswahili), with 20 individuals who had agreed to participate in the clinical trial and 15 who had declined. Interviews reveal that many participants saw now conflict between research and therapeutic goals, that they expected to gain access to state-of-the-art treatment through participation, and that the potential therapeutic benefit may have been overstated by study recruiters. This study highlights some serious problems of therapeutic misconceptions in relation to decisions to participate and/or declining to participate in a clinical trial done in a low income, international setting.

Email address for correspondence: mwangirose2000@yahoo.co.uk

477
Engaging African Communities in malaria research: Evidence from rural Northern Ghana [MIM16701041]
Paulina Onvomaha Tindana, Sunita Bandewar, Renaud Boulanger, Raymond Aborigo, Abraham Hodgson, Jim Lavery

With the growing recognition that communities can suffer research-related harms and exploitation, community engagement (CE) has become an important ethical requirement for research. Despite this recognition, there is little guidance on what makes CE effective in biomedical research and public health practice. In Northern Ghana, the Navrongo Health Research Centre (NHRC) has developed an approach to CE that has contributed to the success of its malaria research activities over the past 20 years. We conducted a case study of the Navrongo NHRC CE approach, as one of a series of 10 case studies, to examine what makes CE effective, from a range of stakeholder perspectives. The study combined case study and grounded theory research methods. We examined NHRC’s CE process through 20 in-depth interviews, 12 focus groups with paramount chiefs, elders, women’s group leaders, and research participants, and observation of five CE activities, with particular emphasis on perceptions of effectiveness of the CE practices. The NHRC’s CE approach blends traditional Kassena-Nankana community practices with modern research requirements in a five-stage process: community entry; a community durbar; compound level meetings; individual informed consent; and feedback meetings during and after the research. These stages represent levels of authorization and acceptance of the research by the community.
and facilitate the building of mutual trust. Our case study of the NHRC's CE practices provides a detailed “map” of the cultural issues that affect CE in this traditional setting. We discuss the transferability of these findings to other research settings.

Email address for correspondence: ptindana@navrongo.mim.com.net

478 High malaria prevalence and risks in women who do not attend Antenatal Clinic [MIM16563564]

K.F.C. Thole, J.M Moyo, Thawani E. Jonazi, Kamoza Chimungu

There are about 6 million episodes of malaria per year in Malawi (4.5 million health facility based), malaria OPD cases range from 200,000 to 350,000 per month, 30% of all OPD cases are due to malaria, 40% of all U/5 children inpatients are due to malaria, 30% of all hospital deaths in U/5 children are due to malaria. Lilongwe malaria prevention study has been examining biases in ANC data for some a year by making comparisons to community data by directly following up women who have not attended ANC recently identified from our population-based study. To compare the malaria cases amongst these women to that amongst ANC attendees in order to quantify the basis arising when using ANC data to estimate population malaria prevalence. As part of our demographic population studies in Lilongwe, women who have not attended ANC within the 2 years prior to interview date are identified. Women are then followed up by female counselors, consented, and interviewed. Those willing are counseled and referred to the nearest health center malaria prevalence differs at every age group when comparing ANC women with ANA. Women who do not attend ANC are at higher risk of malaria (although ANC is major focus of health education efforts).

Email address for correspondence: calebfaith@gmail.com

479 Understanding and improving access to prompt and effective malaria treatment: A combined supply-demand side approach [MIM16668343]

Flora Kessy, Manuel Hetzel, Sandra Alba, Iddy Mayumana, Angel Dillip, Christian Lengeler, Brigit Obrist, Alexander Schulze, Hassan Mshinda

The ACCESS project carried out in two rural districts in Tanzania aims at improving the overall delivery of quality health care using malaria as a tracer condition through three complementary interventions: social marketing to reach community members; improved quality of health care services; and development of high quality commercial drug outlets. Objectives: To determine factors affecting access to prompt and effective malaria treatment at both the community and health care provider levels. Semi-quantitative cross-sectional community surveys were used to investigate disease perception and treatment seeking behaviour, complemented by quantitative and qualitative studies on drug availability and quality of care. Health system factors appeared to be major obstacles to treatment but poverty related problems also influenced negatively the outcome of health episodes. Drug and antimalarial stock-outs had occurred in 60% of all health facilities surveyed. Modern medicine was preferred by most patients and 87.5% of the fever cases in children and 80.7% in adults were treated with one of the recommended antimalarial, reflecting the intensive social marketing and health education campaign. However, an estimation of community effectiveness revealed that only 22.5% of children and 10.5% of the adult received prompt and appropriate treatment despite high health facility usage. This reflects delays in seeking care as a result of mobilizing resources. Access issues are multiple and arise at different levels of the health care system. This calls for a comprehensive approach that moves beyond provision of health services to also include measures that strengthen household economies.

Email address for correspondence: flessy@gmail.com

480 Technical and perceived quality of malaria treatment services of public and private healthcare providers in urban and rural areas of southeast Nigeria [MIM16676099]

Ogochukwu Onwujeke, Benjamin Uzochukwu, Ijeoma Okoronkwo, Eric Obikeze, Edith Ikeh, Obinna Onwujeke

Information about quality differentials across providers is needed to identify and correct problems associated with quality of malaria treatment services. Poor quality of healthcare services is a major contributor to the high societal costs of malaria. The study determined the differential quality of malaria treatment services as well as inequities in quality of malaria treatment services received from a range of public and private healthcare providers. The study was undertaken in three urban and three rural areas in southeast Nigeria. A questionnaire was used to collect information from 225 healthcare providers to assess technical quality. Exit polls were also conducted with at least 10 clients (consumers) of each provider to determine perceived quality of malaria treatment services. The lowest quality of services was found from low level providers such as patent medicine dealers (PMDs). Conversely, public and private hospitals as well as primary healthcare centers had the highest quality of services. Householders were least satisfied with quality of services of PMD and pharmacy shops and were mostly satisfied with services rendered by public and private hospitals. The urbanites were more satisfied with the overall quality of services than the rural dwellers. There was differential quality of treatment of malaria amongst different providers, with responses from both consumers and providers yielding similar results. These findings provide areas for interventions to equitably improve the quality of malaria treatment services.

Email address for correspondence: onwujeke@yahoo.co.uk

481 Monitoring program impact at the health level: A case study from population services international—Laos [MIM16704880]

Saysana Phanalasy (PSI Laos, Research Manager), Malaykham Duangdara (PSI Laos, Research Assistant), Elena Olivi (PSI Laos, Fellow), Rob Gray (PSI Laos, Country Representative), Gary Mundy (PSI, Regional Researcher)

The NGO PSI/Laos is implementing a 5-year malaria prevention program in three southeastern provinces in the Lao PDR, where frequent overnights in forests/ricefields brings villagers into increased contact with the primary malaria-transmitting mosquitoes. The purpose of the program is to prevent malaria among high-risk rural populations by increasing ownership and correct use of long-lasting insecticide-treated nets (LLINs) through interpersonal communication and free LLIN distribution. A household-behavior and parasitology study, conducted between September and December 2008, will allow for analysis of parasite prevalence as compared to self-reported LLIN use. Twenty-nine villages across the intervention area were selected using probability proportionate to size. Seventy respondents were randomly selected from each village (n = 2030). Data were collected.
using a structured questionnaire, and all respondents and their household members were tested for *Plasmodium falciparum* malaria prevalence using RDTs. Respondents’ test results were linked to the questionnaire data via use of a unique identifier code. Data will be analyzed in SPSS using multivariate techniques. Full study findings will be available in mid-2009. Results of this study will be used to: inform the program about the link between LLIN use and parasite prevalence, which is not as definitive in Southeast Asia as it is in Africa, due to early biting behavior of vectors; inform the program about the link between forest/ricefield work and parasite prevalence; provide baseline data against which program efficacy (both in terms of LLIN use and parasite prevalence) can later be assessed, which is unique among NGOs.

Email address for correspondence: sphanalasy@laopdr.com

---

**482**

**Connaissances, attitude et pratiques des mères et gardiennes d’enfants sur le paludisme a Mbuji Mayi, RD Congo [MIM16697192]**

J.C. Mbalabu, L.T. Bobanga

Plus des 3/4 des décès liés au paludisme surviennent dans la communauté. Et cette pathologie est la première cause de morbimortalité en RDC avec plus de 20 millions des cas et près de 140,000 décès. Et cette tendance ne peut s’améliorer qu’en fonction des connaissances sur le paludisme des mères et gardiennes d’enfants. Ainsi une étude sur les connaissances, les attitudes et les pratiques a été mené à Mbuji Mayi qui est zone de paludisme stable. **Objectif:** déterminer le niveau de connaissance des mères sur le paludisme une étude CAP a été menée auprès de 400 mères et/ou gardiennes d’enfants à Mbuji Mayi. Sur les 400 mères interviewées, 27% ont de bonnes connaissances sur la maladie; 26% connaissent les méthodes préventives et 10.5% le traitement du paludisme tel que recommandé par le Programme Nationale de lutte contre le paludisme (PNLP). Parmi ces mères ou gardiennes d’enfants 76.5% ont une bonne attitude vis-à-vis du paludisme de l’enfant particulièrement la fièvre. Seulement 27% ont une bonne pratique en matière de paludisme (traitement du paludisme simple ou référence en cas de paludisme grave. Bien que l’attitude des mères vis-à-vis du paludisme de l’enfant soit bonne dans la plupart des cas, les connaissances et les pratiques doivent être renforcées). Il est donc important que les activités du PNLP soient ciblées vers la communauté si l’on veut changer radicalement l’impact du paludisme.

Email address for correspondence: jeanclaudembt@yahoo.fr

---

**483**

**Accessibilité et utilisation des antipaludiques dans les interventions sous-directives communautaires [MIM16689341]**

Innocent Takouang

Email address for correspondence: itakouang@yahoo.com

---

**484**

**A country-wide assessment of malaria diagnostic capabilities versus a sampling technique [MIM16697573]**

Jane Carter

Kenya’s Division of Malaria Control, supported by USAID’s Improving Malaria Diagnostics Project under the President’s Malaria Initiative, conducted a census of health facilities with a laboratory to identify gaps and develop strategies to improve malaria diagnostic capabilities (staff, equipment, supplies, training and supervision, and quality assurance protocols). One hundred and fifty district laboratory supervisors trained for 2 days collected data from approximately 1600 health facilities with laboratories. A sample, simulating an assessment done according to the lot quality assurance sampling (LQAS) methodology, was drawn, and estimates of precision based on the sample were compared to the preliminary census findings. The census detected 55% of laboratories without a technician recently trained in malaria microscopy. LQAS with a sample size of 19 produced valid responses to this selected variable. Estimates at provincial level made from an LQAS-type assessment may hide much internal heterogeneity that might have emerged from an analysis at district level, but a district level LQAS analysis would require a sample of almost the same size as the census. A national census providing complete information is costlier than an LQAS-based approach. LQAS cannot identify individual laboratories and specific gaps, but can provide estimates of the proportion of laboratories failing to meet minimum standards. Further analysis of the census as the gold standard will compare advantages and disadvantages of cluster, random and stratified sampling of laboratories for annual monitoring of diagnostic capabilities.

Email address for correspondence: jcarte@iconnect.co.ke
486 Coping strategies of public sector health workers during ACT stock-outs [MIM16705848]

Beatrice Wasunna, Caroline O. Jones, Jane Bruce, Jayne Webster, Robert W. Snow

Prompt treatment with an effective antimalarial drug remains essential both to save lives and for effective malaria control. In July 2008, 2 years after changing first-line malaria treatment policy to artemeter lumefantrine (AL), the Kenyan Ministry of Health reported nationwide stock outs of the drug at public health facilities. Understanding the factors contributing to district level stock-outs and health worker coping mechanisms is critical in assessing the consequences of such stock-outs for effective case management. In August 2008, to investigate reported treatment practices at time of AL stock-out, semi-structured interviews were conducted with every health worker performing out-patient consultations in all public health facilities in Bondo district, Kenya (n = 49). Four interviews were undertaken with key members of the District Health Management Team to explore factors underlying district level drug stock-outs. Health workers reported four main coping strategies for uncomplicated malaria: (1) prescription and dispensing of alternative ineffective antimalarials such as amodiaquine and sulfadoxine pyrimethamine; (2) prescription and dispensing of second-line antimalarial drug (quinine); (3) prescription of antimalarials for purchase, particularly amodiaquine as it was affordable in the private chemists; (4) purchase of AL in installments from the private sector. The majority of coping strategies reported by health workers at a time of AL stock-outs severely compromises effective case management of malaria. The broader repercussions of these findings in terms of patient trust and use of the public health system, as well as implications for malaria control in light of the drive towards malaria elimination, are discussed.

Email address for correspondence: bwasunna@nairobi.kemri.wellcome.org

487 Interacting environmental and programmatic status determinants of coverage with larval stage mosquito surveillance in Dar es Salaam, Tanzania [MIM16757336]

Prosper P. Chaki, Nicodemus J. Govella, Bryson Shoo, H. Abdallah, Khadija Kannady, Steven W. Lindsay, Ulrike Fillinger, Gerry F. Killeen

Controlling vector mosquitoes in their larval stages for malaria control is logistically complex. Here we assess the quality of a community-based larval control program by examining interacting environmental and programmatic determinants of coverage with larval surveillance. The Urban Malaria Control Program (UMCP) in Dar es Salaam, Tanzania delegates responsibility for routine mosquito control and surveillance to community members, known as Community Owned Resource Persons (CORPs). A trained mosquito biologist initially conducted an independent cross-sectional quality control assessment of larval surveillance by 64 CORPs. Only 7.8% of the 2965 wet habitats found by the investigator were occupied by any aquatic stages of Anopheles. CORPs had detected almost 66.2% of wet habitats. The detection sensitivity for occupation of habitats by aquatic mosquito stages by CORPs was low, ranging from 15 to 30% but was particularly poor for late stage Anopheles (2.7%; 3/111). The distribution of habitat types varied differed between fenced plots and unfenced plots (χ² = 50.037, d.f. = 10, P < 0.001) and the former drastically reduced detection sensitivity of early stage Anopheles (OR [95% CI] = 0.07 [0.010, 0.728] and all stages of Culex (P < 0.05). One fifth (20.5%) of aquatic habitats occurred in plots with which the CORPs were unfamiliar and this reduced their detection probability (OR [95% CI] = 0.16 [0.13, 0.21]. Although detection levels of habitats by CORPs have improved through vertical management coverage remains incomplete. Moreover, detection of mosquito larvae by CORPs was very poor. Improved supervisory and quality control systems are urgently required to maximize operational impact.

Email address for correspondence: pchaki@ihi.or.tz

488 Understanding the operation of specialized drug shops in four Kenyan districts [MIM14962546]

Francis Wafula, Abdinasir Amin, Timothy Abuya, Catherine Goodman

Studies indicate that a high proportion of people in sub-Saharan Africa visit pharmacies and drug shops for quick, inexpensive and convenient access to treatment. There is increasing interest in how policymakers can work with retailers to facilitate the delivery of malaria combination therapies and other treatments. However, little is known about the operation of these shops, and how this relates to existing regulatory frameworks. This study seeks to describe the operation of pharmacies and drug shops in four Kenyan districts, and explore any discordance between actual practices and regulatory requirements. The study will be conducted in three phases between November 2008 and September 2009. Kenyan laws and policy documents will be reviewed to map regulatory frameworks governing the sale of medicines. Shop attendant surveys and simulated client surveys (SCSs) will be conducted in pharmacies and drug shops in South Bungoma, Kakamega Central, Kisii Central and Rachuonyo districts (estimated 175 shops). SCSs will identify the proportion of shops that still stock and sell dihydroartemisinin (an unlicensed monotherapy treatment for malaria), and attendants who sell sub-therapeutic doses of the drug. Results will be presented on shop characteristics, and knowledge and practices of attendants-in-charge. Patterns of association will be explored between practices of attendants and shop characteristic findings will inform policy on working with retailers to improve the quality and coverage of appropriate malaria treatment.

Email address for correspondence: fwafula@nairobi.kemri.wellcome.org

489 Achieving effective and equitable coverage through a national ITN delivery system [MIM16682428]

T. Marchant, D. Schellenberg, R. Nathan, J. Schellenberg, H. Mponda, C. Jones, Y. Sedekia, J. Bruce, K. Hanson

The health benefits of malaria interventions delivered at scale are compromised by the multiple challenges of delivery systems. We applied an effective coverage framework to the distribution of insecticide treated mosquito nets (ITN) within the Tanzanian National Voucher Scheme. Our aim was to quantify the steps required for ITN delivery and understand how effective coverage could be improved. Data from a national two-stage cluster household survey was analysed. A representative probability sample of 210 clusters, each of 30 households, was selected from 21 districts across mainland Tanzania in 2007. Women pregnant at the time of the survey, and women who reported having had a live birth in the previous 12 months, were interviewed. We analysed the extent to which respondents progressed through the system using an effective coverage framework, and disaggregated findings by socioeconomic status. Six steps were defined and coverage for
each individually was relatively high (60–98%). However, application of the effective coverage framework estimated an effective ITN coverage of only 27%. This figure was compatible with the measured ITN coverage in pregnant women of 23% (95% CI 19–27%). Analysis by socioeconomic status revealed that effective coverage ranged between 13 and 36% in the poorest to least-poor. Delivery of long-lasting-insecticide-treated nets (LLIN) could increase effective coverage from 27% currently to 47% within the existing system. The cumulative effect of modest failures at several steps in a delivery system diminishes the potential for health benefit. This approach is useful to reveal where opportunities for action lie.

Email address for correspondence: tanya.marchant@lshtm.ac.uk

490
Determinants of ACT availability and price in Cambodia: An economic perspective [MIM15761175]

ACTwatch Study Team, Dr Duong Socheat, Dr Kheng Sim

Cambodia was the first country to switch to artemisinin combination therapy (ACT). However, coverage remains low, especially in the retail sector where affordable ACT is rare whilst monotherapies and counterfeit products are common, potentially fuelling multidrug resistance. Little is known about the factors that determine retailers’ stocking and pricing decisions but they are likely to be influenced by what happens further up the supply chain. Data were collected between June and October 2009, from a random sample of wholesalers and distributors operating at different levels of the chain supplying malaria endemic areas with confirmed or suspected multidrug resistance. Quantitative surveys and in-depth interviews were used to identify the influence of key aspects of the antimalarial supply chain on ACT availability and price, with a specific focus on economic factors. Results will be presented on the economic aspects of the supply chain that influence ACT availability and price. Key aspects will include the impact of market structure on price competition and the role of non-price competition, for example through branding and marketing. Strategies to shape providers’ pricing and stocking decisions at different levels of the chain will be identified, in order to improve availability of affordable ACT in the retail sector. Particular attention will be directed towards incentives for providers to supply subsidized ACT and limit supply of ineffective antimalarials.

Email address for correspondence: edith.patiouillard@lshtm.ac.uk

491
The regulatory environment for private sector ACT distribution in five African countries: Implications for a global subsidy [MIM15761567]

ACTwatch Study Team

There is considerable interest in using the private sector to expand access to artemisinin-based combination therapies (ACTs), especially in light of the planned global subsidy. However, the high price of private sector ACTs is a significant barrier to access, and there is concern that the benefits of a subsidy may fail to reach the poorest. The regulatory environment for private retail and wholesale antimalarial sellers may influence the operation of the distribution chain, and therefore the accessibility and affordability of ACTs. We undertook a comparative analysis of the regulatory context of private markets for antimalarials in five sub-Saharan African countries. Qualitative research methods were used to collect information on the regulatory influences on antimalarial markets in Benin, Nigeria, Madagascar, Uganda and Zambia during January–August 2009. In each country we conducted 8 key informant interviews with stakeholders at the central level, and 24 in-depth interviews divided equally among 4 levels of the supply chain. The regulatory environment for private markets for antimalarials in the five countries will be described in terms of requirements for licensing, permits, and registration of retail and wholesale antimalarial sellers; taxes and tariffs paid; regulatory restrictions; and the level of enforcement and compliance. The strength of drug regulatory policies and the roles of national pharmaceutical boards will be compared across countries. The implications of regulations for access and affordability of retail ACT will be identified. Global policy implications for the implementation of a global ACT subsidy will be highlighted.

Email address for correspondence: sarah.tougher@lshtm.ac.uk

492
Spatial indicators for the management of malaria control interventions [MIM16704395]

Natasha Morris, Ishen Seocharan, Rajendra Maharaj

The regional malaria control programme of the Lubombo Spatial Development Initiative (LSDI), a trilateral initiative involving the governments of South Africa, Swaziland and Mozambique, has reported repeated successes since its inception in 1999. Core to this success has been the implementation of integrated spatial decision support systems that allow for monitoring and evaluation of the vector control programme, based in large part on indoor residual spraying (IRS). A comprehensive spatial data repository was developed to support the mapping and analysis of IRS data. Of particular significance was the development of small-scale spatial boundaries for data display and analysis for the southern Mozambique region, facilitated through the use of innovative participatory methods. Spray information being captured into a relational database provided critical decision making outputs regarding spray coverage, progress and insecticide usage. Outputs assisted in planning insecticide requirements for future spray rounds. Small-scale mapping of this data for decision support proved valuable at all levels of management. The success of the programme clearly demonstrated the benefits of using spatially enabled data to inform large-scale field based interventions. The importance of data maintenance and good record keeping has proved a key component of sustainable data collection. Substantial skills transfer to the local malaria control programme was enabled. The quality of spatial visualisation and analysis of data was further improved through systematic review, consultation and feedback.

Email address for correspondence: nmorris@mrc.ac.za

493
The cost-effectiveness of LLIN distribution for malaria control at community level in Enugu State, South East Nigeria [MIM16698024]

C. Ezenduka Charles, E. Onoriode

Provision of long-lasting insecticide treated bed nets (LLINs) has become a key component of prevention strategy to control malaria in endemic areas especially in pregnant women and children under 5 years. The study estimated the economic costs and effects of LLIN distribution at highly subsidized prices to pregnant women and children under 5 years as part the Global Fund for Malaria project at the community level in Enugu State, South East Nigeria. From a provider perspective economic costs and effects of the intervention were estimated on incremental basis reflecting a comparison between the LLIN intervention and a “do-nothing” alternative. Effects were measured as malaria cases averted, deaths
averted. Regional estimates were used to model the malaria cases. Cost data were obtained from financial records and interviews with key stakeholders. Univariate sensitivity analysis was used to test the robustness of the study results. From the provider perspective, assuming equal distribution of shared costs between the LLIN and the other ACT and IPT interventions of the malaria project, the gross cost per LLIN distributed was $6.62, cost per malaria case averted, $10.96 and cost per death averted, $1199.00, without accounting for saved treatment costs. Seventy percent constant utilisation is assumed over the period of study. The cost-effectiveness of the LLITN distribution at the community level falls within the common range obtained with similar studies, underscoring the importance of LLIN as a key strategy in reducing malaria mortality and morbidity in malaria endemic areas.

Email address for correspondence: ezendukacc@yahoo.com

494
Connaissances, attitudes et pratiques des prestataires et utilisatrices des services de traitement préventif intermittent du paludisme [MIM16692517]
Lele Kouawa Albertine, Fotso Fokam Zacharie, Takougang Innocent, Ngassa Pius

Chaque année, 50 millions de femmes enceintes sont exposées au paludisme parmi lesquelles 30 millions dans la région Africaine, entraînant des avertissements, faibles poids de naissance, décès maternel et infantiles. Au Cameroun, la prévalence du paludisme pendant la grossesse varie de 30 à 45%. Trois doses sulfadoxine-pyriméthamine (SP) sont recommandées pour le traitement préventif intermittent (TPI), à administrer sous observation directe lors des consultations prénatales (CPN). Le but de notre étude était de déterminer la couverture en TPI et d’identifier les contraintes relatives à sa pratique. Des formations sanitaires de référence de la ville de Yaoundé ont fait l’objet des investigations qui se sont déroulées du 03 Avril au 18 juin 2008. Le personnel (33) et les utilisatrices des services de CPN (353) ont été soumis à un questionnaire. Moins du quart de femmes enceintes (21.9%) prennent le TPI sous observation directe. Plus de la moitié du personnel de santé (54.5%) n’ont reçu aucune formation sur la pratique du TPI, de même 57.6% n’ont reçu aucune supervision au cours des 6 derniers mois. Les services d’offre du TPI devraient intensifier la formation continue des prestataires des services de CPN à la pratique du TPI et de doter ces services de matériel nécessaire pour la prise de la SP sous observation directe. Mots clés: paludisme, Traitement Préventif Intermittent, Consultations Prénatales, formations sanitaires, utilisatrices des services, prestataires de services.

Email address for correspondence: lelekouawa@yahoo.fr

495
Variations in prescribing practice for antimalarials in the Gambia [MIM14940441]
Joseph Okebe, David Schellenberg, David Conway, Michael Walther

The study describes prescription patterns in the Gambia, evaluating the impact of seasonality and availability of microscopy services on prescription practice. Two health centre-based cross-sectional surveys; during (WS) and outside (DS) the transmission season. Participants with malaria diagnosis made by clinic staff were enrolled. Data on microscopy results were obtained. Blood samples were collected for comparative slide review. Outcome was the proportion of antimalarials prescribed for a confirmed slide negative result. Five hundred and thirteen subjects were enrolled; 215 in the DS, 298 in the WS (213 < 5 years, 5 ≤ 132 ≤ 15 years, 168> 15years). Slide requests were similar between seasons (35% in WS, 32% in DS, p = 0.468). However, the odds of positive slides at the health centre was highest in adults (>15 years) in the wet season (OR 11.625, p = 0.002). Research slides were negative in 95% (209) and 88% (264) of subjects in the dry and wet seasons. Compared to the research slides, sensitivity of health centre slides was 90%, but specificity was 53.9%. In 92% (319) of the patients treated presumptively, the research slide was negative; 97% received an ACT for presumptive treatment. A consistent rate of presumptive treatment in the face of seasonal variations in malaria results in significant over-prescriptions in the dry season. Cost implications of this observation are being evaluated.

Email address for correspondence: jokebe@mrc.gm

496
Cost and cost-effectiveness of intermittent preventive treatment of malaria in infants (IPTi) [MIM16702909]
Alexandra de Sousa, Idriissa Camara, Jobiba Chimkhumba, Mialy Rabarison, Anselm K. Abotsi, Victorin Capo-Chichi, Abdou Diop, Don Mathanga, Jacques Hassan, Alassane Dicko, Leonce Paul Rabarajona, Ebenezer Inokoorn, Jacques Hassan, Eric Ribaira, Rozen Le Mentec

Intermittent preventive treatment in infants (IPTi) is a new malaria control strategy with potential for implementation scale up. UNICEF launched a pilot study involving 6.5 million people distributed in six malaria endemic countries to calculate scale up costs in natural implementation conditions. Additionally, IPTi cost-effectiveness was evaluated from a societal perspective to enable decision makers and implementing partners to justify their economic choices. Implementation scale up cost was calculated by estimating IPTi incremental costs in start-up years (financial costs of IPTi administration and of implementation activities) and in recurrent years (IPTi administration and safety surveillance). To estimate IPTi cost-effectiveness (IPTi net cost per case of malaria averted, per death averted, per year of life saved, and per disability adjusted life years) we calculated the intervention’s economic costs in recurrent years. IPTi incremental financial costs on start up years (53.65 cents/child) were substantially higher than in recurrent years (15.48 cents/child). In routine, the part of programme costs was 14.12 cents/child, and patient costs only 1.36 cents/child. The time needed to administered IPTi was an average of 5 min/child, representing 11% of the time spend by health workers in immunization clinics. The net cost of IPTi per case of malaria averted was $8.85, per death averted $85.55, per year of life saved $3.13, and per DALYs $3.35. IPTi can be scaled up at low financial costs in routine years provided that in start up years implementation activities are appropriately conducted. The strategy is highly cost-effective.

Email address for correspondence: adesousa@unicef.org

497
Antibodies to Plasmodium falciparum merozoite surface protein-1 (MSP1): Characteristics and dynamics in Nigerians naturally exposed to malaria [MIM16756525]

Merozoite invasion is a process central to the pathology of malaria. The immune response to Plasmodium falciparum in humans includes the production of three categories of anti-MSP1 antibodies (processing-inhibitory, blocking and neutral antibodies) in respect
Malaria infection during pregnancy has adverse consequences for both the mother and foetus. This study seeks to assess the impact of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPT-SP) in combination with other preventive measures on placenta parasitaemia and newborn’s immune responses at delivery. Pregnant women who provided informed consent were recruited during their third trimester and information on use of malaria prevention methods was documented. At delivery, maternal peripheral blood, placental biopsy and cord blood samples were collected to determine the presence of malaria parasites and study the functional heterogeneity of CD4+ T cell (Th1 and Th2) in vitro using ELISPOT assay. Recrudescence rates were lower during the 2nd than the 1st pregnancy and pregnancy-associated recrudescence decreased with increasing parity. Hemoglobin levels and the proportion of anemic mice correlated with parity (P<0.001). Correlations were observed with respect to parasitemia (P<0.001 and P<0.009). There was accumulation of the parasites in the kidney and placenta. Placental parasitemia was higher (p<0.001) than peripheral. In primigravidae, except for IL-10 (P=0.16), concentrations of other five cytokines correlated with hemoglobin level (P<0.001). Correlations were observed with respect to parasitemia with the same cytokines (r<0.001). IL-10 (P=0.23). While IL-10 decreased with increasing parity (P=0.004). Other cytokines increased (P<0.001). Elevated IL-10 and decrease of other cytokines significantly associated with PTDS. Iteration in cytokine levels may strongly contribute to poor pregnancy outcome. Our mouse model reproduces.
Differential recognition of *Plasmodium falciparum* MSP-19 antigen by antibodies from subjects residing in a rural malaria endemic area of South west Cameroon [MIM15067603]

D.N. Anong, T. Nkuo-Akenji, S.K. Mbandi, V.P.K. Titanji

The aim this study was to establish the profile of immunoglobulin (IgG) antibody isotypes in immune responses in individuals to MSP-19 antigen a vaccine candidate. Serum samples from 240 individuals were analysed by ELISA for the presence of IgG sub-classes. Individuals were placed in three age groups: 1–5 years, 10–14 years and >18 years. Each age group comprised an equal number of infected and uninfected subjects. In the age group <5 years and 10–14 years the mean IgG1 and IgG3 levels were significantly higher in those who were positive for malaria when compared with those who were negative (P < 0.0001). In the age group 10–14 years, the mean IgG1 and IgG3 levels of malaria negative subjects was higher when compared with the mean values of malaria positive subjects and the difference was also statistically significant (p < 0.05). In adults (≥18), although the mean IgG1 and IgG3 levels were higher in malaria negative adults when compared with malaria positive adults the difference was statistically significant only for IgG1 responses (p < 0.05). Mean IgG2 levels were significantly higher (p < 0.05) in positive than in negative individuals in all age groups. In all malaria positive subjects with fever, there was a negative correlation observed in the mean IgG1 and IgG3 responses with fever while there was a positive correlation between mean IgG2 and mean IgG4 with fever. These data suggest that immune responses to MSP-19 are protective and are mainly cytotoxic antibodies of the IgG1 and IgG3 subclasses. This work was sponsored by WHO/TDR grant 990965 and IPICS CAM-01 project. Email address for correspondence: anongdn@yahoo.com

Subclass antibody responses to *Plasmodium falciparum* differ by antigen in areas of stable and unstable transmission [MIM16676442]

Gregory S. Noland, John M. Vulule, Xinan M. Min, Chandy C. John

Immunoglobulin G (IgG) subclass antibodies to pre-erythrocytic and bloodstage *Plasmodium falciparum* antigens are thought to play a role in protection against clinical malaria. IgG subclass responses to the pre-erythrocytic antigens circumsporozoite protein (CSP), liver-stage antigen 1 (LSA-1), and thrombospondin-related adhesive protein (TRAP), the pre-erythrocytic/blood-stage antigen apical membrane antigen 1 (AMA-1) and the blood stage antigens erythrocyte binding antigen-175 (EBA-175) and merozoite surface protein-1 (MSP-1) were measured by ELISA in residents of stable (n = 116) and unstable (n = 96) malaria transmission areas in Kenya. In the stable transmission area, >60% of residents had IgG1 antibodies and >70% of residents had IgG3 antibodies to each antigen, and these antibodies were acquired by 5 years of age. In contrast, in the unstable transmission area, frequencies of IgG1 and IgG3 responses to all six *P. falciparum* antigens continued to increase with age well into adulthood, except for MSP-1-specific IgG3 frequencies, which were similar in all age groups. IgG1 and IgG3 frequencies and levels were significantly lower at all ages in the unstable as compared to stable transmission area, except for MSP-1-specific IgG1 and EBA-175-specific IgG3 frequencies, which were similar in both sites in individuals >15 years old. IgG2 responses were common in the area of stable but not unstable transmission. IgG4 responses were infrequent in both sites to all antigens except TRAP. In areas of stable and unstable transmission, development of IgG1 and IgG3 responses to pre-erythrocytic antigens and AMA-1 parallels the development of clinical immunity to malaria. Email address for correspondence: nolan103@umn.edu

Cortisol, prolactin, cytokines and the susceptibility of pregnant Sudanese women to *Plasmodium falciparum* [MIM16691634]

Elhassan Elhassan

Understanding the hormonal and cytokine interactions that underlie susceptibility to the disease should be helpful in elucidating the pathogenesis of malaria during pregnancy. The current study was conducted in the Wad Medani hospital, in an area of central Sudan that is characterised by unstable malarial transmission. Its aims were to investigate the roles and interactions of cortisol, prolactin, interferon-c (IFN-c), interleukin-4 (IL-4) and interleukin-10 (IL-10) in pregnant women with *Plasmodium falciparum* malaria. The 82 pregnant subjects who were enrolled either had uncomplicated, *P. falciparum* malaria (the 45 cases) or were apparently uninfected and healthy women (the 37 controls) who were similar to the cases in terms of their mean age, weight, gravidity, gestational age and haemoglobin concentration. Compared with the controls, the cases were found to have significantly higher serum concentrations of total cortisol and IL-10 and significantly lower levels of prolactin and IFN-c (but similar concentrations of IL-4). The hormone and cytokine concentrations measured in the infected primigravidae were similar to those recorded in the infected multigravidae. Among the cases, there was a significant positive correlation between serum cortisol and IL-10 (r = 0.188; P ≤ 0.05) and significant negative correlations between prolactin and IFN-c (but similar concentrations of IL-4). The hormone and cytokine concentrations measured in the infected multigravidae. Among the cases, there was a significant positive correlation between serum cortisol and IL-10 (r = 0.188; P ≤ 0.05) and significant negative correlations between prolactin and both IL-4 (r = 0.175; P ≤ 0.038) and IL-10 (r = 0.186; P ≤ 0.027) but no significant correlation between prolactin and cortisol. During pregnancy, immune responses appear to be influenced by *P. falciparum* infections, irrespective of parity. Cortisol, prolactin and some cytokines appear to be key mediators in the host response to *P. falciparum* infection, although further research on this subject is clearly needed. Email address for correspondence: elhasans@yahoo.com

Alterations in early cytokine-mediated immune regulation to *Plasmodium falciparum* infection in micronutrient deficient Tanzanian children: A cross-sectional survey [MIM15852711]

Elhassan Elhassan

Micronutrient deficiencies are common and are of clinical and public health magnitude in developing countries accounting for significant infectious disease morbidity possibly due to down-regulation of immune responses to infections. Difficulties in linking in vitro observations with in vivo interventions involving micronutrients supplementation especially due to varied direct or indirect effects on inflammatory cells has been an issue of concern. We conducted in vitro stimulation of peripheral blood mononuclear cells from whole blood of 304 children aged 6–72 months using *Plasmodium falciparum* infected red blood cells prepared from in vitro cultures. The study was aimed at assessing the effects of micronutrients deficiencies on early innate immune responses to *P. falciparum* malaria. Preliminary results indicate that, most cells had a good response towards IL-1β production drawing a special
attention for its possible protective role in early innate immune responses to malaria. Correlation between IL-1β and IL-10 is strong in zinc deficiency and is modulated by infection with malaria. Most micronutrient deficiencies appear to induce high TNF-α production as compared to IL-1β and IL-10, the effect that is highly modulated by malaria status. *P. falciparum*-infected red blood cells are critical in vitro surrogate antigens in analysis of human cytokine responses to malaria. With special attention on zinc, magnesium and iron, our findings provide an insight for future inclusion of carefully selected, with modest amounts, of micronutrients rather than single nutrients as part of malaria vaccine intervention programs in endemic countries. In conclusion, the findings from this study show the plasticity in cytokine profiles of monocytes reacting to malaria infection under conditions of different micronutrient deficiencies.

Email address for correspondence: rerasto@yahoo.com

505 Cytokine profiles in peripheral, placental and cord blood in an area of unstable malaria transmission in Eastern Sudan [MIM16715470]


Understanding the cytokine interactions that underlie both control and disease should be helpful when investigating the pathogenesis of malaria during pregnancy. Few data exists concerning pathogenesis of malaria during pregnancy in areas of unstable malaria transmission. **Objectives:** The study was conducted in New Halfa hospital, eastern Sudan, which is characterized by unstable malaria transmission to investigate the cytokine profiles in peripheral, placental and cord blood in parturient women. Enzyme-linked immunosorbent assay was used to measure the concentrations of three cytokines, interferon- (IFN-), interleukin-4 (IL-4) and IL-10, in sera from peripheral, placental and cord blood of 87 Sudanese women. The concentrations of these cytokines were significantly higher in peripheral, placental sera from uninfected women than in sera from infected women. IFN- concentrations were significantly lower in the cord sera from uninfected women in comparison to the infected ones. The levels of these cytokines were not significantly different between the primiparous and multiparous. Cord sera in all groups showed lower levels of these cytokines. Strong positive correlations were observed between peripheral and placental cytokines. The immune responses that occur in placental, peripheral and cord blood were influenced by the malaria infections, irrespective of the parity. The immune response during *Plasmodium falciparum* infection is not different in the peripheral and placental compartments, further studies are required.

Email address for correspondence: nanybayoumi@yahoo.com

506 The Swain Langley and McCoy blood group polymorphisms of complement receptor 1 and severe *Plasmodium falciparum* malaria [MIM16696884]

B.O. Guyah, A.S.S. Orago, M.F. Otieno, V. Thathy, J.A. Stoute

Binding of *Plasmodium falciparum*-infected red blood cells (RBCs) to uninfected RBCs to form rosettes has been associated with severe malaria in Africa. The major parasite ligand that mediates rosetting is a high molecular weight protein of the var gene family, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1). PfEMP1 interacts with several receptors on uninfected RBCs, including complement receptor 1 (CR1). The domain of PfEMP1 that interact specifically with active site of CR1 leading to rosetting has been mapped (DBL1α). In order to understand how single nucleotide polymorphisms (SNPs) on CR1 gene could influence rosetting, we cloned sequences encoding PfEMP1 (DBL1α) and expressed the synthetic constructs as GFP fusion chimeric proteins on COS7 cells. The transiently transfected cells were used in erythrocyte binding assays using erythrocytes genotyped for Swain Langley and McCoy. The binding rates were recorded using fluorescent microscope. Although all the synthetic constructs were positive for GFP at a transfection efficiency of >70%, none of them bound erythrocytes incubated with them. The observed results were opposite of what was expected since previous data has documented that DBL1α binds erythrocytes during rosette formation. This observation could be explained by the lack of surface expression for these constructs. Moreover, the structural conformational changes of these constructs could explain these observations. Understanding the molecular mechanisms of rosetting may provide more insights into the pathogenesis of severe *P. falciparum* malaria and inform the development of therapeutic agents against the disease.

Email address for correspondence: bguyah@wrp-ksm.org

507 Immune recognition of PfRh protein domains and inhibition by naturally acquired antibodies [MIM16692408]

Ambroise Ahouidi

The PfRh protein family members are merozoite invasion ligands that possess large ectodomains involved in binding and invasion of human erythrocytes. In our study, we seek to characterize the humoral immune response to PfRh protein domains in multiple paralogs, using plasma from Senegalese patients. We further seek to determine whether affinity purified antibodies to these PfRh domains can functionally inhibit invasion of field-isolated parasites. Specific PfRh protein domains were expressed as bacterial fusion proteins. Total IgG and IgG subclass responses were measured by ELISA for 564 sera from different regions in Senegal. Affinity purification of PfRh antibodies from highly endemic clinical sera was performed using PfRh–GST fusions conjugated to CNBr–sepharose. Uncultured Senegalese isolates were inoculated with antibodies to inhibit parasite invasion in vitro. Invasion was measured by flow-cytometry and microscopy. Positive IgG responses were identified for all PfRh proteins, with responses to domains within PfRh2a and PfRh2b being the most highly recognized. IgG subclass analysis of positive responders revealed a predominance of IgG1 and IgG3 subclass, with negligible responses of IgG2 and IgG4, implying high a proportion of antibodies with opsonic and cytophilic potential. Functionally, antibodies against PfRh domains were able to inhibit invasion of uncultured Senegalese parasite isolates in vitro. These studies provide descriptive and functional data validating the PfRh proteins as important targets of humoral immunity and have implications for assessing potential vaccine candidate antigens.

Email address for correspondence: ahouidi@hsph.harvard.edu

508 Plasma inhibition of adhesion of *Plasmodium falciparum*-infected erythrocytes to chondroitin sulphate A in vitro [MIM16685003]

Tina Dobrilovic, Michael F. Ofori, Lea Barfod, Lars Hviid

Parasite-encoded, clonally variant surface antigens (VSA) on the surface of *Plasmodium falciparum*-infected erythrocytes (IEs) allow their adhesion to a number of vascular host receptors, and VSA-specific IgG is a key mediator of protective immunity to
P. falciparum malaria. Placenta-specific IE sequestration is the cause of pregnancy-associated malaria (PAM), and is mediated by particular pregnancy-associated VSA (VSAPAM) with specificity for chondroitin sulphate A (CSA). Assaying the capacity of plasma IgG to inhibit IE adhesion to CSA in vitro has been frustrated by problems with patchy IE adhesion and low assay reproducibility. An improved assay of CSA-specific IE adhesion was developed and used to assess the impact of different plasma anti-coagulants on antibody-mediated inhibition of IE adhesion to CSA in vitro. The improved assay overcomes the limitations of current IE adhesion and adhesion inhibition assays. Plasma anti-coagulants affect assays of antibody-mediated inhibition of IE adhesion to CSA in vitro. The improved assay is a convenient and robust alternative for measuring CSA-specific IE adhesion, suitable for use in endemic country laboratories. Its robustness, and knowledge of anti-coagulant impact on assay performance, will facilitate comparison of results regarding plasma IgG-mediated inhibition of CSA-specific IE adhesion obtained in different laboratories.

Email address for correspondence: tinado@sund.ku.dk

509 Cross-reactivity study between two rosetting variants, Palo Alto/VarO and R29/IT4 [MIM16670774]

Vigan-Womas Inès, Guillotte Micheline, Juillerat Alexandre, Baril Laurence, Bentley Graham, Mercereau-Puijalon Odile

The capacity of Plasmodium falciparum-infected erythrocytes (IE) to bind uninfected erythrocytes (rosetting) is associated with severe malaria in African children. Rosetting was mapped to the PfEMP1 DBL1α domain. A key issue for rational intervention design is serological cross-reactivity. We investigated this question for two rosetting clones, VarO and R29 whose NTS-DBL1α domains share 63.4% sequence identity. Mouse antibodies to soluble VarO or R29 NTS-DBL1α recombinant domains produced in Escherichia coli were used to establish homogeneous in vitro cultures of VarO and R29 parasites by panning or sorting. Cross-reactivity of antibodies elicited by individual NTS-DBL1α domains in mice or acquired in humans living in a holoendemic rural setting (Dielmo, Senegal) was studied on the recombinant proteins by ELISA and on the parasite expressed PfEMP1 by Western blot, surface immuno-fluorescence (S-IFA), mixed agglutination assay (MAA) and rosette disruption. The recombinant domain elicited high titers of antibodies reacting by S-IFA and rosette disruption on the autologous variant. Cross-reaction to the heterologous variant was observed by ELISA and Western blot, but there was no cross-reactivity by S-IFA and rosette disruption assays. In a holoendemic rural setting, seroprevalence to each recombinant domain and to the varO- or R29-IE surface by S-IFA was >90%. However, MAA clearly established that there was no cross-reactive antibody to the IE surface. Two rosetting variants expressing a closely related NTS-DBL1α domain do not display cross-reacting surface serotypes, indicating that a vaccine strategy based on PfEMP1 domains will need to combine multiple rosetting serotypes or engineer the antigen to broaden specificity.

Email address for correspondence: ines.vigan-womas@pasteur.fr

510 Mechanisms of malarial anaemia: Potential involvement of the Plasmodium falciparum low molecular weight rhoptry-associated proteins [MIM16658236]

Nancy W. Awah, Marita Troye-Blohmeg, Klavs Berzins, Jürg Gysin

Plasmodium falciparum malaria remains a global health problem. Anaemia is a constant feature of the disease and pregnant women and children below the age of 5 years remain the most afflicted. Its pathogenesis is multifactorial and incompletely understood. Among several factors, the destruction of erythrocytes is the most frequently observed cause of severe malarial anaemia (SMA) and the removal of non-parasitized red blood cells (RBCs) is thought to be the most important, accounting for approximately 90% of the reduction in hematocrit in acute malaria. Previous studies demonstrated that the tagging of normal RBCs with the parasite antigen RSP-2 (also designated RAP-2) due to either failed or aborted invasion by merozoites resulted in the destruction of these cells. The mechanisms mediating the destruction of normal erythrocytes in the development of SMA and the possible involvement of RSP-2/RAP-2 and other members of the low molecular weight rhoptry complex (RAP-1 and RAP-3) were investigated by flow cytometry using mouse monoclonal antibodies to RAP proteins. Antibodies to the rhoptry-associated proteins were found to recognise the surface of normal erythrocytes in a parasitaemia-dependent manner after merozoite release in P. falciparum in vitro cultures. These cells, as well as erythroblasts co-cultured with infected erythrocytes, could then be destroyed by either phagocytosis or lysis after complement activation. Our data suggest that cytophilic antibodies to the RAP proteins mediate the death of RSP-2/RAP-2-tagged erythroblasts in the presence of adherent monocytes. The mechanism of cell death is not yet fully known, but seems to involve both apoptosis and necrosis. These results suggest that the antibody response against RSP-2/RAP-2 and other members of the complex could trigger the destruction of RSP-2-tagged host cells. Taken together it appears that during SMA a defective bone marrow or dyserythropoiesis possibly due to erythroblast cell death, may overlap with the accelerated destruction of normal erythroid cells, either by opsonisation or complement activation further aggravating the anaemia which may become fatal. These observations could therefore have implications in the design, development and deployment of future therapeutic interventions against malaria.

Email address for correspondence: wanancy@yahoo.com

511 Hematological and inflammatory mediator analyses in Kenyan children with Plasmodium falciparum and bacteremia co-infection from a holoendemic malaria region [MIM16012851]

Gregory C. Davenport, Tom Were, Collins Ouma, James B. Hittner, Yamo Ouma, John M. Ong’echa, Douglas J. Perkins

Bacteremia is associated with malnutrition in resource-poor settings and has been shown to exacerbate malarial anemia. We previously demonstrated that children co-infected with malaria and bacteremia had significantly decreased parasitemia, but no exacerbation of anemia. Our objective was to determine the inflammatory mediator profile associated with the reduced parasitemia in co-infected children. Plasmodium falciparum-infected children (n = 192, aged <3 years) were divided into three categories: malaria alone, [Pf+]; Gram(−) bacteremia plus malaria, G[−]; and Gram(+) bacteremia plus malaria, G[+]. Since a pro-inflammatory milieu is known to control parasitemia, but adversely affect erythropoiesis, circulating levels of 16 inflammatory mediators were determined with a human bead-based multiplex assay. Compared to the [Pf+] group, both co-infected groups had lower parasitemia and increased granulocytes. In addition, both co-infected groups had significantly increased IL-4, IL-5, IL-7, IL-15, IFN-α, IFN-γ, and decreased TNF-α levels relative to the [Pf+] group. Additional significant findings included higher IL-1β, IL-1Ra, and lower IL-10 in the G[−] versus the [Pf+] group. The G[−] co-infected group also had significantly higher levels of WBC, granulocytes, IFN-γ, and GM-CSF than the G[+] group. Enhanced immune activation
in co-infected children appears to promote reduced parasitemia without adversely affecting anemia outcomes. By comprehensively examining the inflammatory profile in malaria mono-infected and co-infected children we can begin to elucidate both common and unique inflammatory pathways responsible for differing clinical outcomes in children with malaria and bacteremia.

Email address for correspondence: gdavenport19@yahoo.com

512 Cytokine gene polymorphism analysis in children exposed to malaria and/or helminths infections in Zimbabwe [MIM15047133]


Single nucleotide polymorphisms within the cytokine genes, TNF-alpha (−308 G/A), IFN-gamma (−874 A/T), TGF-β (T/C codon 10 and G/C codon 25) and IL-10 (−1082 G/A and −819 T/C) associated with protection and susceptibility to parasitic infections were examined in samples from school aged children in the Eastern district of Zimbabwe. Whole blood specimens were obtained from 492 children between the ages of 5 and 16 years, of which 27.2% were not infected and 72.8% infected with either malaria and/or different helminths. Genotyping was carried out using the Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR). The prevalence of samples with wild-type TNF-alpha (GG) associated with low cytokine production was 76.1%, while 22.2% and 1.6% were predictors of medium and high production of TNF-α, respectively. For IL-10 (position −819) the distribution of wild-type, heterozygotes and homozygotes was 59.6%, 22.9% and 17.5%, respectively and a similar analysis of the polymorphisms on position −1082 for IL-10 revealed that most of the samples were of the wild-type genotype. For IFN-γ (−874 A/T), 70.5% were wild-type (AA) which is associated with high cytokine secretion, with 4.4% (TT) and 25.1% (AT) associated with low cytokine production. Limited analysis on the sample population also revealed that at the TGF-β locus (T/C codon 10) 88.5% were homozygous (TT) which predicts high production of the cytokine whereas 9.2% were homozygous (CC). Similar analysis at another locus of TGF-β (G/C codon 25) showed that only 2.3% of the sample population was heterozygous (GC) which would also predict high TGF-β production. There were no statistically significant differences in the frequencies of TNF-α, IFN-gamma genotype polymorphisms among children infected with Plasmodium falciparum at baseline and at 6 weeks, 6 months and 12 months follow-up periods. Equal distribution of IL-10 (−819 G/A) and the rare occurrence of allele associated with low IL-10 (−1082 AA) production would suggest moderate to high IL-10 responses in the population analyzed. Finally, the high prevalence of TGF-β genotype (TT) predicting high cytokine production and the existence of homozygotes for IL-10 (high producer) might suggest the dominance of an anti-inflammatory environment when faced with acute P. falciparum infection in the samples analyzed. Studies are in progress to corroborate these preliminary conclusions with actual cytokine measurements. These studies also suggest a complex interaction between various cytokine gene polymorphisms and infections caused by malaria and/or helminths parasites.

Email address for correspondence: mduluza@medic.uj.ac.zw

513 Serum intercellular adhesion molecule-1 (ICAM-1) levels are invariably raised in children with malaria and significantly correlate with parasite density and haemoglobin status independent of age [MIM16699019]

Lungowe Sitali, Cecilia J. Shinondo, James Chipeta

Malaria is highly prevalent in sub-Saharan Africa and is the leading cause of morbidity and mortality accounting for approximately one million deaths annually. Disease severity and parasitaemia have been linked to cytokine levels, but their relationship requires further elucidation. Better understanding of these relationships would herald novel and effective management of malaria. To gain insight into potential relationships between TNF-α, IL-10, IL-12 and ICAM-1 and malaria disease severity, children of ages 12–144 months presenting with severe and uncomplicated malaria (Cases) including healthy children (Controls) were assessed as regard their respective serum cytokine levels, haemoglobin status as well as parasitaemia rate at the time of enrollment and 3 days later after commencement of anti-malaria treatment. TNF-α, IL-10, IL-12 levels and ICAM-1 expression were determined using single monocalonclal antibodies to the respective cytokines using sandwich ELISA at 450 nm wavelength (R and D Systems; TNF-α-DTA00C, IL-10-D1000B, IL-12-H5120 and ICAM-1-BBE1B). IL-12 levels were determined using a similar sandwich ELISA method but at 490 nm wavelength. Parasite densities were determined using Giemsa stained thick and thin blood films while haemoglobin status was assessed using an automated full blood count (FBC) coulter counter. Data was initially entered and computed into Microsoft excel spread sheets, and then converted and analyzed using SPSS software (SPSS version 11.0). Differences among the mean concentration of cytokine in various groups were evaluated using Kruskal–Wallis and Mann–Whitney tests. Correlations between variables were assessed by Spearman’s coefficient. Results were expressed as mean (plus/minus) standard deviation. Of the 102 participants recruited into the study at University Teaching Hospital, Lusaka (UTH) and three peri-urban and rural health facilities (Chongwe and Mpulungu District Referral Rural Health Centres and Mpongwe Mission Hospital), 20 had severe malaria, 55 had uncomplicated malaria and 27 were healthy controls. Parasitaemia rates were found to be relatively similar in children with severe malaria and those with uncomplicated malaria and parasite densities did not correlate to disease severity. However, as also reported elsewhere, levels of TNF-α (picogram per milliliter) were found to be higher in patients compared with healthy controls (31.7 ± 3.4/17.4 ± 16 severe/uncomplicated malaria and 8.9 ± 6.67 in the controls). No significant reduction in these levels was observed after 3 days of treatment. Levels of IL-10 in patients with uncomplicated malaria were not significantly higher as compared to those with severe malaria (602.56 ± 1032.89 and 409.59 ± 627.39, respectively). Furthermore, levels of IL-10 were significantly higher in patients with uncomplicated (602.56 ± 1032.89) and severe (409.59 ± 627.39) malaria than in controls (11.21 ± 28.16). These levels of IL-10 significantly reduced after 3 days of treatment. There was no significant difference in the levels of IL-12 between patients and healthy controls nor was there any substantial and significant reduction in the levels of IL-12 after 3 days of treatment. Finally, ICAM-1 (nanograms per milliliter) levels were significantly higher in patients (447.43 ± 380.53 and 392.60 ± 167.38) than in the healthy controls (196.06 ± 125.19; P value <0.001) and the levels in severe and uncomplicated malaria groups were similar (P value = 0.88). Interestingly, the levels of ICAM-1 remained unchanged in uncomplicated malaria and significantly reduced in severe malaria 3 days after commencement of treatment. In addition, there were correlations observed
between cytokine (TNF-α, IL-10, ICAM-1) levels and temperature, haemoglobin status as well as parasite density underscoring the already existing evidence that cytokines play important roles in the pathogenesis and severity of malaria. In particular, there was a significant association between TNF-α (rho = −0.24, P < 0.05), IL-10 (−0.43, P < 0.001), ICAM-1 (rho = −0.34, P 0.05) and Haemoglobin (Hb) status indicating that when Hb was low, the named cytokines were high. Similarly, there was significant correlation between parasite density and cytokine levels (ICAM-1: rho = 0.45, P<0.001; IL-10: rho = 0.59, P<0.001; TNF-α: rho = 0.23, P 0.029). More interesting and remarkable was the finding that, unlike the other cytokines (IL-10 and TNF-α) age has no significant influence on ICAM-1 serum levels in comparison to parasitaemia and Hb status. This study reveals that serum levels of TNF-α, IL-10, IL-12 and ICAM-1 are invariably and significantly raised in malaria as compared to healthy controls with strong correlation to parasite density, body temperature and haemoglobin levels signifying the important roles that cytokines may play in the pathogenesis of malaria.

Email address for correspondence: lungwesitali@yahoo.com

514 Cohort study of the association of antibody levels to AMA1, MSP119, MSP3 and GLURP with protection from clinical malaria in Ghanaian children [MIM16736495]

Daniel Dodo, Anastasia Aikins, Kwadwo Asamoah Kusi, Helena Lampert, Ed Remarque, Paul Milligan, Samuel Bosomprah, Roma Chilengi, Yaa Dife Osei, Bartholomew Dicky Akanmori, Michael Theisen

The role played by antibody-mediated immune responses is important for natural protection against clinical malaria. Estimates however, of this association from studies in different settings are conflicting. This study assessed the relationship between antibody responses to four malaria vaccine candidate antigens and protection from clinical malaria in a cohort of Ghanaian children. Standardized ELISA protocols were used to measure isotype and IgG subclass levels to AMA1, MSP119, MSP3 and GLURP in plasma samples obtained from 352 Ghanaian children, aged 3–10 years preceding a 9-month malaria surveillance. The incidence rate of malaria was 0.35 episodes per child per year. Isotype and IgG subclass levels for all antigens investigated increased, while the risk of malaria decreased with age. After adjusting for age, higher IgG (MSP119, MSP3 and GLURP) and IgM (MSP119, MSP3 and AMA1) levels were associated with decreased malaria incidence. For IgG subclasses, only IgG1 to MSP119 was associated with reduced incidence of malaria. A combined model including the IgG, IgM and IgG subclass levels for all four antigens showed only IgG1 [(0.80 (0.67–0.97), p = 0.018)] and IgM [(0.48 (0.32–0.72), p < 0.001)] to MSP119 to be independently associated with protection from malaria. Using standardized procedures, the study has confirmed the importance of antibodies to MSP119 in reducing the risk of clinical malaria in Ghanaian children, thus substantiating its potential as a malaria vaccine candidate.

Email address for correspondence: aocran@noguchi.mimcom.org

515 Immunological profile of two synthetics constructs of the CS protein and protection against clinical malaria in malaria endemic setting in Burkina Faso [West Africa] [MIM16696004]

Diarra Amidou, Alfred Tiona, Issa Nebie, Andre Lin Ouendoa, Issiaka Soultoua, Alphonse Ouendoa, Jean B. Yaro, Esperance Ouedraogo, Edith C. Bougouma, Souleymane Sanon, Amadou T. Konate, Adama Gansane, Giampietro Corradine, Sodimon B. Siri

Identification of antigens with reliable and reproducible immune correlates of protection against Plasmodium falciparum infection could be important in the development and testing of malaria vaccine candidates. IgG against two synthetic constructs (MR48 and MR178) representing the N- and C-terminal domains of the CS were used to assess the seasonality and the relationship between antibody levels and the protection against clinical malaria. Study was carried out with children less than 5 years from four villages belonging to Saponé Health District, in Burkina Faso. We performed two clinical and parasitological cross-sectional surveys at the low and the peak of malaria transmission seasons. During each survey, thick and thin blood films were prepared for parasites check and 5 ml of venous blood taken and plasma used for total IgG measurement by ELISA. Children were then actively followed by being home visited twice a week to record malaria cases for a year. No relationship was found between the IgG levels and age for both peptides (P = 0.69 (low transmission) and 0.16 (peak of transmission)) for MR48 and P = 0.86 and 0.93 for MR178). Geometric means of IgG levels to CS N-terminus were similar at the low and at the peak of transmission (1.1, 95% CI: 1.0–1.2 vs. 1.2, 95% CI: 1.1–1.3; P = 0.51 for MR48 and 1.2, 95% CI: 1.0–1.3 and 1.1, 95% CI: 1.0–1.1; P = 0.24 for MR178). IgG to these two constructs may be associated with protection; however investigation on the IgG subclass responses may help to better understand the type of the induced protective immunological responses.

Email address for correspondence: diarra.cnrrf@fasonet.bf

516 IFN-γ and IL-10 levels in patients co-infected with tuberculosis and malaria in Ibadan, Nigeria [MIM15998746]

C.I. Anumudu, C. Ezenamuo, I.B. Cadmus

The increasing prevalence of TB in Nigeria may be due in part, to the emergence of HIV/AIDS and co-infection, especially with malaria. Modulation of the inflammatory response in Mycobacterium tuberculosis co-infection with malaria may be important, because protection against malaria requires type 2 responses. Objectives: This work determined the levels of pro-inflammatory cytokine, IFN-γ and anti-inflammatory cytokine, IL-10, in plasma samples of volunteers with TB and malaria co-infection. Fortnight collections of sputum and blood samples were made from 101 volunteers attending the outpatient departments of four hospitals in Ibadan; who were treated for, or diagnosed with TB. They were recruited from the University Health Centre, University of Ibadan, Adeoyo Maternity Hospital, Yemetu, Government Chest Hospital, Jericho, and Catholic Hospital, Oke-Ofa, Oluyoru. Sputum samples were stained for acid fast bacilli by Ziehls-Nelson stain, and cultured for 8 weeks on LJ medium. Blood was examined for malaria parasites by microscopy, and plasma was used to determine IFN-γ and IL-10 cytokine levels by ELISA. A positive diagnosis for TB was made for 59 (58.4%) cases of the study population, while 21 (20.8%) of the 101 volunteers were positive for Plasmodium falciparum. By culture, 14% (6/43) positive TB cases were diagnosed. IFN-γ levels were higher in patients with malaria infection alone. However IL-10 levels were higher in those with co-infections than in those with either malaria or TB infections alone. The higher levels of IFN-γ in malaria compared to values in both TB and co-infected is consistent with the high levels of
IFN-γ produced by primed T cells in response to secondary malaria infections or molecules from other pathogens such as bacteria and fungus. Which cytokine dominates in co-infection will to some extent depends on which of these parasites was first to infect the host.

Email address for correspondence: cianumudu@yahoo.com

517 Antibody responses to a C-terminal fragment of the Plasmodium falciparum blood-stage antigen Pf332 in Senegalese individuals naturally primed to the parasite [MIM155831053]

The protective role of IgG and its subclasses has been investigated for many malaria antigens, but IgM and IgE has not been well elucidated. Studies have shown that antibodies from humans continuously exposed to malaria recognize the Plasmodium falciparum asexual blood-stage antigen Pf332. Using ELISA, we measured the serum levels of IgM, IgE, IgG and subclasses to both C-terminal fragment of Pf332 (C231) and crude P. falciparum extract from Senegalese individuals (4–87 years). All antibody classes were detected in all sera tested and antibody levels increased significantly with age, in the older age-groups most of the donors displayed antibodies to C231. Anti-C231 antibodies in the different IgG subclasses differed from that shown by crude P. falciparum antigen. IgG1/IgG2 ratio was considerably lower for C231 than for crude antigen. IgG2/IgG3 ratio regarding crude antigen was higher in all age-groups. The IgG2 and IgG3 levels against C231 were similar, except for children aged 4–9 years, where IgG3 was higher. In relation to malaria attacks, analysis showed that individuals who did not experience any malaria attack during follow-up had higher anti-C231 responses (IgM, IgG class and subclass and IgE) than those who did, but no significant association was seen after multivariate analysis, controlling for age, number of infected bites and parasitemia. This study shows that C231 harbours epitopes recognized by antibodies elicited by natural P. falciparum exposure.

Email address for correspondence: halima@imun.su.se

518 The Afro-Immuino Assay network and capacity building project for malaria vaccine development in Africa [MIM16692166]

Afro-Immuino Assay (AIA) network project aims at developing standardized immunological assays that could form part of criteria for validating malaria vaccine candidates, provide baseline information for clinical trials and enhance quality assured laboratory capacity. Phase-1 focused on using harmonized ELISA and statistical methods to assess isotypes and IgG subclass levels against AMA1, GLURP, MSP1-19 and MSP3 from cohort samples in relation to protection from clinical malaria. Harmonized protocols were used to measure isotype and IgG subclass levels to these antigens in plasma samples obtained from 352 Ghanaian and 286 Burkinabe children, aged 6 months to 15 years preceding a 9-month malaria surveillance. Data from the two sites showed IgG1 [(0.80 (0.67–0.97), p = 0.018)] and IgM [(0.48 (0.32–0.72), p = 0.001)] levels to MSP1-19 independently correlated with protection from malaria in Ghanaian children while IgG1 to AMA1 (0.87 (0.78–0.97), p = 0.013) and IgG3 to GLURP (0.82 (0.72–0.94), p = 0.004) were associated with reduced risk to malaria in Burkinabe children. MSP1-19, GLURP and AMA1 antibodies were associated with reduced risk of clinical malaria. Difference between sites may result from differences in malaria transmission. GIAs required to confirm functional roles of antibodies correlated with protection. Current Phase-2 has been initiated in eight African sites (Ghana, Burkina Faso, Mali, Kenya, Tanzania–NMRI, Tanzania–KCMC, Uganda and Sudan). ELISA, T-Cell responses (ELISPOT), intracellular cytokine staining and GIAs to determine functionality of specific antibodies/correlates of protection to clinical malaria will be done. Immunological and genetic factors important for acquiring protection to clinical malaria in African children will be ascertained (meta-analysis), N–S/S-S technology transfer and capacity building will be done across the eight sites.

Email address for correspondence: DDodoo@noguchi.mimcom.org

519 Transmission Reduction Activity (TRA) of Plasmodium falciparum malaria in Senegal [MIM16647266]
L. Gadiaga, A. Gaye, M.O. Ndiath, L. Konaté, C. Sokhna, C. Boudin, J.F. Trape

TRA is the immunity against the sexual forms of malaria parasites found in individuals living in endemic areas. The objective of this study was to measure TRA in two different epidemiological areas in Senegal. We used Direct Membrane Feeding (DMFA) in two ways: (i) We used classical DMFA to measure the TRA of a sample of gametocyte carriers in a hypoendemic area with short seasonal malaria transmission (Thiès). (ii) We used heterologous DMFA to measure TRA in the general population of a holoendemic area with permanent malaria transmission (Diélimo). We measured three parameters: (i) Percents of inhibiting sera: 43.8% (gametocyte carriers of Thiès) and 31.6% (general population of Dielimo). (ii) Average TRA intensity: 0.32 (gametocyte carriers) and 0.38 (general population). (iii) The role of TRA in inhibition total: 0.46 (gametocyte carriers) and 0.33 (general population). Gametocyte density, which is the principal antigenic stimulus, has an impact on TRA in Thiès. In both areas, the age of the individuals seems to have no effect on TRA. Transmission reduction activity has an epidemiologic role in both areas and may depend on the gametocyte density. The TRA estimation in the general population can be useful to assess the efficacy of a vaccine against malaria. The DMFA proved useful. Compared to the SMFA standard technique, it is cheaper, better suited for field work and can be performed in greater number.

Email address for correspondence: gadiagalibass@yahoo.fr

520 Antibody responses to GLURP–MSP3 hybrid antigen and protection from clinical malaria in Ghanaian children [MIM16395432]
Helena Lampyte, Daniel Dodoo, Anastasia Ocran, Michael Ofori, Dominic Edoh, Michael Theisen

In the advent of drug resistance to malaria and insecticide resistant strains, development of a protective malaria vaccine may lead to decrease in malaria morbidity and mortality especially in children. Anti-GURP and MSP3 antibodies are involved in antibody-dependent cellular inhibition that may lead to protective immunity. Antibody responses to these antigens and possibly when combined will be useful in future malaria vaccine. The 200-kDa Glutamate Rich Protein (GLURP) and 48-kDa Merozoite Surface Protein-3 (MSP3) are found on the surface of merozoites and infected erythrocytes. Plasma samples were from a cohort of 273 children, 3–15 years of age. The antibody levels to GLURP and MSP3, and GLURP–MSP3 hybrid antigens were measured by ELISA isotype
and IgG subclass levels for all antigens increased with age, whilst the risk of malaria decreased with age. GLURP IgG1 (p = 0.04) levels correlated with reduced risk of clinical malaria after correcting for age, while that to MSP3 approached significance (0.05). Interestingly, only IgM levels to GLURP and MSP3 were associated with protection in a combined model (P = 0.01). Antibody levels to both antigens were higher in protected than in susceptible individuals and correlated with protection. The study confirms the association of IgG1 levels to GLURP with protection from malaria. The role and mechanisms that IgM to the two antigens play in malaria immunity may be important, since IgM to both antigens in a combined model was strongly associated with decreased risk of malaria. Development of a hybrid GLURP–MSP3 vaccine is therefore suggested.

Email address for correspondence: hnarrey@noguchi.mimcom.org

521
Identifying immunologic markers that protect against malaria infection in an endemic area in Mali [MIM16690015]
Sangaré Cheick Papa Oumar, Niangaly Hamidou, Diallo Nouhoum, Roussilo Christian, Druihe Pierre, Doumbo Ogobara, Dijmde Abdoulaye

This study seeks to identify immunologic responses that protect against malaria. 300 volunteers 12 months or older were recruited in Pongonon, a village where malaria is endemic with seasonal peaks. Baseline, prevalence of malaria and other parasitic infections were measured. The cohort is being followed through cross-sectional surveys (July 2007, November 2007, and May 2008) and the permanent record of all clinical malaria. Plasma samples were collected to measure the prevalence and titers of antimalarial antigens including MSP1, MSP3, GLURP, SERP, LSA1 and AMA1. These immunological data will be correlated with the number of episodes per person. We found low levels of heminthes and schistosomiasis in the population, 2.6% and 0% respectively in the dry season and 8.4% and 7.7% respectively in the rainy season. Malaria parasite prevalence was found to be 40.6% and 80% during the dry and rainy seasons, respectively. In the 1–15-year age group, 56.5% were diagnosed with one or more episode of clinical malaria during the 2-year study compared to only 9.1% in the 16 years and older group (P < 0.05). We found a maximum of seven clinical malaria episodes in the younger group and three episodes in the older group. Preliminary clinical results show that adults suffer from fewer episodes of malaria than do children in this setting. Prevalence and titers of plasma antibodies are being measured by ELISA and will be presented at the meeting.

Email address for correspondence: Oumar@mrtchko.org

522
Impact of exposure to Anopheles bites on the development of human antibody response to Plasmodium falciparum in children living in malaria endemic area [MIM15314350]
J.B. Sarr, B. Samb, I. Dia, S. Senghor, C. Thiam, A.M. Schatch, S. Guindo, F. Simondon, L. Konate, G. Riveau, F. Remoue

Numerous ecological and epidemiological factors could modulate the anti-malaria immunity. Among these factors, the exposure to Anopheles bites and, especially the active components of Anopheles saliva, could play a key role on the development of immune response to Plasmodium falciparum in exposed individuals. The objective of the study was to evaluate the impact of the exposure to Anopheles bites (mainly uninfected), on the development of antibody (Ab) response against P. falciparum in children living in malaria area. A multi-disciplinary study was conducted in two Senegalese villages where the intensity of malaria transmission/exposure to Anopheles species was differed (Mboula = low; Gankette Balla = high exposure to Anopheles bites). In each village, epidemiological, parasitological, entomological and immunological data were followed in children (1–9 years; n = 120). IgC, IgG1, IgG3 response directed to P. falciparum whole schizont extract (WSE) and specific antigens (CSP10, TRAP, SALSA, STAR, GLURP, LSA1, LSA3, MSP1, and MSP2) were determined before (June), at the peak (September) and after (December) the period of transmission by ELISA and multiplex fluorescent microsphere-based assays. In Mboula, the peak of malaria exposure was followed by a considerable increase of anti-WSE IgG levels whereas low and constant specific IgG response was observed through the year in Gankette. Interestingly, anti-WSE IgG1 levels were significantly higher in Mboula whereas specific IgG3 response predominated in Gankette. These results suggest that specific IgG and isotype IgG responses could be regulated according to the nature (Anopheles species) and/or the intensity of exposure to Anopheles bites. In addition, IgG response to several antigens (CSP, TRAP, GLURP, LSA1, and LSA3) progressively decreased from June to December in children negative for malaria infection. It suggested an immuno-suppression of IgG responses to specific antigens during the season of exposure to Anopheles bites. Altogether, this study shows that the development of anti-malaria Ab response was profoundly different according to areas where the exposure is dependent on the intensity and/or species of Anopheles. The influence of Anopheles saliva could be thus involved in the observed immune regulation.

Email address for correspondence: sarrjb@ird.sn

523
Anti-inflammatory, pro-inflammatory cytokines and haematological indices in children with sickle cell disease suffering from uncomplicated falciparum malaria [MIM15091367]
Y.M. Tatfeng, D.E. Agbonlahor

Background: A prospective study carried out on children below 5 years between the months of June 2007 and February 2008, some anti- and proinflammatory cytokines, i.e. Interferon-gamma (IFN-γ), Interleukin-2 (IL-2) and Interleukin-4 (IL-4), Interleukin-10 (IL-10), respectively, and haematological indices of children with sickle cell anaemia suffering from uncomplicated malaria were assessed. The cytokines were determined by Enzyme Linked Immunosorbent Assay, CD4 and CD8 were assessed using Dynabeads T4–T8 quantification protocol and the haematological indices were analyzed using standard haematological techniques. Serum levels of IFN-γ of homozygote SS subjects with malaria was not significantly different from levels in homozygote SS control subjects without malaria (P > 0.05), however IFN-γ was significantly higher in heterozygote AS and homozygote AA genotyped subjects with malaria when compared to the homozygote SS subjects with malaria (P < 0.05). IL-2 was significantly higher AS subjects with malaria than AA and SS subjects with malaria (P < 0.05). On the other hand IL-4 and IL-10 levels in homozygote SS subjects with malaria were not significant when compared to AS and AA subjects with malaria (P > 0.05). The CD4 and CD8 counts in SS subjects with malaria were significantly higher than values obtained in AS and AA subjects with malaria (P < 0.005). There was a significant increase in the total white cell and monocyte count of SS subjects with malaria when compared to AS and AA subjects with malaria. Impaired immunity in sickle cell anaemia has contributed immensely to increased rate of mortality and morbidity due to malaria.

Email address for correspondence: youchtou@yahoo.com
524 Natural resistance against severe malaria in Ghanaian children depends on Toll-like receptors [MIM15068329]

John K.A. Tetteh, Bamela Q. Goka, George Obeng-Adjei, Catherine Jacquemot, William Ekloch, Charlotte Behr, Bartholomew D. Akanmori

Toll-like receptors (TLRs) are involved in innate immune responses against infections by the induction of cytokines. We assessed the in-vitro capacity of leukocytes of Ghanaian children with Plasmodium falciparum malaria to produce TNF-α, IL-6, IL-10 and IL-1ra in response to TLR-mediated ligands. Heparinised blood samples from children with severe malarial anaemia (SA), cerebral malaria (CM) and uncomplicated malaria (UM) were stimulated with tripalmitinoylated lipopeptide (PAM3CSK4)(TLR-2), Staphylococcus aureus (SAC)(TLR-2), Escherchia coli lipopolysaccharides (LPS)(TLR-4) and heat killed Salmonella typhi (HKSal)(TLR-4) and incomplete culture (SAC) (TLR-2), Escherchia coli lipopolysaccharides (LPS)(TLR-4) and heat killed Salmonella typhi (HKSal)(TLR-4) and incomplete culture medium (negative control). The plates were incubated at 37.4 °C and in 5% CO2 atmosphere in a humidified CO2 incubator for 22 h after which the supernatants were tested for the cytokines by ELISA. Significantly higher levels of TNF-α, IL-6 and IL-1ra were measured in response to PAM3CSK4 in severe malaria (SA, CM) patients than in UM patients. Similarly, significantly higher levels of IL-1ra was measured in response to HKSal in children with severe malaria than in UM patients. In addition, significantly higher levels of IL-10 and IL-1ra were measured in response to PAM3CSK4 and SAC in SA than in UM patients. In response to LPS, significantly higher levels of TNF-α, IL-6, IL-10 and IL-1ra were measured only in SA, whereas levels of TNF-α in supernatants of CM were significantly reduced as compared with UM. Our data suggest that TLRs may be involved in the pathogenesis of severe malaria.

Email address for correspondence: jtetteh@noguchi.mimcom.org

525 Maternal and neonatal immune responses to P. falciparum infection [MIM14905094]

Samia Ali Omer, Eltahir Awad Gasim Khalil, Hashim Abdelrahman Ali, Abdalla Hassan Sharief

Plasmodium falciparum infection during pregnancy has severe consequences for both mother and foetus and can lead to the transplacental passage of malarial Ags that are capable of inducing neonatal immune responses. Out of the 836 volunteered women followed monthly during their antenatal period immune responses of 228 Sudanese women and their neonates were studied. The blood was requested for malaria parasite by microscopy and PCR. Neonatal and maternal cytokine and antibody responses to merozoite surface protein-1 (MSP-19) in infant mother pairs were examined. Maternal and cord malarial IgG levels were correlated ($p < 0.001$). Anti-MSP-19 IgG levels in neonates whose mothers had malarial infection during antenatal period were higher than those who were parasite negative. Cord blood cells (CBC) from neonates released significantly less interferon (IFN-γ) and higher amount of interleukin 10 (IL-10) when activated with the PHA into cell culture supernatants than peripheral blood cells (PBMCs) of mothers. In response to P. falciparum antigen PBMC from mothers infected during her antenatal period secreted significantly more IFN-γ than non-infected women ($p = 0.0001$) and decreased IL-10 production ($p = 0.005$). Moreover PBMCs produced significantly higher level of IFN-γ ($p = 0.0001$) than that of the paired cord samples and less IL-10. Anti-MSP-119 IgG was significantly higher in mothers with increased IL-10 ($p = 0.007$). A mixed Th1/Th2 immune response was seen most commonly in women who had confirmed positive blood films P. falciparum infections; on the other hand neonates born of malaria-positive mothers mounted predominantly Th2 type immune responses. The study showed high level of exposure to malaria infection mirrored by the high level of transplacental transfer of malarial antibodies found in the cord.

Email address for correspondence: samiaomer@hotmail.com

526 Effect of placental infection, parity and gender on antibody reactivity to Plasmodium falciparum antigens [MIM14909331]

Alfredo Mayor, Eduard Rovira, Sonia Macheco, Ruth Aguilar, Mauricio H. Rodríguez, Llorenç Quintó, Alfons Jiménez, Betuel Sigaque, Inacio Mandomando, Carlota Dobaño, Chetan E. Chitnis, Pedro L. Alonso, Clara Menéndez

Women are at higher risk of infection and disease when pregnant. Regained resistance to malaria with increasing parity has been explained on the basis of an acquisition of immunity against placental forms of the parasite. Gender-specific and parity-dependent antibody recognition of the surface of infected erythrocytes remains the most unequivocal serological marker of relevance in terms of malaria in pregnancy. The effects of placental malaria infection, host sex and parity were evaluated by measuring IgGs against the erythrocyte surface antigens of mature-stage parasites infecting pregnant and non-pregnant hosts and merozoite recombinant antigens, as well as total and asymmetrical IgGs, in plasma samples from Mozambican pregnant women, men and children. Determinations were made by flow-cytometry and ELISA. Plasma samples from pregnant women with placental malaria were found to have higher levels of antibodies to Plasmodium falciparum antigens as well as total and asymmetrical IgGs, than both pregnant women without placental malaria and men. IgG against maternal ($p = 0.010$), non-maternal isolates ($p = 0.004$) and total IgGs ($p = 0.047$) increased with parity. Recognition of non-maternal isolates was higher for girls than for boys ($p = 0.037$). Parity- and gender-dependent recognition are not serological features exclusive to maternal parasites. The increased susceptibility of primigravidae women to malaria might be explained by pregnancy-associated physiologic factors conferring advantage to malaria infection.

Email address for correspondence: agmayor@clinic.ub.es

527 PfHsIV: Mitochondrial localization of a prokaryotic protease homolog in Plasmodium falciparum [MIM16684497]

Serena Tschan

No abstract.

Email address for correspondence: valerie.dacremont@unibas.ch

528 Multi-faceted impact of MSP-1p42 specific antibodies on blood stages of P. falciparum [MIM16683514]

Elke S. Bergmann-Leitner, Elizabeth H. Duncan, Evelina Angov

Antibodies are the main effector molecules in the defense against blood stages of the malaria parasite P. falciparum. Previous pre-clinical and clinical studies have shown that antibody responses to fragments of MSP1p42 correlate with protection and/or reduced parasite densities. Understanding the mechanisms by which vaccine-induced anti-blood stage antibodies work in protecting
against malaria is essential for vaccine design and testing. The functional activity of anti-MSP1 antibodies was characterized by (1) Giemsa stained blood smears from cultures at various time points in one life cycle, (2) flow cytometric analysis discerning between invasion and growth inhibition, (3) pLDH-based GIA assays, (4) affinity purification of fragment specific immunoglobulins and (5) confocal microscopy. We demonstrated that the mode of action of anti-MSP-1p42 antibodies differs among the various parasite strains: anti-MSP-1p42 antisera act mainly through invasion-inhibitory mechanisms against FVO parasites by either preventing the schizont from rupturing or agglutinating the merozoites upon release. Anti-MSP-1p42 antibodies do not prevent the rupture of 3D7 schizonts; instead they agglutinate merozoites and arrest the development of the young parasite at the early trophozoite stage, thus act by growth- and invasion inhibitory mechanisms. The inhibitory activity on mature schizonts is mediated by antibodies entering the infected erythrocyte as shown by labeling of developing merozoites with fluorochrome-conjugated anti-MSP-1p42 antisera. Anti-MSP1 specific antibodies affect blood stage parasites differently depending on the MSP1 allele. Whether these strain-specific differences are associated with different clinical outcomes and/or vaccination success needs to be determined. Email address for correspondence: agmajor@clinic.ub.es

529
The influence of topography on malaria exposure and malaria sensitivity in the western Kenya highlands [MIM16669878]
C.L. Wanjala, R.S. Shivairo, R.O. Odhiambo, A.K. Githeko, J.N. Waitumbi

Epidemic malaria continues to be a threat to human populations living in the western Kenya highlands. A weather based epidemic prediction model was developed to forecast the risk of out breaks. Recent research has shown that there are other non-climatic conditions such as topography and immunity that can affect the sensitivity of a site to epidemics. This study aims to examine how terrain in the highlands affects the exposure and sensitivity of a site to malaria. The study was conducted in two highland sites, one with a “U” and the other with a V-shaped valley. Exposure to malaria parasites was tested using Circum-sporozoite protein (CSP) and Merozoite surface protein (MSP) immunochromatographic antibody test. Prevalence of infection was tested through the microscopic examination of thick and thin blood smears. The prevalence of the CSP and MSP antibodies in the U-shaped valley was found to be 9.97% at the valley bottom and 1.08% at the hilltops whereas in the V-shaped valley the prevalence of the antibodies was 0.74%. The prevalence of malaria infection was 15.33% at the valley bottom and 2.19% at the hilltops. In the V-shaped valley the prevalence of the antibodies was 0.74%.

530
New analysis of mitosis and the cell division cycle of intra-erythrocytic Plasmodium falciparum infection using improved in situ hybridisation methodology to position chromosomes and telomeres in the nucleus [MIM16705845]

Sulfadoxine–pyrimethamine is used for intermittent presumptive treatment of malaria during pregnancy in the Democratic Republic of Congo. However, the level of resistance is high, and there is a need for alternative drugs. Artemisinin-based combinations are being considered. Unfortunately, little is known about the pharmacokinetics and safety of artemisinin derivatives in pregnancy. This information is crucial for optimal use. We hypothesized that there are lower levels of AS and DHA in pregnant women compared to controls, particularly during the third trimester. Email address for correspondence: dearnot@sund.ku.dk

531
New analysis of mitosis and the cell division cycle of intra-erythrocytic Plasmodium falciparum using improved in situ hybridisation methodology to position chromosomes and telomeres in the nucleus [MIM16705845]
David E. Arnot, Dominique Bengtsson

Clinical malaria is largely a consequence of the repeated rounds of intra-erythrocytic replication of parasitic zoites of a few species of protozoa of the genus Plasmodium. Surprisingly, the nuclear divisions occurring during the malaria parasite’s intra-erythrocytic schizogony remain a very poorly understood part of the cell cycle of this important parasite. An example of the many puzzling features of this process is that these divisions often result in numbers of daughter schizonts (6, 18, 20, etc.) that are difficult to explain as the results of the geometric progression of a simple binary division (1, 2, 4, 8, 16, etc.). While the nature and sequence of mitosis-related events in the Plasmodium cell cycle remain uncertain there is no reliable map on which to position many of the landmarks revealed by molecular biology and transcriptional analysis. The cell biological problems essentially revolve around the uncertain fate of the nuclear envelope during schizogony and the relative timing of the S and M phases during this process. By using laser scanning confocal microscopy and improved nuclear preservation techniques for in situ hybridization with fluorescently labeled oligonucleotide probes for chromosomes and telomeres, we have been able to analyze the behavior of P. falciparum chromosomes in better resolved images of the mitotic nuclear divisions of schizogony. Email address for correspondence: dearnot@sund.ku.dk

532
New analysis of mitosis and the cell division cycle of intra-erythrocytic Plasmodium falciparum using improved in situ hybridisation methodology to position chromosomes and telomeres in the nucleus [MIM16705845]
David M. Menge, Melissa Riedesel, Robert O. Opoka, Chandy C. John

Plasmodium falciparum infection leads to different clinical outcomes namely, cerebral malaria (CM), severe malarial anemia (SMA), uncomplicated malaria (UM) and asymptomatic parasitemia (AP) hence the need to determine the molecular basis of the diversity of clinical outcomes. Email address for correspondence: dearnot@sund.ku.dk

533
Extraction of DNA from frozen blood clots for malaria genotyping by PCR [MIM16768789]
Klara Lundblom, Marianne Lebbad, Anna Färnert

Genotyping of Plasmodium falciparum by PCR has become a well established technique applied in drug trials and in molecular
epidemiological studies. Frozen blood clots are a potential, but often disregarded, source of parasite DNA. Purifying DNA from frozen clotted blood involves challenges since the clot initially needs to be totally dispersed in order to avoid protein contamination and to achieve optimal extraction. We have evaluated different methods for the homogenization of clots for DNA purification. Blood clots with different parasitemias (10–1000 p/μl) were prepared from cultured parasites and stored frozen. Field samples were also included. Three methods for clot dispersion were tested: (i) clot protocol recommended by Puregene; (ii) centrifugation of samples through the plastic sieves Clotspin Basket (Qiagen); (iii) shaking of samples with Mini BeadBeater (Biospec), without adding beads. DNA was extracted using Puregene kits (Qiagen). Quantity and purity of DNA was measured by Nanodrop. PCR sensitivity was tested with a nested PCR for msp2. Blood clots were totally dispersed and easily pipetted after 30 s shaking by Mini BeadBeater. The two other methods failed to dissolve the clot. The sensitivity of PCR detection from clot samples was highest with the Mini BeadBeater (threshold 10 p/μl), compared to the Clotspin method (100 p/μl) and Qiagen clot protocol (800 p/μl). Shaking of clots with Mini BeadBeater resulted in homogenised material and high sensitivity of PCR detection. This simple and fast method minimizes manual handling of individual samples and enables analyses of low parasitemias in stored blood clots.

Email address for correspondence: klara.lundblom@gmail.com

534 Plasmodium ovale exists as two non-recombining species in tropical Africa [MIM16669382]

Colin Sutherland, Colin J. Sutherland, Debbie Nolder, Charlie Jennison, Mary Oguike, Martina Burke, Peter L. Chiodini, Spencer Polley

The burden of malaria due to infection with Plasmodium ovale has been greatly underestimated, particularly in Africa. Studies of ribosomal gene sequence variation in P. ovale world-wide suggest dimorphism into classical and variant forms. We examined four unlinked genetic characters in a sample of 40 parasite isolates from 8 different African countries. Each character was dimorphic, and there was perfect correspondence among the four characters, confirming the absence of recombination between the dimorphic forms. Both forms were sympatric in Uganda, Sierra Leone, Ghana and Nigeria, consistent with continent-wide dispersal of both. We conclude that two sympatric species of the parasite are circulating in Africa and name the classic form P. ovale wallikeri, and the variant form P. ovale wallikeri. We propose that an original speciation hypothesis was tested with a nested PCR for msp2. Blood clots were totally dispersed and easily pipetted after 30 s shaking by Mini BeadBeater. The two other methods failed to dissolve the clot. The sensitivity of PCR detection from clot samples was highest with the Mini BeadBeater (threshold 10 p/μl), compared to the Clotspin method (100 p/μl) and Qiagen clot protocol (800 p/μl). Shaking of clots with Mini BeadBeater resulted in homogenised material and high sensitivity of PCR detection. This simple and fast method minimizes manual handling of individual samples and enables analyses of low parasitemias in stored blood clots.

Email address for correspondence: colin.sutherland@lshtm.ac.uk

535 Introduction des tests de diagnostic rapide du paludisme (TDR) au Sénégal [MIM16689744]


Le traitement précoce et correct des cas de paludisme constitue une stratégie majeure du Programme National de lutte contre le Paludisme (PNLP) au Sénégal. L’objectif d’ici 2010 est la confirmation de 80% des cas de paludisme vus dans les structures de santé. Pour atteindre cet objectif, de nouvelles stratégies ont été initiées afin de relever le niveau de confirmation. Il s’agit entre autre de la mise à l’échelle des TDR après une recherche opérationnelle dans 10 districts sanitaire du pays. Cette mise à l’échelle de l’utilisation des TDR a nécessité un renforcement des compétences de tous les agents de santé: Montrer aux agents de santé la place et l’utilité des TDR; Amener les prestataires à utiliser les TDR dans la prise en charge du paludisme Cette stratégie a permis de mettre en place les TDR dans tous les districts sanitaires Au niveau des centres et postes de santé, les TDR sont mis en place dans tous les points de prestations. Cette stratégie a permis d’obtenir une augmentation significative du taux de confirmation des cas de paludisme. Ce taux est passé de 13% en 2006 à 70% en 2008. Au niveau des centres de santé et des postes de santé, les TDR sont mis en place dans tous les points de prestations. L’adoption de cette stratégie est porteuse de beaucoup d’espoir sur la mortalité et la morbidité liées au paludisme.

Email address for correspondence: haril76@yahoo.fr

536 Burden of malaria, tuberculosis and AIDS in confirmed HIV 1 patients [MIM16692181]


The co-existence of falciparum malaria, human immuno-deficiency virus and Mycobacterium tuberculosis are responsible for staggering morbidity and mortality in sub-Saharan Africa. The epidemics of these diseases affect each other negatively. In view of this, the present study was conducted to assess the prevalence of co-existence in confirmed HIV patients and the assessment of CD4/lymphocyte count for antiretroviral therapy (ART), in confirmed HIV 1 positive cases, attending clinics of General Hospital Owerri, Imo state, Nigeria. X-ray along with Zhiel-Nelsen’s test for (AFB) sputum samples for the confirmation of Mycobacterium tuberculosis, thick and thin smear tests for the presence of P. falciparum and venous blood was used to screen HIV, followed by confirmation test. Of the total of 469 patients, 39.27% were male while 60.73% were females. The frequency differences were significant (x² = 7.815, p > 0.005). More number of female in the age range of 21–40 years showed higher prevalence (x² = 5.991, p < 0.05) than their male counterpart. Use of ART was independent of sex. In 40% of stage III and IV cases showed co-infection of tuberculosis while 27% were infected with malaria parasitemia. 23% were pregnant. Nigeria is an endemic country for tropical diseases and due to poor health surveillance system, the magnitude of their interaction is difficult to assess. Initiatives to control through effective education about these diseases are recommended.

Email address for correspondence: preet.onyeka@yahoo.com

537 Induction of HO-1 during Plasmodium liver infection protects infected hepatocytes by modulating the inflammatory response [MIM16700528]

Sabrina Epiphanio, Sebastian A. Mikolajczak, Lígia A. Gonçalves, Ana Pamplona, Silvia Portugal, Sónia Albuquerque, Michael Golderberg, Sofia Rebelo, Daniel G. Anderson, Akin Akinc, Hans-Peter Vornlocher, Stefan H.I. Kappe, Miguel P. Soares, Maria M. Mo

The clinically silent Plasmodium liver stage is an obligatory step in the establishment of malaria infection and disease. Heme oxygenase (HO) is the rate-limiting enzyme in the catabolism of free heme. We have previously shown that HO-1 (encoded by Hmox1) expression controls susceptibility to cerebral malaria in mice. We now report that HO-1 promotes the establishment of the malaria liver stage of infection. We have used Plasmodium berghei and Plasmodium yoelii rodent models together with transgenic mice, in vivo
RNA interference and overexpression systems to determine the role of HO-1 during Plasmodium liver infection. Gene expression quantification as well as liver histopathology and immunochemistry were used to establish the mechanism behind the observed role of HO-1 in infection. We report that expression of HO-1 is upregulated in the liver following infection by P. berghei and P. yoelii sporozoites. HO-1 overexpression in the liver leads to a proportional increase in parasite liver load, and treatment of mice with carbon monoxide and with biliverdin, each an enzymatic product of HO-1, also increases parasite liver load. Conversely, mice lacking Hmox1 completely resolve the infection. In the absence of HO-1, the levels of inflammatory cytokines involved in the control of liver infection are increased. These findings suggest that, while stimulating inflammation, the liver stage of Plasmodium also induces HO-1 expression, which modulates the host inflammatory response, protecting the infected hepatocytes and promoting the liver stage of infection.

Email address for correspondence: sabrina.epiphanio@gmail.com

538 Clinical and physiopathological features of cerebral malaria in Douala town, Cameroon [MIM14210629]

Pankoui M. Joel, Gouado Innocent, Fotso K Honore, Zambou Odette, Ngueu Pulcherie, Combes Valery, Grau E. Georges, Amvam Z. Paul Henri

Cerebral malaria (CM) is the most severe neurological complication of infection with Plasmodium falciparum. Insights into the processes leading to these severe forms might lead to new interventions that address pathophysiological processes causing malaria's peculiar morbidity and mortality. We set out to investigate the link between some clinical and immunological factors which may be helpful for it better understanding. Throughout the year 2007, children of 0–15 years old were recruited after informed consent in 4 hospital institutions in Douala (Cameroon). Cerebral malaria was defined as impaired consciousness (Blantyre coma score ≤2) not attributable to any other cause in a patient with a positive malaria smear. Clinical, nutritional and laboratory indices were assessed. Later on, microparticles (MP) determination was investigated using flow cytometry. CM patients were significantly younger than those with severe malaria anaemia (SMA) or uncomplicated malaria (UCM), *P*=0.0107. On admission, 36% of CM patients had hyperpyrexia and all were prostrated. None of them had severe undernutrition, however, 48% had mild undernutrition as assessed by the WAZ score. CM patients showed also an important increase in MP levels, particularly from platelets, erythrocytes, endothelium and monocytes. This study highlights peculiarities in clinical presentation and outcome, as well as in some physiopathological parameters of CM patients. This is helpful for a better understanding of the immunologic interactions incidental to CM. Furthermore it could lead to new avenues for prevention and/or therapy and to the investigation of new targets for drugs design.

Email address for correspondence: mfonpankoui@yahoo.fr

539 Taux de letalité imputable au paludisme chez l’enfant de moins de 5 ans hospitalisé en Cameroun [MIM15065366]

Molumba Paul

En République Démocratique du Congo, le paludisme à Plasmodium falciparum est la cause de 47,1% des décès parmi les enfants de moins de 5 ans hospitalisés. Ce chiffre, toute chose égale, est deux fois plus élevé que celui rapporté 19 ans auparavant. OBJECTIF DE RECHERCHE. La présentée étude a été menée en vue d’identifier les causes probables de cette flambée de mortalité. Une enquête transversale était conduite dans 6 des 16 hôpitaux de référence que compte Kinshasa. Elaient inclus dans l’étude, tout sujet de moins de 5 ans hospitalisé pour paludisme grave présumé sans autre pathologie associée. Un échantillon de 1.645 dossiers médicaux des cas hospitalisés du 1er septembre 2006 au 31 décembre 2007, répondant aux critères d’inclusion et d’exclusion fixés, était prélevé systématiquement dans chaque hôpital. Le TL global moyen était de 17,8% (IC95% = 13,9–18,1%). L’analyse multivariée sur la base de régression logistique a montré que les co-facteurs de pronostic retenus étaient différents d’un hôpital à l’autre. Selon l’établissement hospitalier considéré, venait en tête, soit l’âge, le choc, le délai, la pâleur ou la détresse respiratoire; le délai de référence prolongé n’a nulle part été incriminé. Le retard dans la référence des cas n’étant pas en cause, comparativement aux statistiques des autres pays africains, le TL élevé observé dans les hôpitaux de référence de Kinshasa pourrait être probablement expliqué par la baisse générale de la qualité de la prise en charge. MOTS CLES. Paludisme grave – Taux de létalité - hôpitaux de référence - Kinshasa – République Démocratique du Congo.

Email address for correspondence: pmolumba@yahoo.fr

540 Assessment of packed cell volume (PCV) and plasma iron levels following treatment for malaria and helminthic infections in children in rural Muea, Cameroon [MIM16735761]

Moses Samje, Irene Sumbele, Anna Njunda, Elsy Mankah, Lucien Kamga, Theresa Nkou Akenji

In many tropical regions, anaemia, iron deficiency, malaria and helminthic infections co-exist and are interrelated. To investigate the effect of malaria and soil-transmitted helminthic (STH) infections on anaemia and plasma iron levels, 203 children (<15 years) residing in rural Muea were enrolled into a longitudinal study in which they were followed up weekly for 6 weeks between April and October 2006. Malaria parasitaemia was determined microscopically, packed cell volume (PCV) was determined using a haematocrit, and plasma iron levels by spectrophotometry. Stool samples were prepared by the Kato-Katz technique and examined microscopically for the presence and intensity of intestinal helminths. Overall, 99% of the children had malaria parasites while 53.7% were infected with STH. The prevalence of anaemia (PCV <31%) and plasma iron deficiency (plasma iron <50 μg/dL) was respectively, 43.4% and 50.2%. Of the worm-infected children 54.1% and 51.4% were anaemic and iron deficient respectively. Following appropriate malaria and worm treatment mean PCV increased progressively from 31.14 ± 5.24 to 35.23 ± 5.04 and mean plasma iron levels from 65.05 ± 68.56 to 68.57 ± 84.05 on day 0 and day 42, respectively. The difference between pre- and post-treatment mean PCV was significant (*P*<0.05). Our findings indicate that malaria and helminthic infections have an impact on PCV and plasma iron levels. The co-existence of these infections in this community contributes to the severity of anaemia and iron deficiency. The population has to be educated on the benefits of prompt and proper treatment of these parasitic diseases.

Email address for correspondence: msamje@yahoo.com
541
Acquisition of antibodies to Plasmodium falciparum variant surface antigens in distinct malaria pathology [MIM16672720]

N. Kheliouen, N. Tuikue N'dam, P. Deloron, A. Aubouy

In areas of intense Plasmodium falciparum transmission, protective immunity to malaria is gradually acquired during childhood leading to decreased susceptibility at adulthood. This acquired protective immunity to P. falciparum malaria is mediated at least in part, by antibodies against parasite-encoded variant surface antigens (VSA) expressed on parasitized red blood cells. Clinical disease is then thought to be mainly caused by parasites expressing VSA not recognized by pre-existing VSA-specific antibodies in non-immune or semi-immune individuals. This has also been clearly demonstrated for pregnancy-associated malaria (PAM), mostly occurring in primigravidae as they lack antibodies against parasites expressing PAM-specific VSA. The main aim of this study was to investigate VSA-specific antibody acquisition in distinct malaria pathology. P. falciparum parasites were collected from children with cerebral malaria (CM), uncomplicated malaria (UM) and asymptomatic malaria (AM) and also from pregnant women. Plasmas were also collected from these groups and the repertoires of VSA antibodies carried by patients from each category at the time of diagnosis and at convalescence were measured. Data from the current analysis show association between the plasma level of VSA-specific antibody in a given group and the parasite collected from individuals of the same group. We will analyse whether specific malaria pathology could be explained in terms of the total repertoire of VSA antibodies carried at the time of disease and the impact of the disease on resulting acquired repertoire.

Email address for correspondence: nabila.kheliouen@gmail.com

542
Parasite density during Plasmodium falciparum malaria attacks according to drug policies in Dielmo, Sénégal, 1990–2008 [MIM16705731]


Drug policies for malaria treatment may have an impact both on the incidence and the severity of the disease. Here we compare the impact of four different drug regimens used in Dielmo from 1990 to 2008 on the incidence of cases hyper-parasitaemia during Plasmodium falciparum attacks. From 1990 to 2008, we monitored the incidence of malaria morbidity in Dielmo, Sénégal, by daily clinical surveillance and blood testing of patients with fever. Parasite density, i.e. the number of malaria parasites per 100 leukocytes, was measured for all episodes of fever. During the study period, four drug policies were successively deployed for the first line treatment of malaria attacks: oral Quinimax® (QX: 1990–1994), chloroquine (CQ: 1995–2003), amodiaquine + sulfadoxine/pyrimethamine (AQ-SP: 2004–2006), and artesunate + amodiaquine (AS-AQ: 2006–2008). The incidence density of P. falciparum malaria attacks was 1.3-, 2.1- and 3.5-fold higher during the period of CQ administration than during treatment periods with QX, AQ-SP and AS-AQ, respectively. The proportion of malaria attacks with parasitaemia ≥800 trophozoites per 100 leukocytes (≥2% of parasitized red cells) was initially (QX period) at 37% in children 0–4 years and 23% in children 5–9 years. It decreased to 22% and 9% during the period with CQ but increased to 30% and 15% with AQ-SP and 43% and 23% with AS-AQ, respectively. Email address for correspondence: fambayedieyeba@yahoo.fr

543
A stat6 single nucleotide polymorphism is associated with protection against cerebral malaria in Ghanaian children [MIM15252157]


The IL-4|Stat6 signalling pathway could be crucial for Th2 mediated immunity and protection against malaria. Although we and others have previously shown associations between some IL-4 polymorphisms and severe malaria, the role of Stat6 and IL-4Rα polymorphisms in malaria pathogenesis is yet to be established. This study investigated the distinctive and interactive association of known polymorphisms of the IL-4 gene (+33C/T, 590C/T, VNTR), IL-4R gene (Arg551Gln) and STAT6 gene (1570C/T) with total IgE production and subsequently, malaria severity in Ghanaian children. PCR-RFLP was used to genotype all polymorphisms in a hospital-based cross-sectional study involving 290 malaria cases and controls. Malaria cases were categorized into uncomplicated malaria (UM), severe malarial anaemia (SMA), and cerebral malaria (CM). We found that a single nucleotide polymorphism (SNP) (rs3024974) which causes a C→T change in intron 18 of the stat6 gene is associated with protection from cerebral malaria (OR = 0.361, P = 0.0107). All other polymorphisms studied did not show any association with malaria severity except the IL-4 VNTR polymorphism. Our data did not show any association between rs3024974 and levels of total IgE. Data from this study suggest that rs3024974 is associated with protection against cerebral malaria in Ghanaian children. However, this protection maybe mediated by other factors other than total serum IgE. To the best of our knowledge, this study is the first to suggest a role for the stat6 SNP (rs3024974) in malaria pathogenesis. This study was supported by a WHO/TDR/MIM grant: A 11034.

Email address for correspondence: dasakyi@yahoo.com

544
Absolute levels of macrophage migration inhibitory factor, Interleukin-4, Interleukin-10 and the ratio of Interleukin-10/tumour necrosis factor-alpha correlate with severe malaria in Ghanaian children [MIM15080546]

S. Adukpo, A. Kusi, M.F. Ofori, B.D. Akanmori, B. Gyan, D. Dodoo

Plasmodium falciparum-infection induces host inflammatory response necessary for the elimination of parasite but appears to be important factor in the pathogenesis of severe malaria. The inflammatory response is marked by the production of pro-inflammatory cytokines. Imbalance in the production of these pro- and anti-inflammatory cytokines may result in complications associated with malaria disease. In this hospital-based study, levels of pro- and anti-inflammatory cytokines and their ratios that may be involved in the pathogenesis of severe malaria were assessed in the plasma samples of paediatric malaria patients. Acute plasma samples were collected from children suffering from cerebral malaria (CM), severe malarial anaemia (SMA), and uncomplicated malaria (UM). Levels of macrophage migration inhibitory factor (MIF), tumour necrosis factor-alpha (TNF-α); interleukin (IL)-4 and IL-10 were measured using ELISA. High levels of MIF were seen in CM (8.48 ng/ml) compared to UM (6.14 ng/ml, p = 0.008) but not SMA (6.53 ng/ml, p = 0.174). Similarly, IL-4 levels were found to be higher in SMA (71.12 pg/ml) compared to UM (49.21 pg/ml, p = 0.033) but not CM (55.80 pg/ml, p = 0.22). Levels of TNF-α were however similar in the groups (UM = 26.25 pg/ml, SMA = 27.00 pg/ml and CM = 28.80 pg/ml; p = 0.951). IL-10 levels were lower in SMA.
(1426.00 pg/ml) compared to UM (2156.00 pg/ml, p = 0.046) and CM (1873.05 pg/ml, p = 0.018). When the patients were re-grouped into SMA and non-SMA; low levels of IL-10 were again seen in SMA compared to non-SMA (1873.05 pg/ml, p = 0.029). Also, the ratios of IL-10 to TNF-α were significantly lower in SMA (0.020) than non-SMA (0.015, p = 0.032). High levels of MIF are associated with CM while low IL-10 levels and low IL-10 to TNF-α ratios may influence development of SMA.

Email address for correspondence: sadukpo@noguchi.mimcom.org

545
Troubles hématologiques au cours du paludisme de l’enfant en zone sahélienne (Sénégal) [MIM16689627]
Lô Aminata, B. Faye, R.C. Tine, J.L. Ndiaye, O. Gaye

En Afrique, le statut hématologique joue un rôle important dans l’évolution des maladies infectieuses en général et du paludisme chez l’enfant en particulier. Une étude est menée au Sénégal pays sahélien pour déterminer l’importance des troubles hématologiques pouvant influencer l’évolution clinique dès l’accès palustre de l’enfant en zone sahélienne. Nous avons mené une étude transversale comportant 480 enfants de moins de 7 ans souffrant de paludisme simple ont été inclus. Tous ont bénéficié d’une NFS avant traitement dans les 24 heures suivant le début de la symptomatologie. L’anémie et la thrombopénie ont été étudiées en fonction de la densité parasitaire et de l’âge pour déterminer un risque d’aggravation de l’état clinique. Après analyse, il apparaît que la thrombopénie était présente dans 58,9% des patients présentant une densité parasitaire élevée (DP > à 25000 p/μl) contre 11,3% pour les patients présentant une densité parasitaire faible (<5000 parasites par microlitre de sang) et 29,7% pour ceux ayant une DP entre 5000 et 25000 p/μl (RR = 1,98 IC95% [1,15–3,35] p = 0,01). Concernant l’anémie, 85% présentaient une anémie modérée (hémoglobine 6–11 g/dl) et 15% présentaient une anémie sévère (Hémoglobine < à 6 g/dl). Dans les deux groupes, les enfants ayant une forte DP étaient plus touchés (p = 0.001). Les troubles hématologiques sont fréquents en début d’infection palustre chez les enfants sahéliens de moins de 7 ans. Il est important de pouvoir les diagnostiquer à temps car ils peuvent conditionner une évolution défavorable de la maladie malgré un traitement antipaludique adéquat.

Email address for correspondence: amlosn@yahoo.fr

546
Establishment of an external quality assessment programme for blood film interpretation for malaria clinical research laboratories in Africa [MIM16670786]
E.J. Wagar, P.A. Onyor, P.B. Obare, D.S. Walsh, B. Ogutu R

We discuss the establishment of an external quality assessment programme for Malaria Clinical Trials Alliance-supported clinical study sites (MCTA sites). Quarterly, the Malaria Diagnostics and Control Centre of Excellence (MDCoE) sends five malaria blood film (MBF) slides to each of the 14 current MCTA sites, and each site sends to MDCoE five of their own slides plus results for their slides and the MDCoE slides. MCTA site results for parasite detection, species identification, and parasite density are compared with those of expert microscopists at MDCoE. Participation in both arms of the EQA programme averaged 11 of 14 eligible sites over two quarters. Smear and staining quality of the slides submitted to MDCoE was good. For slides sent from the MCTA sites to MDCoE, average concordance for parasite identification (positive/negative for parasites) was 97% and 98% (second and third iterations), species identification was 94% and 96%, and parasite density counts was 90% and 66%. For MBF sent from MDCoE to the sites, concordance for parasite identification was 97% and 99%, concordance for species identification was 84% in both iterations, and concordance for parasite density counts was 76% and 78%. Programme design and supporting logistics are simple, yet robust. Proficiency at parasite and species identification is high, but parasite density counts show room for improvement. Challenges facing the programme include improving the analysis of data and providing results to MCTA sites in a manner that fosters improvement.

Email address for correspondence: ewagar@wrp-ksm.org

547
Use of event related potentials (ERPs) as a marker of cognitive performance in children exposed to severe falciparum malaria [MIM16520887]
Michael Kihara

Plasmodium falciparum is the most common parasitic infection of the central nervous system. It causes neuro-cognitive deficits in about 5–26% cases. There lacks sensitive, culture-fair and robust techniques of data collection in rural settings that would estimate the neuro-cognitive burden of malaria with neurological involvement. We proposed passive auditory and visual event related potentials (ERPs) as a surrogate marker of cognition in rural Kilifi. Fifty children, aged between 6 and 7 years, who had been hospitalized at Kilifi District Hospital (KDH) with severe forms of falciparum malaria were selected and compared with 77 unexposed children randomly selected from the DSS database maintained at KDH. These school-age children had ERPs recorded by a technician who was blinded to their group status. The results showed that the auditory N2 and visual P2 latencies to novelty were significantly longer [N2: 261 ± 49 ms vs 237 ± 18 ms and P2: 273 ± 37 ms vs 250 ± 27 ms, respectively] in children exposed to severe falciparum malaria compared to community controls which is a sign of delayed processing. The results from the ERPs tests suggest that children with a history of severe malaria may have cognitive deficits, probably as a result to their exposure to malarial disease. ERPs are useful in rural settings as they allow for comparison of disease-effects in young, unschooled or uncooperative participants.

Email address for correspondence: mkihara@kilifi.kenmri-wellcome.org

548
Registering and analyzing malaria clinical trials in Africa: The ATM registry initiative [MIM15069103]
Vittoria Lutje, Annette Gerritsen, Nandi Siegfried, Paul Garner

The ATM Registry (www.atmregistry.org), is an African initiative that provides a platform to prospectively register HIV/AIDS, tuberculosis and malaria trials conducted in Africa. The register contains information on completed trials as well as trials in progress or planned. We analyzed published randomized controlled trials (RCTs) of malaria prevention and treatment, conducted in Africa. Objectives: To describe RCTs of malaria prevention (including malaria prophylaxis) and treatment conducted in Africa, and analyze their geographical and temporal distribution and clinical characteristics. Systematic searches of electronic databases (Medline, Embase, CENTRAL, and LILACS) were run to identify all malaria RCTs; African trials were identified by applying a geographic search filter. We analyzed the distribution of trials by country and by decade, a random sample of these trials was further analyzed for
clinical characteristics, and author's affiliation. We obtained 943 records describing malaria trials conducted in Africa. The total number of trials increased over time and since 2000, more trials took place in Africa than in other parts of the world. The highest number of trials was conducted in Kenya, Tanzania and Nigeria. We will present the results of our analysis on types of interventions, trial participants and other clinical characteristics. This retrospective analysis of malaria trials in Africa provides useful information for researchers and policy-makers to plan future trials and complements the prospective trial registration provided by the ATM Registry.

Email address for correspondence: vlutje@liv.ac.uk

549
Evaluation of a FACS-based method using autofluorescence and DNA stain YOYO-1 for parasite quantification in peripheral blood from children in rural Mozambique [MIM16674641]

Augusto Nhabomba, Joe Campo, Jahit Sacarlal, María Belén Jiménez-Díaz, Eusebio Macete, Montse Renom, Ihigo Angulo-Barturen, Pedro Alonso, Carlota Dobano, John Aponte

An increasing volume of clinical trials and epidemiological studies in malaria calls for new and better tools for detection and quantification of *Plasmodium falciparum* infection. A flow cytometry-based method of quantifying parasitemia has been developed in the mouse models of malaria. This method (FACS FL2/YOYO-1) exploits the autofluorescence patterns of fixed blood stained with YOYO-1, a DNA-specific dye, to identify Plasmodium-infected cellular events. This study aimed to assess the FACS FL2/YOYO-1 method in human samples acquired in a malaria-endemic setting and assess its utility in epidemiological studies of malaria. Small volumes of blood were collected from 100 children admitted to the inpatient department of a health center in rural Mozambique. Blood was analyzed for hematology and parasite density by microscopy. 2 µL of blood was fixed in glutaraldehyde, permeabilized, treated with RNase, and stained with YOYO-1. Stained samples were acquired on a FACS Calibur. A unique stage-specific population pattern of infected erythrocytes was identified by adjusting the compensation of FL1 into FL2 (FL2 = %FL1). Regions were placed around the infected erythrocyte and leukocyte populations to quantify parasitemia and parasite density. Assessment of parasite density by the FACS FL2/YOYO-1 method correlated with microscopic assessment of parasite density and increased limits of quantification and detection. The FACS FL2/YOYO-1 method may be a valuable tool for malaria epidemiological studies and clinical trials due to its high range of quantification and detection. Additionally, the high throughput nature of the method makes it practical for large-scale studies.

Email address for correspondence: augusto.nhabomba@manhica.net

550
Evaluation en pratique courante des déterminants de la prise en charge du paludisme simple en Côte d’Ivoire [MIM16643846]

E. Bissagnené, Y.T. Aba, I. Ouattara, F. Ello, S. Eholié, D. Ekouévi, M. San Koffi

Après l’adoption des nouveaux outils de prise en charge du paludisme (TDR, CTA, TPI), il convient de fournir aux programmes nationaux des données actualisées leur permettant d’orienter la promotion de ces outils. Objectif: Apprécier les connaissances et pratiques des prestataires des soins sur les moyens diagnostiques et thérapeutiques du paludisme simple. Enquête réalisée à Abidjan auprès des professionnels de santé prenant en charge le paludisme, d’abord pour noter les moyens diagnostiques et thérapeutiques qu’ils utilisent, puis pour obtenir de ces enquêtés les raisons de leurs choix diagnostiques et thérapeutiques. Dans 9 districts, 131 centres de santé ont été visités par 15 enquêteurs qui ont colligé 1 691 cas de paludisme simple auprès de 20 sages-femmes, 92 infirmiers, 27 médecins spécialistes, 307 médecins généralistes. Comme moyens diagnostiques, la clinique seule demeure la pratique courante (80%) devant la confirmation par goutte épaisse (15%) ou TDR (5%). Comme moyens thérapeutiques, 70% des prestataires prescrivent les CTA (artésunate-amaédoquine), mais 73% ne sont pas formés au nouveau protocole thérapeutique. L’utilisation des nouveaux outils de diagnostic et de traitement est encore limitée, du fait de l’insuffisance de formation des prescripteurs. L’opportunité qu’offre le Fonds Mondial devrait contribuer à corriger ces insuffisances.

Email address for correspondence: bissagnene@yahoo.fr

551
Acceptability of intermittent preventive treatment versus intermittent screening and treatment for malaria in pregnancy: A qualitative study [MIM16673241]

Lucy Smith, Caroline Jones, Rose O. Adjei, Gifty Antwi, Nana A.A. Boateng, Daniel Chandramohan, Harry Tagbor, Jayne Webster

A randomised controlled trial on strategies for protecting pregnant women and their newborns from malaria was undertaken in two districts in Ghana. The trial compared two strategies: intermittent preventive treatment (IPTp) versus intermittent screening with a rapid diagnostic test (RDT) at each ANC visit and treatment of the parasite positives with either sulphadoxine–pyrimethamine (S–P) or artesunate–amodiaquine (AS/AQ). In addition to clinical outcomes, qualitative data were collected on the acceptability of the different strategies to key actors involved in their delivery and uptake. In-depth interviews were conducted with midwives (n = 12), nursing assistants (n = 2), laboratory (n = 4) and pharmacy (n = 4) staff working in the ANC clinics at the 6 facilities involved in the trial. The views of women involved in the trial were collected through focus group discussions (n = 12). Preliminary analysis of the data suggests that the additional attention the women were given (as a consequence of being enrolled in the trial) was more significant to them than any specific tests or treatment they received. From the provider side, the primary concern among the interviewees was the additional workload that might be imposed if all women had to be tested for malaria at each ANC visit. The results are used to develop a broader discussion around women’s knowledge of the services and treatments they receive during their ANC visits and to identify the factors that are likely to influence the acceptability (to users and providers) of services and treatments delivered at ANC.

Email address for correspondence: franck.remoue@ird.fr

www.mimalaria.org
made our fieldwork in religious related health services situated in Sir and Mayo-Ouldémé, villages of the region of Far North from 2006 to 2007. The tools were semi-structured interviews and direct observations. We had interviews with 10 caregivers in charge of antenatal care on their experiences on direct observed treatment and in depth interviews with 80 pregnant women. During antenatal clinics, women are supposed to swallow the Sulfadoxine–Pyriméthamine. Women come far from the health center and they let their home before 07 AM. They do not have the time to take their breakfast. Regarding their experience on women complains of side effects, caregivers prefer to give the medicines for home care. The direct observed treatment should fit the diet habits of users. In addition, the dialogue between public–private sectors of the health system is necessary to minimize the burdens of Malaria amongst pregnant women.

Email address for correspondence: esthelka@yahoo.com

554 Acceptance and safety use of insecticides by the community and spray-men at the gambiæ control project area at Sudan [MIM16698431]

Mostafa M.M. Ibrahim, Osama M.E. Seidahmed*, Suad M. Sulaiman

Indoor Residual Spraying and Larviciding of aquatic breeding sites were the methods used to control malaria vectors and are deployed in the Gambiæ Control Project. This study aimed to evaluate community acceptance and safe use of insecticides by SM the major threats to the sustainability of IRS and Larviciding at GCP in the GCP area at Sudan. The study conducted in 2008 at the red zone of GCP. The total population protected 100,000. Two surveys carried out: cross-sectional household survey and spray-men interviews. Using simple random sampling technique, two pre-designed and pre-tested questionnaires were used to collect data from households and SM after obtaining informed consent to participate in the study. 500 HHs and 30 spray-men (total 40) were interviewed. Only 21 households have malaria case. About 60% of SM mentioned March to June high peak of mosquito. 93% HHs IRS is a useful measure for malaria prevention, 93.4% accepted GCP. Un-spraying HH absence 7.9% and refusal 9% (n = 43) (because allergic cases (n = 21)). While 52% was wall painting before “El Eid”. 86.7% SM identified breeding sites as water pipe breaks, leakages of irrigation canals and wells. 76.7% of SM cover 1–7 km. 72.4% of SM high peak of Anophèles from March to June. 66.7% of SM used pumping sprayer, 1/3 used other spraying method (gallon). 2/3 of SM possessed no protective measures. 42% of SM keeps remained insecticide to other workdays. 72% SM return back unused insecticide. Administrative and educational interventions should be arranged in order to sustain success of GCP.

Email address for correspondence: mostafamohieldin@gmail.com

555 Building household-centered resilience to malaria risk in Kilombo District, Tanzania [MIM16692518]

Iddy Mayumana, Flora Kessy, Manuel Hetzel, Angel Dillip, Sandra Alba, Alexander Schulze, Christopher Mshana, Ahmed Makemba, Brigit Obrist, Christian Lengeler, Hassan Mshinda

Prompt and correct treatment of malaria cases is crucial for reducing malaria morbidity and mortality. However, interlinked factors at the household, health system and policy levels hinder access to prompt and correct treatment. Inspired by the Sustainable Livelihood approach and guided by the Health Access Livelihood framework, our paper outlines an approach to study social resilience to malaria risk, and exemplifies the approach with a small case study. We explored the mutually reinforcing benefits that people can generate by mobilizing, combining and transforming human, financial and social capital and the role of institutions in influencing people’s transformation of assets and their access to health services. The study was conducted within the Ifakara Demographic Surveillance System area between November 2006 and February 2007. Data were collected through qualitative methods such as family portraits and focus group discussions. Empirical data highlighted patterns of transforming livelihood assets resulting in building a thin layer of resilience. The time taken to mobilize social, financial and physical resources for treatment seeking greatly contributed to the fact that most children who were sick during the stay in temporary farm houses were delayed in accessing treatment. This led many children to be in severe conditions at the time of reaching a health facility. The data further identifies three institutions – reciprocity, profit maximization and cost-recovery – which shape these transformations. Programmes need to link health services with income generating activities to strengthen social resilience to malaria risk.

Email address for correspondence: imayumana@yahoo.com

556 Determinants of health care seeking behaviour for malaria treatment in Uganda [MIM16696628]

Patrick Tutembe

Despite being preventable and curable, malaria continues to be one of the global leading killer diseases especially amongst young children and pregnant mothers. Treatment interventions for reducing malaria in Uganda largely focus on drug efficacy; neglecting health
557 Cost-effectiveness of intermittent preventive treatment of malaria in pregnancy in southern Mozambique [MIM16697879]

Elisa Sicuri, Azucena Bardají, Maria Maixenchs, Claire Chaise, Tacitá Nhampossa, Ariel Nhacolo, Delino Nhalungo, Sergi Sanz, Pedro L. Alonso, Clara Menéndez

Knowledge of the economics of malaria is still scarce. Economic evaluation of malaria prevention strategies in pregnancy in different epidemiological settings have been pointed out as of relevance in the malaria research agenda. This study was conducted in the context of a randomised, placebocontrolled trial of intermittent preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) in pregnant women. The cost-effectiveness of IPTp-SP on maternal health and child survival was evaluated. Probabilistic and threshold analyses were undertaken to assess which factors mainly affect the economic outcomes and threshold values beyond which the intervention ceases from being cost-effective. In 2007 US$, the incremental cost-effectiveness ratio (ICER) of the IPTp-SP was of 1.07 (95% CI, 0.46–2.38) per disability-adjusted life-year (DALY) averted, referred to neonatal mortality reduction. Furthermore, the incremental cost-effectiveness ratio of the intervention was 4.46 (95% CI, 2.33–10.16) per number of episodes of clinical malaria averted and 32.10 (95% CI, 15.9–73.6) per DALY averted in the mother. This intervention resulted in savings both at the health system and household levels. Protective efficacy was the factor affecting the most the economic evaluation of IPTp with SP (Pearson Correlation Coefficient always \( > -0.9 \)). Threshold analysis highlighted the presence of quite a wide range for an increase of intervention costs. In this study IPTp-SP has been found to be highly cost-effective, both on neonatal survival and maternal health. The intervention results to be worth especially when evaluated on the reduction of neonatal mortality. Therefore, investing on IPTp is strongly recommended, even in the context of scarce resources available for health care.

Email address for correspondence: elisa.sicuri@cresib.cat

558 Knowledge, attitudes and treatment-seeking behaviour (KAB) towards malaria among adult residents of Bushbuckridge, Mqumalanga Province, South Africa [MIM14922220]

Khumbulani W. Hlongwana, Wondwossen T. Lerebo, Rajendra Maharaj

Highest-risk malaria areas in South Africa share borders with Mozambique, Swaziland and Zimbabwe. Ongoing migration, whereby 30% of the adult population in Bushbuckridge owe its origin to Mozambique, impacts on malaria control interventions. Nevertheless, no malaria KAB study has been conducted in Bushbuckridge This study was undertaken as a descriptive cross-sectional survey. A field-piloted structured questionnaire was administered to 602 randomly selected households. The interviewees (one person per household) were the heads of households, except in their absence whereby responsible adults above 18 years were interviewed. Of 602 respondents (25.1% males and 74.9% females), 80.4% correctly associated malaria with mosquito bites, and no significant gender differences (Odds Ratio = 0.93; 95%CI = 0.5877–1.4792) were noted in their knowledge. However, malaria knowledge decreased with an increase in age (\( P \)-value = 0.0002), and increased with an increase in level of education. Health facility (29.1%) and radio (19.8%) were the main sources of malaria information. Knowledge of malaria symptoms (headache: 59%, high temperature/fever: 33.1% and chills: 45%) was poor. About 98.8% would use health facility for treatment, 82% of whom would do so within 24 h of onset of symptoms. Fair amount of malaria knowledge and positive attitude towards its treatment exist in Bushbuckridge. However, poor knowledge of malaria symptoms poses a serious threat to these gains. Nevertheless, the identification of health facilities as the treatment of choice is commendable, given the fact that most African countries still use non-public sources for treatment.

Email address for correspondence: khumbulani.hlongwana@mrc.ac.za

559 Are there spatial and socio-economic inequities in incidence and burden of malaria? A study in southeast Nigeria [MIM14922220]

Ogoamaka Chukwuogo

Malaria is a leading cause of death and impoverishment in Nigeria and Africa. Thus, the extent of the economic burden imposed by malaria and the coping mechanisms are relevant to government/policy makers for effective allocation of resources towards malaria prevention and treatment. This work explored the socioeconomic and geographic differentials in the incidence, burden and prevention of malaria in Nigeria. A cross-sectional household survey was conducted among 1657 respondents in two urban and rural communities in Anambra State, southeast, Nigeria. Information was collected on: their socio-economic and demographic data, incidence of malaria among respondents or their family members one month preceding the survey, how malaria was diagnosed, duration of the Illnesses, treatment seeking and reasons for choosing a particular care provider. About 38.5% and 35% of the rural and urban respondents had malaria respectively a month preceding the survey. The incidence of malaria was similar across the socioeconomic status (SES) quartiles. Treatment seeking in rural and urban settings did not differ. However there were statistically significant inequities in the use of treated and untreated mosquito nets across the SES quartiles and among the rural and urban dwellers. There is no SES and geographic difference in the incidence and

www.mimalaria.org
treatment seeking for malaria but significant differences exist in use of untreated and treated mosquito nets. Efforts should be made to ensure that all SES groups use mosquito nets.

Email address for correspondence: ogoamakai@yahoo.com

560 Consumer prioritization and risk perception of malaria and intermittent preventive treatment for malaria in pregnancy in southeast Nigeria [MIM16697648]

E.N. Shu, N. Dike, O.E. Onwujekwe, N. Ezumah, P.O. Okonkwo

Malaria infection during pregnancy is a major public health problem in tropical Africa. In Nigeria, intermittent preventive treatment for malaria in pregnancy (IPTp) was adopted to replace chemoprophylaxis with pyrimethamine. The sustainability of such control measure requires knowledge on how the communities prioritize malaria and its prevention/treatment. This study therefore investigated the prioritization and risk perception of malaria and IPTp. The study was carried out in two urban and two rural local government areas (LGAs) in Enugu, Southeast Nigeria. Pre-tested, interviewer-administered questionnaires were used to collect data from 1204 women of childbearing age. A majority of the respondents in each geographical location considered malaria in pregnancy as a high priority illness, and most of the respondents perceived a high risk of contracting malaria during pregnancy. In the urban LGA, most respondents who considered malaria in pregnancy as a medium/high priority illness did not have preference for any health facility. Most respondents in the rural LGA who considered malaria in pregnancy as a high priority illness visited the community-based health workers and maternity homes for prevention/treatment. The use of IPTp for the prevention/treatment of malaria was given a low priority by both urban and rural respondents. Communities regarded malaria in pregnancy as a highly prioritized problem and perceived high risks of contracting it, and this could be used to support sustainable community-based IPTp programmes. However, the low rating of IPTp should be corrected in community health education outreach campaigns for the control of malaria in pregnancy.

Email address for correspondence: enshu1@yahoo.com

561 Primauté de la valeur marchande du médicament sur sa valeur thérapeutique [MIM16699197]

Carine Baxerres, Albert Tingbé-Azalou, Jean-Yves Le Hesran

Depuis l’initiative de Bamako (1987), le médicament tient en Afrique un rôle financier majeur car il contribue largement au financement du fonctionnement des structures de santé publiques. Quelles conséquences sanitaires, notamment dans la prise en charge du paludisme, la mise en avant de la valeur marchande du médicament («commodifications») entraîne-t-elle? Nous avons mené une enquête d’anthropologie sociale (observations, entretiens, suivi) durant 17 mois à Cotonou auprès des différents acteurs intervenant dans l’offre de médicaments -détailleurs, grossistes formels et informels, acteurs institutionnels– ainsi qu’auprès de familles. La vente des médicaments se fait dans le cadre des consultations des centres de santé mais ceux-ci ont aussi, bien qu’ils n’y soient pas tous autorisés, un rôle de distribution quand cette vente n’est pas consécutive à une prescription. De même, les grossistes privés et public distribuent les médicaments à des acteurs et des structures auxquels la loi ne les lie pas. Cette distribution exacerbée souligne la «commodification» du médicament généralisée à l’ensemble du système de santé. Celle-ci prend le pas sur la valeur thérapeutique du médicament. De plus, elle favorise une automédication importante, souvent éloignée des recommandations biomédicales. Cette automédication est encore accrue en matière de «palu», entité nosologique populaire aux contours plus larges que ceux décrits par la biomédicine pour le paludisme. La réaffirmation de la valeur thérapeutique du médicament au centre du système de santé est à la fois indispensable et problématique dans un contexte de précarité.

Email address for correspondence: lehesran@ird.fr

562 Acceptabilité de l’innovation thérapeutique dans le domaine du paludisme par les soignants et les communautés au Sénégal [MIM16672993]

Sylvain L. Faye, Badara Cisse, Jean L. Ndiaye, Babacar Faye, Omar Gaye

Face à la chloroquinorésistance, le Sénégal a initié un changement de politique de traitement du paludisme (ACT) et un renforcement des moyens diagnostics (TDR). Le pays expérimente aussi le traitement préventif intermittent (TPI) chez l’enfant et le nourrisson, dans le but de proposer sa mise à l’échelle nationale. Au-delà de leur efficacité et de leur faisabilité, nous voulons démontrer comment les communautés s’approprient ces innovations, mais aussi de quelle manière les soignants appliquent les normes édictées dans leurs pratiques professionnelles. Nous précisons que les données sont issues de recherches socio-anthropologiques qualitatives accompagnant l’évaluation et l’opérationnalisation des nouvelles mesures, allant de 2007 à 2009. Nous avons utilisé les grilles de supervision, les entretiens approfondis, les focus group et l’observation participante. Les résultats montrent qu’au niveau communautaire, les parents font preuve de bonnes dispositions vis-à-vis de l’innovation. En revanche, leurs attitudes d’adhésion, de rejet et d’observance sont intimement liées à une absence d’informations, à des habitudes singulières d’usage du médicament, à des expériences personnelles (effets secondaires) et à des processus décisionnels au niveau familial, etc. Chez les soignants, si l’utilité et l’impact sur la morbidité de ces mesures sont reconnus, des doutes, des interrogations sont aussi exprimés. Selon le statut du soignant, les mises en actes varient et s’expliquent par des opportunités que présente l’innovation pour une valorisation et une re-négociation statutaire, par une non maîtrise des compétences techniques, un rapport singulier aux convictions établies par les formations initiales ou par la préméditation de la verticalité dans le champ de la santé.

Email address for correspondence: fayesylvain@yahoo.fr

563 Potential acceptability of a rectal route combination (antibiotic–antimalarial formulation) among Lao people and Lao Health staff [MIM16669486]

H. Barennes, Somphavong Silaphet, T. Franchard, S. Inthavilay, Shi Jing, IFMT P10 Study Group, K. Paulin, A. Haiwanmana

Malaria and pneumonia together account for over a quarter of all deaths in the world in young children. These conditions are estimated to kill over 10,000 people each day, but they are difficult to distinguish clinically. Recent studies in Africa showed an interesting acceptability of the rectal route. A combined antibiotic–antimalarial formulation is under development. We evaluate the acceptability of such a combination as a prerereferal treatment among the Lao population and the Lao doctor. We performed a cross-sectional survey of 1100 households randomly
selected through a multi-stage sampling in 12/18 provinces and of 300 heaths staff randomly selected at each administrative level in 2009. Description of traditional and non-traditional practices, acceptability from the population and from the Health staff will be presented. Acceptability of the combination formulation will be compared with that of the antimalarial rectal route. Best choice for antibiotic from the doctors counterpart will be presented. Understanding the perception related to the rectal route of the potential users is a key issue that might help to better implement of a potent prerreferal treatment and avoid common pitfalls.

Email address for correspondence: barenesshub@yahoo.fr

Trials of transmission blocking of P. falciparum with single dose primaquine in villages of Solomon Islands [MIM16723361]

Akira Ishii, Nobuo Ohta, Makoto Owhashi, Makoto Kawabata, Don-Il Chung, Albino Bobogare, Bernard Bakotee

Early diagnosis and proper treatment are essential prerequisites to combat malaria. However, without finishing the big reserve of asymptomatic malaria carriers in the villages, malaria control will be an endless effort. Transmission blocking is the final goal of malaria control. We have attempted to block transmission in the villages of Solomon Islands. We introduced ICT to detect malaria infection in the villages and treated positives with not only schizonticides but also single dose of primaquine. We introduced newly invented G6PD test (WST-8) to prevent possible adverse effect of primaquine. Now we can use ICT and G6PD test in the village. We repeated this operation once a year for several years. We used mathematical epidemiology model to analyse and assess our trials. In 2009, we obtained good results of decrease of prevalence in trial villages. It is revealed to attain high coverage of people is very important to attain the elimination.

Email address for correspondence: ishiiaki@jichi.ac.jp

Antiplasmodial and immunomodulating activity of some Sudanese herbal medicine with emphasis on pristimerin as antimalarial agent [MIM16689520]

M. Idris A. elT, G.M.H. Satti, T. Theander, S.B. Christensen, S.A. Khalid

The Sudan is being the largest country in Africa, covering an area of one million square miles with the different meteorological and polythetic, with a diverse flora. Most people in rural areas rely on traditional medicine for the treatment of many infectious diseases. Objectives: WHO has recently advocated the use of traditional medicine where appropriate health services become inaccessible, therefore, the study aims to investigate the potential antimalarial, antileishmanial activity of some medicinal plants and to detect their effect on human lymphocytes proliferation which may imply the ability to potentiate the human immune system. 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. Thirty-four methanol extracts (50%) exhibited significant activity against 3D7 with IC50 values ≤50 μg/ml, while twenty-one extract (57%) showed antimalarial activity on Dd2 with IC50 values ≤50 μg/ml. On the other hand, thirteen extracts (22%) and ten extracts (18%) only showed an activity with IC50 values ≤5 μg/ml on 3D7 and Dd2, respectively. Human lymphocytes treated with the most of extracts demonstrated a minimum level of toxic inhibitory effect at concentration ≥100 μg, whereas Sonchus cornatus, Balanites aegyptica, Acacia nilotica and Tamarindus indica enhanced lymphocytes proliferation. Bioactivity directed fractionation of the chloroform extract of the root bark of Maytenus senegalensis resulted in the isolation and characterization of the quinonemethide triterpene, (20α)-3-hydroxy-2-oxo-24-nor-friedela-1(10),3,5,7-tetraen-carboxylic acid-(29)-methylster (pristimerin). The structure was elucidated by spectroscopic techniques. The in vitro antimalplasmodial activity of the isolated compound against chloroquine-resistant strain (Dd2) of Plasmodium falciparum was IC50 = 0.5 μg/ml and its in vitro antileishmanial activity performed on promastigotes of Leishmania major was IC50 = 6.8 ± 0.8 μg/ml while the cytotoxicity on lymphocyte proliferation model was detected at IC50 = 6.8 ± 0.8 μg/ml. The promising response of Acacia nilotica and Mayenus senegalensis conclude that some Sudanese plants used in traditional medicine possess a potent antimalarial activity with minor effects on lymphocytes proliferation. These plants have been subjected to long-term clinical trials in folk medicine and hence we propose that these plants should be further investigated.

Email address for correspondence: ahmedeltahir@hotmail.com

Probability of emergence of antimalarial resistance in different stages of the parasite life cycle [MIM15991987]

Understanding the evolution of drug resistance in malaria is a central area of study at the intersection of evolution and medicine. Artemisinin combination therapies (ACTs) are now recommended worldwide as first line treatment for uncomplicated malaria, and losing them to resistance would be a disaster for malaria control. Studying the emergence and spread of antimalarial drug resistance in the context of different scenarios of antimalarial drug use is essential for the development of strategies protecting ACTs. We review the basic mechanisms of resistance emergence and estimate the probabilities of de novo resistance mutations at three stages of the parasite life cycle: sporozoite, hepatic merozoite, and asexual blood stages; we discuss the factors that affect parasite survival i.e. antimalarial drug use, immunity, and parasitaemia. In the absence of drug effects, and despite very different parasite numbers, the probability of resistance emerging at each stage is very low and similar in all stages (for example per-infection probability of 10-10-9 given the per-parasite chance of mutation is 10-10 per asexual division). However, under the selective pressure provided by antimalarial treatment and particularly in the presence of hyper-parasitaemia, the probability of resistance emerging in the blood stage of the parasite can be approximately five orders of magnitude higher than in the absence of drugs. The simple calculations do provide a framework for comparing probabilities at different stages of the parasite life cycle. Detailed models built upon these basic methods should allow us to predict the emergence of artemisinin resistance and design appropriate treatment strategies.

Email address for correspondence: pan@tropmedres.ac

Treatment seeking behavior of populations located in areas of identified drug resistance in Cambodia [MIM16689001]

Sochea Phok, Duong Socheat, Diane Freeman, Dianna Long, Chris Jones, Kheng Sim, Chea Nguon

The overall malaria prevalence decreased from 4.4% in 2004 to 2.9% in 2007. However, another phenomenon has appeared—drug resistance. It is influenced by the behavior of drug abuses, availability
and affordability of quality of antimalarial drugs, and knowledge of malaria treatment. In November 2008, a baseline study with the objective of monitoring consumer testing and treatment seeking behavior and volumes of specific anti-malarials consumed was conducted. A stratified, multi-stage cluster sample was conducted. The eligible respondents are to have a fever episode in the 2 weeks prior to survey. An interview administered questionnaire addressed socio-economic status, treatment seeking behavior, confirmed malaria, source and type of treatment as well as attitudes and beliefs around diagnostic testing. Caregivers completed the interview for eligible adolescents/children. Multivariate analysis will be conducted on data to address socio-economic variances and determinants of diagnostic testing. Psychometric tests will be performed on scaled determinants. A total of 1520 were included in the sample. All of respondents are adult with 75.4% female and 24.6% male. When someone in their family has got the fever, 52.5% of them bought drugs from the shop. The detailed results will be presented by socio-economic quintiles. Determinants of diagnostic testing will also be shown. Evidence will be used to inform access of populations living in Containment Areas to antimalarials, with specific focus on use and adherence to ACTs. Information will be used to inform national policy and complement existing evidence. Email address for correspondence: psochea@psi.org.kh

569
High risk of severe anaemia after chlorproguanil-dapsone + artesunate antimalarial treatment in patients with G6PD (A−) deficiency [MIM14819078]


Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common inherited human enzyme defect which provides some protection from clinical malaria, but can also cause haemolysis after administration of drugs with oxidant properties. G6PD deficiency in a secondary analysis of an open-label, randomized clinical trial [1] on the safety of chlorproguanil-dapsone + artesunate (CD + A) and amodiaquine + sulphadoxine-pyrimethamine (AQ + SP) for the treatment of uncomplicated Plasmodium falciparum malaria was evaluated. 702 children, treated with CD + A or AQ + SP and followed for 28 days after treatment were genotyped for G6PD A− deficiency. In the first 4 days following CD + A treatment, mean haematocrit declined on average 1.94% (95% CI 1.54–2.33) and 1.05% per day (95% CI 0.95–1.15) respectively in patients with G6PD deficiency and normal patients; a mean reduction of 1.3% per day was observed among patients who received AQ + SP regardless of G6PD status (95% CI 1.25–1.45). Patients with G6PD deficiency recipients of CD + A had significantly lower haematocrit than the other groups until day 7 (p = 0.04). In total, 10 patients had severe post-treatment haemolysis requiring blood transfusion. Patients with G6PD deficiency showed a higher risk of severe anaemia following treatment with CD + A (RR = 10.2; 95% CI 1.8–59.3) or AQ + SP (RR = 5.6; 95% CI 1.0–32.7). CD + A showed a poor safety profile in individuals with G6PD deficiency most likely as a result of dapsone induced haemolysis. Screening for G6PD deficiency before drug administration of potentially pro-oxidants drugs, like dapsone-containing combinations, although seldom available, is necessary (ClinicalTrials.gov identifier: NCT00461578). Email address for correspondence: skarema@gmail.com

570
Couverture et utilisation des moustiquaires impregnées d’insecticide après la campagne de distribution gratuite à Kinshasa, RD Congo [MIM16697676]

L.T. Bobanga, E.S. Umesumbu, J. Musenga

Le paludisme demeure encore un problème de santé publique en RDC avec près de 150 000 décès annuels dont la majorité est constituée des enfants de moins de cinq ans. Ainsi, le gouvernement avec l’appui financier de la banque mondiale a pris l’option de distribuer gratuitement les moustiquaires imprégnées d’insecticide (MII) dans le but de réduire l’impact de ce fléau. Près de 2 millions de MII ont été distribuées à Kinshasa avec comme objectif une couverture à 80%. Objectifs La présente étude avait comme objectif de déterminer la proportion des ménages possédant MII, de déterminer la proportion des ménages ayant installé la MII; la proportion des femmes enceintes et des enfants dormant sous MII. Une enquête transversale a été menée 1 mois après la campagne de distribution auprès de 2400 ménages dans la ville. Sur l’ensemble des ménages visités près de 79% disposaient d’au moins une MII. Email address for correspondence: lblehman@yahoo.fr

Le paludisme demeure encore un problème de santé publique en RDC avec près de 150 000 décès annuels dont la majorité est constituée des enfants de moins de cinq ans. Ainsi, le gouvernement avec l’appui financier de la banque mondiale a pris l’option de distribuer gratuitement les moustiquaires imprégnées d’insecticide (MII) dans le but de réduire l’impact de ce fléau. Près de 2 millions de MII ont été distribuées à Kinshasa avec comme objectif une couverture à 80%. Objectifs La présente étude avait comme objectif de déterminer la proportion des ménages possédant MII, de déterminer la proportion des ménages ayant installé la MII; la proportion des femmes enceintes et des enfants dormant sous MII. Une enquête transversale a été menée 1 mois après la campagne de distribution auprès de 2400 ménages dans la ville. Sur l’ensemble des ménages visités près de 79% disposaient d’au moins une MII. Email address for correspondence: lblehman@yahoo.fr
571
Automedication antimalarienne en milieu universitaire a Kinshasa [MIM16696817]

Kamitalu Kabongo, Maciste Kakesa, Louise Guruza, Michel Aloni

La nouvelle politique de traitement du paludisme initiée par l’OMS à travers le Programme National de Lutte contre le Paludisme (PNLP) recommande l’utilisation de l’association artésunate/amodiaquine en première intention, en République Démocratique du Congo. La quinine étant réservée aux formes résistantes ou graves du paludisme une étude prospective a été menée en milieux étudiantin à l’Université de Kinshasa du 1er janvier 2008 au 30 avril 2008 au Centre de Santé Universitaire. Les éléments anamnestiques, cliniques et biologiques ont été recherchés. N’étaient retenus que les cas de paludisme confirmé avec une goutte épaissie positive. Cent trente trois étudiants sur 458 suivis en consultation pendant cette période ont été retenus, soit (29,0%). Sur les 133 patients, on a enregistré 28 cas d’automedication soit 21,1%; sur les 28,12 (42,9%) ont pris les dérivés de l’artémisine, 9(32,1%) l’association sulfadoxine/pyriméthamine, 5(17,9%) la quinine, 1(3,6%) l’artémisine/amodiaquine et 1(3,6%) artésunate/luméfantrine. Aucun patient n’a pris une posologie exacte avec ces différents anti-malariens et toutes les gouttes épaisse étaient restées positives. L’automedication reste un problème préoccupant en milieu universitaire à Kinshasa. Les recommandations du PNLP ne sont pas suffisamment connues au sein de notre communauté étudiante. La prise de doses incorrectes expose cette catégorie de la population au développement des résistances.

Email address for correspondence: ramses.kamitalu@yahoo.fr

572
The efficacy of Malartin/Sulphadoxine-Pyrimethamine (Fansidar) combination in the treatment of uncomplicated falciparum malaria in a rural setting of the Mount Cameroon Region [MIM16496593]

Helen Kimbi, Theresa Nkou Akenji, Mesante Ntoko, Nelson Ntoni-for, Emmaculate Lun, John Egbe

The WHO now recommends the use of artemisinin-based combination therapy in the treatment of malaria in order to slow down the development of drug resistance against the parasite. The aim of this study was to assess the in vivo efficacy and tolerability of a combination of Malartin (artesunate) and Fansidar in the treatment of uncomplicated falciparum malaria in Dibanda, a rural setting in Southwest Cameroon. 197 subjects were recruited into the study, after meeting the inclusion criteria. They were then administered the appropriate doses of the drugs for 3 days and followed up on days 3, 7 and 14. A total of 174 subjects were successfully followed up. The drug combination was effective in clearing parasitaemia, fever and improving on the anaemia status of the patients. The overall success rate (ACPR) was 92.5% (161/174), and therapeutic failure was experienced in 07.5% (13/174) of the subjects. Parasite density decreased during the follow-up period in the different age groups and sexes. The prevalence of anaemia was 23.0% at enrollment and decreased to 10.0% on day 14. The drug combination was well tolerated as most of the side effects were self-limiting and disappeared by day 14. This study demonstrated that a combination of Malartin and Fansidar is effective and well tolerated in the treatment of uncomplicated falciparum malaria in this part of Cameroon. This confirms that artemisinin derivatives remain very potent and rapidly acting antimalarials to which the malaria parasite has not yet developed resistance.

Email address for correspondence: hkimbi@yahoo.co.uk

573
Evaluation of Rapid Diagnostic Tests, Optimal-IT (Diaimed) and malaria Plasmodium falciparum Rapid Test Device (Acon), for malaria diagnosis in children at Oyem and Owendo [MIM16425140]


Suspicion of malaria requires a rapid and accurate diagnosis to optimize the disease outcome. To confirm malaria infection, routine diagnosis is based on microscopy. But, its processing and interpretation need appropriate equipment and trained technicians not always available in endemic areas. New methods, Rapid Diagnosis Test (RDT), have been developed as an alternative. Our objective was to evaluate the diagnostic value of two RDTs (Optimal IT® and Malaria P f Rapid Test Device®) for malaria diagnosis in comparison with microscopy, in two areas of Gabon (Oyem and Owendo). Children from 0 to 10 years, with fever or history of fever, were included at Community Health Center of Owendo (CSCO) and at the regional hospital of Oyem. For each child, a thick blood smear and both RDTs, were realised for malaria diagnosis. From February 2008 to January 2009, 2125 children were included: 1436 (68%) at CSCO and 689 (32%) at CHRO. Four hundred and seventy-six children (22.4%) had positive blood smears whom 181 (13%) at CSCO and 295 (43%) at CHRO. Prevalence of positive tests was 27% for both RDTs. Sensitivity was 88% and specificity 91% for both RDTs. Sensitivity and specificity analysis of RDTs indicates that Optimal-IT sensitivity is variable. Previous studies showed variation from 24 to 98% depending on malaria species and study site. Specificity is mostly above 98%. No previous studies have been performed with Malaria Pf Rapid Test Device, no data are available about specificity and sensitivity for this test.

Email address for correspondence: dpmawili@hotmail.com

574
Knowledge about malaria and its relationship to net ownership and use in women of child bearing age in south-western Chad, Central Africa [MIM16598344]

Dr. Djékdoum Ndilta, Dr. Johannes Schäfer

Insecticide treated nets (ITN) are an important tool for the prevention of malaria in pregnancy. As part of a programme of subsidised distribution of ITNs to women a survey was conducted to assess knowledge about malaria and patterns of net ownership and use in the target population. A random cluster survey using standardised questionnaires was conducted among women of child bearing age in ten rural health zones. Among the 239 women interviewed there was a high rate of illiteracy (36%). Only one-third named mosquitoes as a cause of malaria, followed by exposure to cold (31%), supernatural causes (25%) and lack of hygiene (23%). Nets for prevention were mentioned by only 28%, next to vaccination (28%) and hygiene (26%), 25% could not name any method of prevention. 79% of the households owned at least one mosquito net, of these 58% were long-lasting insecticide treated nets, but less than 50% had used them the previous night. Utilization rates were significantly higher in those who mentioned nets as a means of prevention. Most ITNs were obtained from a health centre (90%), ITN ownership was higher in those living close to a health centre. Rates of ITN ownership were higher than previously reported for Chad; however the level of utilisation was low. Knowledge about malaria and its prevention were poor and there were numerous misconceptions. In addition to improving access to ITNs health there is a need to develop effective health education strategies targeting the needs of women.

Email address for correspondence: ndilta@yahoo.fr

www.mimalaria.org
575  
Lipid peroxidation and variation of some antioxidant enzymes and major antioxidant vitamins in Plasmodium falciparum malaria infected patients [MIM16745650]

Z. Ndongmo, S. Tiyong Ifoue, W. Abia, I. Couado

Malaria infection is accompanied by increased production of reactive oxygen species (ROS) that are produced both by the parasite and the human host. This production of ROS leads to the induction of an oxidative stress on the host cells. These biochemical injuries caused by oxidative stress represent a key factor in the pathophysiology of malaria. The measurement of the activity of antioxidant enzymes as well as the status of antioxidant vitamins may give the possibility of a specific prevention of malaria based on nutrition. The objective of this work was to investigate the possible alterations in antioxidant enzymes superoxide dismutase (SOD) and catalase, and in some major antioxidant vitamins (E and C). Plasma thiobarbituric acid reactive substances (TBARS) were quantified as malondialdehyde (MDA) level. Activities of SOD and catalase were measured and the total antioxidant capacity (TAC) was estimated to determine the status of the antioxidant vitamins. Plasma MDA level was significantly increased in malaria patients compared to controls. Activities of SOD and catalase as well as the antioxidant vitamins E and C were decreased significantly in malaria patients when compared to healthy controls. The general depression in antioxidant levels in malaria patients suggest that nutritional improvements of antioxidant capacities may be a therapeutical strategy to prevent the occurrence of oxidative stress and thus a concomitant decrease in severity of malaria. Hence, a significant reduction of the risk of lost of the over 2 million lives to malaria in sub-Saharan Africa every year.

Email address for correspondence: ndongmozita@yahoo.fr

576  
Connaissances, attitudes et pratiques face au paludisme: Enquête dans les formations sanitaires de Libreville, Gabon [MIM16986700]


La lutte contre le paludisme passe en partie par la prévention et l’application des recommandations de l’OMS sur la prise en charge de la maladie. Des directives ont été adoptées et introduites au Gabon en 2003, mais sont effectives depuis 2005. L’objectif de cette étude est d’évaluer les connaissances, le comportement des praticiens dans la prise en charge du paludisme trois ans après l’application de ces directives. Il s’agit d’une enquête descriptive transversale conduite de septembre à décembre 2008. Les prescripteurs enquêtés appartaient aux services publics et privés. Un interrogatoire à l’aide de deux questionnaires (agent de santé et structure sanitaire) a été effectué auprès des enquêtés. Au total, 220 prestataires ont été inclus dont 152 dans le public et 68 dans le privé. Les enquêtés étaient repartis en médecins (35%), infirmières/infirmiers (26%), étudiants-stagiaires (20,9%), sages-femmes (14,1%) et techniciens supérieurs de santé (3,2%). La moyenne d’âge des prescripteurs était de 31 ans ±7,3 ans. 35,2% des cas n’avaient pas pris connaissance des directives nationales et 24,3% ne les respectaient pas. Parmi ceux qui l’appliquaient (94,1%), les CTAs étaient le traitement antipaludique de première intention dans 93,7% des cas. Selon notre étude, les recommandations sur le traitement du paludisme sont appliquées dans la majorité des cas dans les secteurs public et privé. Toutefois, il est nécessaire de réaliser une étude de faisabilité afin de vérifier la concordance entre la réalité des prescriptions auprès des malades et ces résultats.

Email address for correspondence: ngoungou2001@yahoo.fr

577  
Age of participating mothers and IPT interventions [MIM16764839]

Sunny Oyakhirome, Julian Gabor

Intermittent preventive treatment (IPT) is an effective malaria control intervention for pregnant women [IPTp] and young children [IPTi] in Africa. The report from the IPTi intervention trial in Lambaréné, Gabon noted that the proportion of subjects that took first doses of anti-malaria drug was 85% and 51% took the third dose “IPT uptake Gap”. The efficacy of IPTi intervention was also lower in the Lambaréné trial compared to that reported by Schellenberg and colleagues. One reason for IPT uptake gaps is non-compliance to pre-natal care especially among young at first pregnancy. The potential impact of poor uptake of the IPT regime on effectiveness of IPT interventions is still being elucidated. We evaluated recruitment data of IPTi trial in Lambaréné Gabon. Twenty-seven percent (281) of women whose babies were recruited into the IPTi trial in Lambaréné were younger than 20 years of age and their babies had 30% higher odds of dropping out of IPTi intervention compared to babies of older mothers. The proportions that took dose 1 of the IPT regime were different in the two groups (0.03) but the significance increases at subsequent dosing (dose 2, p = 0.005; dose 3, p = 0.007). Age demography of mothers in IPT target population might have a significant impact on the effectiveness of IPT interventions and therefore need to consider in translating the IPT intervention results.

Email address for correspondence: drsunnysmcn@hotmail.com

578  
Validation of a new rapid diagnostic test for children with asymptomatic malaria: Implications for malaria control strategies [MIM16690216]

Samuel Wanji

Rapid and correct diagnosis of malaria is considered an important strategy in the control of the disease. However, it remains to be determined how well these tests can perform in those who harbour the parasite, but are asymptomatic, so that rapid diagnostic tests (RDTs) could be used in rapid mass surveillance in malaria control programmes. Microscopic and immunochromatographic diagnosis of malaria were performed on blood samples from the hyperendemic Mount Cameroon region. Thin and thick blood films were stained with Giemsa and examined under light microscopy for malaria parasites. The RDT was performed on the blood samples for the detection of Plasmodium species. In addition, the performance characteristics of the test were determined using microscopy as gold standard. Results revealed 40.32% to be positive for microscopy and 34.41% to be positive for the RDT. Parasites were detected in a greater proportion of samples as the parasite density increased. Plasmodium falciparum was the predominant Plasmodium species detected in the study population either by microscopy or by the RDT. Overall, the test recorded a sensitivity and specificity of 85.33% and 95.05% respectively, and an accuracy of 91.40%. The sensitivity and specificity of the RDT increased as parasite densities increased. The Hexagon Malaria CombiTM test showed a high sensitivity and specificity in diagnosing malaria in asymptomatic subjects and so could be suitable for use in mass surveillance programmes for the management and control of malaria.

Email address for correspondence: swanji@yahoo.fr
High levels of drug resistance to sulphadoxine-pyrimethamine (SP) have been reported from north-eastern Tanzania. This study compared the in vivo efficacy of SP in symptomatic 6–59-month children with uncomplicated malaria and in asymptomatic 2–10-month-old infants. An open label single arm (SP) standard 28 days in vivo WHO antimalarial efficacy protocol was used in 6–59 months old symptomatic children and a modified protocol used in 2–10 months old asymptomatic infants. Enrolment stopped early (87 in the symptomatic, 25 in the asymptomatic studies) due to the high failure rate. Molecular markers were examined for recombination and markers of drug resistance and a review of literature of studies looking for the 581G dhps mutation was carried out. In symptomatic children PCR-correction early treatment failure was 38.8% (95% CI 26.8–50.8) and total failures by day 28 were 82.2% (95% CI 72.5–92.0). There was no significant difference in treatment failures between asymptomatic and symptomatic children. 96% of samples carried parasites with mutations at codons 51, 59 and 108 in dhfr and 63% carried a double mutation at 437 and 540. 55% carried a third mutation at codon 581 in dhps gene. This triple:triple haplotype maybe associated with earlier treatment failure. In northern Tanzania SP is a failed drug for treatment and its utility for prophylaxis is doubtful. The study found a new combination of parasite mutations that maybe associated with increased and earlier failure.

Email address for correspondence: sgesase@yahoo.com

### 580 Adherence to artemether-lumefantrine (ALu) as first-line treatment for uncomplicated malaria and training on pharmacovigilance in rural African settings: The experience of ALIVE [artemether/lumefantrine in vulnerable patients: Exploring health impact] (MIM16671867)

Abdunoor M. Kabanywanyi, Nathan Mulure, Christian Lengeler, Raymond Schlienger, Aggrey Malila, Blaise Genton

To assess patients’ adherence and perceptions regarding ALu and pharmacovigilance under programmatic conditions. 552 patients recruited in the adherence and perception survey were randomized for a follow-up and impromptu visit at home after either ALu dose 2, 3, 4, 5, or 6 (patient blinded to assessed dose) and administered a structured questionnaire. For children <13 years (64%), questionnaires were administered to relatives/care takers. In the pharmacovigilance program ~40 health workers had a 1-day training on reporting and recording of SAEs and adverse drug reactions. Based on patients’ responses and blister pack checks, none missed a dose. Overall 95% of 112 patients assessed after Dose 2 took dose at 8 ± 1 h (recommended 8 h), and >80% of the remaining 440 patients took subsequent doses at the correct ±4 h. Overall, 92% patients found the pictogram on the ALu pack useful to take doses at the appropriate time. During the 6-month observation period, 17 AEs (14 after ALu), 7 SAEs (3 after ALu) and two deaths were reported: one due to progression to severe malaria and another due to pneumonia complicating malaria. Most frequently reported AEs after ALu intake were vomiting (5) and itching/rash (4). Adherence to standard ALu regimen was excellent and may indicate good understanding of pictorial dosing instructions on packaging. Additionally, the ALIVE experience puts forward the importance of dedicated training set-up in the frame of collaborations between research institutions, health authorities, industry and health care workers in order to ensure adequate reporting of AEs in rural African settings.

Email address for correspondence: ajithkumar.vasudevan@novartis.com

### 582 ARQ: An integrated multi-country monitoring program for a new combination bed net (PermaNet® 3.0) and insecticide resistance of malaria vectors (MIM15050289)

Tessa Knox, Helen Pates Jamet, Michael Stanley Peterson, Navneet Garg

PermaNet® 3.0 combines different technologies and fabrics for a long-lasting bed net with greatly improved bioefficacy and durability. It is suitable for application in areas where pyrethroid resistance is present. In order to evaluate household use and bioefficacy of this promising public health tool, the manufacturer (Vestergaard Frandsen) has established a long-term monitoring program to be applied in multiple sites in malaria-endemic areas. The monitoring program (termed ARQ) assesses: (a) user acceptability through focus groups and questionnaires, (b) insecticide resistance of vector populations in areas where this bed net has been distributed, and (c) quality performance in terms of physical structure and active ingredient content of nets. The program will continue at each site for a
minimum of three years after PermaNet® 3.0 distribution in order to evaluate temporal trends. Data collected will contribute to current public knowledge on the emergence of insecticide resistance. Country-specific reports provide feedback on the performance and utility of LNs under location-specific conditions. Results from this monitoring program will provide further insight into differences in net-use behaviour in a variety of ecological settings and detailed information regarding the impact of the first Combination Net in areas of pyrethroid resistance.

Email address for correspondence: tk@vestergaard-frandsen.com

583
Low quality of routine microscopy for malaria at different levels of the health system in Dar es Salaam [MIM16737338]
J. Kahama-Maro, V. D’Acremont, D. Mtasiwa, B. Genton, C. Lengeler

In malaria endemic areas, WHO recommends Rapid Diagnostic Tests (RDTs) for malaria in dispensaries, but not in health centers and hospitals. We evaluated the quality of routine microscopy for malaria and effect of RDT implementation at three levels of the health system. The positivity rate (PR) of routine microscopy was compared with routine RDTs in 3 hospitals, 3 health centers and 3 dispensaries. Routine blood slides were randomly picked in each of the 9 health facilities (HF) and 3 control HF. Sensitivity and specificity were measured using expert microscopy as reference. From April to December 2006, the mean PR using routine microscopy was 41% in hospitals, 52% in health centers and 47% in dispensaries. From April to December 2007, mean PR using RDTs was 6%, 6% and 7% respectively. PR of routine microscopy remained the same (72%) in control HF over these two years. Two surveys confirmed that the PR was low in Dar es Salaam: 13.6% in the HF and 1.9% in the corresponding community during rainy season and 3.3% in the HF during dry season. Sensitivity of routine microscopy was 71% whereas specificity was 47%. As a result 53% of blood slides were reported as positive by the HF, against 2% by expert microscopy. The quality of routine microscopy was poor in all health facilities, regardless of the type of facility. Over-diagnosis was massive. RDTs should replace microscopy as first-line diagnostic tool for malaria in all HF, especially in hospitals where potential for saving lives is greatest.

Email address for correspondence: judykmaro@yahoo.co.uk

584
Rapid diagnostic tests in the private health sector in Uganda: Randomised trial among drug shops to evaluate impact on antimalarial drug use [MIM16646708]
Anthony Mbonye, Richard Ndumugyenyi, Kristian Schultz Hansen, Clare Chandler, Pascal Magnussen, Siân Clarke

Most malaria deaths occur within 48 h of onset of symptoms, and in rural areas with poor access to health facilities, home management of malaria (HMM) can improve the timeliness of treatment and reduce malaria mortality by up to 50%. In order to maximize both coverage and impact, ACTs should be deployed in HMM programmes. It is widely recognised that up to 80% of malaria cases are treated outside the formal health sector and shops are frequently visited as the first (and in some cases only) source of treatment. Strategies to deploy ACTs in Africa thus also need to examine the role of shops in home management of malaria and to ensure that the drugs sold are appropriate. The current practice of presumptive treatment of any febrile illness as malaria based solely on clinical symptoms without routine laboratory confirmation, results in significant over-use of antimalarial drugs, and with ACT being a more costly regimen, it is important to be more restrictive in its administration. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in remote locations lacking electricity and qualified health staff. A randomised trial to evaluate the feasibility, acceptability, and cost-effectiveness of using RDTs to improve malaria diagnosis and treatment in registered drug shops and pharmacies is being undertaken in Mukono District, Uganda, 2009–2011. Findings from formative research to explore diagnostic processes and drug sales in the commercial sector, and attitudes towards diagnostic blood testing among private providers and patients will be presented.

Email address for correspondence: vpadmn@infocom.co.ug

585
Evaluation of the Kenya division of malaria control's indoor residual spray campaign in an area of high malaria transmission and high insecticide treated net use [MIM16697471]
Peter Otieno, John Gimnig, Philip Onyona, Kayla Laserson, Simon Kariuki, John Vulule, Laurence Slutsker, Mary Hamel

Insecticide treated nets (ITNs) and indoor residual spraying (IRS) are the two main malaria prevention strategies used in sub-Saharan Africa. There has been debate about the relative merits of ITNs and IRS with some claiming IRS to be more effective than ITNs while others argue that IRS is cost-prohibitive and unsustainable. One question which remains unanswered is whether the combination of these interventions further reduces malaria transmission beyond the levels that would be achieved by one of these interventions alone. If ITNs and IRS have synergistic effects when applied in combination, then this strategy may be an effective way to reduce transmission. However, if there is no added effect of combining ITNs and IRS, then implementation of both is an inefficient use of resources. We conducted cluster-sample cross-sectional surveys at household level in Rachuonyo (IRS) and Nyando (non-IRS) districts before and after IRS, to assess the combined effectiveness of ITNs and IRS in an area of high malaria transmission in western Kenya. From the on-going data analysis, we intend to show whether or not malaria parasitemia and anemia declined in Rachuonyo compared to Nyando before and after the IRS and to demonstrate whether IRS has an added benefit to ITNs. Data generated will provide critical information to malaria control programs on whether to continue and scale up the integrated vector control program, combining ITNs and IRS, or whether to conserve scarce resources and continue with the previous practice of promoting ITN use in all malarious areas, and conducting IRS campaigns in epidemic prone areas only.

Email address for correspondence: potieno@ke.cdc.gov

586
Overuse of artemisinin-combination therapy in Mto wa Mbu (river of mosquitoes), an area misinterpreted as high endemic for malaria [MIM15032522]

Adequate malaria diagnosis and treatment remain major difficulties in rural sub-Saharan Africa. These issues deserve renewed attention in the light of first-line treatment with expensive artemisinin-combination therapy (ACT) and changing patterns of transmission intensity. This study describes diagnostic and treatment practices in Mto wa Mbu, an area that used to be hyperendemic for malaria, but where no recent assessments of
transmission intensity have been conducted retrospective and prospective data were collected from the two major village health clinics. The diagnosis in prospectively collected data was confirmed by microscopy. The level of transmission intensity was determined by entomological assessment and by estimating zero-conversion rates using anti-malarial antibody responses. Malaria transmission intensity by serological assessment was equivalent to <1 infectious bites per person per year. Despite low transmission intensity, > 40% of outpatients attending the clinics in 2006–2007 were diagnosed with malaria. Prospective data demonstrated a very high over-diagnosis of malaria. Microscopy was unreliable with <1% of slides regarded as malaria parasite-positive by clinic microscopists being confirmed by trained research microscopists. In addition, many ‘slide negatives’ received anti-malarial treatment. As a result, 99.6% (248/249) of the individuals who were treated with ACT were in fact free of malaria parasites. Transmission intensity has dropped considerably in the area of Mto wa Mbu. Despite this, most fevers are still regarded and treated as malaria, thereby ignoring true causes.

Email address for correspondence: sshekaghe@yahoo.com

587
The management of febrile patients drastically improved after introduction of Rapid Diagnostic Tests for malaria in health facilities of rural Tanzania [MIM16692723]
R. Tyllia, V. D’Acremont, N. Swai, B. Genton, C. Lengeler

Presumptive treatment with antimalarials is often considered the safest strategy for children presenting with fever in highly endemic areas, the reason being that no malaria case is left untreated. We assessed the impact of routine RDT introduction on the quality of management of malaria patients in health facilities. After training of health workers of 2 health-centers and 4 dispensaries, RDTs were introduced. Consultation processes were observed before and 12 months after RDT initiation. Data on antimalarial use were extracted from each health-facility drugs-books. Before RDT-implementation, proportion of febrile patients tested for malaria with microscopy was 43% only. 23% were reported as having a negative microscopy result and, among them, 22% still treated with antimalarials. Among non-tested patients, 48% were not treated with antimalarials at all. Preliminary results from the survey done one year after RDT initiation show that the proportion of febrile patients tested increased while the one of patients not-tested and not-treated decreased drastically. Since RDT-performance was much better than that of the former routine microscopy, proportion of reported positive-results decreased from 63% to 35% after RDT initiation. This led to a drop of 1.8-fold in the overall antimalarials’ consumption. With RDT, clinicians stopped leaving half of febrile patients untreated and untreated for malaria. The strategy of using RDTs in routine management of febrile patients is clearly much safer than that of presumptive treatment. Instead of a random sample of patients treated with antimalarials, true malaria cases are treated with an antimalarial-drug and not negative patients.

Email address for correspondence: robert_tillya@yahoo.com

588
Comparison between district based and centralized (IDI based) method of delivering the integrated team-based training on improving malaria case management of health care workers in Uganda [MIM16696780]
Umaru B. Ssekabira, Senjovu K. Dan, Alex Ojaku, Allen Namagembe, Priscilla Omwangangye, Patrick Eyal, Marcia R. Weaver, Lydia Mpanga Sebuyira, Adoke Yeka, Alex Coutinho

Malaria case management in Africa is characterized by presumptive treatment and substantial overtreatment. Health workers often do not request diagnostic tests for patients suspected to have malaria. When tests are performed, health workers prescribe antimalarials despite a negative test result. We compared the effectiveness of an integrated team-based training program delivered by national trainers at 1 government clinic with that delivered by district trainers in 2 government clinics in Uganda. Health center staff: clinicians, laboratory staff, and records clerks were trained. Training covered general aspects of malaria, evaluation and treatment of febrile patients, preparation of blood smears, microscopy skills, and medical record keeping. Two support supervision visits were made 6 and 12 weeks after training. Outcomes were measured using a surveillance system. Data collected for 64 days before and 87 days after training was compared. Diagnostic accuracy of field microscopy before and after training was compared using expert microscopy as the gold standard. The two trainings were associated with significant increase in proportion of patients with suspected malaria referred for blood smears (from 41.5% to 58.5% (P < 0.001) for central site from 68.1% to 75.3% (P < 0.040) for district sites), decreased proportion of patients with negative blood smears prescribed antimalarials (reduced by 32.6% (P < 0.001) for central site and by 38.6% (P = 0.0064) for district sites). The diagnostic accuracy for microscopy significantly improved in the district based sites. The improvement in performance of clinicians and laboratory staff in all sites; implies that the two training methodologies are effective in transferring knowledge for improved malaria case management.

Email address for correspondence: ussekabira@idi.co.ug

589
Artemether-lumefantrine versus dihydroartemisinin-piperazine for uncomplicated malaria: A longitudinal randomized trial in young Ugandan children [MIM16202244]

Artemisinin-based combination therapies (ACTs) are now widely advocated for the treatment of malaria. There is debate as to the optimal ACT and limited data in very young children. Artemether-lumefantrine and dihydroartemisinin-piperazine were compared in a longitudinal randomized clinical trial. 351 children aged 1.5–12 months were provided insecticide treated nets and followed for all their health care for a median duration of 289 days. Children at least 4 months of age and 5 kg diagnosed with their first episode of uncomplicated malaria (fever and positive blood smear) were randomized to artemether-lumefantrine or dihydroartemisinin-piperazine. The same therapy was given for all subsequent episodes. 113 children were randomized to artemether-lumefantrine and 119 children to dihydroartemisinin-piperazine, resulting in 320 and 351 treatments, respectively. After 28 day follow-up, artemether-lumefantrine had a significantly higher risk of recurrent parasitemia unadjusted by genotyping (35% vs. 11%, p < 0.0001), but the risk of recrudescence was low if both treatment arms after adjustment by genotyping (1.0% vs. 0.3%, p = 0.24). Extending follow-up to 63 days resulted in a similar risk of recurrent malaria and malaria incidence following randomization was similarly high in both arms (4.82 vs. 4.61 total episodes per person year, p = 0.63). These treatments were safe and well tolerated. Both treatments were safe and efficacious in terms of preventing treatment failures due to recrudescence in very young children. Compared to dihydroartemisinin-piperazine,
There is an urgent need to assess the safety of artemisinins in early pregnancy.

Methods: Between June 2006 and October 2008, the safety of artemisinins during early human pregnancy was assessed in central-eastern Sudan. Pregnant women in the first or second trimester who were attending antenatal-care clinics at the Wad Medani, Gadarif and New Halfa hospitals were interviewed. Each was asked if they had had malaria in the first trimester of the index pregnancy and, if so, what treatment they had received. The women who had received artemisinins were then followed-up until delivery and their babies were followed-up until they were 1-year olds. Overall, 62 of the pregnant women reported receiving artemisinins – artemether injections (48), artesunate plus sulfadoxine–pyrimethamine (11) or artemether plus lumefantrine (three) – during the first trimester. Only nine (15%) of the 62 women given artemisinins had not known that they were pregnant when treated. Two of the treated women (both given artemether injections in the first trimester) had miscarriages, one at 20 weeks of gestation and the other at 22 weeks, each while receiving quinine infusions for a second attack of malaria. The other 60 women who had received artemisinins delivered apparently healthy babies at full term. No congenital malformations were detected, there was no preterm labour, no maternal deaths were recorded during the follow-up, and none of the babies died during their first year. It therefore appears that artemisinins may be safe to use during early pregnancy, although further study is clearly needed.

Email address for correspondence: ishagadam@hotmail.com

Quality assurance of rapid diagnostic tests for malaria in routine patient care in rural Tanzania [MIM15578545]

I.M. Masanja, M. McMorrow, S.P. Kachur, E. Kahigwa, S. Abdulla

Histidine rich protein II (HRP2)-based malaria rapid diagnostic tests (RDTs) have shown high sensitivity and specificity for detecting Plasmodium falciparum malaria in variety of study settings. However, RDTs are sensitive to heat and humidity which may affect their useful life in field use. Currently, RDT manufacturers provide no instructions for quality assurance (QA) of RDTs. We evaluated sensitivity and specificity of RDTs during routine use for malaria case management in rural Tanzania by comparing RDT results to reference microscopy. From September 2006 to April 2007, we introduced HRP2-based Paracheck® RDTs for suspected malaria in patients greater than five years of age in nine health facilities with existing microscopy. Facility laboratorians performed thick blood smears on patients receiving RDTs for reference microscopy. In the second implementation, December 2007 to October 2008, HRP2-based ParaHIT® RDTs were introduced in twelve facilities without microscopy. Thick blood smears were collected for 2–3 days per week from patients receiving RDTs for reference microscopy. In the initial implementation, assessment of RDT sensitivity and specificity was difficult due to poor quality of blood smear staining. Operational mean sensitivity was 64.8% and specificity was 87.8%.

In the second implementation, the overall RDT sensitivity improved to 90.8% and specificity decreased to 78%. QA is essential to the adequate performance of any laboratory test. Successful implementation of RDTs was achieved in peripheral health facilities with adequate training and supervision. However, this level of QA is not sustainable nationally. There is an urgent need for widely implementable QA guidelines for RDTs.

Email address for correspondence: imasanja@ihi.or.tz

Synergistic effects of Artemisia annua on in vitro cultures of P. falciparum [MIM15015997]

Lucy Kangethe

Malaria is an infectious disease that continues to be associated with considerable morbidity and mortality and significant social and economic impact on developing societies. In humans a protozoan of the genus Plasmodium causes it. Four species are implicated; these are Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax and Plasmodium malariae. Approximately 300 million people worldwide are affected and between 1 and 1.5 million people die from it every year. The research dealt with Artemisia annua a herb native to China and aimed at understanding the role of different fractions in the antiplasmodial activity of the chemical blends of the herb. The plant A. annua has been used for many
centuries in Chinese traditional medicine as a treatment for fever and malaria. Upper foliage 3.5 kg was collected, dried for 2 weeks, ground to powder form, and sequential extraction followed using dichloromethane (DCM) and methanol. The effect of the crude extract was tested on in vitro cultures of *P. falciparum*. The extract was the fractionated using high performance liquid chromatography (HPLC). The fractions and different blends of these were tested on *P. falciparum* to determine if there is any additive or synergistic effects between them. Nine different fractions of *A. annua* were tested against D6 (CQ sensitive isolate) and W2 (CQ resistant isolate). 2 fractions (22.2%) had an activity above 250 mg/ml, 4 fractions (44.4%) had an activity of less than 3.9 mg/ml whereas the remaining 3 fractions (33.3%) had an activity of between 4.77 and 14.76 mg/ml against isolate D6 and isolate W2. After blending the activity in all the blends was below 27 mg/ml. The fractions were run together with the convolucrational drugs and their IC50 compared.

Email address for correspondence: fkkateera@yahoo.com

594  
Efficacy, safety and tolerability of amodiaquine-artesunate verses artemether-lumefantrine for the treatment of uncomplicated malaria in HIV-infected children in Kampala, Uganda [MIM16667695]
Fredrick Kateera, Grant Dorsey, Jane Achan, Theodore Ruel, Edwin Charlebois, Diane Havlir, Philip Rosenthal, Moses R. Kamya, Anne Gasasira

Artemisinin-based combination therapies have been adopted as first-line antimalarial treatment in most African countries. However, there are limited data on the safety and tolerability of these therapies in HIV-infected populations. We compared the efficacy, safety and tolerability of artesunate-amodiaquine (AS/AQ) and artemether-lumefantrine (AL) for the treatment of uncomplicated malaria in HIV-infected Ugandan children. Antiretroviral therapy (ART) was prescribed according to national guidelines. AS/AQ was used for malaria treatment between August 2006 and June 2007 and AL between July 2007 and December 2008. Patients treated for malaria were followed for 28 days using standard WHO guidelines. Complete blood counts were measured on days 0 and 14. Multivariate analysis was used to identify independent predictors of adverse events classified according standardized criteria. Seventy-four episodes of malaria (37 treatments with AS/AQ and AL each) occurred in 47 children. ART was concomitantly used in 50% of antimalarial treatments. Both AS/AQ and AL were highly efficacious with only one late parasitological failure, due to a new infection, occurring in the AS/AQ group. The risk of common adverse events following antimalarial treatment included neutropenia (28%), anorexia (18%), malaise (18%), and abdominal pain (12%). Compared to AL, AS/AQ was associated with a higher risk of neutropenia (RR = 2.1, 95% CI = 1.1-4.0, p = 0.03) and anorexia (RR = 5.0, 95% CI = 1.3-19.3, p = 0.01). After adjusting for repeated measures, age, and ART use, AS/AQ and AL were highly efficacious in treating uncomplicated malaria in HIV-infected Ugandan children; however, AL was safer and better tolerated than AS/AQ.

Email address for correspondence: fkkateera@yahoo.com

595  
Impact of *Plasmodium falciparum* infection on hematological parameters in children living in Western Kenya [MIM16495244]
Robert Maina

Malaria is the commonest cause of childhood morbidity in Western Kenya with varied haematological consequences. In this report we sought to elucidate the haematological changes in children infected with malaria that can impact improved diagnosis and therapy for childhood malaria. We performed a retrospective audit of hematological parameters in 961 children (+, n = 523 and −, n = 438) living in Kisumu West District, an area of malaria holoendemic transmission. The following parameters were significantly lower in malaria positive children: platelet, lymphocyte, eosinophil, RBC counts, and Hb, while absolute monocyte, neutrophils counts, and mean platelet volume were higher in comparison to malaria negative children. Children with platelet counts of <150,000 µL⁻¹ were 15 times (odds ratio) more likely to have malaria. A platelet count of <150,000 µL⁻¹ was found in 49% of malaria positive cases and was associated with high parasitemia levels, low Hb levels, increased mean platelet volume (MPV) and platelet aggregate flag. Platelet aggregates were more frequent in the malaria positive group (25% vs. 4%, p < 0.0001) and associated with thrombocytopenia rather than malaria status. *Plasmodium falciparum* malaria in children is associated with important changes in some hematological values. Low platelet count and hemoglobin levels are the two most important predictors of malaria infection in this population. When used in combination with other clinical and microscopy parameters, they could improve malaria diagnosis in sub-patient cases.

Email address for correspondence: rmjunguna@wrp-ksm.org

596  
Malaria treatment in the private drug sector in Tanzania [MIM16668458]

Throughout Africa, the private (drug) sector is an important source of antimalarial treatment, complementing formal health services. Accredited Drug Dispensing Outlets (ADDOs) were introduced in the Kilombero and Ulanga districts in 2006 to improve the performance of drug selling shops. In August 2007 ADDOs started selling highly subsidized artemether-lumefantrine (ALu). The present study, conducted in the frame of the ACCESS Programme, aimed to assess the performance of drug selling shops before and after implementation of ADDOs. We conducted yearly cross-sectional shop surveys (2004–2007) to record drug stocks, complemented by mystery shopper studies (2004–2007) for assessing quality of advice and treatment. From 2004 to 2007 the number of villages without drug shops decreased from 14 to 6. From 2004 to 2005 the number of shops increased by 64% but then stayed stable from 2005 to 2007 after the introduction of ADDOs. Overall, less antimalarials were dispensed in drug shops after the introduction of ADDOs. Before ADDOs, amodiaquine and quinine were dispensed as often as sulphadoxine-pyrimethamine (SP), while after ADDOs, SP was mostly sold (as long as subsidized ALu was not yet available). The percentage of customers who got correct advice and treatment increased after the introduction of ADDOs. The quality of malaria case management in the retail sector has improved since the introduction of ADDOs in the study districts. However, the overall availability of antimalarials did not increase and there are still villages without commercial access to drugs.

Email address for correspondence: makemba_am@yahoo.co.uk
Community perceptions of clinical trials and their effect on participation in a trial of intermittent preventive treatment for malaria in infants (IPTi) in North Eastern Tanzania [MIM15051291]

Peter Mangesho

With the recent push to reduce the mortality and morbidity of malaria in sub-Saharan Africa, larger scale community based intervention studies are being planned and carried out. For interventions to be tested in generalisable way relevant segments of the community must be involved. During recruitment into a randomized placebo controlled trial of safety and efficacy of intermittent preventive treatment of malaria in infants (IPTi) we noticed that despite district, village and individual sensitization approximately 30% of sensitized mothers did not return for enrolment into the study. We did a qualitative study to explore factors influencing participation and non-participation in the IPTi trial. Semi-structured questionnaires and in-depth interviews were used. Non-participation among those who were sensitized but did not enrol, those who later dropped, was linked to fear of drug side effects, unmet expectations, misconceptions about the aim of the project and refusal by a husband or father of the child. Those who agreed to participate did so mainly to obtain prevention and cure for malaria or to receive free services. Participation was influenced mainly by rumours. This study suggests that sensitization of malaria trials should involve the whole community including fathers and it should be a never ending process throughout the trial period.

Email address for correspondence: mangeshop@gmail.com

Lessons from plan Tanzania's long lasting insecticide treated net (LLINs) project [MIM15080786]

Louisa Masayanyika

Malaria is the leading cause of morbidity and mortality in children below five years of age in Tanzania. LLINs are among effective tools for reducing this situation. The health of children being one of our priorities, we decided to assist communities with the provision of subsidized LLINs through a voucher system for children below five years starting July 2006 to June 2008. A baseline survey assessing ownership and use of nets among children below five years was conducted in Kisarawe, Mwanza and Geita districts in June 2006. Following the survey, we collaborated with District Medical Offices in registering children and distribution of vouchers by community health workers. Vouchers were exchanged with LLINs to nearby appointed retailers with a top up of Tshs 1500. After a year’s intervention a follow up study was conducted assessing success and challenges of the project. 90.4% of voucher redemption was achieved. The project increased overall under five net ownership (44% compared to 28.6% in comparison wards) and use. Morbidity due to malaria decreased from 48.6% to 43.8%. Majority of nets in the intervention wards were found to be from the project as compared to comparison wards where most nets came from other sources. Children from poorest households both in intervention and comparison wards remained with the lowest net ownership indicating that a different approach needs to be adopted to reach this particular target group.

Email address for correspondence: louisa.masayanyika@plan-international.org

Improvements in the quality of care for prompt and effective malaria treatment [MIM16692316]

Dominic Mboya, Ahmed Makemba, Flora Kessy, Sandra Alba, Alexander Schulze, Christian Lengeler, Brigit Obrist

The Quality Improvement and Recognition Initiative (QIRI) aims to improve the quality of services available at health facilities. Gaps are identified by comparing services available at health facilities with the expectations on these services, as defined by (1) the national standards of care and (2) community preferences. We aimed to assess the quality of care provided in health facilities, with emphasis on proper malaria case management. A baseline assessment was conducted in 75 health facilities in the Kilombero and Ulanga districts in southern Tanzania using a QIRI tool with quality indicators on six major areas. Of the 151 health care providers that we observed, 78% were able to show malaria treatment guidelines. 85% of 60 providers who treated patients enquired about history of fever in malaria suspected patients, but only 65% gave correct treatment classifications. The recommended first line drug (ALu) was available in 82% of health facilities over the past 3 months and ALu treatment algorithms were displayed in 80% of health facilities. 88% of health facilities had the second line drug quinine in stock. 72% of the surveyed facilities had SP for Intermittent Preventive Treatment. 54% of providers were trained on malaria but only 35% on IMCI. The findings show adherence to preventive and curative treatment guidelines and good availability of antimalarial drugs. The level of malaria and IMCI management training is low and this calls for tailor made training on malaria case management to untrained providers and continuous supportive supervision.

Email address for correspondence: mdmansu@yahoo.com

High efficacy of artemether-lumefantrine three years after use as first line drug for the treatment of uncomplicated falciparum malaria in Wondo Genet, South-central Ethiopia [MIM16323253]

Moges Kassa, Afework H/mariam, Markos Sileshi, Woldemariam Girma, Francis Kimani, Ambachew medhin

In Ethiopia, artemether-lumefantrine (AL) was officially adopted as the first line treatment for uncomplicated Plasmodium falciparum malaria in 2004. This study measures the efficacy of AL in a malarious area of the country where there is no information on its efficacy was available. Between October 2007 and January 2008, in vivo efficacy study was conducted in Wondo Genet (South-central Ethiopia). 103 patients aged 1 year and above with uncomplicated P. falciparum malaria were treated with standard dose of AL and responses were assessed over a 28-day follow up period. Outcome of treatment was defined following the 2003 WHO guidelines. Recurrent parasitaemia were genotyped by polymerase chain reaction (PCR). Analysis was by intention-to-treat (ITT) and per protocol (PP). Day 28 PCR unadjusted adequate clinical and parasitological response (ACPR) rates in the ITT (n = 99) and PP (n = 96) population were 96.0% and 95.8%, respectively. The corresponding rates after PCR adjustment were 96.9% (95/98) and 96.8% (92/95), respectively. Most patients had cleared their fever (93.9%) and parasite (96.0%) on day 3 and there was no patient with gametocyte by day 7. However, the mean haemoglobin did not show significant change between baseline (12.2 g/dL) and day 28 (12.5 g/dL) (P = 0.173). No drug related serious adverse events were reported AL was highly effective for the treatment of uncomplicated falciparum malaria 3 years after use as first line in this rural village. Whether a similar
pattern is followed in other malarious areas of the country need to be investigated.

Email address for correspondence: eyobmk@yahoo.com

601 Real-field stability of two brands of malaria Rapid Diagnostic Tests (RDTs) at a tropical area in Sudan [MIM16699090]
Muneir M.N. Mohamedein, Osama M.E. Seidahmed, Afrah A. Elsir, Eldirdieri S. Ahmed

Reliance on malaria Rapid Diagnostic Tests (RDTs) is considerably subject to the appraisal of their effectiveness under tropical-field conditions of storage and performance real-field stability of two brands (HRP2-based and pLDH-based) of RDTs was assessed in terms of validity and accuracy at a tropical area in Sudan. Kits of the RDTs were kept under field conditions within cupboards at six health dispensaries along six months. Temperature above 30 °C and relative humidity less than 40% were repetitively recorded in the storage cupboards throughout the study period. Core Malaria Pf™ devices (HRP2-based) were remained valid (control band was visible) at these storage conditions. Whereas, a gradual decrease on stability of OptiMAL IT™ devices (pLDH-based) – which is associated with the absence of control lines – was negatively correlated with the monthly increase of mean temperature, but positively correlated with the mean of humidity. However, effect of the field conditions on accuracy of the two brands of RDTs was unobserved. In conclusion, we appraised distribution and use of HRP2-brands of RDTs in tropical areas. Logistical arrangements such as improving transport and storage conditions (e.g. developing cool chains) and shortening storage periods at the periphery health system (particularly during summer season) should be considered in order to avoid damage of RDTs.

Email address for correspondence: mmohamedein@gmail.com

602 Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar—A cross-over validation study [MIM16670486]
Mwinyi Msellem

No Abstract

Email address for correspondence: mmwinyi@hotmail.com

603 Utility of a point of care test for lactic acidosis in children admitted to hospital for severe malaria in endemic area of Tanzania [MIM16697461]
George Mtove, B. Nadjm, Ben Amos, Hugh Reyburn

Metabolic acidosis largely contributes to mortality in children with severe malaria. Its recognition is important to guide blood transfusion in children with intermediate anaemia and possibly to treat presumptively for co-existent bacterial disease. Tanzanian guidelines recommend blood transfusion for children with haemoglobin between 4 and 6 g/dl accompanied by acidosis (deep breathing or respiratory distress) but these signs are often not sought in African hospitals. We evaluated the use of low-cost lactate-meter in detecting lactic acidosis and guiding treatment in children with severe malaria. Children 2 months to 13 years with recent or current fever were enrolled over 1 year. Clinical information was recorded on standard form and blood collected for serum lactate (Lactate-Pro™), pH, blood culture and malaria slide. Of 884 children with WHO criteria of severe malaria, 85 (9.6%) died. Blood lactate >5 mmol/l identified additional 121 children, 5% of whom died while children with lactate <5 mmol/l and no other signs of severe malaria only 0.42% died. Among children with severe malaria, 353 had haemoglobin between 4 and 6 g/dl and 28 died with no other indication for blood transfusion. The odds of bacterial co-infection were increased (OR 1.38, p = 0.02) for blood lactate >5 mmol/l. Compared to venous pH using iStat™, the sensitivity and specificity of Lactate-ProTM were 19.5% and 81.4% respectively. bedside blood lactate is effective means of identifying children with mild-moderate acidosis who would not have been detected clinically. This information can be used to improve targeting of blood transfusion and antibiotics in children with severe malaria.

Email address for correspondence: mtoveg2002@yahoo.co.uk

604 Community acceptability of the use of rapid diagnostic tests for malaria by community medicine distributors in Ilanga District, Uganda 2008 [MIM16669815]
D. Mukanga, J. Tibenderana, J. Kiguli, G. Pariyo, S. Peterson, K. Kallander

Uganda is currently moving towards introduction of an artemisinin-based combination at community level. Introduction of this efficacious but expensive treatment with reliance on presumptive diagnosis alone, may lead to excessive use, increased costs and risk of development of resistance. If used correctly by community medicine distributors (CMDs), rapid diagnostic tests (RDTs) for malaria could potentially improve diagnosis and the quality of care. We assessed community acceptability of the use of RDTs by CMDs. through focus group discussions using a pre-designed interview guide, we interviewed care-takers of under-5 children identified through purposive sampling. The selection criterion was based on having ever had or taken care of a child with fever. Thematic content analysis was used. Community members had mixed feelings about blood and a finger prick. Fears ranged from concerns that the blood could be used to test people for HIV or for witchcraft. Some participants wondered why test for malaria when the signs are clear to everyone. Participants said that given their CMDs past experiences they would actually be able to take on the new task of conducting an RDT provided they are trained, equipped and facilitated. “She will manage because she is well educated and can speak English well. She talks well to even us who did not go to school and tells you what is going on” although some community members have misgiving about drawing blood, there was consensus that CMDs if trained and supported would be able to take on this new task.

Email address for correspondence: dmukanga@afenet.net

605 Evaluation of home-based management of fever with artemether-lumefantrine in urban Ugandan children: A randomized trial [MIM16349257]
Norah Mwebaza, MBChB, Moses R. Kamya, MMed, Tamara D. Clark MPH, Grant Dorsey MD, PhD, Philip J. Rosenthal, MD, Christopher J.M. Whitty, FRCP, Sarah G. Staedke, MD

Artemisinin-based combinations (ACTs) are being introduced into home management of malaria (HMM) programmes; however, no data on the clinical impact of HMM using ACTs are available. We evaluated the impact of home delivery of artemether-lumefantrine (AL) for management of febrile illnesses on the
incidence of antimalarial treatment and other clinical measures, compared to the current standard of care in Kampala, Uganda. Households were randomised following a pilot period and children were followed for 12 months. Of 437 children, 225 were assigned to HMM and 212 to standard care; 365 (84%) completed follow-up. Significantly more febrile episodes were treated within 24 h with an effective antimalarial in the HMM arm than in the standard care group (444/862 [58%] vs. 30/570 [8%], respectively, RR 7.19, 95% CI 4.58–11.27, p < 0.0001). At the final evaluation, the proportion of participants in the HMM arm with parasitaemia was lower than in the standard care arm (2% vs. 10%), but no other differences in standard malariometric indices, including anaemia, were seen. The HMM group received nearly twice the number of antimalarial treatments per person-year as the standard care group (4.66 vs. 2.53, IRR 1.72, 95% CI: 1.43–2.06, p < 0.0001), and approximately five times the number administered for microscopically confirmed cases of malaria in a comparable cohort of children (4.66 vs. 1.03, IRR 5.19, 95% CI: 4.24–6.35, p < 0.0001). In this urban setting, HMM with AL improved prompt effective treatment of fever, but had a modest impact on clinical outcomes, at the cost of substantial over-treatment.

Email address for correspondence: nmwebaza@med.mak.ac.ug

606 Pharmacokinetics of paracetamol in children with fever associated with severe malaria [MIM15295477]


Paracetamol is often used as an antipyretic in children with falciparum malaria. The currently recommended dosages of paracetamol for children have been based on Western children with viral illnesses, rather than on any pharmacokinetic–pharmacodynamic considerations. Although a paracetamol plasma concentration of 10 mg/L is the steady state plasma concentration (Css) reported to be appropriate for antipyretic effect, it is uncertain whether this target concentration is achieved with the currently used doses in children with malaria. We evaluated the pharmacokinetics of paracetamol in children with fever associated with severe malaria. Thirty-five children aged 30 (range: 12–115) months with severe malaria and fever (≥38°C) received paracetamol orally (15 mg/kg 6 hourly) for 24 h. Blood samples were collected at various times over 24-h period after drug administration. Plasma paracetamol concentrations were measured using Abbott TDx FLx polarization immunoassay analyzer. Plasma concentration–time data were used to simulate for dosing regimens that would be required to achieve a target Css ≥ 10 mg/L, using TOPFIT™ program. Median (range) paracetamol Css of 8.2 (2.7–22.4) mg/L was achieved within a median Tmax of 2.0 (0.5–6) h. Only 13 (37%) children achieved paracetamol Css > 10 mg/L. Simulations suggested that a Css ≥ 10 mg/L would be achieved with a paracetamol loading dose (30 mg/kg) followed by maintenance doses (15 mg/kg 6 hourly). Administration of paracetamol at the currently recommended dose (15 mg/kg 6 hourly) achieved sub-therapeutic plasma paracetamol concentrations, suggesting that this dosage regimen needs to be re-evaluated.

Email address for correspondence: sndirangu@kilifi.kemri.wellcome.org

607 What if nets are not enough? Examining malaria prevention practices and health outcomes in Mvomero, Tanzania [MIM16771985]

Elizabeth H. Shayo

Malaria control policies in Tanzania and elsewhere place a heavy emphasis on the importance of households’ ownership and use of insecticide-treated mosquito nets. While there has been intense debate over how nets should be distributed, researchers and policymakers seem united in their belief that once net use reaches a high enough level, malaria rates will drop substantially. Additional operational research is needed to assess whether or not this is the case, with particular attention to how human behavior affects use of mosquito nets. Household surveys administered to 408 households in Mvomero District, Tanzania, collected data on households’ ownership and use of mosquito nets, self-reported malaria illness, and socioeconomic variables. Net ownership and use appear quite high, with between 83 and 93% of households owning at least one mosquito net, and 74–88% of children under five sleeping under nets. More than half of nets were reportedly retreated with insecticides in the 6 months prior to the survey. Despite high levels of net use, self-reported malaria rates in this area are also quite high: 53% of all individuals and 69% of children under five had a reported malaria case in the 3 months prior to the survey. This paper suggests that high rates of malaria illness can persist in any area even when use of mosquito nets has reached a very high level. While additional operational research is needed to determine nets’ effectiveness in real-world settings and mosquito biting behavior complementary malaria control strategies also need to be developed.

Email address for correspondence: bshayo@yahoo.com
609 Evaluation of Artemisia annua infusion efficacy for the treatment of malaria [MIM16152545]

Magnus A. Atemnkeng, Bantuzeko Chimanuka, Biekke Dejaegher, Yvan Vander Heyden, Jacqueline Plaizier-Vercammen

The efficacy of artemisinin (AR) against malaria has prompted its use as a tea drink in many endemic regions. Such arguments have favoured the propagation of Artemisia annua L. plant cultivation and its use as an infusion. However, there is controversy surrounding its effectiveness in this form. The aim of this study was to assess the efficacy of A. annua infusion in plasmidium infected mice. OF1 mice infected with Plasmodium chabaudi chabaudi were treated twice daily for 6 days by administration of: water (control group), A. annua infusion (tea group), 0.022 mg AR (AR-equiv. group) and 0.8 mg AR on the first day and 0.4 mg the following days (AR-WHO group). Thin Layer Chromatography was used to quantify the artemisinin concentration in the plant material. Initially, the parasitaemia increased in all groups. On day 4 it reached 75, 72, and 0.8 mg AR on the first day and 0.4 mg the following days (AR-WHO group). Thin Layer Chromatography was used to quantify the artemisinin concentration in the plant material. Initially, the parasitaemia increased in all groups. On day 4 it reached 75, 72, 50 and 3% in the control, AR-equiv, tea and AR-WHO groups respectively. Mice treated with A. annua tea died after 11 days, while 83% of those that received the AR-WHO dose survived. The concentration of artemisinin in the plant was found to be 1.15 g per 100 g of dried leaves. The tea does not decrease the parasitaemia fast enough. We suggest that the artemisinin concentration in the plant should be standardized and large scale clinical trials conducted in humans are necessary to determine its efficacy in the standardized tea. Additionally, other treatment options should be considered.

Email address for correspondence: magnusajong@yahoo.com

610 Erythropoietin in children with cerebral malaria: A potential for resuscitation [MIM16512151]

Anne-Lise Bienvenu, Salimata Konate, Sibiri Sissoko, Abdoulaye Barry, Elisabeth Diarra, Karidiatou Bamba, Abdoulaye Djimde, Ogebbara Dumboo, Stephane Picot

Cerebral malaria carries an unacceptable case fatality rate in children despite timely and adequate chemotherapy. To improve the survival rate, adjunctive therapies previously tested mainly focused on the modulation of the inflammatory response, without definitive effect in humans. In this context, we proposed a new adjunctive strategy using a neuroprotective drug: erythropoietin. An open-labelled study including cerebral malaria children (Blantyre coma score below 3) was conducted in Mali. The first objective was to assess the safety of 3 days erythropoietin high doses (1500 U/kg/day) combined to quinine. The second objective was to prove the non-inferiority of erythropoietin combined to quinine in terms of cases fatality rate at day 6 post-admission. 35 patients with unrousable coma were included in the study. None of expected or unexpected side effects of erythropoietin were observed. A case fatality rate of 9.7% was obtained compared to 27% in other studies in similar endemic areas. These data provide the first evidence of the safety of erythropoietin high doses combined to quinine, and its potential for resuscitation. Clinical registration number: ClinicalTrials.gov ID: NCT00697164

Email address for correspondence: anne-lise.bienvenu@recherche.univ-lyon1.fr

611 An algorithm for estimating the proportion of febrile children receiving prompt and effective treatment: Extending national estimates to inform national strategies for effective treatment [MIM16696810]

Jane Bruce, Lucy Smith, Sylvia Meek, Caroline Jones, Jayne Webster

The proportion of children receiving prompt and effective treatment for malaria is one of the core RBM indicators. Obtaining the information required to estimate this indicator accurately can be complex. Also, current guidelines as used in a Malaria Indicator Survey (MIS) do not address source, dose of treatment or diagnostics in detail. We propose an algorithm that can be used to obtain national estimates not just for country comparisons but also for national policy planning. Guided by an algorithm, with the correct sequence of questions we can determine if a febrile child has received prompt and effective treatment or not, identifying children with and without malaria parasites, and treatment status of those not tested. Household surveys in Ghana, Kenya and Senegal through the Mobilize Against Malaria (MAM) project were used to refine and validate the algorithm. Data from the surveys in the MAM project demonstrate the application of the algorithm to obtain overall estimates. They also illustrate the integration of the use of diagnostics for malaria and how they enable a focus on the treatment received by children who are positive and those who are negative for malaria parasites, depending upon their source of treatment seeking. This algorithm provides a model that can be used for the evaluation of a program targeting the treatment of febrile children; to obtain a national level estimate of the core RBM indicator by source of treatment and to estimate the type and magnitude of treatment provided to children who are RDT negative.

Email address for correspondence: jane.bruce@lshtm.ac.uk

612 Efficacy of aquatain, a monomolecular surface film, against the malaria vectors Anopheles stephensi and An. gambiae s.s. in the laboratory [MIM16670632]

Tullu Bukhari, Bart G.J. Knols

Aquatain forms a monomolecular layer when applied over the water surface and lowers the surface tension. The water surface can partly or wholly harbour every mosquito life stage due to its surface tension. Consequently any change in the surface tension can affect all stages. Aquatain has been designed for treating large water bodies which makes it suitable for areas where irrigation canals, dams and rice paddies play an important role in malaria transmission. Aquatain mosquito formulation (AMF), designed particularly for mosquito control, contains additional 2% eucalyptus oil. Bioassays were conducted against larval and pupal stages for different concentrations (ml/m²) of both AMF and Aquatain. Choice and no-choice oviposition experiments were designed for gravid females. At the dose of 1 ml/m², LT50 for late larval instars was 3.02 (95% CL, 2.76–3.25) and 0.98 (95% CL, 0.75–1.20) days for An. stephensi and An. gambiae s.s., respectively. None of the treated larvae pupated. Pupal mortality reached 100% within 2 h. AMF repelled gravid females to oviposit in untreated oviposition cups. Without the choice of an untreated cup, the lowered surface tension caused most females to drown while attempting to oviposit. Aquatain differed only in the repellent effect. As Aquatain is efficient against the immature stages and ovipositing females, it is a potential control tool for reducing both mosquito density and longevity. Field trials, however, will be carried out in an irrigation scheme for a realistic insight into Aquatain’s control potential and effect on non-target organisms.

Email address for correspondence: tullu.bukhari@wur.nl
Intermittent preventive treatment in infants (IPTi) is a promising malaria control strategy. However, the relative importance of the treatment effect (clearing existing parasites) and post-treatment prophylaxis for the protective efficacy of IPTi is unclear, delaying rational development of IPTi drug regimens. We investigated the duration of protection against malaria given by IPTi using sulfadoxine-pyrimethamine (SP) in Ghana, where SP resistance was moderate, and using SP, chlorproguanil-dapsone and mefloquine in Tanzania, where SP resistance was high. Incidence rate ratios were calculated for defined time periods after IPTi doses to examine protective efficacy against malaria over time. In Navrongo, Ghana, SP protective efficacy (PE) against malaria was 75% (95% CI: 66%, 82%) up to 4 weeks after IPTi; significant protection remained until the sixth week (PE: 38% (10%, 57%)). In Korogwe, Tanzania, SP provided PE of 65% (11%, 86%) for 1 month but there was no difference in cumulative malaria incidence by 12 months of age compared to the placebo group, possibly because infections with SP-resistant parasites were suppressed but not eliminated. Long-acting mefloquine provided high protection for 2 months (PE: 73% (24%, 91%); 73% (0%, 93%)), whereas short-acting chlorproguanil–dapsone gave no protection. Post-treatment prophylaxis appears to be the major mechanism by which IPTi protects children against malaria. Consequently, long-acting antimalarials will be most efficacious for IPTi. Since monotherapies are vulnerable to development of resistance, combinations of long-acting antimalarials should be investigated for IPTi.

Email address for correspondence: matthew.cairns@lshtm.ac.uk

Role and effectiveness of rapid diagnostic tests in home-based management of malaria: A comparative study in two areas of high and low transmission in Uganda [MIM16646083]

Richard Nydumugyenyi, Anthony Mboine, Kristian Schultz Hansen, Clare Chandler, Pascal Magnussen, Siân Clarke

Most malaria deaths occur within 48 h of onset of symptoms, and in rural areas with poor access to health facilities, home management of malaria (HMM) can improve the timeliness of treatment and reduce malaria mortality by up to 50%. In Uganda, ACTs are being deployed in the National HMM Programme in Uganda, as well as in formal health facilities, in order to maximize both coverage and impact. However the current practice of presumptive treatment of any febrile illness as malaria based solely on clinical symptoms without routine laboratory confirmation results in significant over-use of antimalarial drugs, and with ACT being a more costly regimen, it is important to be more restrictive in its administration. Rapid diagnostic tests (RDTs) provide a simple means of confirming malaria diagnosis in remote locations lacking electricity and qualified health staff. A cluster-randomised trial to evaluate the feasibility, acceptability, and cost-effectiveness of using RDTs to improve malaria diagnosis and treatment by community drug distributors will commence in Kabarole and Hoima Districts, Uganda in 2009–2011. The trial will evaluate and compare the accuracy of RDTs, and impact of diagnostic testing on the proportion of children who receive appropriate ACT treatment and referral in low and high transmission areas. The study will also evaluate the acceptability and cost-effectiveness of this approach. Findings from formative research to explore treatment-seeking and diagnostic processes in home-based management in Uganda, and attitudes towards diagnostic blood testing among community drug distributors, patients and government health staff will be presented.

Email address for correspondence: sian.clarke@lshtm.ac.uk

Etiology of fever in children from urban and rural Tanzania [MIM16671706]


Previous studies have looked at the proportion of either malaria, pneumonia, diarrhea, or bacteremia among fevers, but none at the overall spectrum of etiologies. We aimed at investigating precise causes of fever episodes in children attending outpatient clinics in urban/rural settings in Tanzania. All consenting children aged 2 months to 10 years with temperature >38°C were recruited, except those requiring immediate support. Detailed medical history and clinical examination were done and blood taken to perform rapid tests for malaria and typhoid, blood cultures, serological and molecular-analyses. All had nasal/throat swabs taken for viral molecular investigation, urine when no obvious cause was found and stools when diarrhea was present. Chest X-rays were performed when IMCI criteria for clinical-pneumonia were met. Each diagnosis was assigned a probability level (high/moderate/low) based on pre-defined criteria. 1010 (510 Dar es Salaam, 500 Ifakara) children were recruited. Preliminary results (prior to molecular-analysis) on the causes of fever of high-probability were: 43% acute-respiratory-infection (ARI) (30% URTI, 6% clinical-pneumonia, 7% X-ray-confirmed), 12% malaria, 9% diarrhoea (3% rotavirus, 6% bacterial/unknown), 8% urinary-infection, 4% typhoid, 2% skin-infection, 1% occult-bacteremia and 21% unknown at this stage. 8% had more than one diagnoses (high-probability). These results provide for the first time an accurate picture of the diversity of causes of fever in African children. ARI (mainly URTI) contributed to the largest burden of disease. Results of molecular analyses will provide further insight on respective contribution of bacteria versus viruses, a critical issue for appropriate management of fever and rational use of antibiotics.

Email address for correspondence: valerie.dacremont@unibas.ch

Cochrane systematic reviews in malaria [MIM15067175]

Group Editors (Hasifa Bukirwa, Sarah Donegan, Michael Eisenhut, Paul Garner, Hellen Gelband, Patricia Graves, Katharine Jones, Harriet MacLehose, Heather McIntosh, Martin Meremikwu, and Piero Olliaro, Madhukar Pai, Mical Paul)

Systematic reviews are important for translating research into policy and practice, and for identifying research priorities. The Cochrane Infectious Diseases Group has been preparing and updating such reviews over the last 15 years. The Cochrane Infectious Diseases Group methods draw on standards set by The Cochrane Collaboration. Each review requires a team and starts with a referred and published protocol that specifies the methods for applying inclusion criteria and quality assessment. The subsequent full reviews are peer reviewed before publication in The Cochrane Library (an electronic journal), and are updated as new research becomes available. The Group is now a global network consisting of over 200 authors, 13 editors, and full time administrative and
editorial staff in the U.K., India, Nigeria and South Africa, and are responsible for over 35 systematic reviews in malaria. The WHO Technical Expert Group on malaria chemotherapy and national governments use the reviews to help inform policy. We will present a summary of our current portfolio, reviews in progress, and the lessons we have learnt. Cochrane reviews based on rigorous, objective and continually improving standards of systematic reviewing and robust meta-analysis methods can guide policy and research priorities.

Email address for correspondence: pgarner@liv.ac.uk

617 Predicting protective efficacy of SP-IPTi using dhfr and dhps mutation prevalence rates in a simple model [MIM16686720]
Jamie Griffin, Azra Ghani, Cally Roper, David Schellenberg, Brian Greenwood, Daniel Chandramohan, Roly D. Gosling

Intermittent Preventive Treatment of malaria in Infants using sulfadoxine-pyrimethamine (SP-IPTi) is efficacious in areas with low to moderate SP resistance but not in areas of high resistance. To aid policy makers to assess where to implement we have investigated IPTi protective efficacy (PE) and prevalence of mutations implicated in SP resistance and developed a model to predict SP-IPTi PE if mutation prevalences are known. Data from seven published SP-IPTi trials was compared to contemporaneous studies of SP treatment failure and molecular studies reporting mutation prevalence in dhfr and dhps genes. We fitted a model that predicts SP-IPTi protective efficacy. A model using dhfr triple and dhps double mutations reproduced the observed PE at each site well for both PE during 35 days after the 9-month dose of SP and PE to 12 months of age. The prevalence of the dhps double was too low at most sites to allow precise estimation of the predicted PE against a background of this mutation. However, the estimated PE to 12 months old is 0.652 (0.425, 0.900) for no mutations and 0.002 (−0.249, 0.234) for 100% triple mutation. A simple model was able to explain the widely differing results of the seven trials, although other factors may also be at work. The model can be used by policy makers to estimate the likely PE of SP-IPTi when prevalence of resistance mutations in P. falciparum dhfr and dhps are known.

Email address for correspondence: roly.gosling@gmail.com

618 Plasmodium falciparum amodiaquine resistance in Afghanistan is not associated with pfmdr1 mutations [MIM16677961]
Khalid Beshir, Rachel Hallett, Ioannis Merinopoulos, Naeem Durrani, Toby Leslie, Mark Rowland, Colin Rowland

Amodiaquine (AQ) is an important partner drug in artemisinin-based combination therapies (ACTs) for Plasmodium falciparum malaria. We and others have shown that, in Africa, pfcr7-76 allele CVIET and pfmdr1 mutations are selected by AQ treatment. We examined these genetic polymorphisms in samples from a clinical trial in Afghanistan. P. falciparum DNA was extracted from pre and post-treatment samples from 83 patients treated with AQ in Jalalabad in 2002/3. A combination of molecular techniques was used to assess the parasite genotype at pfcr7-76 and pfmdr1 86, 184, 1034, 1042 and 1246. AQ resistance was high in this population; adequate clinical and parasitological response by day 42 was 9%. The pfcr7-76 haplotype was SVMNT in all samples tested. Pre-treatment prevalences of AQ resistance-associated mutations pfmdr1-86Y, 184Y and 1246Y were 18%, 45% and 0% respectively. Preliminary analysis of post-treatment pfmdr1 genotypes shows no evidence of directional selection by AQ. However, a novel mutation, pfmdr1-N86F was detected in 1 pre-treatment and 3 post-treatment samples. Its association with AQ resistance will be further investigated. This study provides in vivo data to support the reported correlation between the pfcr7 SVMNT allele and increased AQ resistance. In contrast to African studies, we did not identify any within-host selection of pfmdr1 mutations after AQ treatment failure. The data suggest that SVMNT alone is sufficient to confer a high degree of AQ resistance, whereas parasites with the African haplotype CVIET may require additional pfmdr1 mutations to reach a similar level.

Email address for correspondence: rachel.hallett@lshtm.ac.uk

619 A simple colourimetric test for the rapid detection of type 2-pyrethroids on bed nets and on sprayed walls [MIM16647708]
Harparkash Kaur, Teunis A. Eggelte

Insecticide-treated nets and indoor residual spraying of insecticides (IRSSs) are used as the major modes of intervention in the fight against malaria. Measuring the actual amount of deposits of insecticides on the bed nets and on the walls is essential for evaluation of quality control of the applied intervention as per instruction. Currently such information can only be provided by costly, chromatography techniques or through technically demanding bioassays both requiring skilled staff and sophisticated laboratory facilities. We have developed the first rapid, field friendly/cost effective colourimetric test that uses three chemical reagents and can be carried out by individuals without specialized scientific training to estimate the amount of type 2 pyrethroids (deltamethrin, α-cypermethrin and λ-cyhalothrin) on the bed nets as well as to check for the compliance of IRS. Our simple test is performed in situ and leads to the formation of an orange-red colour whose depth will indicate in a semi quantitative manner the amount of type 2 pyrethroid on the bed net and, has been validated by measuring the amount the extracted insecticide from parts of bed nets on HPLC using our published procedure [Med. & Vet. Entomol., 2005, 19, 72–83]. No interference to the formation of this colour has been found from soaps, possible degradation products of deltamethrin, insecticide binders, non-fast colour bleeding off the nets or charcoal. Prototype KITs of our test have recently undergone field evaluation in Tanzania and will be widely available in the near future.

Email address for correspondence: harparkash.kaur@lshtm.ac.uk

620 Durable residual wall lining (DL) installation concepts and acceptability as an IRS replacement tool for malaria vector control [MIM15007804]
Marie Louise Larsen, Torben Lenau

Timing of indoor residual spray (IRS) applications for the start of the transmission season is critical. Sustainability of such programs is difficult because campaigns must be repeated annually or semi-annually in some regions. Durable residual wall lining (DL) is an innovative technology that will eliminate the need for repeated spraying of walls over a period of 3–4 years after installation. The objective of this project was to evaluate a variety of methods for attaching DL to various wall surfaces found in traditional, rural African housing. Assessments were made as to the strength of attachment under various stresses and appearance after installation. The experiments took place in Anwona village near Obuasi, Ghana with the support and cooperation of AngloGold Ashanti’s Malaria Control Center. 55 mechanical and adhesive products were tested for
their ability to hold a static load (simulating long-term installation); weight concentrated in a small area (simulating a child hanging on the DL); ease of pulling or peeling the DL from the wall; impact on the wall when DL was removed; and finished look of DL once installation was complete. Results showed 38% of the nails, 90% of the adhesive tapes, and 70% of the glues failed to meet minimum standards for fixing and holding DL in position. The best solution, across all characteristics measured, was the use of a plastic nail cap (20 mm diameter) with local nails app. 3.5 cm in length having a head 3 mm to lock the cap in place. Findings from the experiment and local observations were included in the DL Installation Manual, the final delivery of this project.

Email address for correspondence: s032377@student.dtu.dk

### 621

**Prescription d’antimalariques: Des pratiques à améliorer**

*MIM16647850*

Sophie Sarrassat

L’efficacité des stratégies antimalariques passe par une bonne application des recommandations officielles. L’objectif de notre enquête était l’identification des écarts aux directives dans les prescriptions d’antimalariques. L’enquête s’est déroulée au centre de santé d’Oussouye, au sud du Sénégal. L’AQ + SP était recommandée. La prise en charge intégrée des maladies de l’enfant <5 ans (PICIME) préconisait le traitement présomptif des fièvres. Les registres des consultations ont été analysés en 2004 et 2005. Le déroulement des consultations a été observé et des entretiens avec le personnel ont été menés. 4924 enfants ont consulté. 17% (196/1144) des enfants <5 ans sont repartis sans antimalarique malgré leur fièvre. Chez les enfants ≥5 ans, 13% (119/950) ont été traités par antimalarique malgré un diagnostic autre que paludisme et 84% (142/169) ont été traités malgré une goutte épaissie négative. Les infirmiers ont prescrit l’AQ + SP dans 74% (2063/2789) des cas. Les injections IM de QN ont été le traitement de second choix. Les causes de ces écarts non conformes ont été un manque de personnel au laboratoire, une formation inadaptée aux situations pratiques et logiques de prescription des infirmiers, l’existence de messages ambigus en cas de goutte épaissie négative et un manque d’outils d’aide à la pratique. Notre enquête a révélé des écarts aux directives trop souvent ignorés qui, pourtant, constituent des obstacles au succès des stratégies antimalariques. Les causes de ces écarts montrent qu’il est difficile d’intégrer les recommandations officielles dans les pratiques quotidiennes des soignants.

Email address for correspondence: sophiawa00@hotmail.com

### 622

**From fever to anti-malarial: The process of seeking and receiving appropriate treatment in rural Senegal**

*MIM16671406*

Lucy Smith, Jane Bruce, Lamine Gueye, Rodio Diallo, Babacar Gueye, Caroline Jones, Jayne Webster

National and international targets of 80% of malaria cases receiving appropriate treatment are not being met. There are several stages involved in receiving appropriate treatment. Understanding at which stage children are lost from the appropriate treatment process is key to developing effective interventions. This paper reports on a cluster-based household survey to investigate the current treatment-seeking practices of caretakers of children under 5 years with fever in the last 2 weeks in southeast Senegal. Multivariate logistic regression is used to investigate determinants of action at different stages in the process of seeking and receiving treatment. Overall 61.6% of children under 5 years with fever were given any treatment; 17.0% an anti-malarial and 9.2% artesunate-amodiaquine (ASAQ, the national first-line ACT). Although the proportion of febrile children seeking treatment at a community-level delivery point was only 7.8% (compared to 31.5% and 17.0% accessing a formal public health facility or informal private source, respectively), the likelihood of these children receiving an anti-malarial was 4 times greater than those that first sought care in the formal public sector (OR: 4.20; 95% CI: 1.49, 11.8; p = 0.007); findings were similar for receiving ASAQ (OR: 4.18; 95% CI: 1.37, 12.7; p = 0.004). These findings are used as the basis for a discussion of the determinants of receiving appropriate and effective treatment, depending upon where this treatment is sought. Thereby providing an evidence base for district level strategic planning and focussing of resources.

Email address for correspondence: lucy.smith@lshtm.ac.uk

### 623

**Age- and weight-based dose regimens for the fixed-dose combination of dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria in Asia, Africa and Latin America**

*MIM16761677*

D.J. Terlouw, D.J. Hayes, K. Barnes, P.L. Olliaro, N.J. White, S. van Buuren, F.O. ter Kuile

Dihydroartemisinin-piperaquine (DHA-PPQ) is currently available as a fixed-dose combination with a tablet strength of 40 mg DHA and 320 mg PPQ. The manufacturer recommended target dose is 2.25 mg/kg DHA and 18 mg/kg PPQ once daily for 3 days. In field trials in Asia and Africa this co-formulation is efficacious and well tolerated when accurately dosed by body weight. In practice however, antimalarial treatments are often dosed by age, resulting in a proportion of patients receiving doses outside of the therapeutic range. We defined practical, programmatically appropriate regional age-based dose regimens using a representative weight-for-age database of >1.3 million individuals from malaria endemic regions around the world. Smoothed regional reference growth curves were generated from the reference database using an extended version of the LMS method with the R GAMLSS package (R2.7.0). Based on the associated weight-for-age centiles, we compared the proportions of patients predicted to receive doses within newly defined therapeutic dose range for different age-dose categories in Asia, Africa and Latin America. Therapeutic dose ranges were established based on available pharmaco-kinetic, efficacy and safety data, and applied to predict the optimal age-based regimen for available DHA-PPQ formulations. Results will be presented with particular attention to inter-regional differences of optimal age-based dose regimens, the challenges posed by the current formulation, drug ratio and packaging of DHA and piperaquine and the lack of a paediatric formulation. The specific challenges with dosing of DHA-PPQ, the need for appropriate paediatric formulations and remaining gaps in knowledge will be discussed.

Email address for correspondence: dj.terlouw@liv.ac.uk

### 624

**Three-month follow-up of a randomised controlled trial of Argemone mexicana versus artesunate-amodiaquine in the home-based management of presumptive malaria**

*MIM16694119*

Merlin L. Willcox, Bertrand Graz, Chiaka Diakite, Jacques Falquet, Florent Dackuoc, Oumar Sidibe, Sergio Gianie, Drissa Diallod

Artemisinin Combination Therapies (ACTs) are proposed for the home-based management of malaria (HMM), but are not yet...
widely available for this. We compared in a randomised controlled trial in south Mali two strategies for HMM: a validated local herbal medicine (Argemone mexicana—AM) as first line treatment versus the standard artemesunate/amodiaquine (ACT). Within the first 28 days, AM was well tolerated and correlated with good clinical results. We extended the follow-up to 84 days, to evaluate incidence of uncomplicated and severe malaria and anaemia. 294 of 301 patients (median age 5 years) included were followed to day 84. Between days 29 and 84, a similar proportion of patients had a new episode of uncomplicated malaria (AM 39.4%, ACT 30.7%) and coma/convulsions (1.9% of children <5 years in each group). Incidence of other forms of severe malaria (vomiting, severe anaemia) was greater in AM (5.7%) than in ACT (1.9%) over the 3 months. Prevalence of moderate anaemia (haematocrit <24%) was 1.1% in both groups at day 84. A first-line treatment with the locally produced Argeomexicana decoction was equivalent to ACT in terms of incidence of uncomplicated malaria, coma/convulsions, and anaemia at 3 months. The incidence of severe malaria was kept to a lower level than reported in other studies of home-based management of malaria. Argemone mexicana could be proposed as first-line treatment of uncomplicated malaria in semi-immune patients (children ≥5-year old and adults) in high transmission areas, and as first aid when modern antimalarials are not immediately accessible.

Email address for correspondence: merlinwillcox@doctors.org.uk

625
The use of routine health information to evaluate the effectiveness of IPTi in Southern Tanzania [MIM16677382]
Barbara Willey, Joanna Schellenberg, Kizito Shirima, Werner Mayokola, David Schellenberg

We describe the use of routine health information within a community-randomised study to evaluate the effectiveness of intermittent preventive treatment for malaria in infants (IPTi). Infants attending clinics for routine immunizations within intervention areas received IPTi as a single dose of sulfadoxine-pyrimethamine at 2, 3 and 9 months of age. Attendance at 11 sentinel clinics (6 in intervention areas) was monitored by electronic data capture from routine health records, including age, diagnostic and treatment information. Malaria was defined by clinical diagnosis, a positive blood slide and a combination of both (definitive definition). Clinic attendances in infants aged 2–11 months between April 2006 and March 2007 were used in an intention to treat analysis of first or only episode of malaria. Age-adjusted incidence rates were calculated using Poisson regression. Rates in intervention and comparison areas were compared adjusting for clustering by clinic, using techniques appropriate for small numbers of clusters. In 9880 infants, with 3374 person years follow-up, 2848 episodes of clinically diagnosed, 974 episodes of slide-confirmed, and 763 episodes of definitive malaria were recorded. Age-adjusted rates of slide-confirmed and definitive malaria were lower in intervention areas (IRR = 0.69 and IRR = 0.72). Rates of clinically diagnosed malaria were similar between areas. On adjustment for clustering by clinic, none of the differences between intervention and comparison areas reached statistical significance. Results are compatible with the reduction in malaria reported by individually randomised trials of IPTi. This study illustrates the potential of consolidated routine health information for measuring effectiveness and safety of malaria control and other public health interventions.

Email address for correspondence: barbara.willey@lshtm.ac.uk

626
Costs and consequences of large scale vector control in sub-Saharan Africa [MIM16559517]
Josh Yukich, Fabrizio Tediosi, Christian Lengeler

Insecticide treated bed nets (ITNs) and indoor residual spraying (IRS) are effective malaria control tools. Policy makers and donor partners need better evidence to support investment decisions. In order to generate comparable and policy-relevant evidence we reviewed seven large scale programs in Africa; for ITNs: subsidized distribution in Malawi; free distribution in Eritrea; EPI-linked free distribution in Togo; a public–private partnership in Senegal; public–private partnerships including discount vouchers in Tanzania; and for IRS: South Africa and southern Mozambique. Operational descriptions of the seven programs were compiled. Costs were compiled for both the set-up and maintenance phases. The emphasis was on the provider perspective, but direct costs of ITN procurement to the end user were included where appropriate. Child deaths prevented were estimated using published indicators of effectiveness and sensitivity analyses were conducted. Results indicate that there are significant differences in cost per output for different strategies but that all of the interventions are attractive public health interventions. Long-lasting ITNs had a lower cost per person protected and were more cost-effective than IRS under most scenarios. The main sources of variation in cost were the type and usage rate of nets, and the frequency and insecticide type for IRS. IRS distribution strategies varied significantly in sources of financing and the speed of delivery but were always highly cost-effective under base case scenarios. IRS appeared to be at its most cost-effective where only one round of spraying would be necessary per year.

Email address for correspondence: jyukich@gmail.com

627
Neuroprotective roles and in vivo significance of neurogobin in cerebral malaria [MIM16616184]
B. DellaValle, C. Hempel, J. Kurtzhals, M. Penkowa

Cerebral malaria (CM) is a life-threatening complication of Plasmodium falciparum malaria and is presently without neuroprotective treatment. The pathogenesis of CM is associated with the sequestration of erythrocytes in the cerebral microcirculation, blood–brain barrier dysfunction, and cerebral inflammation. Neuroglobin (Ngb) is a recently discovered globin thought to function as an endogenous neuroprotective protein in the brain. This study was designed to investigate the in vivo significance of Ngb in the pathogenesis of CM. C57BL/6j mice were divided into four groups: uninfected control mice; uninfected mice receiving i.p. erythropoietin alpha (EPO); mice infected with P. berghei ANKA parasitized red blood cells; and infected mice receiving EPO. Experimental CM was diagnosed by the clinical presentation of specific neurological symptoms. These symptoms developed in the malaria-infected mice lacking EPO treatment. Brains were analyzed by immunohistology. In the malaria infected mice both + and – EPO, the expression profile of Ngb was different than that of the control mice. Additionally, neuronal expression of Ngb was lower in uninfected mice receiving EPO. To date this is the first study on the role of Ngb in CM. The results of this study suggest that Ngb responds to experimental CM pathology in a novel way not previously reported in other in vivo Ngb studies. Further characterization of the mediators involved in this response is necessary and may reveal potential targets for the treatment of CM.

Email address for correspondence: briandellavalle@hotmail.com
628 Effects of routine use of ACT on malaria parasitaemia in rural Tanzania: Repeated household surveys [MIM16335830]

Rashid Khatib

The Interdisciplinary Monitoring Programme for ACT in Tanzania (IMPACT-Tz) evaluated the effectiveness of sulfadoxine pyrimethamine (SP) + artesunate (Art) versus SP monotherapy in reducing malaria prevalence in 3 rural districts of Tanzania with perennial malaria transmission. The project implemented SP + Art combination for uncomplicated malaria cases in all health facilities in Rufiji District between 2003 and 2006. Household surveys were conducted in 2002, 2004, 2005 and 2006 in Rufiji. Parallel surveys were conducted in Kilombero/Ulanga districts where uncomplicated malaria cases were treated with SP monotherapy per national guidelines. Household surveys were conducted in two Demographic Surveillance System (DSS) sites. Each year, we selected a sample of households from each DSS database using simple random sampling. Every registered member of the selected household available during the visit who agreed to participate in the study was examined for malaria parasitaemia. Participants were also questioned on the use of insecticide-treated nets (ITNs). Compared to 2002 baseline household survey and after adjusting for age, malaria parasite prevalence decreased by 35.2% in 2004, 36.3% in 2005 and 47.3% in 2006 in Rufiji District. In Kilombero/Ulanga, parasitaemia increased by 12.5% in 2004; but dropped by 49.1% in 2005 and 40.7% in 2006. ITN use among study participants in Rufiji ranged from 3% in 2002, 10% in 2004, 22% in 2005 and 30% in 2006. In Kilombero/Ulanga ITN use for similar period was 10%, 26%, 34% and 36%. Routine use of SP + Art appeared to be primarily responsible for the sharp decline of malaria parasite prevalence in Rufiji district in 2004. Increased ITN use should account for additional decline observed after that period.

Email address for correspondence: rkhatibu@ihi.or.tz

629 Bioassay-directed isolation of steroidal alkaloids in Physalis angulata by HPLC coupled with UV detection [MIM15927494]

Larry C. Okpako, Colin W. Wright

Malaria still remains the major tropical disease worldwide, with more than 300 million people being affected, and 3 million deaths annually. The search for new antimalarial drugs from plant source constitutes a promising strategy as exemplified by the use of quinine and artesinin. Extracts or infusions from Physalis angulata L. (Solanaceae) have been used for the treatment of inflamed livers, asthma, hepatitis, fevers and malaria in various countries. Bioassay-directed fractionation of the chloroform extract of P. angulata was achieved by a combination of chromatographic techniques. HPLC coupled with UV detection was employed for the separation of closely related alkaloids present in the samples. Antiplasmodial activity was assessed against Plasmodium falciparum (3D7 and K1) using the parasite lactate dehydrogenase assay. The CHCl3 extract was the most active with IC50 values of 15.63 and 31.50 µg/ml against the 3D7 and K1 strains, respectively. The 1H NMR data suggested that the major compounds isolated are steroidal alkaloids. Result of the bioassay-guided fractionation and structure elucidation of new compounds using IR, UV, MS and NMR-based methods are presented. This work reports the separation of the main alkaloids found in P. angulata and this is the first report of steroidal alkaloids from P. angulata. The antiplasmodial activity of these compounds has not been previously reported.

Email address for correspondence: l.c.okpako@bradford.ac.uk

630 Impact of the introduction of artemether-lumefantrine as first line treatment policy on malaria transmission in two rural districts of Tanzania [MIM16669995]


The deployment of artemisinin-based combinations (ACT) is supposed to reduce malaria transmission. Until now, there is no proven demonstration of such an effect in highly endemic areas of Africa. As part of the ALIVE project [Artemether-Lumefantrine (AL)], in Vulnerable patients: Exploring health impact, we aimed at assessing the transmission impact of the introduction of AL as first line treatment for uncomplicated malaria on parasite prevalence and anaemia. Two cross-sectional surveys were conducted in two rural districts during the sulfadoxine-pyrimethamine era (2005 and 2006) and one 18 months after AL introduction (2008). Information on demography and bednet use was collected and a blood sample was taken to measure parasitaemia and hemoglobin. 5903 persons were assessed in 2005, 6324 in 2006 and 4557 in 2008. Parasite prevalence in the whole population was 11.4% in 2005, 13.6% in 2006 and 11% in 2008. Prevalence of anaemia in children under five was 17.8% in 2005, 9.7% in 2006 and 10.1% in 2008. Use of impregnated treated bednets was 35%, 36% and 44%, respectively. These data show no impact of AL on malaria transmission 18 months after its introduction. Possible explanations might be that the level of endemicity had already been reduced substantially by high ITN use, or that coverage of AL was not high enough or sustained long enough to affect transmission. Longitudinal data over several years are needed to reliably assess transmission trends after ACT implementation. Results of gametocyte rates and of the 2009 survey will also be presented.

Email address for correspondence: blaise.genton@unibas.ch

631 Malaria RDTs in active case detection [MIM15038971]

Luis Benavente

Strategies to deliver antimalarials to—asymptomatic or symptomatic—parasitemic individuals include intermittent preventive treatment, passive detection, focalized treatment of index cases contacts, active case detection (ACD), selective population therapy in high-prevalence areas and mass drug administration without screening. ACD is often defined as active search and treatment of febrile individuals, with parasitologic confirmation sought in those above 4 years of age. The Bioko Island Malaria Control Project (BIMCP) pilot-tested ACD on the North-West quadrant of Bioko Island, Equatorial Guinea, to contribute reducing malaria's human reservoir, increase access, utilization of effective antimalarials, and deliver educational messages. BIMCP's ACD campaign used CDC's malaria case definition: any symptomatic or asymptomatic individual with malarial parasitemia as per a rapid diagnostic test (RDT) administered by nurses to all age groups in their homes. Cases were treated with artesinin-based combination treatment (ACT). On 32 workdays, 12,490 individuals were screened, 3872 tested positive. Only 0.2% refused to be screened. Parasitemia prevalence peaked among children <15 years (39%). In 98% of cases age matched the ACT dose. Adherence to a 3-day ACT course was 80% in a revisited cohort. ACD delivered about three times more ACT than all combined health facilities that used passive case detection. If given at the proper interval (the higher entomological inoculation rate, the shorter the interval) ACD may reduce the magnitude of malaria human reservoir. As artesunate is not 100%
effective as gametocidal drug, ACD cannot be a standalone malaria control strategy but to complement effective vector control activities.

Email address for correspondence: lbenavente@mcd.org

632
Community directed intervention to deliver malaria in pregnancy services in Nigeria [MIM16708278]
William R. Brieger, Bright Orji, Joseph Okeibunor

Akwa Ibom State, Nigeria has high malaria transmission but is late in receiving malaria interventions. Jhpiego, with support from ExxonMobil Foundation and the State Ministry of Health is working to reduce burden of malaria in pregnancy (MIP) using a two-pronged approach to reach pregnant women: improving antenatal care (ANC) service quality and community involvement through community-directed distributors (CDDs). A 2007 baseline survey was conducted in the catchment areas of 39 LGAs clinics that provide ANC. Intervention began in July 2008 in all 15 ANCs; the remaining 21 were controls. 135 ANC staff were trained on MIP; 32 were trained on community directed intervention (CDI). Communities selected 734 CDDs. Monitoring of ANC clinic and CDD statistics took place. At baseline 6% of recently pregnant women had received two doses of intermittent preventive treatment (IPTp). 23% slept under an insecticide treated net (ITN). 14% slept with their new baby under the net the previous night. Only 17 clinics offered IPTp1, and 2 provided ITNs to pregnant women. In 6 months of intervention, ANC clinics distributed 1349 doses of IPTp1, 659 doses of IPTp2, but none before intervention. Village census identified 7300 pregnant women. The CDDs under ANC staff supervision delivered 10,985 doses of IPTp1, 8185 doses of IPTp2 and 5000 ITNs. The CDI approach has reached under-served populations with ivermectin for onchocerciasis control.

Preliminary results in Akwa Ibom State show this process can address other health problems. CDDs are important partners in the delivery of MIP control services when they are linked with local ANC clinics. NOTE: A follow-up survey is planned for October 2008, and preliminary results from that may be available for the conference.

Email address for correspondence: bbrieger@yahoo.com

633
Sulfadoxine-pyrimethamine, sulfadoxine-pyrimethamine+ artesunate, and AL for uncomplicated malaria infection [MIM16682034]
Julie Gutman, Abdunoor Mulokozí, Deborah Sumari, Allan Malisa, Peter B. Bloland, S. Patrick Kachur, Salim Abdulla, John R. MacArthur

In 2001, the Tanzanian government adopted sulfadoxine-pyrimethamine (SP) as the first line antimalarial treatment. Continuous monitoring of antimalarial efficacy is crucial in light of increasing parasite resistance to antimalarials. We measured in vivo efficacy of SP alone versus SP+artesunate (SPAS) or artemether-lumefantrine (AL) 5 years after SP introduction and prior to widespread deployment of AL. Patients <5 years old with uncomplicated P. falciparum mono-infection were enrolled and randomized to receive either SP, SPAS, or AL. Using the standard WHO 28-day protocol. PCR genotyping was used to distinguish recrudescence from re-infection and characterize known molecular markers of antimalarial drug resistance. We enrolled 361 patients: 121 in the SP arm, 122 in the SPAS arm, and 118 in the AL arm. The uncorrected cure rates were 51%, 64%, and 86% in the SP, SPAS, and AL groups, respectively. PCR corrected cure rates are pending. This represents a significant decrease in efficacy for SPAS since 2004, when respective uncorrected cure rates were 58%, 78%, and 80%. In comparison to AL, treatment with both SP and SPAS resulted in a significant increase in the hazard ratio for infection (4.6, 95% CI: 2.7–8.0 for SP and 2.9, 95% CI: 1.7–5.2 for SPAS). Both SPAS and AL were significantly more efficacious for treatment of uncomplicated malaria than is SP, however, the efficacy of SPAS is rapidly decreasing. SP should no longer be used for treatment in Tanzania, either as monotherapy or as part of artemisinin combination therapy.

Email address for correspondence: fift2@cdc.gov

634
The Malaria Transmission Consortium [MIM16672921]
Neil Lobo, Frank Collins

The Bill and Melinda Gates Foundation funded Malaria Transmission Consortium (MTC) comprises a network of research groups associated with established malaria control and evaluation programs. Evidence from field trials has demonstrated the protective efficacy of malaria control interventions (i.e., insecticide treated nets, indoor residual spraying, etc.). However, there is a limited range of epidemiological environments in which these trials were conducted and a paucity of information on the impact of combinations of these interventions in areas of differing intensities of transmission. The ability of control program managers to make decisions about program design is limited by the difficulty of accurately measuring rates of malaria transmission and of monitoring the impact that interventions have on transmission. MTC seeks to address this critical gap by (1) developing standardized cost-effective methods for defining the intensity of malaria transmission through entomologic, parasitologic or serologic techniques, (2) evaluating primary transmission-reducing malaria control techniques, both alone and in various combinations across a range of malaria transmission environments, (3) look at the impact of entomological factors such as the emergence of insecticide resistance and behavioral changes and, (4) develop malaria transmission models and simulations. Information garnered will be critical in enabling malaria control professionals to decide on the optimal and most cost-effective malaria control strategies to use across the full range of transmission conditions. Models and simulations developed will be used to better understand transmission dynamics. Our goal is to develop and evaluate methods that will be useful to the malaria control community.

Email address for correspondence: nlobo@nd.edu

635
Development of a portable microfluidic chip device for malaria diagnosis and speciation by PCR [MIM16680559]
Brian Taylor, Alex Stickel, John Booth, John Crabtree, Jutta Preiksaitis, Chris Backhouse, Linda Pilarski, Stephanie K. Yanow

In the absence of confirmation from a diagnostic test, malaria can be over-diagnosed and mis-diagnosed. Accurate diagnosis is essential to ensure appropriate patient care and to maintain the therapeutic lifespan of antimalarial drugs. As such, we are developing a sensitive, low-cost, portable device to diagnose malaria infections at the point of care. The test is based on the extraction of parasite DNA from whole blood followed by real-time PCR and melt-curve analysis on a microfluidic platform. The result is positive or negative detection of Plasmodium and identification of the infecting species. Tests are performed on a glass and polydimethylsiloxane (PDMS) microfluidic chip. DNA bound to the intercalating dye
LCGreenPlus is detected by a photodiode. We have successfully performed PCR of malaria DNA on-chip. Amplicons from a conserved region of the 18S gene of all four species of Plasmodium result in a discrete peak by on-chip melt-curve analysis. Furthermore, clear discrimination of species-specific amplicons of the 18S gene is observed on-chip with the following Tm values: P. malariae (72 °C), P. vivax (75 °C), P. ovale (82 °C), P. falciparum (85 °C). Current work is focused on seamless integration of parasite DNA extraction, real-time PCR, and melt-curve analysis on a single chip. Our results demonstrate in principle that the development of an integrated microfluidic-based device for the diagnosis of malaria is achievable. With further miniaturization, this platform will make malaria testing more widely accessible and overcome the barriers of complexity, cost, and time that currently limit the use of conventional technologies. REQUEST FOR ORAL PRESENTATION PLEASE

Email address for correspondence: s.yanow@provlab.ab.ca

636
Understanding of connection between taking full course of treatment and clearance of malaria parasites in communities in Tanzania and Uganda [MIM16771008]

James Moloney

Patients stop taking antimalarials (AMs) when they feel better-treating symptoms, not disease. This practice contributes to development of resistance to AMs. Providers often tell patients to finish treatment, most do not give reasons. Two studies were conducted to assess comprehension, acceptability and usefulness of 2 versions of AM packs to inform how (pack 1) and how and why (pack 2) to take a full course, and assess potential influence on adherence. These pretesting studies used a flexible action research methodology. Instructional cards were tested using semi-structured interviews and checklists ($n=104$ Tanzania, $n=116$ Uganda). Purposive sampling with >80% women 15–45 years; 25% had 0–3 years schooling, 40–60% 4–6 years. Pack 1 shows user, symbols of sun, moon and tablets over 3 days. Pack 2 shows connection between taking full course and killing all parasites. Qualitative analysis combined inductive and deductive approaches; quantitative calculated simple proportions for key outcome measures. Images of parasites and connection to full cure was seen immediately by 67% in Tanzania, 27% in Uganda. After explaining these were parasites, a further 20% and 63% saw connection; 13% and 10% respectively did not understand. In Tanzania, all but two would use pack 2 to explain reasons for adherence to neighbour; in Uganda, all but one ($N=45$). Many commented on usefulness, some were scared of parasites but still preferred this pack to explain reasons for adherence. Instructons on use of AMs which give users visual logical reason to finish course may influence adherence.

Email address for correspondence: moloney.james@hotmail.com

637
Critical policy issues to be considered when changing treatment protocol for uncomplicated malaria falciparum: An example from Timor-Leste [MIM15206217]

Joao Soares Martins

Timor-Leste is an endemic malaria country; the country changed its treatment policy for uncomplicated falciparum malaria from sulphadoxine-pyrimethamine to Coartem in 2007. Objective: To examine the policy process and actors’ involvement in the formulation of the new treatment protocol and to document challenges in the implementation. This study used a mixed method approach involving 27 in-depth interviews and 11 focus group discussions with policy makers and health workers, and a survey on the utilization of Coartem at 14 Community Health Centers and 4 hospitals. The rise of the clinically malaria trends and honoring the political commitment promised to WHO SEAR Region were the main reasons driving the change of treatment policy. A participatory approach involving wider stakeholders was used in developing the protocol. The new treatment protocol was signed off in June 2007 and the implementation began in 2008. Although Coartem was already adopted, most of health facilities still used sulphadoxine-pyrimethamine. factors that contributed to this are: lack of training, confusions over the new anti-malarial drug and rapid test diagnosis, lack of monitoring in the implementation, the availability of the sulphadoxine-pyrimethamine at health facilities, and the non-involvement of private sector. Significant challenges are noted from both the process of the protocol development and its implementation. The change of treatment policy appeared to be a top down decision and drew stakeholder to participate in the formulation process. Lack of policy direction provided by policy makers to health workers at implementation level hampered the implementation of the new treatment protocol. To improve the implementation the impeding factors need to be addressed adequately.

Email address for correspondence: cmanyo@yahoo.com

638
Safety profile of artemether-lumefantrine (AL; Coartem®) compared with suludaxine-pyrimethamine (SP) in pregnant women with symptomatic malaria: preliminary results of an observational study [MIM15067265]

Christine Manyando, Rhoda Mkandawire, Lwipa Puma, Moses Sinkala, Eric Njunju, Melba Gomes, Raymond Schlienger, Verena Walter, Mailis Virtanen, Anne Claire Marrast

Safety data on Artemisinin Combination Therapy (ACT) in pregnancy are limited. A prospective observational study was conducted in Zambia to compare safety of AL and SP in pregnant women with symptomatic falciparum malaria. The primary objective was to evaluate perinatal mortality rate (i.e. stillbirth or neonatal death ≤7 days post-birth). Mothers and live newborns were followed up to 6 weeks post-delivery. Data from 1001 pregnant women (AL 495, SP 506) and fetuses/newborns (AL 470, SP 477) were analyzed. There were no clinically relevant differences in rates of perinatal mortality (AL 4.2%, SP 5.0%), neonatal mortality (both groups 3.0%), stillbirths (AL 1.8%, SP 2.5%), preterm deliveries (AL 14.1%, SP 17.4%) or gestational age-adjusted low birth weight (AL 9.0%, SP 7.7%). Seven spontaneous abortions occurred in the AL and 5 in the SP group. Birth defect rates were 4.9% (AL) and 2.6% (SP), mainly umbilical hernia (3.7% and 1.5%, respectively). No major malformations were reported, except for 2 chromosomal aberrations. Of 6 maternal deaths (AL 1, SP 5), 3 were due to comorbid infections (pneumonia, viral encephalitis, sepsis; all SP). Most common adverse events were premature delivery (AL 13.7%; SP 17.2%), stillbirth (AL 1.8%; SP 2.6%), abortion (AL 1.2%; SP 1.0%) and infections (malaria [AL 3.4%; SP 6.7%], syphilis [AL 4.8%; SP 4.0%], respiratory tract infection [AL 1.8%; SP 1.0%]). These results indicate that the incidence of perinatal death, spontaneous abortion, neonatal mortality, premature delivery, stillbirth and low birth weight is similar after pregnancy exposure to AL or SP.

Email address for correspondence: cmanyando@yahoo.com
639 Impact of indoor residual spraying program in Nkhotakota District, Malawi [MIM16728869]

Don Mathanga, MBBS, PhD, Carl H. Campbell, Jr, MPA, Themba Mzilahowa, PhD, Henry Chakaniza, MBBS, Rabson Kachala, MBBS, John A. Chiphwanya, MSc, Katherine Wolf, MPH

As part of a multi-pronged approach to reduce the burden of malaria, the Malawi National Malaria Control Program (NMCP) with support from the President's Malaria Initiative in Malawi is scaling up indoor residual spraying (IRS). The NMCP implemented an IRS pilot program in approximately half of Nkhotakota District using lambda-cyhalothrin capsule suspension during the 2007/8 rainy season. A pre and post intervention household survey was carried out, comparing anemia (<8 g/dl) in 6–30 months old children before and after the first round of spraying. Vector density was assessed using window traps. 91.1% of targeted structures were sprayed during the pilot. The prevalence of anemia in the whole district was 25.9% pre-IRS and 18.6% (p = 0.001) post-IRS. In areas which received IRS, the prevalence of anemia decreased from 21.7% to 12.2% (p < 0.001) representing a 44% reduction. In non-IRS areas, the prevalence of anemia decreased from 29.1% to 27.4% (p = 0.063). ITN ownership (households owning at least one net) in the non-IRS areas was 65% pre-IRS and 62.1% post-IRS compared to the IRS areas where ownership was 70.5% pre-IRS and 60.3% post-IRS. Post IRS, the mean number of anophele mosquitoes per house was significantly lower in IRS areas compared to non-IRS areas (4 in IRS areas compared to 53 in non-IRS areas). High coverage of IRS in rural Malawi is achievable with leadership from the District Health Management Team. IRS can rapidly reduce anemia, decrease competent vector densities and achieve the goals of the NMCP.

Email address for correspondence: ccampbell@cdcmalaria.org

640 Prevalence of HIV-1 and malaria co-infections among children presenting at University Teaching Hospital, Lusaka, Zambia [MIM16696712]

James Chipeta, Chipeco Kankasa, Mable Mwale Mutengo, Pascalina Chanda, Lars Hviid, Phillip Thuma

Elucidation of interaction between Human Immunodeficiency type 1 Virus (HIV-1) and malaria infections is important as it may herald novel and effective ways of managing these diseases especially in populations where the two diseases are co-endemic. However, to date, studies on HIV-1 and Malaria co-infection have mainly been among adults. Little is known of HIV-1 and malaria disease interaction among children as very few studies have been conducted in this age group. In this pilot study, we aimed to elucidate the clinical interactions of HIV-1 and Plasmodium falciparum malaria among children co-infected with these infections. One hundred children aged between 0 and 15 years presenting with malaria at University Teaching Hospital (UTH), Lusaka, Zambia were prospectively recruited to the study from August 2005 to April 2007. Upon enrolment to the study a comprehensive clinical assessment was done on all children including documentation of presenting symptoms and signs as well as complete haematological evaluation. Malaria parasitaemia at presentation was determined and all recruited children were evaluated for HIV-1 infection by two serological antibody tests and or HIV DNA PCR. For the purposes of data analysis recruited children were grouped into two main categories. The HIV-1-malaria Co-infected children as study cases and the HIV-1 none infected malaria children as controls. Databases for the study were created on both Epi-Info (version 6.04d) and Excel (Microsoft Office Excel 2003) soft wares for statistical analysis. Comparison of various clinical features between the two study groups was made by analysis of simple variance calculations using Student’s t-test and or Fisher exact test. Assessment of impacts of HIV-1 on malaria was performed by matching respective data variables for similarities and differences using Pearson correlation coefficients. P-values <0.05 were considered statistically significant. There was 11% prevalence of HIV-1 and malaria co-infection among the 100 malaria children recruited to the study. In comparison to their HIV-1 negative counterparts, the HIV-1 and malaria co-infected children tended to have atypical constitutional malaria symptoms and signs at presentation with lower mean axillary temperatures (37.97 ± 1.5 °C versus 38.26 ± 1.2 °C; P = 0.26812), less incidence of history of headache (12% versus 44%; P = 0.04351), higher incidence of fever of more than a week (11% versus 9%; P = 0.00140) and less incidence of jaundice (0% versus 11%; P = 0.00220). The HIV–malaria co-infected children also tended to be older than their counterparts (mean age: 49.27 ± 46.26 months versus 30.44 ± 14.66 months; P = 0.208537) with no nutrition status differences between the two groups (mean Z-score: −2 ± 0.8 versus −2 ± 0.9; P = 0.79564). Evaluation of disease morbidity and mortality revealed higher parasiteaemia burden in HIV–malaria co-infected children (mean parasiteaemia rate 373392 parasites/μL versus 240785.3 parasites/μL; P = 0.708724). There was no statistically significant difference in haemoglobin status (7.08 ± 2.53 g/dL versus 5.97 ± 2.7 g/dL; P = 0.222315) and hospital stay (4.5 ± 1.4 versus 5.56 ± 3.4; P = 0.087609) between the two groups and anemia was 100% prevalent in both groups. The results of this pilot study support the growing body of evidence of intricate clinical interactions between HIV-1 and Plasmodiumfalciparum malaria. The study reveals higher parasite disease burden in HIV–malaria co-infected children with tendency of atypical clinical presentation in contrast to their HIV negative counterparts.

Email address for correspondence: damaseke@yahoo.com

641 The prevalence of SP and CQ resistance markers following the introduction of artemunate plus SP for the treatment of uncomplicated malaria in Maputo Province, Mozambique [MIM16645676]

Jaishree Raman, Rajendra Maharaj

The artemisinin-based combination therapy (ACT), artemunate plus sulphadoxine-pyrimethamine (SP) was phased into Maputo Province, at district level between April 2004 and November 2006. A major motivating factor for the shift to ACTs, is the hypothesised reduced risk of the emergence and spread of drug resistance markers as a consequence of ACTs short half life and their impact on reducing malaria transmission. We investigated the effect ACT introduction had on SP and chloroquine (CQ) resistance marker prevalence in Maputo Province from 2005 to 2008. Fingerprick blood spots from malaria positive children, aged between 2 and 15 years, where collected during annual parasite prevalence surveys at 26 different sentinel sites. Plasmodium DNA was chelex extracted and then subjected to mutational analysis. Point mutations in the dhfr, dhps and pfcrt genes were investigated. Prior to artesunate plus SP introduction in 2004, prevalence of the dhps double and quintuple mutations were well below 20%, while the dhfr triple mutation prevalence was almost at fixation within the population. By 2008 the dhps double and quintuple mutation prevalence had increased to well over 75%. Over the same period the dhfr triple and K76T mutation prevalence remained above 90%. Artesunate plus SP appears to select for or at the very least does not restrict the spread of SP resistance markers. Given the high prevalence of quintuple mutation, associated with SP treatment failure in southern Africa,
the therapeutic lifespan of artesunate plus SP in Maputo Province appears very limited.

Email address for correspondence: jaishree.raman@nrc.ac.za

642
Time sensitive surveillance to detect residual foci of malaria transmission following implementation of malaria control in southern Zambia [MIM16596996]
Aniset Kamanga, Sungano Mharakurwa, Phil Thuma, Timothy Shields, Gregory E. Glass, William J. Moss, Clive Shiff

In an area where accelerated malaria control is implemented, symptomatic cases of malaria likely do not occur in isolation but within the context of a larger population sustaining asymptomatic infection. These asymptomatic community members are often gametocyte carriers, who can infect vector mosquitoes and thus sustain malaria transmission. One strategy for malaria elimination is to identify these carriers in a timely manner and treat them with a gametocidal drug to interrupt transmission. Locations of the 14 rural health centres (RHC) in the Macha area of Zambia were mapped. Medical personnel at each RHC were provided with resources to cover costs for use of short messaging system (SMS) to report malaria Rapid Diagnostic Test (RDT) results on a weekly basis to our database. RDT results were mapped based on the RHC. As a pilot study the identified positives from one RHC were geo-positioned by individual village to allow for subsequent community screening and treatment of everyone found RDT positive. Results of these efforts will be presented. With the use of this inexpensive surveillance system, we demonstrate that timely identification and treatment of asymptomatic carriers of malaria can be achieved, allowing a targeted method of active case detection to help eliminate the threat of malaria outbreaks.

Email address for correspondence: aniset.kamanga@miam.org.zm

643
In vivo chloroquine efficacy trial and correlation between treatment outcome and presence of mutations in Pfcrt and Pfmdr1 genes in two Zambian sites [MIM14650151]
Justin Chileshe, Eric M. Njunju, Boston S. Mbewe

Malaria remains a disease of public health importance in Zambia. Control of malaria has been affected by increasing resistance to cheaper antimalarial drugs. The main objective of this study was to establish correlation between treatment outcome and presence of mutations in pfcrt and pfmdr1 genes. In vivo efficacy assessment of Chloroquine was done in two Zambian sites in Children under five. PCR amplification and hybridization of the amplified products with radiolabelled allele-specific probes method was employed to detect point mutations at pfcrt 76 and pfmdr1-86 positions. Association of clinical outcome and genotype was examined using Fisher’s exact test. TF was recorded in 64.5% (35.5% ETF and 29% LTF) of the 47 children who completed the study respectively in Mpongwe and Chibombo. There was no association between Pfcrt genotype and clinical outcome (Mpongwe = 0.31). There was significant association between Pfmdr1 genotype and clinical outcome (Mpongwe P = 0.0078; Chibombo P = 0.035), and the combination of Pfmdr1 and Pfcrt double mutant genotype was significantly higher in the TF group (Mpongwe P = 0.0069; Chibombo P = 0.038). Chloroquine became ineffective in the treatment of P. falciparum malaria. TF rates together with presence of mutations in P. falciparum malaria helped change Zambian malaria treatment policy. Pfcrt plays key role in chloroquine resistance but not predictive of treatment failure in these sites. Pfmdr1 is useful indicator of potential treatment failure, in combination with the Pfcrt 76T genotype.

Email address for correspondence: jcbchile@yahoo.com

644
Distribution of ITNs using IRS teams in Botswana: A potential model for achieving universal coverage in malaria-endemic areas [MIM16696908]
Ron Masendu, Simon Chihanga, J.P. Nogues, Tjantilii Mosweunyane, D.S. Ntebela, Bosiela Segogo, Linda Zou, Oliver Sabot

Botswana has set a national goal of eliminating malaria transmission by 2015, with an associated target of achieving at least 80% coverage of insecticide-treated bed nets (ITNs) in malaria-endemic districts. However, despite years of implementing a cost-recovery model to sell ITNs at subsidized prices, usage rates remain below 10%. The National Malaria Control Programme is therefore testing a new model, in which ITNs are freely distributed and hung by indoor residual spraying (IRS) teams during house-to-house visits. Key outcomes of this project are ITN ownership and usage within the Okavango district. A 2007 survey of 460 randomly selected households provides the baseline against which to measure the project’s impact. IRS teams will conduct door-to-door delivery and hanging of ITNs in February and March 2009, with the aim of providing on average one net per every two people and every one sleeping space. After distribution, community-based volunteers will conduct follow-up visits approximately once per month per household to encourage sustained usage. A May 2009 survey will provide robust measures of the initial impact on ownership and usage outcomes; a March 2010 survey will measure longer term impact. Ownership of at least two ITNs was 8.9% among surveyed households in the district at baseline, and less than 6% of individuals used these nets the previous night. Results presented in discussion are expected to show significant gains towards universal coverage of at-risk populations with ITNs. will also focus on the cost efficiency of combining ITN distribution with IRS campaigns in comparison to alternative mass distribution campaigns conducted in other countries and the cost-recovery model currently employed in Botswana.

Email address for correspondence: masenduron@yahoo.co.uk

645
Phase III, randomized, non-inferiority trial on efficacy and safety of dihydroartemisinin/piperquine versus artemether/lumefantrine in African children [MIM16597260]
Michael Nambozi, Quiche Bassat, Halidou Tinto, Patrice Viola, Stefan Borrmann, Clara Menéndez, Modest Mulenga, Innocent Valéa, Carolyn Nabasumba, Philip Sasi, Antonella Bacchieri, Marco Corsi, Michael Ubben, Ambrose Talisuna, Umberto D'Alessandro

Artemisinin combination therapies (ACTs) are the best option for treating uncomplicated malaria. Dihydroartemisinin-piperquine (DHA-PQP) is a promising fixed-dose ACT but little information on its safety and efficacy in African children with malaria is available. The non-inferiority of DHA-PQP versus artemether-lumefantrine (AL) in children 6–59 months old with uncomplicated P. falciparum malaria was tested in five African countries (Burkina Faso, Kenya, Mozambique, Uganda and Zambia). Patients were randomised (2:1) to receive either DHA-PQP or AL. Non-inferiority was assessed using a margin of –5% for the lower limit of the one-sided 97.5% confidence interval on the treatment difference (DHA-PQP vs. AL) of the polymerase chain reaction (PCR) corrected day 28 cure rate. Efficacy
The two treatments were compared. The lower limits of the 97.5% CI of the difference between (PP) in the DHA-PQP group, and 90.0% (ITT) and 95.7% (PP) in the AL group. The risk of recurrent infection at day 28 was significantly lower in the DHA-PQP (ITT 12.3%; PP 7.1%) than in the AL (ITT 23.3%; PP 18.6%) group. Dihydroartemisinin-piperaquine is as efficacious as AL in treating uncomplicated malaria in African children from different endemicity settings, and shows a comparable safety profile. It shows also a statistically superior efficacy in preventing new infections.

Email address for correspondence: michaelnambozi@yahoo.com

646 Increasing IRS acceptance using a communication strategy, Zambia’s experience [MIM15097849]
Pauline Wamulume
No abstract received
Email address for correspondence: paulinekw@yahoo.com

647 Impact of intermittent preventive treatment in infants (IPTi) on the prevalence of the molecular markers of resistance to sulfadoxine-pyrimethamine in south of Senegal [MIM16689556]
Annie Abiola

Intermittent preventive treatment in infants constitutes a new strategy which consists to administrate a half tablet of sulfadoxine-pyrimethamine (SP) during the Expanded Program of Immunisation. However, the recent studies showed an increase in the mutation on the level of genes DHFR and DHPS respectively for resistances to the sulfadoxine and the pyrimethamine. The aim was to evaluate: the impact of IPTi using SP in the prevalence of molecular markers (DHFR and DHPS) in four districts of Senegal A cross-sectional survey was carried out between intervention district and district control before (2007) and after intervention (2008). Filter papers were collected in all the children who were enrolled. Prevalence of mutation Pfddhr-51, -59, -108 and Pfddhps-437 and -540 were analyzed. Before intervention 85% (138/163) of the blood spots analysable present a mutation at least one of DHFR genes or DHPS versus 75% (90/120) after intervention. In 2007, 12% carry a double mutation, 23% triple and 4% quadruple mutation in the intervention district. In these same districts, 17% present the double mutation; 16% triple and 6% the quadruple mutation in 2008. In control district before intervention, 5% present the double mutation, 4% triple and 1% the quadruple. After intervention 3% of the samples carry the double, 10% triple and 2% the quadruple.

We did not detect the quintuple mutation. After 1 year of intervention, IPTi did not increase the prevalence of resistance molecular markers of SP.

Email address for correspondence: annie_abiola@yahoo.fr

648 Pharmacovigilance of artesunate/amodiaquine treatment of P. falciparum uncomplicated malaria. Experience in Casamance (Senegal) [MIM16764044]

The World Health Organisation recommends the use of artemisinin derivatives based combination therapy (ACT) for treatment of uncomplicated malaria in most malaria endemic countries. Consequently, setting up an appropriate pharmacovigilance (PV) system becomes essential to detect and monitor unexpected adverse events or serious adverse events to ACTs. The study was conducted from 2000 to 2008 in the 4 health centres of Oussouye district in Casamance (Senegal). All fever cases were screened using smear and/or RDT. All uncomplicated P. falciparum positive malaria cases were treated with artesunate/amodiaquine (AS/AQ), except children <5 kg and pregnant women. Treatments were supervised for 3 days and seen on day 28 for clinical assessment. Treatment emerging signs and symptoms (TESS) were recorded for each patient. Out of 24,297 fever episodes with smear, 8679 (35.7%) were positive and 3893 patients treated with either a loose combination or a co-blister of AS/AQ. Among them 3766 were assessed. No severe or life-threatening adverse event was observed and 181 patients (4.8%) presented 230 TESS. Among them, 140 (9.2%) treated with a co-blister and 41 (2.1%) with a loose combination (p < 0.0001). The risk of suffering of 1 or more TESS was 4.4 (95%CI = [3.0; 6.1]) times higher in patients treated with a co-blister. TESS appeared within day 1 (72%) and 75% were mild or moderate. Several attempts to set up a PV system in Africa are in process. Their role is crucial for AS/AQ whose use is largely extended in Africa to ensure anticipation of the risk and to provide recommendations.

Email address for correspondence: brasseur@ird.sn

649 Molecular markers of resistance to sulfadoxine-pyrimethamine after 1 year of pilot implementation of malaria intermittent preventive treatment in infants in Mali [MIM16689994]
Alasanne Dicko, Issaka Sagaara, Abdoulaye Djimde, Mariam Traore, Siddy Toure, Souleymane Dama, Abdoubakri I. Diallo, Amadou Barry, Mohamed Dicko, Oumar M. Coulbily, Alpha T. Diallo, Alexandra de Sousa, Ogobara Dumbo

Recent studies have shown that Sulfadoxine-Pyrimethamine (SP) given in Intermittent Preventive Treatment in infants (IPTi) during routine vaccinations is efficacious in preventing malaria disease, without interaction with the vaccines. However there is a fear that IPTi will increase rapidly the resistance of P. falciparum to SP and this study aimed to evaluate the impact this strategy on the resistance to SP. The 22 health areas in the district of Kolokani were randomized into two groups in 1:1 ratio and IPTi was implemented for 12 months from December 2006 haft areas while the other half served as control. In December 2007, a cross-sectional survey was conducted in children aged 0–5 years randomly selected. Thick smears and filter paper blood dots obtained by fingerprick and dhfr and dhps mutations associated with resistance to SP were analyzed by PCR. About half of the children harbour each of 3 dhfr mutation in both the intervention and non-intervention zones and 20% and1% of the children in each zone harbour parasites with mutation 437 and 540 on DHPS, respectively. There was also no difference in frequency of quadruple mutants (triple + dhps 437), between the two zones 11.6% versus 11.2%, OR = 1.04 (95% CI 0.51–2.12) p = 0.90. This study shows a relatively low frequency of quadruple
mutants and no evidence of increase in frequency of molecular markers of SP resistance in areas where IPl was implemented for 1 year.
Email address for correspondence: adicko@mrtcbko.org

650 Geographic inequities in provision and utilization of malaria treatment services in southeast Nigeria: Diagnosis, providers and drugs [MIM16659805]
Ogobukwu Ezoke, Obinna Onwujekwe, Benjamin Uzochukwu

The difficulties in treating malaria in Nigeria may be, in part, a result of geographic inequity in access to appropriate services. The link between the utilization of different types of providers with geographic location of the consumers is not well established. The study examined the levels of geographic inequities in household's choice of providers, mode of diagnosis and drugs for the treatment of malaria. Interviewer-administered questionnaire was used to collect information from 2250 randomly selected 2250 respondents from six malaria-endemic communities in southeast Nigeria. A comparison of data between urban and rural areas was used to examine geographic inequities in treatment seeking. There were geographic inequities in the use of different providers and drugs for the treatment of malaria. The urbanites used more of private hospitals/clinics and specialist hospital, whilst the rural dwellers used more of drug sellers (patent medicine dealers (PMD) and pharmacy shops (PS)). The rural dwellers were prescribed the cheaper drugs whilst the urbanites were prescribed the more costly drugs. The geographic inequities in malaria treatment are tilted against the rural people. Everybody is seeking care the private sector for treatment of malaria but the rural dwellers are using mostly the most informal parts of the sector.
Email address for correspondence: mypaskie@yahoo.co.uk

651 Malaria situation in southern Benin after the national ITNs distribution [MIM16689197]
B.G. Damien, V. Corbel, A. Djénontin, F. Chandre, M. Akogbéto, D. Kindé-Gazard, M.-C. Henry

The malaria situation in Benin needs to be assessed after the national distribution of the Insecticide-Treated Nets (ITNs) in order to appraise the efficacy of these preventive measures that have been set up. From December 2007 to August 2008, the importance of malaria was measured in southern Benin. We clinically monitored 463 children aged 0–5 years randomly selected in 7 villages for 36 days (in 6 periods of 6 consecutive days every 6 weeks). During each survey a systematic blood sample was taken from every sick and asymptomatic child. The parasitological indexes of asymptomatic children showed that the prevalence rate of P. falciparum was 23.3 (95% CI 21.5–25.1%). It increased significantly with age and did not vary with the seasons. The parasite density in the parasite positive children was 576 (95% CI 495–670) P. falciparum asexual forms/µl of blood. The prevalence of P. falciparum gametocytes was 3.3 (95% CI 2.5–4) %. The clinical malaria incidence was 1.5 (95% CI 1.1–1.9) episodes per child per year. The risk of malaria attacks did not vary with the seasons. More than 90% of children had an ITN. However the use of the ITN decreased strongly during the dry season. Our study was the first observation of the malaria situation after the national distribution of the ITNs to children aged less than 5 years. The absence of the seasonal variations of the parasitological indexes and the clinical incidence may be explained by a large seasonal variation in using the ITNs, which obviously needs sensitization of the populations.
Email address for correspondence: barikiss2000@yahoo.fr

652 Management of malaria in children [MIM15424023]
B.N. Orajaka, O.C. Nwaorgu

Home management of malaria has been identified as a key practice in most communities in Africa, hence the need to identify the herbs and home made therapies used by mothers in treating malaria in various communities. An already pretested questionnaire was used to elicit information from mothers on the type of herbs and home made remedies used by mothers for management of malaria in children 1–10 years old and their perception about malaria and where they take their children to when they have malaria. 800 women were involved in the study. Questionnaires were administered through the women council heads in each of the study communities. Eighty three percent of the mothers reported that their children suffered from malaria, which they identified as fever. However, 85.12% (681) report that malaria, was treated at home while 14.9% (119) took their children to the hospital. One hundred and one (84.8%) out of (119) mothers who reported visiting the hospital/health centre actually visited patent medicine vendors to buy drugs while 28.6% of the mothers took their children to hospital/health centres. With regard to the type of home or herbal remedies used by mothers, dogoyaro leaves (neem) was ranked as the first (71.1%) followed by lemon grass (70.6%), then paw-paw leaves (48.3%) and mango leaves (41.9%). Other remedies used include garlic (43%), ginger (13%), guava leaves (33.9%) avocado pear leaves (8.9%) bitter leaves (11.1%) and unripe pineapple (13.9%). There is need for intensified health education on home management of malaria for mothers in various communities and where possible blister packet of malaria drugs should be used for the purpose. This will ensure that children not only receive the right treatment dosage but also that the appropriate malaria treatment is received.
Email address for correspondence: bnoraajaka@yahoo.com

653 Field evaluation of durable residual wall lining (DRWL) as an alternative to indoor residual spraying (IRS) for the control Anopheles gambiæ in rural villages of Obuasi, Ghana [MIM16704069]
J.B. Stiles-Ocran, S.P. Knowles, M.D. Wilson, D.A. Boakye, J. Thomas

DRWL is a new technology that will provide a long lasting solution to the retreatment difficulties associated with IRS. It utilizes a similar concept as the long lasting insecticide net, which involves controlled release of a suitable insecticide impregnated in textile with the ability of remaining effective for 3–4 years. This study was conducted to evaluate the acceptability, durability and residual activity of DRWL in two rural villages of Obuasi. The two trial villages were Anwona and Mmenmiriwa 1 located at the periphery of Obuasi. At 3 weeks post-installation, a survey was made of user impressions of the DRWL appeal and appearance. The residual activity of deltamethrin-impregnated DRWL against susceptible An. gambiæ s.s was assessed monthly using the WHO cone bioassay kits on both cement and mud surfaces and compared with IRS using K-Othrine. Twelve houses, six representing each surface, were selected for each intervention at each village. The installation of DRWL and IRS treatments were conducted in August 2008. About 95% (58/61) of respondents expressed interest in the DRWL.
At 6 months post-installation, the DRWL installation was generally in good condition. 100% mortality of An. gambiae s.s was observed for DRWL installed on both surfaces, 91.6% mortality for the IRS-treated cement surface, and 57.1% mortality on IRS-treated mud surface with a residual life of 4 months. The high acceptability and residual activity of DRWL observed makes it ideal for use in the control of Anopheles vector especially in rural areas where houses are constructed with mud.

Email address for correspondence: jstiles-ocean@anglogoldashanti.com

654 Impact of private sector involvement on malaria control interventions in Nigeria: Results from baseline outlet and household survey [MIM16705207]
K.Y. Yakubu, B.M. Audu, E. Arogundade

In 2005, Nigeria revised the national first-line malaria treatment policy to Artemisinin-based Combination Therapy (ACT). By July 2006, a total of 114 ACTs and Artemisinin based monotherapies were registered and these antimalarials are accessible without prescription. The private sector plays a major role in the delivery of health care and treatment in Nigeria. In 2007, the drug regulatory body stopped the registration of artemisinin monotherapies to safeguard efficacy of ACTs. Household and outlet surveys will evaluate the impact of this significant change in policy. A one-staged, stratified longitudinal outlet survey was conducted in December 2008 and July 2009 to measure the levels and trends in the availability, price, quality, volume, retailer perceptions, and knowledge of antimalarial medicines at different points of sale. Within the selected geographical areas, a baseline population-based household study was conducted to recruit a representative sample of caregivers with children under five (n = 2260) who had recently experienced a fever. A structured questionnaire was used to gather information on malaria treatment-seeking behavior, type of treatment and source. Survey results from these studies will provide data around changes in availability and price of antimalarials. Data from the household and outlet survey will be used to describe access to malaria treatment. We will also present findings related to awareness of ACTs, behavioural patterns related to treatment-seeking, source and type of antimalarials. Results will be used to illustrate and document the role of the private sector as model for improving malaria control in Nigeria.

Email address for correspondence: kyakubu@sfnigeria.org

655 Effect of concomitant artesunate administration and cytochrome P4502C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria [MIM16695712]

Artesunate (AS) is used in combination with amodiaquine (AQ), as first line treatment for uncomplicated malaria in many countries. There is little information on the pharmacokinetics of the AS+AQ combination. A two-compartment model was fitted to plasma concentrations of AQ metabolites from 103 Ghanaian children aged 1–14 years with uncomplicated malaria treated with AQ (n = 15), or with AS+AQ (n = 88) and analyzed, using a population approach. Alleles of the gene of the main enzyme responsible for AQ metabolism (CYP2C8) were identified, using a PCR-RFLP method. There was a non linear relationship between the population clearance (CL) of AQ metabolites and the covariate, body weight (BW), which could be described by the following equation: CL = 29*BW/18/([0.992 + (BW/18)]. Concomitant AS administration increased (p < 0.001) the central volume of distribution of AQ metabolites. Peak plasma concentrations of AQ metabolites were lower (p < 0.01) in the AS+AQ group. There was a high incidence of the nonwild type CYP2C8*2 allele, and levels of plasma AQ metabolite concentrations were different, though not significantly, in subjects with different CYP2C8 alleles. AQ-associated adverse effects occurred more often in children aged 12 years and above. In children with uncomplicated malaria, dosing of AQ should be based on body weight up to 30 kg, and thereafter AQ should be administered as a fixed dose. The pharmacokinetics of antimalarials administered in combination with artemisinin derivatives are influenced by the artemisinin, possibly through its rapid parasite clearance. This could have effect on medium term effectiveness of artemisinin-based combination therapies.

Email address for correspondence: goadjei@yahoo.com

656 Innovative collaboration among healthcare providers, for the control of childhood malaria, in a developing country [MIM16695263]
D.O. Akinbode, O. Fawole, O. Oshowole, O.M. Bolaji, C.O. Falade, J.D. Adeniyi

Health care providers, patent medicine sellers and traditional healers treat childhood malaria in developing countries. Members of each group discriminate against the other groups. When complications arise during treatment, they do not disclose administered drugs or refer patients to orthodox healthcare facilities. These lead to death of children. The objectives of this study were to reduce infant mortality due by malaria among patients who do not respond to the treatment of patent medicine sellers/traditional healers and to establish referral procedures from both groups, to orthodox health care providers. This study was conducted in both rural and urban local government areas in Nigeria. Comprehensive 4 days educational programs were separately conducted for each of the three target groups. Addressed were causes, transmission, symptoms and control of malaria and also the advantages of collaboration between the target groups, and the importance of referral information. Group members established collaborative links to prevent infant mortality due to malaria. They agreed to invite members from other groups to attend their periodical meetings and exchange information on malaria treatment. Post-inauguration visits to study sites revealed adherence to terms of agreement. Educating target groups about collaborative methods of saving the lives of children who did not respond to the treatment of traditional healer or patent medicine sellers, was a remarkable innovation in the treatment of childhood malaria. It is recommended that such experimental studies be conducted in other developing countries, to advance the management and control of childhood malaria and other diseases.

Email address for correspondence: mumsiec@yahoo.com

657 Assessment of treatment pattern of uncomplicated malaria in pediatric patients in a teaching hospital in NorthWest Nigeria [MIM16762554]
A.I. Oreagha, R. Gbadamosi, I.O. Ishola

This study was conducted to assess the treatment pattern of uncomplicated malaria in children below 5 years as a result of change in the National Antimalarial treatment guidelines in
southwest Nigeria. A prospective study was carried out between January and March 2008 among caregivers until a target population of 200 children with uncomplicated malaria were obtained. After which a self-administered structured questionnaire were filled by the prescriber. In the retrospective study, this spanned for a period of 6 months between January and June 2006 to determine the degree of compliance of prescribers to the antimalarial treatment guideline. A total of 342 prescription forms randomly selected among patients between the age groups of 0–5 years were assessed. This study revealed that out of 200 children with uncomplicated malaria, 72% received ACTs, 6% received chloroquine, 2% received SP, 10% received ACT + CQ, 10% received other antimalarial drugs. Analgesic and antibiotics were also indicated. In the retrospective study, prescriptions with oral antimalarials were 52.6%, injectable antimalaria were 2.9%. The proportion of ACTs prescribed was 60%. There were no statistically significant difference in the frequency of ACTs in the prospective and retrospective study. In addition 66% of physicians reported that they prescribe ACTs while 34% reported prescribing chloroquine as first line of antimalarial drug. This study showed that the pattern of treatment of uncomplicated malaria reflects high compliance with the change in the antimalarial drug treatment policy at the pediatriac out-patients department in southwest Nigeria.

Email address for correspondence: ibn20032002@yahoo.com

659

Practice of malaria prevention among school adolescents in Calabar-South, Nigeria [MIM1669678]

A.J. Etokidem, N.E. Udonwa, A.N. Gyuse

An African child dies of malaria every 30 s. The problem of malaria among adolescents has been overshadowed by the huge burden of the disease among the under-fives, leading to paucity of research and information. Malaria leads to school absenteeism and affects adolescents’ performance in school. The objective of this study was to determine the malaria prevention practices of school adolescents in Calabar-South, Nigeria. Four hundred adolescents were randomly selected out of the 4565 in 5 randomly selected schools to participate in the study. A semi-structured questionnaire was administered on them. Data from the study was analysed using EPI-Info software 2002. Nine (2.25%) respondents correctly defined malaria. Seventy-seven (19.25%) respondents did not know the mode of transmission of the malaria parasite while 37 (9.25%) responded that the vector transmits the parasite when it is swallowed. One hundred and thirty-five (34%) respondents obtained information about malaria prevention mainly from radio while 45 (11%) obtained the information from their teachers. Fifty-four (13.5%) respondents would prevent malaria attack by clearing the vegetation in the peri-domestic environment while 103 (25.7%) would use ITNs. The study shows a wide gap in knowledge of malaria prevention among school adolescents in Calabar-South. This could be because attention on malaria among adolescents has been diverted by the huge burden of HIV/AIDS among them, leading to their having access to so much information about HIV/AIDS and so little about malaria.

Email address for correspondence: anietokidem@yahoo.com

660

Cost-effectiveness analysis of use of RDT, microscopy and clinical examination/history in the diagnosis and treatment of malaria, implications for ACT treatment policy in Nigeria [MIM14818968]

Benjamin S.C. Uzochukwu, Eric N. Obikeze, Obinna E. Onwujekwe, Ulla Griffiths

The diagnosis and treatment of malaria are often based on the clinical presentation of malaria symptoms and microscopy examination of blood films. These methods have their inherent problems that make them unreliable. Rapid diagnostic tests (RDTs) have improved malaria diagnosis in the field. Despite this improvement, the cost effectiveness of this new diagnostic option in malaria endemic communities in Nigeria remain uncertain. A total of 638 patients with fever (presumptive malaria) were recruited for examination with RDT and microscopy and questionnaire survey. Decision tree model and probabilistic sensitivity analysis was applied to these patients. Costs and effects encompassed those for both patients positive on RDT and who received artemisinin-based combination therapy (ACT) and febrile patients negative on RDT who received an antibiotic treatment. Cost-effectiveness estimates were done using TreeAge programme 2008. Interventions were defined as cost-effective if they were less costly and more effective or had an incremental cost per disability-adjusted life year averted of less than US$ 150 RDT is cost saving when compared to other malaria diagnostic strategies at malaria prevalence of 43.1% found in this study. Cost-effectiveness is affected by malaria prevalence level; ACT adherence level; cost of ACT, antibiotics and RDT; proportion of Non-Malaria Febrile Illness cases, microscopy and RDT sensitivity. RDT is more cost-effective than the other strategies. There is need for patients to adhere to the treatment procedures. RDT should be
made accessible at all levels of health healthcare in the light of ACT treatment policy in Nigeria.

Email address for correspondence: bscuzochukwu@yahoo.com

661 Local definition of childhood malaria treatment failure among mothers of under-five children in Offa, Nigeria [MIM16677991]

Amzat Jimoh

One of the major strategies in Rolling Back Malaria is the scaling up of home management of malaria (HMM) but malaria treatment failure (MTF) is a major setback. MTF contributes to the social burden of malaria in terms of additional treatment costs, hospital admissions, indirect loss of productivity, psychological trauma and loss of confidence in therapeutic measures. Up to 70% of malaria episodes are first treated at home and that effective management of malaria at home level will reduce the burden of malaria at facilities and save a lot of lives. It is highly significant to understand behavioural issues surrounding malaria treatment failure among mothers in order to impact behavioural intervention in this regard. Therefore, this study examines definition of childhood malaria treatment failure among mothers of under-fives. The study was conducted among selected mothers of under-five children in Offa using qualitative methods involving 24 in-depth interviews [12 young mothers (<35 years old) (6 literates and 6 illiterates), 12 old mothers (>35 years old) (6 literates and 6 illiterates)], 8 focus group discussion [4 among young mothers (2 each among literates and illiterates), 4 among old mothers (2 each among the literates and illiterates)]. Data were analysed using content analysis and ethnographic summary. The study found that experience of treatment failure is pervasive among the mothers of under-five in HMM with both herbal remedy and modern drugs. Treatment failure is also experienced at the official health sector. It usually takes 3–4 days before perceiving treatment failure and thus seeking for further help especially out of the home from other health care providers. Treatment failure is basically defined in terms of illness characteristics (such as progression of illness to severe form and persistence of signs and symptoms), duration of illness, mothers’ beliefs and consultation inputs. This definition (of MTF) is greatly influenced by the local understanding of malaria. The study observed that most deaths occurring from malaria are due to treatment failure as mothers do not act against malaria at home level. It is only the failure of such action that could result in child mortality. The study concludes that home treatment will continue to be a useful response to the treatment of childhood malaria. Efforts should be made to enhance appropriate HMM in order to minimize treatment failure and encourage referral practice.

Email address for correspondence: greatjoa@yahoo.co.uk

662 Community intervention to reduce malaria-related morbidity and mortality in the Shime sub-district in Ghana [MIM16706996]

Collins Ahorlu, Kwadwo Koram

Prompt and effective treatment of malaria cases is a goal desired by all as we wait for the imminent arrival of an effective and affordable vaccine to the population at risk, especially the vulnerable groups of children under five and pregnant women. IPT has recently been accepted as an important component of the malaria control strategy. Recent trials in perennial malaria transmission areas have shown that IPT given at the time of childhood vaccinations reduced the incidence of first episode of malaria and severe anaemia by more than 50% during the first year of life (Dicko et al., 2008; Schellenberg et al., 2001; Massaga et al., 2003). Objective: To demonstrate the feasibility and effectiveness of Community Assistants’ delivery of IPTc combined with timely febrile malaria-related illness management at home. The study combined home-based delivery of intermittent preventive treatment for children (IPTc) aged 6–60 months and home treatment of suspected febrile malaria-related illness within 24h. All children aged 6–60 months received home-based delivery of intermittent preventive treatment using Amodiaquine + Artesunate, delivered at home every 4 months (3 times in 12 months). Malaria parasite prevalence surveys were conducted before and after IPTc was given three times in the community. Community Assistants delivered the interventions. In our study, prevalence reduced from 25–3%, which is about 80% reduction in prevalence. At baseline, 13.8% and evaluation, 2.2% of the children were febrile (auxillary temperature of ≥37.5). However, 42.0% and 7.6% of the caretakers reported fever in within 7 days prior to baseline and evaluation parasite surveys, respectively. The evaluation result indicates that IPTc given three times in a year (every 4 months) combined with timely treatment of febrile malaria illness, could reduce malaria prevalence significantly.

Email address for correspondence: cahorlu@noguchi.mimcom.org

663 Extended high efficacy of the combination sulфadoxine–pyrimethamine with artesunate in children with uncomplicated falciparum malaria on the Benin coast, West Africa [MIM16693029]

Alain Nahum, Annette Erhart, Daniel Ahounou, Désiré Bonou, Cantal Van Overmeir, Joris Menten, Martin Akogbeto, Marc Coosemans, Achille Massougbodji, Umberto D’Alessandro

A study carried out in 2003–2005 in Southern Benin showed a day 28 sulfadoxine–pyrimethamine (SP) monotherapy failure rate greater than 40%, while for SP combined with artesunate (SP–AS) the failure rate was 5.3%. Such a large difference could be explained by the relatively short 28-day follow-up period, with a substantial number of recurrent infections possibly occurring after day 28. This paper reports the treatment outcome observed in the same study cohort beyond the initial 28-day follow-up. After the 28-day follow-up, children treated with either chloroquine alone (CQ), SP or SP–AS, were visited at home twice a week until day 90 after treatment. A blood sample was collected if the child had fever (auxillary temperature ≥37.5°C). Total clinical failure for each treatment group was estimated by combining all the early treatment failures and late clinical failures that occurred over the whole follow-up period, i.e., from day 0 up to day 90. Pre-treatment randomly selected blood samples were genotyped for the dhfr gene (59) and the dhs gene (437 and 540) point mutations related to SP resistance. The PCR-corrected clinical failure at day 90 was significantly lower in the SP–AS group (SP–AS: 2.7%; SP alone: 38.2%; CQ: 41.1%) (log-rank p < 0.001). The most prevalent haplotype was dhfr Arg59–with the dhps Gly437 mutant and the dhps 540 wild type (85.5%). The dhps 540 mutation could be found in only three (8.3%) samples. Combining artesunate to SP dramatically increased the treatment efficacy, even when extending the follow-up to day 90 post-treatment, and despite the high percentage of failures following treatment with SP alone. Such a good performance may be explained by the low prevalence of the dhps 540 mutation, by the rapid parasite clearance with artesunate and by the level of acquired immunity.

Email address for correspondence: danahoun@yahoo.fr

www.mimalaria.org
664 Comparative efficacy study of dihydroartemisinin alone and dihydroartemisinin plus mefloquine combination in children with uncomplicated *Plasmodium falciparum* malaria in Lagos State, Nigeria [MIM16714707]

O.O. Aina, P.M. Emeka, P.U. Agomo, A. Akintonwa

Nine hundred and twenty children, between the ages of 2 and 13 years attending the Outpatient Department of Massey Street Children Hospital Lagos Island and Ijede Health Center, Ikorodu, Lagos State were screened for malaria parasites. Patients that were positive and fulfilled the inclusion criteria were allotted to one of the two treatment groups after informed consent from parent or guidance and ascertain from the child. One group was treated with dihydroartemisinin (DHA) alone (2 mg/kg) and the second group was treated with combination of dihydroartemisinin plus mefloquine (MQ) (DHA 2 mg/kg and MQ 15 mg/kg). All patients in dihydroartemisinin alone and dihydroartemisinin plus mefloquine combination groups had a rapid initial response to treatment. Their parasite clearance time (PCT) was 34.3 ± 12.83 and 36.0 ± 16.97 h, respectively. The fever clearance time (FCT) in dihydroartemisinin alone and dihydroartemisinin plus mefloquine combination was 15.7 ± 9.27 and 20.0 ± 6.93 h, respectively. This shows that dihydroartemisinin alone had a faster FCT and PCT than when combined with mefloquine in this study. There was no significant difference in the FCT and PCT between dihydroartemisinin alone and combination of dihydroartemisinin plus mefloquine group (P>0.05). The subjects were monitored for 28 days; there was no report of any recrudescence or resistance in dihydroartemisinin group and dihydroartemisinin plus mefloquine group within the 28 days of monitoring the subjects.

Email address for correspondence: gbengaaina2003@yahoo.com

665 Intermittent preventive treatment use among pregnant women attending antenatal clinics in primary health care centers in a rural Local Government Area in southwest, Nigeria: A cross-sectional study [MIM15183722]

Stella Akinleye, Catherine O. Falade, Ikeoluwapo O. Ajayi

Intermittent preventive treatment (IPT) using sulfadoxine–pyrimethamine (SP) has been shown to be effective in reducing the adverse effects of malaria during pregnancy. Despite this, the uptake and coverage of IPT during pregnancy in Nigeria are low. Main Objective: To assess the use of IPT among pregnant women attending primary health centres for antenatal services. In a cross-sectional study 209 pregnant women attending antenatal clinics in primary health care centres in a rural Local Government Area of Ekiti State, Nigeria were interviewed. Respondents were selected by systematic random sampling technique. Information was obtained using an interviewer administered questionnaire. One hundred and nine of 209 (52.2%) respondents have heard about IPT but only 26 (12.4%) were able to define IPT. Fifty-seven (27.3%) reported to have received at least one dose of IPT during the index pregnancy. Twenty-one of these (36.8%) took the SP in the clinic while 3 of the 21 were supervised by a health worker giving a compliance rate with the DOT scheme of 14.3%. Some pregnant women reported that there were periodic shortages of the drug in the clinics. In this study, IPT use among pregnant women was very low. More effort should be made to increase awareness on IPT among women and health workers should be trained and monitored for compliance. The factors militating against successful use of IPT should be addressed.

Email address for correspondence: stellakinleye@yahoo.com

666 Efficacy of three chemoprophylaxis regimens to adequately prevent placental malaria and low birth weight in pregnant women living in a rural area of Burkina Faso [MIM16698095]


The weekly chemoprophylaxis of malaria during pregnancy with chloroquine (CQ) has become problematic with the increasing resistance of *Plasmodium falciparum* to this drug. This study compares the efficacy of two IPT regimens (using chloroquine or sulphadoxine/pyrimethamin (SP)) with the classical chemoprophylaxis regimen using CQ in reducing the adverse outcomes of malaria infection for the mother and the fetus. Pregnant women attending the first antenatal care visit were randomly assigned to one of the three treatment regimens. They were subsequently followed up to delivery. Maternal, placental and cord blood samples were obtained upon delivery for *P. falciparum* infection detection. A total of 648 pregnant women were enrolled in the study. Delivery outcome were available for 423 of them. Peripheral maternal *P. falciparum* infection at delivery was found in 25.8% of the women. The proportion of women with maternal infection was significantly lower in the IPTp/SP group than in the CQ group (P<0.000). The prevalence of placental malaria was 18.8% in the CWC/CQ group; 15.9% in the IPTp/CQ group and 10.6% in the IPTp/SP group. The incidence of LBW (weight <2500 kg) was significantly higher among infants of mothers in the CWC/CQ group (23.9%) as compared with those of mothers in the IPTp/CQ (15.6%) and IPTp/SP (11.6%) groups (p=0.02). Intermittent preventive treatment with SP has shown clear superiority in reducing adverse outcomes at delivery, as compared with intermittent preventive treatment with CQ and classical chemoprophylaxis with CQ.

Email address for correspondence: t.alfred@fasonet.bf

667 Presumptive diagnosis or microscopy under any circumstances? Outpatient case management of malaria in a rural African district [MIM16701495]

Evelyn Ansah

To evaluate the accuracy of presumptive diagnosis and routine malaria microscopy under normal operational conditions at health facilities in rural Ghana. A cross-sectional survey was carried out over a 1-month period at three public sector facilities and one private clinic. Expert microscopists assessed the accuracy of presumptive diagnosis and local microscopy by means of a double-read research slide taken at the same time. The analysis covered 602 patient contacts by 22 clinicians in four health facilities. Of the 602 patients, 542 complained of fever at presentation. Only 128 out of 542 patients (23.6%) with a complaint of fever were febrile on examination. Laboratory tests were requested for 15.1% of 602 patients, one patient with a positive result from local microscopy on examination. Laboratory tests were requested for 15.1% of 602 patients. One patient with a positive result from local microscopy did not receive an antimalarial whilst 21.9% of those with a negative test result did so. 48.1% of those who had no laboratory test done, also received an antimalarial. The sensitivity, specificity, positive and negative predictive values of presumptive diagnosis were 99.3%, 4.2%, 24.7% and 95%, respectively. For local microscopy, sensitivity and specificity were 75% and 37.3% whilst positive and negative predictive values were 20.3% and 87.5%, respectively. Slide positivity rate according to local microscopy was 64.8% while that of expert microscopy was 17.6%. Whilst presumptive diagnosis results in over-diagnosis of malaria, poor quality microscopy does not improve the standard of clinical practice. The use of alternative
Efficacité thérapeutique de trois CTA administration répétée dans la prise en charge de paludisme non compliqué au Mali [MIM16694578]


Les monothérapies ont été remplacées par les combinaisons thérapeutiques à base d’artémisinine (CTA) en Afrique. Les différents CTA testées sur 14 ou sur 28 jours ont montré une bonne efficacité thérapeutique mais leur efficacité en traitement répété est mal connue. De 2005 à 2007 nous avons mené un essai clinique randomisé de phase IV à Bougoula Hameau au Mali. Nous avons utilisé le protocole OMS 2003 pour comparer l’efficacité de artésunate-amodiaquine (AS/AQ), artésunate-sulfadoxine-pyriméthamine (AS/SP) et artéméther-luméfantrine (AR-L) lors du traitement d’épisodes consécutifs de paludisme. Dès qu’un patient était randomisé à un bras de traitement, il recevait la même CTA durant toute l’étude. 780 participants ont été inclus dont 260 par bras de traitement. Au jour 28, les trois groupes étaient comparables après la correction moléculaire RCPA ≥98% pour chaque bras et quelque soit la période de l’année. Sans la correction moléculaire de Janvier à Mars nous trouvions 100%, 100% et 98% de RCPA respectivement dans les bras AS/AQ, AS/SP et AR-L. D’Avril à Juin les taux de RCPA etaient de 79%, 91% et 75% dans le même ordre puis 75%, 85% et 57% et d’octobre à Décembre 80%, 94% et 58%, respectivement dans les bras AS/AQ, AS/SP et AR-L (p < 0,001 (Chi-2)). Les trois CTA demeurent efficaces après correction moléculaire. Nous constatons des variations saisonnières de l’efficacité non corrigée.

Email address for correspondence: ddembele@mrtcbko.org

Missed opportunities for intermittent preventive treatment [IPTp] in among pregnant women attending antenatal clinic (ANC) in a secondary health care facility in Calabar, Nigeria [MIM15728685]


Intermittent preventive treatment of malaria in pregnancy (IPTp) strategy, implemented through the administration of sulfadoxine/pyrimethamine (SP) under direct observation, has been adopted in Nigeria as a key intervention package for making pregnancy safer. Preliminary findings indicate that many eligible pregnant women do not receive SP during ANC visits. This study determines the magnitude and contributory factors for missed opportunities for SP administration among pregnant women in a secondary health care facility in Calabar, Nigeria, as a basis for developing an intervention strategy to increase IPTp uptake. Exit interviews were carried out on all pregnant and postnatal women who attended the ANC clinic for 2 weeks in November 2008. Data were analyzed using SPSS statistical software, version 15. A total of 161 eligible women were interviewed. Mean gestational age at booking and current gestational age were 19 ± 5.5 and 30 ± 5.9, respectively. The prevalence of HIV among the pregnant women was 14.3%. The overall prevalence of missed opportunity was 69.6% and 60.9% for IPTp1 and IPTp2, respectively. The prevalence among those living with HIV/AIDS was 87.0%, 48.0% and 74.0% for IPTp1, IPTp2 and IPTp3, respectively. Factors responsible for missed opportunities include unavailability of SP in the ANC clinic, failure to prescribe and supervise intake of medication, poor record keeping and staff lack of knowledge of current schedule of IPTp. The findings establish the need to educate health workers and pregnant women about current schedule/procedures for IPTp and for making SP available in ANC clinic.

Email address for correspondence: bamideleedet@yahoo.com

The potential for successful delivery of ACT through Licensed Chemical Sellers in Ghana [MIM16729295]

David Ofori-Adjei, Kwame Adogboba, Sofo Ali-Akpajjak, Nana Eny-imayew, Bertha Garshong, Kwadwo Koram

Licensed Chemical Sellers (LCS) are often the first point of call for persons seeking medical care in Ghana. There are currently no clear policies on their role in health services delivery, however, there is an increasing interest in using them for managing malaria in
the community. Objectives: To determine the knowledge and practices of LCS for management of malaria and to better understand how to use their full potential, and to inform the ongoing policy dialogue. Structured questionnaires were administered to a regionally representative sample of LCS in the Ashanti Region of Ghana. 161 randomly selected LCS were interviewed. Most LCS had secondary or technical school education (77.1%). Their shops were often located in urban areas (77%) and served more than 20 clients a day (78.3%). All shops sold anti-malarial drugs but only 40% stocked Artemisinin-based combination therapy (ACT). Nearly all LCS knew the correct symptoms and signs for malaria but only 38.0% knew the approved treatment to be ACT. Initial training of prospective LCS by the Pharmacy Council was inadequate, their monitoring was infrequent and their tenure was short-lived. LCS and health sector managers alike realise the need for adequate training and other forms of support. LCS contribute significantly to the management of malaria in Ghana, but realising their full potential to provide timely and safe treatment requires more focused training, financial and material support, regular monitoring, and an enabling policy environment. Email address for correspondence: nana_enyimayew@yahoo.co.uk

673 Pattern of health seeking behaviour of women on malaria treatment during pregnancy in rural and urban Nigeria [MIM16649591]
U.E. Ezeoke, E.N. Shu, E.O. Onwujekwe, P.O. Okonkwo, B.C. Ozumba

Malaria in pregnancy is a major public health problem. Studies have shown that malaria is often not perceived as severe but rather as mild, self-limiting illness which does not require immediate treatment. Women can delay or even avoid accessing Antenatal care services in public health facilities due to some socio-cultural reasons. It becomes necessary to ascertain the health seeking behaviour of pregnant women in rural and urban Nigeria on malaria in pregnancy. The study was carried out in two urban and two rural local government areas (LGAs) in Enugu State, Nigeria. Four focus group discussions (FGDs) were held with pregnant women. A pre-tested interviewer-administered structured questionnaires were used to collect data from 601 pregnant women. Respondents in both areas resort to self-medication for malaria in pregnancy and visit hospitals only when home treatment fails. Majority of the urban respondents visited private hospitals/clinics 391 (65.1%) and general hospitals 249 (41.4%). Most of the rural respondents visited health centres/posts 246 (40.8%), and general hospitals 146 (24.2%). There is significant association between socio-economic status and health facilities visited in the two areas. The women take to self-medication for malaria in pregnancy and seek for care when home treatment fails. It is recommended that an intensive health education be instituted for the women on the need to seek prompt health care whenever they have malaria in pregnancy. Email address for correspondence: ezeokeuche@yahoo.com

674 Variations in serum lactate dehydrogenase and acid phosphatase activities in patients with acute, uncomplicated falciparum malaria: potential application as biomarkers of human malaria infection [MIM16779524]
Ibrahim H. Garba, Ubom G. Abraham

Total serum lactate dehydrogenase (LDH), acid phosphatase (ACP), two ACP isoenzymes, tartrate-resistant acid phosphatase (TRAP) and erythrocyte-specific acid phosphatase (ESAP) activities were assayed in 76 adult male and female patients each (age range, 18–40 years), presenting with acute, uncomplicated falciparum malaria infection (parasite density = 1000–10,000 asexual forms/ml of blood) and a control group of 82 healthy adult male and females each, matched for age. The mean LDH activity was found to increase by nearly three-fold from 247.10 ± 19.01 IU in the control group to 789.40 ± 35.01 IU in the male patients and 634.0 ± 35.1 IU in the female patients, p < 0.05. Both the total and isoenzyme forms of ACP were also found to increase significantly in malaria patients relative to the control. Total serum ACP was 2.20 ± 0.06 IU and 2.35 ± 0.13 IU in the male and female patients, while the respective male and female controls were 1.40 ± 0.07 IU and 1.30 ± 0.04 IU, p < 0.05. Serum TRAP activity was 1.08 ± 0.06 IU and 1.22 ± 0.13 IU in the male and female patients, while among the respective male and female controls, the values were 0.83 ± 0.07 IU and 0.79 ± 0.04 IU, p < 0.05. ESAP activity was 0.94 ± 0.02 IU (male patients) and 1.07 ± 0.05 IU (female patients). The synergistic effect of the two major pathological events in falciparum malaria infection, acute hepatocellular injury caused by invading sporozoites and merozoites-induced erythrocyte haemolysis could account for the observed elevation in LDH and ACP/ACP isoenzymes’ activities during malaria infection. These results indicate the potential of using the changes in serum LDH and ACP activities as biomarkers of malaria infection in humans, especially in the absence of other complicating diseases known to be associated with an elevated serum LDH and ACP activities apart from the promise it holds as a key to providing further insights into the mechanisms of the pathogenesis of malaria infection. Email address for correspondence: ihgarba2002@yahoo.com

675 Impact of artemisinin-based combination therapy intermittent preventive treatment on malaria linked morbidity in elementary school students in Mali [MIM16694540]
Hamm a Maiga, Breanna Barger, Oumar Bila Traore, Mamadou Tekete, Antimbe Timbine, Antoine Dara, Zoumana Isaac Traore, Soren Gantt, Ogobara Doumbo, Abdoulaye Djimde

Among school-aged children, malaria causes symptomatic illness and anemia, resulting in absenteeism and impaired cognitive development. Intermittent preventive treatment (IPT) is effective for children under 5 years, but few studies have included school-aged children. We conducted an open randomized controlled trial of seasonal IPT among students from 6 to 13 years of age in Kolélé, Mali. The study began in September 2007 and completed follow-up in May 2008. Students were randomized to one of three study arms: sulfadoxine-pyrimethamine plus artesunate (SP/AS), amodiaquine plus artesunate (AQ/AS), or vitamin C. All students received two full treatment doses, given 2 months apart during the season of high transmission from September to December. Groups were compared with respect to incidence of febrile malaria, asymptomatic parasitemia, and blood hemoglobin concentration. 296 students were randomized, and retention in the study was 99.3%. Febrile malaria incidence in the SP/AS and AQ/AS arms was reduced by 66.6% and 46.5%, respectively, versus vitamin C (p < 0.001). There were fewer acute clinic visits for any cause among the children receiving SP/AS or AQ/AS (p = 0.024). The prevalence of asymptomatic parasitemia was >5-fold higher in the vitamin C arm than either SP/AS or AQ/AS at each post-treatment evaluation (p < 0.001). At the end of the transmission period, children treated with IPT had lower rates of anemia (SP/AS 17.7%, AQ/AS 16.0%, vitamin C 29.6%; p = 0.039). IPT reduced the rates of febrile malaria, all-cause acute clinic visits, asymptomatic parasitemia, and anemia among school-aged children. Email address for correspondence: hmaiga@mrtcblko.org
676 Randomized study comparing artesunate plus amodiaquine to artemether plus lumefantrine in repeated treatment of Plasmodium falciparum uncomplicated malaria attacks occurring in Senegalese cohort during 2 years [MIM16700256]


The use of artemisinin combination therapy (ACT) is currently recommended for treating uncomplicated Plasmodium falciparum malaria. We carried out a study that aimed to validate the efficacy and safety of fixed-dose combinations of reiterated administration of artesunate + amodiaquine (ASAQ) in comparison with a fixed-dose combination of artemether–lumefantrine (AL) in consecutive episodes of P. falciparum malaria. A randomized, investigator-blinded, open comparative study was conducted in a rural community in center of Senegal from August 2007 to January 2009. Patients suffering from uncomplicated P. falciparum malaria were randomized to receive orally ASAQ once daily (ASAQ), or artemether–lumefantrine twice daily (AL) for 3 days. Drug doses were determined according to body weight. Treatment for first episodes were supervised and unsupervised for consecutive ones. The primary outcome was adequate parasitological and clinical response rate after PCR correction on Day 28 for the first episode. Audiometric and cardiac’s effects of the two treatments were measured for all patients aged equal and over 12 years. After 2 malaria transmission seasons 840 patients were screened, 365 patients were enrolled in the two groups and from them 60 presented second and third episodes during the follow up. We noticed two severe adverse events considered by investigators not treatment related. Analysis is on-going and all results will be presented at the conference.

Email address for correspondence: jlndiaye@yahoo.com

677 Evaluation of different techniques including Partec Cyscope® for the detection of Plasmodium spp. in human blood [MIM16759220]

Bernard Nkrumah, Alex Agyekum, Samuel E.K. Acquah,Yaw Adu-Sarkodie, Frank Huenger

Correct initiation of malaria treatment largely depends on good laboratory-confirmed diagnosis. In many endemic countries, treatment decision is often made only on clinical diagnosis as laboratory techniques to confirm the clinical suspicion are labour-intensive and costly. The screening of blood slides by microscopy is still considered to be the gold standard. A variety of new diagnostic tests have been developed for the rapid diagnosis of malaria. Aim: The aim of this study is to evaluate the recently developed Partec Cyscope® for the detection of Plasmodium spp. in human blood from patients in an endemic area and compare the results with other techniques such as giemsa stained blood smears and PCR in terms of sensitivity and specificity. 486 samples from patients attending the under five clinic and out patient department of the Agogo Presbyterian Hospital in the Asante Akim North District were examined with three different tests independently. The test methods employed in the study were Giemsa stain which was taken as the gold standard, Partec Cyscope® unspecific fluorescent stain (DAPI) which detects intraerythrocytic plasmodial DNA and real time PCR developed by the BNITM, Hamburg, Germany. Out of 486 patients, 181 (37.2%), 183 (37.7%) and 297 (61.1%) of the total population were positive for giemsa, cyscope and PCR respectively whilst 305 (62.8%), 303 (62.3%) and 189 (38.9%) were negative for giemsa, cyscope and PCR respectively. Compared to Giemsa stain the sensitivity of cyscope test was 93.4% whilst the specificity was 96.7%. 121 (24.9%) and 127(26.1%) of the total population were positive for PCR but not giemsa and cyscope respectively. Giemsa stain was used as the gold standard for the study. The cyscope method uses unspecific fluorescent stain which detects any intracellular DNA that may be present in erythrocytes and thus artifacts might be misinterpreted as plasmodial DNA. This method can be used as an alternative method when giemsa staining is not possible. The advantages of this test are that results can be obtained quicker and less labour intensive than with the giemsa stain. Disadvantages are that the method to some extend is prone to produce false positive results and lacks discrimination of parasite species. PCR had more positive results due to its high sensitivity. However were necessary, giemsa stain can be used as a confirmatory test and for species identification.

Email address for correspondence: nkrumah@kccr.de

678 Improving rational treatment of malaria: perceptions and influence of RDTs on behaviour of health workers in southeast Nigeria [MIM16674107]

B.S.C. Uzochukwu, O.E. Onwujekwe, K.E. Nwala, N. Ezuma, E.N. Obikeze, N. Ugurou, O. Chukwugo

Developments in rapid diagnostic tests (RDTs) have opened new possibilities for improved remote malaria diagnosis that is independent of microscopic diagnosis. However, the health workers’ perceptions of RDTs and its potential influence on their malaria treatment practices are unknown. The study was in rural and urban health centers in Enugu east local government of Enugu state, Nigeria. All the 32 health workers in the health centers where RDTs were deployed were interviewed by the field workers. Information was sort on their perception of symptoms based, RDT-based and microscopy-based malaria diagnosis. In addition prescription analysis of 200 prescriptions was carried out 6 months after the deployment. Majority of the health workers were of the opinion that RDT is more effective for malaria diagnosis than microscopy and clinical diagnosis. They also felt that the benefits of RDT included increased use of RDTs in the facilities, tendency to prescribe more ACT and less of chloroquine. It also led to fast diagnosis. However, some of the health workers experienced some difficulties in the process of using the kits. Their prescriptions were irrational in the pre RDT era and more rational 6 months after RDT deployment. RDTs-supported malaria diagnosis reduces over-prescription of antimalarial drugs that are not first-line drugs and improved appropriate use of ACTs. More rational anti-malaria drug use, in turn, could be expected to reduce the rate of increase in resistance to these drugs. In addition, more accurate diagnosis could result in more timely treatment of non-malarial illnesses.

Email address for correspondence: kelestonkcdonald@yahoo.com

679 Acceptability and willingness to pay for intermittent preventive treatment (IPT) of malaria amongst pregnant women [MIM16650082]

C.I. Okoli, E.N. Shu, O.E. Onwujekwe, N. Dike, N. Ezuma, P.O. Okonkwo

In Nigeria, malaria is mainly the cause of hospital attendance in all age groups and 48.2% of the ailments experienced by pregnant women have been classified as malaria. Currently, intermittent preventive treatment during pregnancy is practiced by most providers. This study therefore aims to investigate the acceptability and willingness to pay for IPT of malaria among pregnant women in the rural community of Senegal from August 2007 to January 2009. Patients suffering from uncomplicated P. falciparum malaria were randomized to receive orally ASAQ once daily (ASAQ), or artemether–lumefantrine twice daily (AL) for 3 days. Drug doses were determined according to body weight. Treatment for first episodes were supervised and unsupervised for consecutive ones. The primary outcome was adequate parasitological and clinical response rate after PCR correction on Day 28 for the first episode. Audiometric and cardiac’s effects of the two treatments were measured for all patients aged equal and over 12 years. After 2 malaria transmission seasons 840 patients were screened, 365 patients were enrolled in the two groups and from them 60 presented second and third episodes during the follow up. We noticed two severe adverse events considered by investigators not treatment related. Analysis is on-going and all results will be presented at the conference.

Email address for correspondence: jlndiaye@yahoo.com
Southeast Nigeria. The study was conducted in two purposively selected urban and two randomly selected rural areas in Enugu State. Four focus group discussions were held with groups of women and pre-tested interviewer administered questionnaires were administered to 600 (urban) and 600 (rural) women of child bearing age. The knowledge of IPT for malaria in pregnancy amongst urban and rural dwellers was 48% and 40%, respectively. FGD results revealed that there is no consensus whether IPT would be acceptable to many women due to cost of drugs and religious beliefs. Also, result shows that 88.8% and 79.4% of respondents in urban and rural communities were willing to pay for IPT for themselves. WTP across the SES quartiles showed that highest SES quartile (Q4) in both urban and rural communities were willing to pay for IPT for themselves than lowest SES quartile (Q1). There is poor knowledge of IPT in urban and rural communities. WTP is dependent on the SES of the respondents. There is need for sensitization, drug subsidy, increased availability and accessibility of publicly owned healthcare services for the provision of near and appropriate treatment of malaria.

Email address for correspondence: okolichji Coke@yahoo.com

680
Control of malaria among people living with HIV/AIDS in Calabar, Nigeria [MIM16673378]
P.E. Samson-Akpan, RN, MPH; O.B. Edet, RN, MPH, E. Asuquo, BN, SC, M.A. Mgbelem, BSc, MSc., I. Ojong, RN, MSc., I. Akpabio Ph.D.

Roll Back Malaria (RBM) is one of the strategies aimed at reducing mortality due to malaria by half in 2010. People living with HIV/AIDS (PLWA) are at higher risk. The study aimed at examining the RBM programme's efforts at controlling malaria among PLWA and their perception of the programme's control strategies. The study was a descriptive survey involving guided interviews of top managers of RBM programme and structured questionnaire administered to 82 PLWA attending an HIV/AIDS clinic in a Secondary Health Facility in Calabar between 24th November and 5th December 2008. Data were analyzed using descriptive statistics. Thematic analysis revealed that RBM programme strategies include effective case management; promotion of long acting insecticide treated nets (ITN), intermittent preventive treatment (IPT) and integrated vector management (IVM). Complementary results showed that 50 (61%) accepted accessibility of malarial treatment. Although 58 (70.7%) of PLWA have ITN, only 50 (61%) use them. Majority of the respondents (70 (85.4%) have not heard of indoor /outdoor residual spraying (IRS) while inaccessibility of IRS was listed by 72 (76%) of the respondents as a barrier to its use. The barriers to using ITN as affirmed by the respondents were lack of funds 28 (34.2%) and exposure to strong odors 50 (61%). Malarial treatment was moderately accessible to PLWA. The barriers to the use of ITN and IRS could be addressed through free distribution of odorless ITN and IRS to PLWA. Higher rates of utilization of the products can be achieved through behavioral change communication.

Email address for correspondence: patedoho2005@yahoo.com

681
A study of intermittent preventive treatment and home based management of malaria in a rural area of the Gambia [MIM16199001]
Sanie Sesay

Malaria remains an important cause of mortality and morbidity among young children. The global malaria control strategies include prompt treatment with an effective antimalarial drug, vector control using ITNs or curtains or indoor residual spraying (IRS) and intermittent preventive treatment (IPT). However, individually these interventions provide only imperfect protection. Thus, there is a need to investigate whether additional control measures provide added benefit in reducing mortality and morbidity. Thus, during the 2008 malaria transmission season, xxx children under 5 years of age were randomly allocated to receive IPT or placebo from village health workers (VHWs) based in primary health care villages. Treatment with a single dose of sulfadoxine/pyrimethamine plus three doses of amodiaquine or placebo was given to all study subjects at monthly intervals on three occasions during the peak malaria transmission season (September, October, and November). In addition, VHWs were trained to administer treatment with Coartem to children if they develop symptoms compatible with malaria during the malaria transmission season. The primary end point was incidence of clinical attacks of malaria detected by passive case detection during the study. Results of the trial will be presented. Will follow results.

Email address for correspondence: ssesay@mrc.gm

682
Evaluation of a hrp 2 rapid diagnostic test of malaria in paediatric hospital in Dakar, Senegal [MIM16689945]

To ensure a rational use of ACT, accurate diagnosis should precede malaria treatment in Africa. HRP2 rapid diagnostic test is one of RDT which can be implemented at all levels. The HRP2 antigen is a hydro soluble protein and can pass in the spinal fluid. This study aimed to test the sensitivity and the specificity of these tests in paediatric hospital areas in peripheral blood and spinal fluid. An exploratory study was conducted into Albert Royer paediatric hospital in Dakar from November 2006 to May 2007. All the patients less than 15 years presenting symptoms of malaria were included. A thick film and commercial RDT Paracheck®PF was performed and for patients with neurological symptoms RDT was performed on spinal fluid. On the 1223 screened patients, 137 were found positive by paracheck and 136 by the thick film. The sensitivity and specificity are respectively, 98.5% and 99.7%. The positive and negative predictive values were respectively, 97.8% and 99.8%. Antigen HRP2 was never detected in the spinal fluid on the 107 patients with clinical severe cases. The paracheck applied to blood in the children is thus very sensitive and specific to Plasmodium falciparum. Antigen HRP2 not found in the spinal fluid is probably due to its molecular weight or the paracheck®PF unable to detect it in the spinal fluid. The paracheck®PF constitutes a useful tool for malaria diagnosis but microscopic examination remain the gold standard.

Email address for correspondence: doudsow@yahoo.fr

683
Etude comparée de l’efficacité et de la tolérance de la combinaison Artésunate-Amodiaquine (Camoquin plus®) versus Artheméter-Luméfantrine (Coartem®) dans la prise en charge du paludisme non compliqué à Plasmodium falciparum: essai multicentrique Sénégal [MIM16689534]

La prise en charge actuelle du paludisme simple fait appel aux combinaisons thérapeutiques à base de dérivés d’artémisinine. Nous avons réalisé un essai clinique phase IV comparant l’efficacité, la tolérance de la combinaison Artésunate-Amodiaquine (Camoquin plus® au Coartem® (Arthémether-Luméfantrine) dans le traitement...
du paludisme simple à Plasmodium falciparum en zone de transmission modérée (Sénégal) et en zone de transmission forte (Côte d’Ivoire). Il s’agit d’un essai multicentrique, randomisé, ouvert, chez des patients âgés de plus de 7 ans au Sénégal et en Côte d’Ivoire utilisant le protocole OMS 2005. Le critère de jugement principal de l’efficacité était la réponse clinique et parasitologique après 28 jours de suivi. La tolérance des médicaments était évaluée par l’incidence des événements indésirables cliniques et biologiques. En ITT, la RCPA à J28 après correction par PCR était de 96,9% pour le groupe Camoquin plus® versus 97,5% pour le groupe Coartem® (p = 0,97). En PP, la RCPA à J28 après correction par PCR était 100% pour le groupe Camoquin plus® versus 98,1% pour le groupe Coartem® (p = 0,25). Aucun événement indésirable grave n’a été observé. Certains événements indésirables mineurs (gastralgies, nausées/vomissements, vertiges) étaient significativement plus fréquents dans le groupe Camoquin plus®. L’étude des paramètres biocliniques n’a pas montré de variations statistiques significatives. Cette étude a permis de montrer la bonne efficacité et la bonne tolérance de la combinaison Artésunate-Amodiaquine (Camoquin plus®) comparativement au Coartem® dans le traitement du paludisme simple.

Email address for correspondence: khadimesylla@yahoo.fr

684 Socio-economic and geographic differences in consumers’ preferences for improved provision of malaria treatment services in southeast Nigeria [MIM16645490]

Nnenna Tasiie, Nkoli Uguru, Obinna Onwujekwe, Benjamin Uzochukwu

To explore the socio-economic and geographic differences in consumers’ preferences for improved treatment of malaria in Southeast Nigeria. This study was undertaken in Anambra state, South-east Nigeria in three rural and three urban areas. A total of 2250 randomly selected householders were interviewed using a pre-tested interviewer administered questionnaire. A socio-economic status index was used to examine for socio-economic differences, whilst urban–rural comparison of key variable was used to examine for geographic differences in key variables. It was found that 30.5% of the respondents stated that improved quality of services in public hospitals was the best strategy for improving treatment of malaria. Training of mothers and improved PHC centres were next highly preferred strategy for improving treatment of malaria. Traditional healers were the least preferred strategy for improving malaria treatment (4.8%). The least poor SES group preferred public hospitals (37%) compared to the most poor (31.9%). The rural dwellers preferred herbalists (6.3%) more than urbanites (3.5%). Consumers mostly preferred that improved malaria treatment services should be through the Public Health System. Hence the government should increase availability of public health facilities so as to increase access to appropriate malaria treatment services.

Email address for correspondence: tasienina@yahoo.com

685 Malaria treatment perceptions and practices of hospitals and other providers in southeast Nigeria: Identifying loci for interventions to improve malaria treatment provision [MIM16645525]

Nkoli Uguru, Obinna Onwujekwe, Benjamin Uzochukwu, Nkem Dike, Emmanuel Nwobi, Elvis Shu

People seek treatment for malaria from various sources. There are a lot of problems with existing practices of treatment provision in hospitals and among other providers. This paper examines the knowledge, pattern of treatment provision and factors influencing the behaviour of hospitals and other healthcare providers in the treatment of malaria. A pre-tested structured questionnaire administered to the heads of selected public and private providers, was used to collect data from 225 providers about their malaria knowledge, treatment practices and factors that influence their provision and treatment in southeast Nigeria. Data from hospitals and other providers were compared to determine if there were systematic differences between them. All hospitals and 87.4% of other providers stated that malaria was a serious health problem (p < 0.05). Malaria diagnosis was based mostly on recognition of symptoms by 82.4% of hospitals and 87.4% of other providers. 73.5% of hospitals and 34.5% of other providers used microscopy to diagnose malaria (p < 0.05). Ability to pay bills (35.2%), already existing relationship (9.4%) and body mechanism (35.2%) of the patient was considered before malaria treatment was provided. Requests by patients, the patients SES, their idea of what the patients want and drugs available were the major determinants of the amount charged for treatment. Ensuring adequate supply of appropriate drugs, proper diagnosis of malaria and improving the knowledge of providers about malaria are interventions that could be used to improve malaria treatment provision.

Email address for correspondence: nkuguru@gmail.com

686 Hypothetical and actual consumer choices for malaria treatment services: Implications for improving appropriate use of malaria treatment services [MIM16671237]

Ugenyi Unimna, Ogoamaka Chukwugo, Uche Ezeoke, Benjamin Uzochukwu, Obinna Onwujekwe

Improving appropriate use of malaria treatment services in Nigeria is dependent mainly on identifying and modifying factors which create a discrepancy between the hypothetical and actual consumer choices in malaria treatment services utilized. Pre-tested interviewer administered structured questionnaires were administered to 450 households respectively in 2 communities (1 urban, 1 rural) in Anambra State, South-East Nigeria. Data was collected on socio-demographics, consumers’ preference of malaria treatment services, determinants of choice of treatment and price expectations. Private and public hospitals were found to be most preferred malaria treatment service providers for both rural (31.6% and 44.6%, respectively) and urban (25.7% and 24.7%, respectively) dwellers with traditional healers least favoured (5.3% rural and 3.5% urban). However, when respondents actually had malaria, treatment was sought mainly at patent medicine dealers/vendors (41.3% rural and 55.3% urban). Price expectations were higher in the rural community for malaria treatment services. A definite discrepancy between consumers’ stated and actual choices was found. Patent medicine dealers enjoyed patronage of greater number of respondents seeking treatment for malaria, whilst they stated that they would have preferred to have used hospitals. Formal training of patent medicine dealers on rational prescribing and dispensing of drugs is recommended, as well as instituting intervention to increase actual use of hospitals and other formal health facilities.

Email address for correspondence: ugenyi_u@yahoo.co.uk
687 First line therapies of uncomplicated falciparum malaria in Burkina Faso: Efficacy and tolerance [MIM16689555]

Issaka Zongo, Noel Rouamba, Fabrice Somé, Jean Eric Ouédraogo, Jean Bosco Ouédraogo

Burkina Faso switched to Arthemeter–Lumefantrine and Amodiaquine–Artesunate as first line therapy of uncomplicated falciparum malaria in 2005. We evaluated the efficacy and tolerance of these ACT in Bobo-Dioulasso. We enrolled 342 patients 6 months or older with uncomplicated falciparum malaria in Bobo-Dioulasso, Burkina Faso. Patients were randomly assigned to receive standard doses of either Arthemeter–Lumefantrine (175 mg) or Amodiaquine–Artesunate (167 mg) over 3 days and followed up for 28 days. The primary endpoint was the risk of treatment failure unadjusted or adjusted by genotyping to distinguish recrudescence and new infections. The compliance rate was 96.8% (331/342). We analyzed data using intention to treat and Per Protocol method and went to the same results. No treatment failure occurred up to 14 days of follow up. From that day, the risk of treatment failure was higher in Arthemeter–Lumefantrine group (29.2% versus 19.1%, risk difference 10.1%, p < 0.001). The genotyping work is ongoing. All regimens were well tolerated. Three years after the new policy adoption, there are concerns about the decrease level of these ACTs efficacy. More studies need to be done countrywide to allow a better capture of the drug’s efficacy and to give alert to the National Malaria Control Program. The evaluation of the impact of strategies (Home based managements, bed nets, IPT) is urgently required for malaria effective control.

Email address for correspondence: zongo_issaka@yahoo.fr

688 Mothers’ use and perceptions of artemisinin-based combination therapy for treating malaria among under-five children in Ibarapa central local government area, Nigeria [MIM15192795]

Osuolale Adekunle, King Odor

The adoption and promotion of Artemisinin-based Combination Therapy (ACT) in Nigeria are influenced by the increasing prevalence of chloroquine resistant malaria. However, little is known about the adoption and perceptions of nursing mothers regarding ACT. The study therefore assessed the perceptions and pattern of use of ACT among mothers of under-five children in Ibarapa Central Local Government Area, Oyo State. The study was a cross-sectional survey involving the use of a 5-stage random sampling technique to select 720 participants from households. A validated questionnaire with a 6-point knowledge scale was used for data collection. Descriptive and Chi-square statistics were used to analyze the data using Epidemiology Package Information software. The participants’ mean age was 29 ± 5.3 years. Their levels of education were as follows: no formal education (26.0%), primary (50.7%), secondary (18.2%) and higher institution (4.9%). Thirty percent (30%) of participants had ever heard of ACT and their main sources of information include health facility (69.0%), physician (11.0%), nurses (11.0%) and pharmacy (4.0%). Participants mean knowledge score relating to ACT related drugs was 1.2 ± 2.0. Out of the maximum of 6 points, the mean scores based on their level of education were as follows: no formal education (0.8 ± 1.7), primary (1.2 ± 2.0), secondary (1.5 ± 2.2) and polytechnic (1.5 ± 2.2), p < 0.05. Twenty-seven percent of participants had ever used ACT drugs, while 10.0% are current users of coartem which is the most popularly used (24.2%) among the ACT drugs. The levels of education of the current users were as follows: no formal education (17.1%), primary (49.7%), secondary (22.5%), higher institutions (10.7%), p < 0.05. Majority (90.6%) obtained the drugs from government hospitals where they are distributed free to under-five children. These participants were of the opinion that the ACT drugs are not expensive. Only 27.0% of the participants were of the view that ACT was more effective than chloroquine while 80% of the current users share the same opinion. Fifty-nine percent (59%) of the current users which represents 18.0% of the total participants stated that the drugs were readily available, while 78.0% of the current users could correctly state how they are used for treating under-five children. Seventy-five percent of the current users are of the opinion that ACT drugs have lesser side effects compared to chloroquine. Nonetheless, chloroquine was still the first-line drug of choice for treating children with uncomplicated malaria among majority (59.0%) of the current users of coartem in the home management of malaria. The reasons adduced for this included the following: ready availability (30.2%), they are commonly prescribed by doctors and other health workers (27.8%), chloroquine taste suits my children (17.0%) and chloroquine is very cheap (12.4%). Despite the positive attitude of the population that are aware of ACT and its effectiveness, the awareness and accessibility as well as its use for the management of malaria in under-five children are still low among nursing mothers. Advocacy, social marketing and subsidization of ACT drugs especially in the private sector are needed to address the problem.

Email address for correspondence: odorking_cv@yahoo.com

689 Natural selection maintains a stable polymorphism at the circumsporozoite protein locus of Plasmodium falciparum in a low endemic area [MIM16665456]

Chaturong Putaporntip, Somchai Jongwutiwes, Austin L. Hughes

The circumsporozoite protein of Plasmodium falciparum (PfCSP) has been studied intensively as a vaccine candidate. The gene encoding PICSP can be divided into three distinct regions: a 5′ non-repeat region (5′NR), a central region encoding >40 repeats of the four amino acid motif NANP or a close variant, and a 3′ non-repeat region (3′NR). Single nucleotide polymorphisms, most of which are non-synonymous, are clustered in the 3′NR while the repeat regions differ among allelic sequences with respect to both the number of repeats and their nucleotide composition. The complete circumsporozoite protein gene sequences of P. falciparum were determined from clinical isolates collected a decade apart from a single endemic area in Thailand Estimation of a coefficient of identity between repeat arrays has shown that the repeat units were more similar within the same haplotype than between haplotypes, supporting the hypothesis that repeats arrays evolve by a process of concerted evolution. There was evidence that natural selection has favored amino acid changes in the Th2R and Th3R T-cell epitope regions. One haplotype in these epitopes, designated *5/*1 by at least two amino acid replacements; and divergence in the epitopes was correlated with divergence in the repeats. These patterns are most consistent with balancing selection driven by interactions with the immune system of the vertebrate host, probably involving both T-cell recognition of the Th2R and Th3R epitopes and antibody responses to the repeats.

Email address for correspondence: p.chaturong@gmail.com
690 Immunisation of mice with MSA2 on Lactococcus lactis cell walls [MIM15849021]

Sivagovry A.V. Moorthy, Ranjan Ramasamy

Lactic acid bacteria are safe vectors for oral and perhaps other mucosal vaccinations. We report on the immunisation of different mouse strains with the Plasmodium falciparum merozoite surface antigen (MSA2), a putative vaccine candidate. PMSA2 was covalently attached to the cell walls of live Lactococcus lactis (MSA2cP) and non-covalently attached to L. lactis cell wall ghosts (MSA2cA), and then used to orally and intranasally immunise ICR, Balb/c, C57 Black and C3H/Hef1 mice. Serum IgG antibodies to MSA2 were elicited in a strain and immunogen-dependent manner. Balb/c and C3H mice responded better to MSA2cP and MSA2cA on L. lactis, respectively. The isotypes of the antibodies in different mouse strains reflected the differential influence of TH1 and TH2 cells. The IgG response to native MSA2 on the surface of P. falciparum merozoites was best in outbred ICR mice. Serum and faecal IgA anti-MSA2 antibodies were also detected as well as serum anti-lactococcal antibodies. Antigen specific IFN-γ producing T cells were detectable in spleens of all inbred mouse strains immunised with MSA2cA and in C57 mice immunised with MSA2cP. The results suggest that mucosal immunisation with pathogen molecules on lactic acid bacterial surfaces merits more detailed investigation as a potentially widely applicable vaccination method.

Email address for correspondence: gowry@nsf.ac.lk

691 Age variation in antibody responses and histopathological changes in mice immunized with Lactococcus lactis expressing a malaria parasite protein [MIM16258757]

S.G. Yasawardene, R. Ramasamy

Gram positive food grade bacteria like Lactococci have significant advantages over attenuated pathogens as vaccine delivery vehicles because of their inherent safety. Plasmodium falciparum merozoite surface antigen 2 (PfMSA2) was expressed in recombinant Lactococcus lactis in a form that was partially covalently anchored to the peptidoglycan of the cell wall (MSA2cP). Recombinant L. lactis strain was delivered oro-nasally for mucosal immunisation to Balb/c mice of ages 1 week (neonates), 6 weeks (young adults) and more than 25 weeks (old adults). Non-recombinant L. lactis was used as control. The serum antibody response was investigated by ELISA using recombinant MSA2 as antigen and by immunofluorescence assay. Histopathological changes in gut associated lymphoid tissue were investigated. Serum IgG anti-MSA2 responses were significantly higher in young adult Balb/c mice, after oral and nasal delivery, compared to old mice and neonates. Antibodies elicited in young mice reacted with native MSA2 in the surface of P. falciparum merozoites in an immunofluorescence assay. Enlargement of mesenteric lymph nodes and increased lymphatic infiltration of the lamina propria were noted in both recombinant and non-recombinant L. lactis immunized mice. The gastro-intestinal tract was otherwise normal in oronasally immunised mice. The spleen showed periarterior lymphoid aggregation in immunized mice. Recombinant L. lactis is a suitably safe vector for subunit vaccines. The antibody responses to recombinant L. lactis were markedly weaker in extremes of age. The histological changes of spleens of older mice support weak antibody response seen. These findings are relevant for further developing L. lactis to deliver vaccines mucosally for use in humans of different ages.

Email address for correspondence: surangiy@hotmail.com

692 Cytokine response to Plasmodium falciparum multistage antigen (MB2) in adults living in areas of varying malaria transmission in Western Kenya [MIM16675602]

Lyticia V. Ochola, Gideon M. Ng’wena, Ayub V. Ofulla, Gregory S. Noland, John M. Vulule, Chandy C. John

Malaria immunity in areas of stable, perennial malaria transmission differs considerably from that in epidemic-prone areas with unstable transmission. Plasmodium falciparum MB2 antigen is a novel protein expressed in various stages of the parasite’s life cycle and could be exploited as a vaccine candidate. We evaluated the interferon gamma (IFN-γ) and interleukin-10 (IL-10) responses in PBMC culture supernatants to MB2 by ELISA and ELISPOT in 177 adults residing in either Kipsamoite, a highland site in Western Kenya with unstable malaria transmission, or Kanyawegi, a lowland site with stable malaria transmission frequencies of individuals with a positive IFN-γ response to MB2 by ELISA were similar in the unstable-transmission (27.5%) and stable-transmission areas (18.8%; P = 0.33). Mean levels of IFN-γ were also similar between unstable 17.57 pg/ml (range 0–1064.22 pg/ml) and stable 113.91 pg/ml (range 0–2896 pg/ml) transmission areas (P = 0.26). Likewise, MB2-specific IL-10 frequencies (22.5% vs. 25%; P = 0.78) and mean levels (39.56 pg/ml [range 0–353.4 pg/ml] vs. 65.31 pg/ml [range 0–506.9 pg/ml]; P = 0.55) were similar between unstable and stable transmission areas. Frequencies of IFN-γ−secretory cells measured by ELISPOT were similar in both areas (12.5% and 12.3%, respectively, P = 0.97). These results indicate that MB2 induces significant antigen specific cytokine responses that do not differ with malaria transmission intensity. Further studies are required to investigate whether IFN-γ or IL-10 responses to MB2 correlate with protection from infection or disease.

Email address for correspondence: lytie4ever@yahoo.com

693 Immunological characterization of Plasmodium falciparum using GLURP [MIM14690679]

Munsoor Munsoor

A total of 570 samples of both sexes were collected from malaria-suspected patients who attended Medani Pediatric and adult teaching Hospital (Gazira State, Central Sudan) during August–December 2005. That period marked the peak of malaria transmission season in Gazira region. Out of these, 155 samples were detected to be positive for malaria and 140 cases (90%) out of the positive samples were due to Plasmodium falciparum and the rest, which were excluded from the study, (15 patients) were due to other plasmodium species. The main goal of this work was the investigation of the immune response of glutamate rich protein among the study population as an indicator for acquisition of protective immunity. This is achieved by estimation of IgG, IgG1, IgG3 and IgM antibodies directed against GLURP-R0, GLURP-R1 and GLURP-R2. The results indicate comparatively high titres of these antibodies to the three fragments of GLURP, although R2 and R0 encountered with significantly higher concentrations of the antibodies. The results also showed that IgG3 was presented with high concentration followed by IgG1 suggesting their protective role and their major participation in acquisition of immunity to malaria. The results of association between immune response and different strain of Plasmodium population isolated in this work reveal that some strain are encountered with high immune response and severity of malaria. This association supports the speculation of the presence of strain-specific immunity and pre-munition among inhabitants of malaria endemic areas. The results
also re-enforce the ongoing announcement that any future malaria vaccine should include GLURP fragments notably GLURP-R2 and GLURP-R0.

Email address for correspondence: mun.2000@yahoo.com

694 Vaccination with Plasmodium knowlesi apical membrane antigen 1 induces high levels of functional antibodies and protects against blood-stage challenge in rhesus macaques [MIM14905392]

Muzamil M.A. Mahdi, Edmond J. Remarque, Leonie M. van Duivenvoorde, Clemens H.M. Kocken, Bart W. Faber, Alan W. Thomas

*Plasmodium falciparum* apical membrane antigen 1 (*PfAMA1*) is a leading blood stage vaccine candidate. Phase 1b/2a clinical trials to evaluate product safety and immunogenicity are currently ongoing. We have tested the efficacy of the *P. falciparum* AMA1 orthologue, *Plasmodium knowlesi* AMA1 (*PkAMA1*), as a vaccine in a rhesus macaque immunization-challenge model. The protein was produced using the *Pichia pastoris* expression system, similar to the *PkAMA1* product now under clinical evaluation. Six healthy rhesus monkeys were vaccinated three times with 50 (g *PkAMA1* formulated in novel water in oil adjuvant containing immune stimulant with 4 weeks intervals. A blood stage challenge was subsequently performed 2 weeks after the last immunization with 10^4 *P. knowlesi* parasites. All vaccinated monkeys had a delay of greater than 2 days in the onset of the parasitaemia and slower parasitaemia development, correlating with ELISA titers and parasite growth inhibitory levels, compared to the control group comprising *PfAMA1* vaccinated rhesus monkeys. One rhesus controlled the parasitaemia, while the other five had to be cured with chloroquine. The self-cured animal had the highest functional antibody titer and a high *PkAMA1* dependent IFNγ production. After 225 days (4½ months), all animals were re-boosted with either *PkAMA1* or *PfAMA1* and re-challenged again 2 weeks later. Now, four of the six rhesus monkeys were able to fully control the parasitaemia compared to the ones in the control group. This study shows that correlates exist between high inhibitory levels and the day of onset, and that high efficacy of the vaccine can be achieved in the second challenge upon re-boosting.

Email address for correspondence: mz.mahdi@yahoo.com

695 Determination of natural antibodies to merozoite surface protein-142 (MSP-142) in children from an holoendemic malaria transmission area of Western Kenya [MIM16728569]

Clifford Obuya, Willis Okoth, Joram Siangla, Ismail Mahart Bashir, Bernard Ogutu, John Waitumbing, Ann Stewart

MSP-142 is an asexual blood stage falciparum malaria vaccine candidate. More than one allele of recombinant MSP-142 has been expressed in *Escherichia coli*. MSP-142 is highly immunogenic and antibodies to MSP-1 have been shown to block parasite invasion of erythrocytes in vitro. To prepare for an investigative vaccine trial using this antigen, we carried out a longitudinal survey of antibodies to MSP-142 among children from the malaria holoendemic Lake Victoria basin of western Kenya. Sixty participants who completed all their scheduled visits out of 300 total, were selected for detailed longitudinal evaluation of anti-MSP-142 antibody levels using the Enzyme linked Immuno–Sorbent Assay (ELISA). The assay endpoint was defined as the calculated serum dilution yielding an optical density (OD) of 1.0, and results were quantified by comparison to a standard curve and expressed in (g/mL. Our data indicate that titers are quite variable in this population, both between volunteers and between time points in any given volunteer.

Email address for correspondence: cobuya@wrp-ksm.org

696 Molecular analysis of *P. falciparum* MSP-119 haplotypes in children participating in MSP-142 vaccine trials in Kombewa division, Western Kenya [MIM16704466]

Amos Mbugua

Phase I and II clinical trials were conducted in Western Kenya between 2003 and 2006 to test the safety, immunogenicity and efficacy of a Merozoite surface protein 142 (MSP-142) vaccine comprising both the MSP-133 and MSP-119 segments. Molecular evaluation of malaria vaccines is essential for the determination of vaccine efficacy. Our objectives were: (a) to validate a Real Time quantitative PCR (RT-qPCR) method for discriminating MSP-119 alleles by comparison with direct sequencing; (b) to compare MSP-119 haplotype diversity in the vaccine and control groups 1 month following vaccination (parasite-positive children) and in the subset of children with clinical malaria following vaccination. Eight different MSP-119 haplotypes were identified from the sequence data (EKNG, EKSG, EKSR, ETNG, ETSG, QKNG, QKSG and QKSR) and by RT-qPCR (EKNG, EKSR, ETNG, ETSG, QKNG, QKSG, QTNG and QTSR). EKNG (49%) and QKNG (40%) were the most prevalent haplotypes. There was no significant difference in haplotype prevalence between the two treatment groups among parasite-positive children at 1 month post-vaccination and among children with clinical malaria during the entire 6 months follow-up period. There was however a tendency toward a significantly shorter duration (P=0.08) in the time to first clinical episode among sick children where ETSR (vaccine haplotype) was the dominant inflicting haplotype. RT-qPCR was a suitable substitute for direct sequencing providing greater sensitivity and throughput. The indication of a vaccine haplotype-specific delay in the time to first clinical malaria episode would suggest the generation of strain specific immunity by this vaccine.

Email address for correspondence: akungu@gmail.com

697 Search for of molecular signatures of selection in a Kenyan population in newly described *P. falciparum* merozoite protein coding genes [MIM15496269]

L.I. Ochola, K.K.A. Tetteh, L.B. Stewart, K. Marsh, D.J. Conway

Several *Plasmodium falciparum* merozoite antigens have been shown to be under diversifying natural selection, and are targets of allele-specific immunity, signalling their possible role as vaccine candidates for either multiple allele or conserved domain formulation. The genetic data set on Plasmodium Genome Resource, PlasmoDB, was exploited to identify uncharacterised merozoite genes. As part of a search for additional *P. falciparum* merozoite proteins that are polymorphic and show such molecular signatures of selection, five different single locus genes were chosen for population genetic analysis in an endemic area in Kenya. Genes were first chosen based on maximum expression time (＞30 h in the asexual blood stage cycle) and polymorphism (>10 SNP/kb among parasites that have full or partial genome sequences). then an estimate of nucleotide diversity (θ) was calculated and the 5 genes with the highest (θ) values were sequenced from 90 isolates in an endemic Kenyan village. Sequence data from the population sample and an outgroup reference (*P. reichenowi*) allowed tests for departure from neutrality using Tajima’s D, Hudson-Kreitman-Aguade (HKA) and
Acquisition of antibodies to merozoite surface protein 3 among residents of Korogwe, North Eastern Tanzania [MIM165295480]
Method Segeja
Merozoite surface protein 3 (MSP3) is a polymorphic malaria parasite antigen that may have a role in Plasmodium falciparum parasite invasion of erythrocytes. It has been demonstrated that, MSP3 is a strong candidate for a vaccine against asexual blood stage parasites of P. falciparum. Currently, this vaccine is underdevelopment. The main objective of this study was to determine antibodies response to MSP3 associated protection. The specific objectives of this study were to determine the pattern of malaria in the study area and to assess natural acquisition of antibodies to MSP3. Information obtained from this study will provide useful data for planning malaria vaccine trials. Plasma samples collected from 487 individuals aged 0–19 years in five villages of Korogwe were analyzed using indirect enzyme linked immunosorbent assay (ELISA) to determine immunoglobulin M (IgM), total immunoglobulin G (total IgG), immunoglobulin subclasses 1 and 3 (IgG1 and 3) reactivity to MSP3. Results from this study indicated that, as altitude increased malaria indices diminished. Levels of antibodies increased as age increased. Levels of total IgG and IgM were observed to be high in the highland stratum whilst levels of IgG1 and IgG3 were observed to be high in the lowland stratum. MSP3 could be used as one of malaria vaccine candidates against P. falciparum malaria.

Immunoprotection potential of a 34-kDa recombinant fragment of the interspecies conserved antigen Pfp70 of Plasmodium falciparum [MIM16522283]
Peter C. Vuzzi, George W. Lubega, Mats Wahlgren
The 34-kDa recombinant (p34) is a fragment of the interspecies conserved antigen (Pfp70) of Plasmodium falciparum believed to play a role in parasite invasion and exit from cells. We tested the immunogenicity in rabbits and sero-reactivity with sera from malaria endemic area. We tested the inhibitory properties of rabbit antiserum from endemic area. We amplified, cloned and expressed a 34-kDa recombinant in E. coli. Tested immunogenicity of the recombinant in rabbits by determining titres of the antibodies produced. Determined in ELISA reactivity of p34 with endemic sera and profiles of IgG isotypes. Correlated levels of IgG isotypes to parasite density and to age of serum donors. Test the ability of rabbit anti-p34 antisera and human sera to inhibit parasite growth in growth inhibition assays. Recombinant elicited strong immune response (antibody titre = 106), was widely recognized by (>66%) of endemic sera. CTyphic isotypes predominate and levels significantly correlated with to age and parasite density ($r = 0.63$, $p < 0.0001$ and $r = 0.71$, $p < 0.0001$). Rabbit antisera inhibited growth by 50% and invasion (66%). Serum from endemic area inhibited invasion by 68%. Immunogenicity of p34 could be improved by use of novel adjuvants. Rabbit anti-p34 serum has anti-parasite activity. Antibodies to p34/Pfp70 are produced during natural malaria infection. Abundance of cytophilic isotypes correlated with PD and age, signifying role in controlling parasitemia. This study underlines the potential of p34/Pfp70 as candidate for malaria vaccine requiring further investigation.

Mapping epitopes in multi-domain PfEMP1s involved in severe malaria [MIM16645818]
Pernille Andersen, Anja Bengtsson, Louise Joergensen, Gorm Andersen, Thor G. Theander, Lars Hvid, Ole Lund, Anja T.R. Jensen
Previous results indicate that the PfEMP1 protein PFD1235w (VAR4) is involved in severe malaria pathogenesis, and often recognized by IgG from serum samples of African children and adults. The protein belongs to the group A var genes, and the adhesion receptor for VAR4 remains to be identified. VAR4 and similar proteins are comprised by several DBL and CIDR domains, however little is known about the structures of the individual domains and the overall structure of the protein. Mapping of surface-exposed epitopes in the overall protein can give more insight into regions of the protein structure, which could be targeted by protective antibodies and involved in adhesion. Structures of PFD1235w domains were modeled using the HHpred server. Rats and rabbits were immunized using protein expressed in E. coli and insects cells. The surface reactivity of animal and human antibodies was tested using flow cytometry analysis. Depletion and affinity purification was used to isolate surface reactive antibodies. Epitopes were further mapped by measuring binding to peptides and domains of the protein. The structure models of the VAR4 domains suggest that the DBL domains harbour long inserts predicted as surface-exposed variable loops. Epitopes of surface reactive sera could be mapped to different domains of the protein, and the mapped reactivity was often found in linker and loop regions of the models. Some epitopes seem to be comprised by several loops on the surface of the protein, which lead to the conclusion that a number of epitopes are conformational.

Development of a malaria vaccine based on the Merozoite Surface Protein (MSP)-1 from Plasmodium falciparum [MIM16653776]
The merozoite surface protein 1 (MSP-1) complex, a major component at the surface of the RBC-invading form of the parasite, plays an essential role in the parasite’s life cycle and is considered a prime malarial vaccine candidate. MSP-1 is synthesized as a ~190-kDa GPI-anchored precursor, which is proteolytically processed into four major fragments that remain non-covalently associated. This complex interacts with further parasite surface proteins such as MSP-6 and MSP-7. Recombinantly produced MSP-1 subunits, MSP-6 and MSP-7 were used for the structural and immunological analysis of the MSP-1 complex. In order to investigate MSP-1 as an experimental vaccine candidate a GMP-compatible process for the production of recombinant full-length MSP-1 antigen has been developed. Antibodies directed against each subunit of MSP-1 and in addition against MSP-6 and MSP-7, can inhibit parasite growth in vitro indicating that protective epitopes are found throughout the entire MSP-1 complex. Conditions for the production of full-length
MSP-1 have been established in detail on lab scale and the product already fulfills many key criteria of clinical grade material. Recombinant MSP-1 in combination with different adjuvants shows high immunogenicity in mice and rabbits and antibodies against MSP-1 are capable of inhibiting growth of several parasite strains in vitro. Full-length MSP-1 is an important target of the humoral as well as cellular immune response and therefore constitutes a promising vaccine candidate. The availability of recombinant full-length MSP-1 for clinical studies should make it possible to test the potential of MSP-1 as a vaccine against malaria.

Email address for correspondence: c.epp@uni-heidelberg.de

702 Dynamics and placental tropism of Plasmodium falciparum genotypes during pregnancy [MIM16672676]

J. Guitard, S. Gnidehou, S. Sow, J.Y. Le Hesran, P. Deloron, N. Tuikue Ndam

Pregnancy-associated malaria is of major concern in sub-Saharan Africa causing maternal anaemia, low birth weight and still births due to the accumulation of infected erythrocytes in the placenta. This accumulation is due to the interaction between the VAR2CSA protein on the surface of the infected erythrocytes (IE) and chondroitin sulfate A (CSA) in the placenta. Pregnant women gradually develop protective anti-VAR2CSA IgG over successive pregnancies. However infected multigrid women seem to harbour parasite expressing distinct variants of VAR2CSA as compared to infected primigravidae. The aim of this study was to determine the dynamics of Plasmodium falciparum genotypes during pregnancy in relation to acquisition of anti-VAR2CSA immune response. To study the dynamics of P. falciparum population during pregnancy, msp2 genotyping and analysis of the var2csa DBL5 sequence were performed. ELISA was used to measure the level of VAR2CSA DBL5-specific IgG and their levels were analyzed in relation to infection. The results available now highlight the selection of some parasite genotypes able to persist over several weeks and, still present in the placenta at delivery. Anti-VAR2CSA antibody response is associated to the clearance of some but not all genotypes. We will continue exploiting these results to determine: (i) the possible existence of variant specific immune response against VAR2CSA; (ii) whether parasites infecting primigravidae and multigravidae express different VAR2CSA DBL5 variants; (iii) whether placental tropism is restricted to particular VAR2CSA variants.

Email address for correspondence: julietteguitard@yahoo.fr

703 Immunological memory induced by attenuated Plasmodium berghei parasites [MIM16696599]

Krystelle Nganou Makamdop, Geert-Jan van Gemert, Adrian J.F. Luty, Rob Hermse, Robert Sauerwein

There is an urgent need for a malaria vaccine. A key condition in the development of an effective vaccine is immunological memory, but naturally acquired immunological memory in malaria is poorly understood. Recent studies have revealed that attenuated parasites can induce protective immunity. The concept of genetic attenuation of sporozoites (GAS), based on the disruption of genes essential for liver stage development, has successfully been applied in murine models. Successful immunization studies, performed either with radiation attenuated sporozoites (RAS) or by sporozoite infection concomitant with chloroquine chemoprophylaxis (CPS), have been conducted in both mouse and man. Although the three approaches exhibit different degrees of pre-erythrocytic development, they all induced complete protection. Therefore, these approaches taken together provide powerful tools to study immunological memory in malaria. Mice are being immunized via GAS, RAS and CPS. Both cellular and humoral memory are being assessed in blood, liver and spleen collected from immunized and non-immunized mice before and after challenge infections. Flow cytometric analyses give insight into the composition of the memory pool taking into consideration various cell subsets. With ex vivo stimulation assays, the functionality of the established memory is addressed. Results to be presented comprise: (i) CD4+ and CD8+ T-cell memory with regards to central and effector memory cells; (ii) B-cell memory and antibody levels as a result of immunization; (iii) functionality of T- and B-cell memory response assessed by means of ex vivo assays. We will discuss the results in the context of existing knowledge of T- and B-cell memory in malaria.

Email address for correspondence: k.nganoumakamdop@ncmls.ru.nl

704 A simulation study of the epidemiological impact and cost-effectiveness of malaria vaccines [MIM15074974]

Melissa Penny, Fabrizio Tediosi, Nicolas Maire, Alain Studer, Tom Smith

Many vaccine candidates against Plasmodium falciparum are currently in pre-clinical or clinical development. These candidates vary in characteristics, but none is likely to provide long-lasting sterile immunity. There is a need to specify minimal vaccine profiles, to define appropriate transmission settings and deployment strategies, and to predict cost-effectiveness. Using previously published stochastic simulation models we predict costs and population effects of deployment of each of a range of malaria vaccines on malaria transmission, morbidity and mortality in realistic epidemiological and health-system settings. We simulate pre-erythrocytic (PEV), blood stage (BSV) and combinations with or without mosquito-stage transmission blocking vaccines (MSTBV), considering a range of endemic malaria settings, deployment strategies, vaccine efficacies, and half-life of protection. Our results indicate greatest benefits and cost-effectiveness of PEV in low endemic settings but at high transmission could even lead to increased disease incidence. BSV are predicted to be most useful and cost-effective at high transmission. Combination vaccines are more effective and cost-effective compared to single-component vaccines when deployed via EPI with mass vaccination. Adding booster doses to EPI is unlikely to be a cost-effective alternative. A minimum half-life of 2–3 years is required for substantial epidemiological effects. Significant herd immunity and reduction in transmission are predicted with moderately effective vaccines when deployed through mass campaigns, especially when combined with MSTBV. Clinical development programs need to consider the appropriateness of vaccine types for different transmission settings; to assess effects on transmission and duration of protection; and to consider different deployment strategies.

Email address for correspondence: melissa.penny@unibas.ch

705 Malaria vaccine rational design strategy: example of msp3 family-based vaccine [MIM15093742]

Corine Demanga Galamo, Lena Juliette Daher, Eric Prieur, Catherine Blanc, Jean-Louis Pérignon, Pierre Druihle

Plasmodium falciparum MSP3, identified from clinical observations as main target of protective immunity against malaria, belongs to...
a multi-gene family of six proteins with similar structural organisation particularly in their highly conserved C-terminal regions. Therefore it was of interest to design a vaccine construction combining carefully chosen regions from these proteins. Immuno-epidemiological studies were performed on recombinant antigens and a series of peptides designed from the C-terminal part of each MSP3 protein. Furthermore the biological anti-parasite activity of their corresponding antibodies was assessed. A majority of malaria exposed individuals harbour antibodies to each MSP3 antigen, with a dominance of cytophilic IgGs. The designed peptides were almost all antigenic, defining epitope relevant of native proteins, with distinct IgG isotype pattern for each and an overall predominance of the IgG3 subclass. Human antibodies affinity-purified upon each peptides exerted a P. falciparum killing effect in cooperation with blood monocytes (ADC1) in most cases as strong as that of IgG from protected African adults. Two regions “b” and “d” showing sequence homologies were found to generate in humans a broad network of cross-reactive antibodies with various affinities. These findings suggest that all the six members of MSP3 family complement each other and play collectively an important role in protection. A first MSP3 multigenic construct designed according to these findings and those from immunogenicity studies in mice was shown to have valuable immunological properties. These findings pave the way towards tailoring vaccine constructs based on knowledge.

Email address for correspondence: cdemanga@pasteur.fr

706

Natural immunization against malaria by pre-exposure prophylaxis [MIM16670413]

Johannes Friesen, Olivier Silvie, Elyzana Putrianti, Kai Matuschewski, Steffen Borrmann

Experimetal immunization with live attenuated Plasmodium sporozoites remains the gold standard for anti-malaria vaccine development. However, major hurdles towards implementation in disease-endemic countries include sustainable and affordable production and delivery of purified live attenuated parasites to the population at risk. We hypothesized that pathway delivery upon natural exposure to malaria transmission combined with causal-prophylactic treatment reproduces the potency of whole organism vaccines. In such an infection/treatment approach, medical intervention is reduced to implementation of drug coverage. We immunized mice with varying numbers of P. berghei wild-type sporozoites under prophylactic drug treatment. Mice were challenged with 10,000 sporozoites at least 4 weeks after the last immunization. The mode of action of the drugs of interest was studied in vitro by immunofluorescence assays. The in vivo behavior was studied by employing quantitative Real-Time PCR on mouse liver homogenates. We show that immunization with sporozoites under causal-prophylactic treatment results in swift development of long-lasting and uniquely potent immune responses against re-infection. We propose that causal-prophylactic drugs could act as a natural needle-free anti-malaria vaccine in addition to their immediate preventive and curative activities.

Email address for correspondence: johannesfriesen@hotmail.com

707

Molecular characterization of distinct VAR2CSA DBL5ε variants from placental isolates [MIM16673954]

S. Gnidehou, J. Guitard, P. Andersen, C. Ermont, A. Salanti, O. Lund, P. Deloron, N. Tuikue Ndam

Pregnancy-associated malaria (PAM) is precipitated by accumulation in the placenta of infected erythrocytes (IE) containing a distinct population of Plasmodium falciparum expressing VAR2CSA protein. The relatively conserved VAR2CSA molecule expressed on the surface of IE, which belongs to the otherwise highly variable P. falciparum erythrocyte membrane protein 1 (PfEMP1) family, is the main parasite ligand for placental binding. Since high levels of anti-VAR2CSA antibodies are associated with protection against PAM, this molecule is thought to contain B-cell epitopes to be included in an anti-PAM vaccine. VAR2CSA contain molecular signatures associated with parity in some of its domains. Therefore, in the perspective of developing an efficacious VAR2CSA-based vaccine it is critical to identify sequence characteristics of this molecule that can interfere with immune response. In this study 43 placental and 14 database VAR2CSA DBL5ε sequences were analyzed as part of a general effort of characterizing each of the six VAR2CSA DBL domains employing a combination of experimental and in silico tools. The results available now show that sequence variation generally occurs in regions subjected to diversifying natural selection that are mainly located on unstructured areas of the proteins predicted to form flexible loops. Like for the previously characterized VAR2CSA DBL3x domain, we identified DBL5ε sequence motifs characterizing parasites isolated from primi- and multigravidae, respectively. We will continue fine analysis of the various parasite variants to identify areas of cross-reactivity and experimental investigations will also target variants from parasites displaying different ability to CSA binding in vitro.

Email address for correspondence: gcarine@yahoo.com

708

Malaria vaccine investment and impact model [MIM15076081]

Vicky Cardenas, MHS, PhD, JD

Learning objectives: To gain an understanding of, and an appreciation for, the need, the inputs required and sample outputs generated from a return on investment modeling for a new vaccine. Background: There is no malaria vaccine available today. In part, this may be attributed to the lack of collecting good data and the ability of that data to answer questions related to the returns expected from a vaccine. Specifically, good data can illustrate the potential health impacts of a vaccine on the public sector and the financial returns expected to the private sector in developing a malaria vaccine. Policy overview and relevant issues: A vaccine return on investment case requires information about (1) market demand for the vaccine, (2) health impacts anticipated from estimated demand, (3) how health impacts from the vaccine relate to impact from other interventions, (4) costs to develop and produce the vaccine, including risks of failure and borrowing resources over time and (5) costs to purchase and deliver vaccine based on demand. Current efforts and implications: The Malaria Vaccine Initiative, in collaboration with three partners, has developed a return on investment model that can be used by (1) malaria vaccine development funders, e.g. USAID, MVI, EMVI, and others on MALVAC in deciding the best use of donor funds, (2) vaccine purchasing organizations, e.g. GAVI, GFATM, in deciding the optimal allocation of funds, (3) country-level stakeholders with responsibility for decision-making around the implementation of malaria vaccines, e.g. Ministries of Health and Ministries of Finance, (4) public health practitioners who work with other disease models as well as those working in the malaria field and (5) private industry partners for quantifying the likely returns they could expect from investing in a malaria vaccine. A well-formulated investment analysis can provide a reconciliation of risks and incentives between public and private sectors. Integrated analyses are a critical tool to bridge the public and private sectors, develop new interventions and achieve health impact.

Email address for correspondence: vcardenas@path.org
PADRE restores humoral response to a protective mouse-model-prototype SAPN malaria vaccine modified for administration to humans [MIM16681791]

Stephen Kaba, Clara Brando, Qin Guo, Ian McWilliams, Christian Mittelholzer, Senthilkumar Raman, Peter Burkhard, David E. Lanar

We have previously shown that immunization of mice with a prototype self-assembling polypeptide nanoparticle (SAPN) displaying a B-cell determinate from the Plasmodium berghei CS protein (PbCSP-SAPN) induced 100% protection against lethal challenge with P. berghei sporozoites, in the absence of any extraneous adjuvant. Protection was T-cell dependent and could be transferred with serum. The SAPN building block linear polypeptide is comprised of a pentameric coiled-coil domain and a trimeric coiled-coil domain separated by a linker; multimerization via the coiled-coil sequences results in the assembly of an icosahedral particle consisting of 60 polypeptides. The pentameric sequence of the prototype SAPN was derived from COMP, a rat protein having a human ortholog. For a human-use vaccine it had to be change to another sequence. This change resulted in lost of antibody induction and protection. The inclusion of the sequence for the pan-allelic DR epitope (PADRE) restored antibody induction and protection against challenge. The COMP sequence was replaced with de novo designed tryptophan zipper sequence, to make the SAPN T81c-PbCSP. PADRE, a universal CD4 Th epitope was also engineered into the new SAPN construct designated T81c-8-PbCSP. To investigate the effect of these manipulations, serum from mice immunized with T81c-PbCSP and T81c-8-PbCSP were tested by ELISA. After immunization mice were challenged. Replacement of the pentameric coiled-coil sequence with the Tryptophan zipper sequence abrogated antibody induction; inclusion of PADRE sequence restored it. T81c-PbCSP induced low antibody titers and only 20% protection; T81c-8-PbCSP induced high antibody and 100% protection. A universal T-helper CD4 epitope, PADRE, can be used in SAPN making the platform more versatile for vaccine development.

Email address for correspondence: stephen.kaba@amedd.army.mil

Developing, optimizing and validating ELISA’s for PfMSP142 FVO and 3D7 for evaluating candidate malaria vaccines [MIM16822300]


The PATH Malaria Vaccine Initiative (MVI) and the U.S. Agency for International Development (USAID) are working together to enhance the ability of assays and other laboratory tools to evaluate the potential of new vaccine candidates. As part of this initiative, MVI and USAID are supporting the expansion of the Malaria Serology Laboratory (MSL) within the U.S. Military Malaria Vaccine Program (USMMP) to an International Reference Center that performs enzyme-linked immunosorbent assays (ELISAs) for malaria antigens. The assays are initially developed according to standard ELISA protocols. However, each assay is then validated, implementing rigorous standard operating procedures, and continuous quality assurance and controls under good laboratory practice (GLP) conditions while testing clinical trial samples. For example, in order to run real-time test samples on standardized human ELISAs, each ELISA operator must complete a rigorous qualification process. The process includes completing eight consecutive, quality-controlled ELISA runs and maintaining a nine-sample QC panel within previously established accuracy and precision limits. The MSL ELISAs produce results that are extremely robust and highly reproducible. Here we report the results of developing, optimizing and validating ELISAs for PfMSP142 (FVO) and PfMSP142 (3D7) for the purpose of evaluating the immunogenicity of current and future candidate malaria vaccines and discuss the positive impact of an International Reference Center on global malaria vaccine research.

Email address for correspondence: nancy.richie@amedd.army.mil

Assessing protective efficacy of Plasmodium falciparum circumsporozoite protein-based vaccines in animal models using transgenic parasites [MIM16670596]

Saurabh Dixit, Guan-Hong Song, Lynn Lambert, Rita Tawari, Angela Lungar, Sachy Orr-Gonzales, David Jones, Matt Plassmyer, David Narum, Kazuoto Miura, Andrea Crisanti, Louis Miller, Yimin Wu

The sporozoite challenge model has been used for evaluating protective efficacies of liver stage vaccines in Phase 2a clinical trials. For preclinical evaluation of CSP-based vaccines in various formulation platforms, such challenge model will help to down-select of the most promising product in development path. However, there is no animal malaria sporozoite challenge model available due to the host specificity of Plasmodium species. We are employing as well as developing animal challenge models, using transgenic parasites that express P. falciparum CSP on their sporozoite surface. A chimeric P. berghei parasite (rodent malaria) expressing a human PfCSP transgene (CSP9) has been developed and may be used to evaluate the P. falciparum CSP-based vaccines in mice. We are also developing a P. knowlesi parasite (monkey malaria) expressing a human PICSP transgene. The rodent challenge model has been tested for suitable mouse strain for challenge studies, sporozoite production in mosquitoes, number of sporozoites needed to establish 100% infection, and pre-patent duration. Protection of CSP and CSP-conjugates are being evaluated for protection against the chimeric parasite. For primate challenge model, a construct containing transgene target cassette and selectable cassette has been created. Transfection of P. knowlesi parasites, selection of chimeric P. knowlesi expressing human PICSP transgene is currently underway.

Email address for correspondence: yiwu@niaid.nih.gov

Evaluation of immune responses to two P. vivax CS-based vaccines in rhesus macaques [MIM16681719]


Eradication of malaria cannot be achieved without controlling P. vivax, which is responsible for a large number of malaria cases around the world and is the primary cause of malaria outside of Africa. We have developed two P. vivax CS-based vaccines. The constructs produced as a recombinant protein in E. coli (VMP001) or as a hepatitis B surface antigen fusion particle expressed in S. cerevisiae (CSV-S,S) contain both VK210 and VK247 repeats. Malaria-naive rhesus macaques were immunized three times with either 50 µg or 15 µg of VMP001 or with 50 µg or 10 µg CSV-S,S. Both vaccine candidates were formulated in the GSK proprietary Adjuvant System AS01B. Monkeys were bled two weeks post-each immunization.
Immunization based on native non-codon optimized DNA of single and double domains consistently gave high antibody response. Sera were obtained so far and the results available show that DNA electroporation significantly increases antibody response in mice and that these antibodies are able to recognize native VAR2CSA on the surface of placental isolates. Constructs inducing highly cross-reactive antibodies on placental isolates are observed. We will continue fine functional analysis of the various antibodies generated to identify those carrying unambiguous inhibitory activity.

Email address for correspondence: nicaise.ndam@ird.fr

---

713
A survey of genetic diversity of Plasmodium falciparum in Mpongwe [MIM14487153]
Sydney Mwanza

Mpongwe, a rural town in Zambia, has been characterized for malaria vaccine development. Antigenic diversity related to pf polymorphism is one main limitation to the development of a vaccine against pf. Blood collected on filter paper from consenting parents of microscopy positive children, dried at room temperature and extracted for DNA. Msp-1 block 2, Msp-2 block 3 and GLURP were amplified by nested PCR, each amplification with conserved or family-specific primer pair, being done separately. PCR products were electrophoresed on 1.5% ethidium bromide stained agarose gels, visualized by ultraviolet transillumination. Fragments obtained were compared by size to purified genomic controls amplified and electrophoresed similarly and to the DNA ladder. Among the 81 samples, at the msp-1 locus 62 harbored K1 parasites; 60 had RO33 parasites and MAD 20 was 41. For the msp-2 locus, 53 (65.4%) FC27 alleles ranged from; and 63 3D7 (77.8%) alleles ranged from. At each locus, family distribution was 38.04%, 36.81% and 25.15% for K1, RO33 and MAD 20 of msp-1; and 45.68% and 54.32% for FC27 and 3D7 of msp-2. 79.01% (64 parasites) harbored GLURP parasites. The proportion of patients having at least 2 strains of parasites was found to be 98.7%, with MOI averaging 5.3. Mpongwe has a highly genetically diverse population of pf as seen from the extensive polymorphism mainly in the MSP1 and MSP2 antigens and the high MOI (5.3).

Email address for correspondence: sydneywmwanza@yahoo.co.uk

714
Induction of functional anti-VAR2CSA antibodies in mouse using in vivo DNA electroporation [MIM16674984]
N. Tuikue Ndam, M. Quiviger, P. Bigey, D. Scherman, P. Deloron

Pregnancy-associated malaria (PAM) is of major concern in sub-Saharan Africa and is principally associated to low birth weight babies. Studies have underlined the key role of a P. falciparum protein VAR2CSA, expressed on erythrocytes, in placental sequestration of infected erythrocytes (IE). Evidence is also accumulating that specific immune response against VAR2CSA acquired during first pregnancies reduces the harmful effects of PAM during subsequent pregnancies. These observations strongly suggest that it is possible to develop a new preventive strategy based on vaccination. VAR2CSA is a large polymorphic protein that current technological constraints do not allow recombinant expression of the entire molecule. Thus, the challenge for vaccine development is to define smaller parts of the molecule, which induce antibodies that inhibit CSA binding of IE. DNA vaccination in mice using constructs comprised of single and multiple domains of VAR2CSA proteins originating from laboratory-adapted and fresh placental isolates are used to identify cross reactive epitopes able to drive a protective immune response against the different parasite variants.

---

715
Analysis of IgG responses against PfGPI and kinetic of TNFα serum levels in adults Senegalese with severe malaria [MIM16734787]
Babacar Mbengue, Aïssatou Toure, Bacary Diatta, Birahim Niang, Channe D. Gowda, Ronald Perraut, Alioune Dieye

The induction of neutralizing immunity to Plasmodium falciparum glycosyl phosphatidyl inositol by vaccination has been proposed as a preventive strategy to limit the severity of malaria. PfGPI can induce TNF production by murine macrophages in vitro. However, definitive proof that PfGPI is pathogenic in human malaria is presently not available. Our study analyses the association between levels of IgG responses against PfGPI, kinetic of TNFα serum levels evaluated by ELISA and clinical outcome, in 110 Senegalese patients with severe malaria (8–74 years), including 27 fatal cases. Sera were collected in days 0, 1 and 2 of hospitalisation when available. 72 patients with uncomplicated malaria (10–77 years) were recruited. From the admission to the third day, means of TNFα levels decreased: 70.29–42.21 pg/ml. According to the outcome, TNFα levels are more higher in death patients than recovered cases at the admission: 92.54 pg/ml vs 63.06 pg/ml (day 0), and inversely at day 1: 16.59 pg/ml vs 53.25 pg/ml (P = 0.037) and day 2: 17.38 pg/ml vs 48.29 pg/ml (P = 0.032). 24% of patients showed positive IgG responses against PfGPI and antibodies levels are lowest in death patients at day 0 (P = 0.026) and day 1 (P = 0.014). Negative correlation is found between TNFα and IgG levels only at the day 2 (rho = -0.460, P = 0.014). These findings provide insights into the implication of high levels of TNFαs and PfGPI in the pathogenesis of SM. We find a discriminative result of anti-PfGPI IgG level for fatal cases. Anti-PfGPI IgG play a role in protecting against disease progression and fatality.

Email address for correspondence: m.babacar@voila.fr

---

716
Validation of assays relevant to assessment of immunogenicity during a malaria vaccine trial [MIM15220542]
Dorothy Anum, Kwadwo Koram, William O. Rogers, Bartholomew D. Akanmori, Gregory Raczniak, Martha Sedegah, Daniel Dodoo

The emergence of multiple drug-resistant parasites and insecticide-resistant mosquitoes has shifted focus to the development of an anti-malaria vaccine. Validated assays are required for immunogenicity assessment during malaria vaccine trials. IFN-g ELISPOT ASSAY: CSP peptide pools, positive control class 1 peptide pool and un-stimulated wells were included in each assay. Volunteer PBMC were stimulated for 36 h and the released IFN-g stained and enumerated by the automated ELISPOT reader. ANTIBODY RESPONSE AGAINST PFCSP: IFA: serially diluted sera were tested in IFA against air dried sporozoites and data reported as endpoint titre at which fluorescence is detected. ELISA: Quadruplicate serially diluted sera were tested against recombinant CSP protein in ELISA assay. Pooled
The erythrocyte-binding antigen 175 (EBA-175) is a dimorphic antigen expressed on the merozoite of \textit{P. falciparum} and a potential candidate for vaccine development. Its functions and potential effects however, remain unclear. This study investigated the relationship between its dimorphism and clinical outcome of malaria and also the presence of any sequence polymorphism in the F2 domain of the EBA-175. A nested polymerase chain reaction (PCR) was used to genotype \textit{P. falciparum} strains that exhibit this dimorphism and F2 domain polymorphism in severe and mild cases and asymptomatic malaria controls from the Kassena Nankana District (KND), an area been characterised for future vaccine trail in northern Ghana. The F2 PCR products were sequenced, translated and aligned with the amino acids of published \textit{P. falciparum} Camp strain to determine any sequence diversity A total of 232 samples were used. These include 75 severe and 76 mild cases and 81 asymptomatic controls. The severe samples, had a distribution of 29 (38.2%), and 5 (6.6%); the asymptomatic controls: 59 (73.8%), 24 (32%) and 7 (9.3%); the mild samples: 42 (55.3%), 20 (26.3%), and 1 (1.23%) of the F, C and CF EBA-175 genotypes respectively. A chi-square test revealed that the mixed genotype (CF) was associated with severe malaria (\(p=0.046\)), whereas the F genotype was associated with reduction to the risk of having severe malaria (\(p=0.044\)). Polymorphisms observed in the F2 domain of the EBA-175 in all 20 \textit{P. falciparum} isolates will be further discussed.

Email address for correspondence: begyir@noguchi.mimcom.net

718 Impact of the environmental and gene factors in \textit{Plasmodium falciparum} malaria immunity: Implication for vaccines constructions [MIM16735326]

M. Ndiaye, G. Aribot, A. Tall, P. Drulilhe, M. Thiesen, D. Dodoo, A. Touré

Dielmo and Ndiop are two neighbouring villages in Senegal with holo and mesoendemic transmission. A long-term prospective study of naturally acquired immunity to \textit{Plasmodium falciparum} has been conducted for 10 years providing longitudinal clinical, parasitological and entomological data. In this study we analysed the risk of malaria attack in relationship with immune responses to two \textit{P. falciparum} antigens GLURP and MSP3 and with other factors as transmission, age, gender, hemoglobin type. Blood samples were obtained from 99 children in Dielmo (4.9–17.9 years) and 100 children (3.6–16.7 years) in Ndiop. The distribution of hemoglobin HbAS was in Dielmo 4% and Ndiop 18%. The relationship between the risk of malaria attack from 6 months or 1 year after the blood sampling and the different factors was tested using a Poisson regression model. In Dielmo, age, IgG1/GLURP and IgG3/MSP3 were associated to malaria attacks reduction but not Hb type. In Ndiop, the analysis using this model, showed that age and Hb type AS was the major factors associated to reduction of the risk of malaria attack with such a weight that other factors as immune responses do not appear as significantly associated with protection. But using Mann–Whitney and Kruskal–Wallis tests, immune responses IgG/MSP3, IgG3/MSP3, IgG/GLURP, IgG1/GLURP appeared to be significantly higher in subjects that have no or lesser malaria attack. Our data suggested that environmental and genetic factors are important factors that should be considered when analysing the relationship between protection and malaria immunity and selecting malaria vaccine candidates.

Email address for correspondence: muyideenk@gmail.com

720 Urban malaria and it’s control in India [MIM14578003]

Sharma Rajander

Malaria in urban areas was considered to be a marginal problem restricted to mega towns only and was considered that local...
bodies are capable of handling it. Therefore while launching the National Malaria Eradication Programme in 1958, Urban Malaria was not included. By 1970s, incidence of rural malaria came down drastically i.e. 0.1–0.15 million cases per year but the urban town reported rising trend. Madhok Committee in 1970, investigated the problem and assessed that 10–12% of total cases were contributed by urban areas. The committee recommended anti larval measures for containment of urban malaria, because it was feared that proliferation from urban to rural may spread and nullify the gains already made. Malaria in urban areas is contributed by large scale rural–urban migrations triggered by urban “push” (for earning livelihood) and urban “pull” (for availing both Medicare/educational opportunities) phenomenon. Demographic and societal changes, unplanned urbanization, completion of projects in total disregard of health impact assessment and incorporation of non eco-friendly technologies all contributed to increased vector breeding potential. Insufficient capacities of the civic bodies to deal with water supply, sewage and solid waste disposal led to an all round disruptions. Intermittent water supply led to increased water storage practices which resulted in extensive breeding of An. stephensi, vector of urban malaria and Aedes aegypti, vector of DF/DHF in all house holds.

Rural migration led to the establishment of “urban slums” with poor housing and sanitary conditions. These areas have heavy breeding potential of An. stephensi and An. culicifacies and Culex quinquefasciatus and have earned the reputation of peri-urban malaria paradigms. This is a difficult paradigm because of enormity of the diverse breeding sites. These slums are inhabited by 30–40% poor and marginalized people. Today urban malaria can be stratified into five distinct sub-paradigm, i.e. urban center, peri-urban, construction sites, industrial estates, and weekly market sites. These sub paradigms have variable receptivity and vulnerability and potential for malaria outbreaks. Control strategies therefore have to be paradigm specific and evidence based in an Integrated Vector Management (Mode). An. stephensi control strategy will yield collateral benefit of control of Aedes aegypti, the vector of DF/DHF, because of sharing breeding habitats with An. stephensi. Similarly An. culicifacies control will also yield control of C. quinquefasciatus for similar reason.

Email address for correspondence: rssharmanamp@gmail.com

721

MIM conference [MIM16691144]

Eveline Nsango

Malaria remains a major health problem in Sub-saharan Africa. Novel strategies to control the disease require extensive knowledge of different that shape mosquito immune responses. Most studies have focused on the Anopheles gambiae—Plasmodium berghei model, and identified a series of genes that control parasite numbers and therefore represent potential targets to block parasite development within the mosquito. Moreover, boosting expression levels of these genes or basal immunity was sufficient to block completely P. berghei development. However, it is essential to validate observations under laboratory conditions by studying mosquito interaction with the human malaria parasite P. falciparum. In this study, we investigated by RNAi knockdownd the effect of candidate genes in P. falciparum infections. We found that TEP1 has a broad anti-parasitic activity against rodent and human malaria parasites. Boosting basal immunity by Cactus depletion did not affect P. falciparum survival in contrast to P. berghei survival. Surprisingly, we observed a specific sensitivity of P. falciparum to a wounding-induced immune response. These results implicate that P. falciparum or P. berghei are susceptible to distinct arms of the mosquito immune responses.

We further investigated the role of larval habitats in the establishment of basal immunity of wild mosquitoes. QRT-PCR analysis revealed differential levels of expression of TEP1 and two antimicrobial peptides Defensin and Gambicin in distinct larval breeding sites. Our preliminary results indicate that basal immunity affects P. falciparum development in the natural populations of A. gambiae.

Email address for correspondence: nsango2002@yahoo.fr

722

Détermination des espèces et des formes moléculaires du complexe Anopheles gambiae en République Démocratique du Congo [MIM16698599]

L.T. Bobanga, R. Beach, E. Dotson, W. Hawley, Lee Ann, K. Tshima

Contrairement aux autres pays africains, peu d’informations sont disponibles sur les membres du complexe Anopheles gambiae s.l. vecteur majeur du paludisme en République Démocratique (RDC) y a été décrit il y plus de 60 ans. En RDC le paludisme est la première cause de morbidité mais très peu de travaux sont disponibles sur les vecteurs. Objectifs: Discriminer A. gambiae de Anopheles arabiensis et déterminer les formes moléculaires pour A. gambiae s.s. Recherche du gène de résistance Kdr dans ces populations d’anophèles. Des collectes des Anophèles ont été réalisées dans 8 sites avec écosystème différent à travers le pays. Près de 600 moustiques ont été collectés et analysés par PCR-RFLP et le gène Tous les anophèles analysés étaient de l’espèce A. gambiae s.s. Des moustiques analysés 80% étaient de forme moléculaire M contre 20% de forme moléculaire M. Pour le gène Kdr, sur les moustiques qui ont pu être analysés, il a été trouvé dans 4 sites avec une fréquence qui n’a pas dépassé les 5% La répartition des formes moléculaires de A. gambiae ss était liée au site de collecte des Anophèles. Ainsi dans très peu de site de collecte nous avons rencontré les 2 formes moléculaires. Cette étude veut combler les lacunes sur A. gambiae s.l. en RDC

Email address for correspondence: lggbobanga@yahoo.fr

723

Kdr based insecticide resistance in Anopheles gambiae from Cameroon; origin, spread and level of resistance [MIM16698153]

Josiane Etang, Jose L. Vicente, Philippe Nwane, Mouhamadou Chouaibou, Isabelle Morlais, Virgilio E. Do Rosario, Frederic Simard, Parfait Awono-Ambene, Jean Claude Toto, Maureen Coetzees, Joao Pinto

Knockdown resistance (kdr) mutations in Anopheles gambiae s.s. populations from Cameroon were first reported in 2003. This report questioned of the origins, spread and levels of insecticide resistance induced in the local mosquito populations. Surveys were conducted between 2005 and 2007, to (1) update the geographic distribution of kdr genes and their allelic frequencies, (2) to assess the levels of conferred resistance and explore the number of mutational events originating kdr alleles. Females A. gambiae s.l. were sampled, from 17 sites located across the main geographic areas and used for molecular analysis: species and molecular form identification, kdr depiction and sequencing of intron 1 of the voltage gated sodium channel gene. Adults from larvae collections were used for WHO susceptibility tests. Both 1014S and 1014F kdr alleles were widely distributed in the S-form (frequencies up to 0.87). They were also found in the M form, but at lower frequencies (0.02–0.4). Most of the populations showing high frequencies of kdr alleles displayed high level resistance to DDT and pyrethroids. Bio assays using synergists (4% PBO, 8% DEM, 0.25% DEF) highly suggested co-involvement of detoxifying enzymes (oxidases, esterases or
C-reactive protein levels, microcytemia/hypochromia and response to antimalarials in Yaoundé - Cameroon [MIM14578457]

Justin Komguep, Olivia Achouduh, Priscille Flore Kanouo, Palmer Netongo, Akindeh Nji, Irenée Domkam, Sarah Riwom, Wilfred Mbacham

As an acute-phase reactant, C-reactive protein (CRP) levels can provide a simple measure of disease severity, the efficacy of therapy and the severity of complications. Hemoglobin “Constant Spring” mutation (16p13.3-17q21, TAA142CAA) is one of the most wide-spread α-thalassemia variant with a relatively severe impact on the homozygous carrier and an in vitro decreasing effect on the sensitivity of malaria parasites, infecting the erythrocytes with such carrier. The relationship between levels of CRP, presence of Hb “CS” mutation and response to antimalarials in patients recruited at the same site for monotherapy and artesinin-based clinical trials respectively was determined. D0 serum from 180 malaria patients recruited for SP/AQ clinical trial was evaluated for levels of CRP using immunoturbidimetric kits. Human genomic DNA extracted from 46 patients recruited for COARTEM® and COARINATE® clinical trial on the basis of the persistence of microcytemia (MCV < 86 fl) and hypochromia (MCH < 27 pg) was analysed by PCR-RFLP to detect the presence of the Hb “CS” mutation. There was no significant correlation between CRP levels, the age of patient, parasitaemia and temperature on D0 and D3 (p = 0.05). However, the level of CRP was observed to vary slightly with temperature above 39°C on D3 after treatment. Of the two (4.35%) patients who showed heterozygoty for the Hb “CS” mutation, none of these was associated with a particular treatment response (p > 0.05). Absence of correlation could be due to interaction with other factors which influence inflammatory response during an infection.

Email address for correspondence: oliafa@yahoo.com

Evaluation of wall linings and curtains as an option to IRS in Equatorial Guinea [MIM16696921]

Abraham Matias Arnez, Luis Segura, Chris Schwabe, Richard Allan

Since 2003 indoor residual spraying (IRS) and use of LLINs has become established methods of malaria prevention in Equatorial Guinea (EG). Significant reduction in malaria prevalence has been achieved but requires significant financial and operational investment annually to maintain this. In this study, durable (insecticide treated) residual wall linings (DL) and durable (insecticide treated) curtains (LL ITC) were assessed as sustainable options for malaria prevention. In a comparative study in rural EG villages received one of the following treatments: (1) DL, installed in all sleeping areas, covering the surface of the walls and eaves. (2) LL ITC installed in all windows and external door ways. (3) IRS. Feasibility, acceptability, residual insecticide and material durability were monitored pre- and post-installation and at 1, 3, 6, 9 and 12 months. This study confirms that DL and curtains are feasible to install, are highly acceptable to households. The DL and curtains retain effective levels of residual insecticide for significantly longer than conventional IRS and are materially durable. Early results indicate that DL may be an effective long lasting option for malaria control where IRS has previously been relied upon and LL curtains can be a useful complimentary vector control method. Key to household acceptability and maintenance was the perceived home improvement and decoration benefits of these tools in addition to insect control.

Email address for correspondence: amatiflebo@hotmail.com

Bionomic and population genetic structure studies on Anopheles nili group of malaria vectors in Africa [MIM15065579]

Cyrille Ndo, Christophe Antonio-Nkondjio, Parfait H. Awoono-Ambene, Diego Ayala, Anna Cohuet, Pierre Kenge, Pierre Ngassam, Isabelle Morlais, Didier Fontenelle, Frédéric Simard

Mosquitoes belonging to the Anopheles nili group are recognized as important human malaria vectors in tropical Africa. However, despite their important epidemiological role, few studies have been conducted on this species. Here, we report data on bionomic and genetic studies from vector populations collected in Central and West Africa. Mosquito blood feeding preferences and infectious status were determined by ELISA. Eleven microsatellite loci were used to compare the level of genetic diversity and differentiation between 16 populations from Cameroon, Senegal, Burkina Faso, Ivory Coast, Nigeria and Democratic Republic of Congo. An. nili was found to be highly anthropophilic and exophilic and was the only member of the group collected above Cameroon. Conversely, An. ovengensis and An. carnevalei were highly exophilic and exophagic. All these species were found infected by Plasmodium falciparum, with IS ranging between 0.7 and 6.1. All 11 microsatellite loci were successfully amplified within An. nili samples while eight and nine loci were amplified in An. carnevalei and An. ovengensis populations respectively. Allelic richness and heterozygosity were high for all An. nili populations but low for An. carnevalei and An. ovengensis. High and significant pairwise genetic differentiation estimates were recorded between An. nili, An. carnevalei and An. ovengensis populations (0.19 ≤ Fst ≤ 0.53, P < 0.001). Within An. nili, populations from Kenge (DRC) and Moloundou (Cameroon) appeared more differentiated from the rest. This study confirms An. nili as an important human malaria vector in Africa. Genetic structure analysis fully support morphologic descriptions and provide further evidence for recent taxonomic classification within this group.

Email address for correspondence: cyrndo@yahoo.fr

Validation of plant repellents used to control malaria vectors in a rural setting in Cameroon [MIM13892198]

Corine Ngufor

No abstract received.

Email address for correspondence: corine_for@yahoo.com

La lutte antivectorielle dans la zone minière de Fungurume, Katanga, RD Congo [MIM16312424]

Makan Paul

No abstract.

Email address for correspondence: paulmawaw@gmail.com
729 Impact of intensive larval control and monitoring on malaria vectors in a rural area of western Kenya with high ITN coverage [MIM16679813]

A. Adinani, N. Bayoh, G. Olang, M. Ombok, J. Gimnig, J. Killeen, M. Hamel, J. Vulule, E. Walker

Integrated control of vectors of human malaria in Sub-Saharan Africa relies on strategic targeting of mosquito vectors. We implemented an anti-larval control study in an area of high insecticide treated bed net (ITN) coverage in rural western Kenya with perennial malaria transmission. The goal was to determine whether larval control would provide added benefit to ITNs in reducing malaria transmission. Larval control using Bti was implemented in a 2 km × 2 km zone in Asembo, western Kenya. A neighboring 2 km × 2 km zone was identified as non-intervention zone. Larviciding and monitoring was done weekly in all potential habitats within the intervention zone. Larval and adult An. gambiae populations were sampled fortnightly in both zones for a period of 7 months. An. gambiae larval density in the intervention zone was reduced by 79% (1–RR) for all instars and 97% for the late instars (3rd, 4th instar and pupae). We observed 39.5% fewer female Anopheles mosquitoes in the intervention zone after controlling for household ownership of bednets (RR = 0.605, p < 0.001). However, in limited pre- and post-intervention collections, data suggest a pre-existing trend in lower mosquito numbers in the intervention zone. Preliminary results from this study indicate that there was a decline in malaria vectors in an area of intensive larviciding. However, further analysis is needed to determine whether reduced densities were due to pre-existing differences in the two zones. Costs of the larviciding program and the feasibility of scaling this intervention up in rural African settings will be discussed.

Email address for correspondence: allyadinani@gmail.com

730 Dynamics of malaria transmission in the Sekyere East and Ashanti north districts, Ashanti region of Ghana [MIM16395042]

Kingsley Badu, Thomas Kruppa, Ruth Brenya, Rolf Garms

An entomological study was conducted in the forest zone of Ghana, to investigate the transmission patterns of malaria, and to assess the annual entomological inoculation rates. Four sites with different ecological conditions were selected. A total of 152 night catches were carried out by human landing catches from November 2003 to September 2005. Mosquitoes caught were identified, dissected and microscopically examined to determine their parity and infection rates with sporozoites in the salivary glands. The dissected mosquitoes were later tested for the presence of circumsporozoite (CS) antigens by using the enzyme-linked immunosorbent assay (ELISA). The majority of the mosquitoes caught belonged to the A. gambiae complex (97%), and A. funestus (3%). Members of A. gambiae complex present were only A. gambiae s.s. as identified by PCR. Considerable seasonal changes of biting activities occurred at all sites with highest numbers of anophelines caught during the main (March and July) and minor (August and October) rainy seasons. Infection rates of mosquitoes with sporozoites markedly varied at the four study sites. Of the total of 1356 Anopheles females which were caught an average of 2.7% were estimated to be positive by gland dissection. 10.0% by ELISA (range 6.4–19.2%). Malaria transmission was observed to occur all year round at the study sites as observed elsewhere. Conclusion: Annual entomological inoculation rates of 147 at Abotanso, 226 at Gyidim, 81 at Hwidiem and 96 at Low Cost respectively are clear indicators of an intensive transmission of P. falciparum in the two districts.

Email address for correspondence: kingsleybadu@yahoo.com

731 Insecticide treated nets can reduce malaria transmission across entire communities even when they confer minimal personal protection [MIM16758812]

Nicodem J. Govella, Fredros O. Okumu, Gerry F. Killeen

Insecticide treated nets (ITNs) represent an excellent means of protection against malaria but recent behavioral adaptations by mosquitoes to avoid them threatens to undermine their efficacy. Here we adapt a model mosquito behaviour to show that ITNs can suppress malaria transmission across entire communities even when they only confer modest levels of personal protection. Where half of bites occur outside of normal sleeping hours, personal protection is predicted to achieve only modest reductions (1.78-fold) in relative rate of exposure (RRE = 0.56). In contrast, much greater reductions (3.4-fold) resulting from communal protection are predicted (RRE = 0.29) at 50% coverage. It therefore appears plausible that community-wide use of ITNs can even protect against malaria transmission by vectors which avoid them.

Email address for correspondence: gkilleen@ihi.or.tz

732 Biochemical basis of permethrin resistance in Anopheles arabiensis from Lower Moshi, North-Eastern Tanzania [MIM16198475]

Johnson Matowo

Despite many efforts to control malaria it remains a major public health problem due in part to the development of resistance to different classes of insecticides. Improved understanding of resistance mechanisms in vector populations may facilitate the development of novel strategies to prevent or minimize the spread of resistance. In this study, metabolic-based mechanisms conferring pyrethroid resistance were investigated in the Anopheles arabiensis, the predominant malaria vector in Lower Moshi, North-Eastern Tanzania. Permethrin resistance in Anopheles arabiensis was detected using WHO diagnostic bioassay kits. The levels and mechanisms of permethrin resistance were determined by using piperonyl butoxide and s,s,s-tributyl phosphorothioate in bottle bioassays and by microplate assays. Anophelines arabiensis from the study area was found to be partially resistant to permethrin by showing only 87% mortality in WHO test kits. Resistance ratios at KT50 and KT95 were found to be 4.2 and 4.3 respectively. The permethrin resistance was partially synergized by s,s,s-tributyl phosphorothioate and by piperonyl butoxide when these were mixed with permethrin in bottle bioassays and was fully synergized when used together. The levels of oxidase and β-esterase activity were significantly higher in Anopheles arabiensis from Lower Moshi than in the laboratory susceptible strain. There was no difference in α-esterase activity between the two strains. The observed permethrin resistance may be caused by elevated levels of detoxification enzymes activity resulting from agricultural use of insecticides, especially in the rice fields, as permethrin treated nets are not widely used in Lower Moshi for protection against mosquitoes.

Email address for correspondence: johnntowo@yahoo.com

733 A new experimental hut assay to test behavioural and toxic effects of vector control tools [MIM16698121]

Sarah J. Moore, Jason D. Moore, Nicodem Govella, Benjamin Fuhrer, Fredros Okumu, John Grieco, Nicole Achee, Gerry Killeen, Tanya Russell

www.mimalaria.org
New kit-form experimental huts with reversible entry–exit traps fitted to the eaves and windows (Ifakara Interception Traps: IITs) were evaluated to measure mosquito deterrence, contact irritancy and mortality. Baseline experiments were conducted in four huts using balanced 4 × 4 Latin square to compare *Anopheles gambiae* s.l. numbers in interception traps to CDC Light traps (CDC LT). The huts collect consistent data with low variability compared to local houses. IITs as ENTRY TRAPS collected 1.58 times more *An. gambiae* (p = 0.039) than CDC LT, although window traps caught 0.465 times fewer mosquitoes than CDC LT (p = 0.001). This is consistent with *An. gambiae* s.l. entry behaviour. The IITs also caught 10% fewer mosquitoes. Therefore, some fed mosquitoes are entering huts to rest. Other experimental hut designs assume that all blood-fed mosquitoes have fed on occupants of the experimental hut and may therefore over-estimate feeding success. Comparison of IITs as EXIT TRAPS showed that the IIT captured 1.8 times more mosquitoes than CDC LT (N.S.). The eave exit traps collect equal numbers of mosquitoes to a window trap (N.S.), traditionally assumed to be the best of trapping exiting *An. gambiae* s.l. However, it is known that insecticides increase mosquito movement towards the eaves. The new assay is superior to the “Verandah Trap” used in insecticide evaluation. The IITs are flexible and may be used to measure multiple parameters including entrance, exit and timings thereof which is a significant improvement on previous design.

Email address for correspondence: munga_os@yahoo.com

---

### 734 Evaluation of insecticide treated wall lining materials used in traditional rural African houses [MIM16697253]

Stephen Munga, John Vulule, Richard Allan

Insecticide treated nets and indoor residual spraying (IRS) have been widely promoted as the main malaria prevention tools. However, achieving high net coverage and sustained usage is challenging and IRS normally requires regular repeats. In this study, we assessed the efficacy of insecticide treated wall linings (ITWLs) as an optional control tool to control malaria transmission. ITWL is treated with deltamethrine and provides long term controlled delivery of insecticide. Ten paired villages were used for the study; five villages each were used as control and treatment respectively. ITWLs and untreated wall linings were installed in the bedrooms of houses and used to cover the eaves in the treatment and control villages respectively. Further, the materials were installed on each wall surface to cover all mosquito resting sites. Baseline indoor resting densities of mosquitoes and malaria prevalence was established prior to and post installation of materials together with user acceptability. Initial results show that these insecticide treated wall linings are highly acceptable to households and do not require behavior change to achieve their impact. ITWL have significant potential for malaria control. The residual insecticides in ITWL are durable and maintain control of insects for significantly longer than IRS and may provide an effective alternative or additional vector control tool to ITN’s and IRS.

Email address for correspondence: munga_os@yahoo.com

---

### 735 Landscape characteristics and anopheline habitat productivity in western Kenya highlands [MIM16697359]

Stephen Munga, Tom Ouna, Andrew Githeko, Guiyun Yan

We investigated whether landscape characteristics was related to anopheline larval habitat productivity and further compared two methods for estimating habitat productivity in western Kenya highlands. In a 5 km × 4 km study area, we sampled and categorized all aquatic habitats with respect to five land cover types in the long rainy season and dry season. The location and elevation of each habitat was recorded using the global positioning system in differential mode. Occurrence and density of anopheline larvae was examined at each habitat using a standard dipper. Further, we compared habitat productivity using emergence traps and pupal counting methods. Additionally, we generated digital elevation model using topographic maps of the study area. Logistic regression analyses showed that altitude and could account for 56% of the productivity of anopheline larval habitats. Most of the habitats occurred in the valley bottoms. Comparison of the two methods showed that emergence traps had significantly (P < 0.01) more adult *An. gambiae* s.l. (2.1 mosquitoes/m²/day) compared to pupal counting method (1.3 mosquitoes/m²/day). These results suggest that topographic features may be important determinants of the focal distribution of malaria in western Kenya highlands.

Email address for correspondence: fashile2@gmail.com

---

### 736 Evaluation of long-lasting organophosphate indoor residual spray (IRS) formulations for control of susceptible and pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* in Benin and Tanzania [MIM16696688]


The Pan-African Malaria Vector Research Consortium (PAMVERC) is a partnership of three African research sites specializing in development and evaluation of malaria vector control products and interventions. PAMVERC sites in Tanzania and Benin are evaluating long-lasting IRS formulations of an organophosphate (OP) developed by Syngenta in collaboration with the Innovative Vector Control Consortium. The aim is to develop an OP with residual lifespan of 6 months or more to combat the growing problem of pyrethroid resistance and serve as a viable replacement for DDT. A variety of sprayed surfaces are tested against pyrethroid susceptible and resistant *Anopheles gambiae* and *Culex quinquefasciatus* every 4 weeks to evaluate the effective lifespan of the new formulations. Pyrethroids, DDT and existing OP formulations are used as controls. Field trials using experimental huts in West and East Africa are running concurrently to compare residual efficacy against wild mosquitoes. Initial results show the new formulations are killing a high proportion of all species and strains tested. At least one of the formulations has a longer residual lifespan than existing OP formulations. This research is a prime example of successful collaboration between research sites in Africa, northern institutions, chemical industry, and international consortia that foster public–private partnerships to develop new insecticide products for malaria control. Early results show potential for a new long-lasting insecticide showing no cross-resistance to pyrethroids being added to the limited arsenal. Such collaborations are essential to bring forward new vector control products and sustain current gains in malaria control.

Email address for correspondence: raphael.n’guessan@lshmt.ac.uk

---

### 737 A rigorous assay to measure spatial repellency for mosquito control using experimental huts and tent-traps [MIM16698314]

Sheila Ogoma, Jason Moore, Fredros Okumu, Sarah Moore

The mode of action mosquito-control insecticides may be divided into two classes: (1) toxicants and (2) those that act as irritants and repellents. Both of these actions may control malaria through...
Insecticide treated eave curtains are potential tools for integration with insecticide treated nets for malaria prevention in sub-Saharan Africa. Newer long-lasting treatment products greatly increase the feasibility of this tool. While eave curtains have been evaluated as a stand-alone intervention, they have never been tested in combination with insecticide treated nets. Insecticide treated nets (DuraNetsTM) were distributed to cover all sleeping spaces in 97 pairs of houses. The houses were matched by size and construction. One house in each pair was also fitted with insecticide treated eave curtains from the same material as the nets. Entomological evaluation was done by pyrethrum spray catch in May 2008. WHO cone bioassays as well as surveys to assess damage to nets and curtains were done at one and two years post-distribution. Houses with insecticide treated curtains had 49.0% fewer mosquitoes than houses with nets alone. Cone bioassays conducted 1 year post-distribution indicated >98% mortality on both nets and curtains. There were errors observed in the installation of the curtains resulting in noticeable gaps. However, the curtain material itself did not show significantly more damage than the nets. These results suggest that curtains may be a feasible tool to integrate with insecticide treated nets. Curtains afford protection to all household residents and by employing durable LLIN material, curtains may last several years reducing their annual cost and making them a relatively cost-effective tool for malaria prevention.

Email address for correspondence: mombok@ke.cdc.gov

740
Laboratory and semi field evaluation of ICON® MAXX “a treatment kit for converting mosquito nets into long-lasting insecticide-treated nets [MIM16394507]


Insecticide-treated nets are an important method of preventing malaria. To remain effective, they need to be re-treated with a pyrethroid insecticide at least twice yearly. Systems for re-treating nets in Africa are limited, and the vast majority of nets currently in use have never been treated or were treated only once. To overcome this problem, Syngenta, Switzerland, has as manufactured ICON® MAXX which is a “dip-it-yourself” treatment kit which can be used to convert mosquito nets into long-lasting insecticide-treated nets. Treatment of the net is done post-manufacture under field conditions, after which no re-treatment will be required. We evaluated for the efficacy and wash fastness of ICON® max in semi field condition against wild free flying malaria vector mosquitoes. The evaluation of efficacy of ICON® MAXX was conducted in laboratory bioassays and in experimental huts following the standard WHOPES Protocol (WHO 2006, WHO/CD/C/NTD/WHOPES/GCDPP/2006.3). The main evaluation criteria were based on mortality score and blood feeding inhibition effects. We evaluated and compared the following treatment arms: unwashed ICON® MAXX; ICON® MAXX net washed 20 times; Lambda-cyhalothrin 15 mg/m² treated net (ITN) washed 20 times; Lambda-cyhalothrin 15 mg/m² treated net (ITN) washed to cut off point (4 times). Unwashed ICON® MAXX net and ICON® MAXX net washed twenty times inflicted 75.8% and 72.7% mortalities against An gambiae, respectively, whereas ITN washed to cut off scored only 36.2% mortality. Blood feeding success was scored as 24% with Icon max net washed twenty times and that of ITN washed to cut off was 40%. Similar trends were observed with An funestus results. Net treated with ICON® max and washed twenty times or twenty seven times outperformed ITNs washed to cut off point. This finding raises the prospect of conventional polyester nets being converted into LN nets through simple dipping in the community or at home; hence presumably solving the problem of low re-treatment consequently increasing LN coverage.

Email address for correspondence: smagesa@hotmail.com
741 Characterization of Anopheles gambiae s.l. and their insecticide resistance profile relative to physicochemical properties of their breeding habitats within Accra metropolis, Ghana

Bilali I. Kabula, Paul K. Attah, Michael D. Wilson, Daniel A. Boakye

Anopheles gambiae complex is established to contain the main vectors that transmit malaria in Ghana. This study was conducted to characterize this complex and determine their pyrethroid resistance profiles relative to physicochemical properties of their breeding habitats in urban Accra. Eight aquatic habitats containing Anopheles larvae were studied. From each habitat, A. larvae and water were sampled. Adult An. gambiae reared from larvae were morphologically identified and tested for permethrin (0.75%) and deltamethrin (0.05%) resistance using WHO bioassay method. An. gambiae s.s. found were identified to their molecular levels and kdr mutation detected using PCR-based methods. Twenty-nine physicochemical parameters of each water sample were measured and their levels linked with the proportions of An. gambiae s.s. molecular forms in the habitats. A total of 2257 mosquitoes were morphologically identified as An. gambiae s.l. All 224 processed for PCR were identified as An. gambiae s.s., of which 56.46% and 43.54% were M- and S-forms respectively. Both forms occurred in sympatry in larval habitats and no S/M hybrids were detected. However, M-form larvae were in high proportion in polluted habitats than S-form. An. gambiae s.s. were highly resistant to both deltamethrin and permethrin with mortality rates of 42.98%–70% and 6.5%–20% respectively. The frequency of kdr mutation was 60.50% (n = 195), occurring in both S- and M-forms. The adaptation of An. gambiae s.s. in polluted water coupled with occurrence of insecticide resistance is quite alarming particularly for urban malaria control. This needs further exploration in a wider context.

Email address for correspondence: bika72@yahoo.com

742 The influence of larval stage and density on oviposition site-selection behaviour of the Afro-tropical malaria mosquito Anopheles gambiae Giles sensu stricto (s.s.) [MIM16334839]

Victor Mwingira

This study investigates the influence of age structure and densities of conspecific larvae on the oviposition strategy of the malaria mosquito Anopheles gambiae s.s. Gravid female mosquitoes were exposed to: (i) larvae in the first developmental stage; (ii) larvae in the fourth developmental stage; (iii) larvae in first and fourth developmental stages; (iv) larvae conditioned water; (v) screened larval cups. Gravid An. gambiae deposited 30 times more eggs in cups with 100 first-instars larvae than in cups with water only (p < 0.01). Gravid An. gambiae laid 22 times more eggs in control cups compared to cups with 50 fourth-instars larvae (p < 0.05). In a choice test between 50 first against fourth-instars larvae, no eggs were laid in cups with fourth-instars (p < 0.01). Water taken from larval bowls neither attracted nor deterred mosquito oviposition significantly. When cups were covered with filter paper, An. gambiae laid 10 times more eggs in cups with 100 first instars compared to cups with 100 fourth-instars (p < 0.05) and 4 times more eggs in control cups compared to cups with 100 fourth-instars larvae (p < 0.05). This study demonstrates the ability of gravid An. gambiae to detect the presence and development stage of conspecific larvae and to estimate larval density among potential sites. We suggest that young instar produces attracting chemical substances while older instars produce repelling chemicals. Thus, the larval stages can manipulate their parents’ oviposition behaviour and enhance the fitness of their generation. These findings are useful in designing a novel malaria control strategy.

Email address for correspondence: mwingirafamily@yahoo.com

743 Predicting and mapping malaria vectors emergence and geographical distribution cause by climate change [MIM16494708]

Henri E.Z. Tonnang, Richard Kangalawe, Madaka Tumbo, Elikana Kalumanga, Pius Yanda

Temperature rises provoked by climate change is extending the habitats of malaria vectors. This has a great impact on health situation in Africa where malaria remains one of the main causes of mortality. Better climate change adaptation for malaria requires good understanding of the flow of its vectors. The development of the model was carried out through calibration of CLIMEX parameters. CLIMEX help in estimating the potential geographical distribution and seasonal abundance of a species in relation to climate. The factors related to malaria vector species were used in determining the Ecoclimatic Index (EI). The EI was used to generate maps for the potential new distribution of anopheles species. The study provides maps showing the flow and emergence of malaria vectors in Africa. These maps could serve for National Programme of Malaria Control, WHO/AFRO and RBM in developing and implementing sustainable policies on malarial based vector control tools. The maps will be helpful at various levels of decision making in setting up early warning and sustainable strategies for climate change and climate change adaptation for malaria vectors control programmes in Africa and other part of the world.

Email address for correspondence: htonnang@daad-alumni.de

744 Insecticide susceptibility and vector status of natural populations of Anopheles arabiensis from Sudan [MIM13588971]

H. Abdalla, T.S. Matambo, L.L. Koekemoer, A.P. Mnzav, R.H. Hunt, M. Coetsee

Species composition, blood meal source, sporozoite infection rate, insecticide resistance and the kdr mutations were investigated in the Anopheles gambiae complex from 13 sentinel sites in central Sudan. Species identification revealed that 89.5% of 960 specimens were A. arabiensis. Of 310 indoor resting females, 88.1% were found to have fed on humans, while 10.6% had fed on bovines. The overall sporozoite infection rate from the five localities tested was 2.3%, ranging from 0 to 5.5%. Insecticide susceptibility bioassay results showed 100% mortality on bendiocarb, 54.6–94.2% on permethrin, 55.4–99.1% on DDT and 76.8–100% on malathion. The kdr analysis by PCR and sequencing revealed the presence of the Leu–Phe mutation in both permethrin and DDT bioassays. There was no significant difference in the frequency of kdr (P > 0.05) between dead and surviving specimens. These findings have serious implications for the malaria control programmes in Gezira and Sennar states.

Email address for correspondence: hiba_mohamed@hotmail.com

745 Dynamics of malaria vectors and insecticide resistance in a highland village in northern Ethiopia [MIM16363773]

Meshesha Balkew, Ibrahim Elhassen, Muntaser Ibrahim, Howard Engers, Abraham Aseffa, Teshome Gebre-Michael

Malaria is highly prevalent in the Lake Tana region in north Ethiopia where occasional epidemics claim a number of lives. The most
recent out break occurred in the 2003/2004 malaria transmission season. In order to understand the vector dynamics and insecticide resistance of the malaria vectors, entomological studies were conducted in 2006 and 2007 in a highland village, Gorgora. Adult anophelines were collected from human habitations, cattle sheds and out door resting locations. Enzyme linked immunosorbent assay was applied to detect infection and blood meal sources. Standard insecticide susceptible WHO test kits were employed to detect physiological resistance to DDT, permethrin and deltamethrin and determine phenotypes of susceptible and resistant individuals. The gene responsible for resistance was detected from An. arabiensis using PCR assays and gene sequences. Overall 8776 anophelines belonging to eight species were sampled. An. pharoensis and An. arabiensis (the only member of the An. gambiae complex) were the dominant species. Very few mosquitoes were found resting in houses. More than 1,000 An. arabiensis and 800 An. pharoensis were analyzed for infection of P. falciparum, P. vivax polymorphs of 210 and 247 but none was found with infections. Blood meal analysis of An. arabiensis showed higher propensity to human blood than cattle. Both knockdown and mortality rates showed lower susceptibility of both An. arabiensis and An. pharoensis to DDT and the pyrethroids. The West African type of knock down resistance (kdr) mutation was detected. As elsewhere in the country, An. arabiensis is the major vector in the study area. Collections of small number of mosquitoes from indoors are suggestive of avoidance of human dwellings, as a result of behavioral response probably developed a long time ago, influenced by indoor insecticide sprays. In the last two transmission seasons, mosquitoes were devoid of infections, a result corresponding to the low infection rate observed in humans. Both An. arabiensis and An. pharoensis were shown to be highly resistant to the insecticides used for indoor residual sprays and treating mosquito nets. The detection of the kdr gene in An. arabiensis might implicate the role of this gene for resistance to DDT and the pyrethroids.

Email address for correspondence: meshesa_b@yahoo.com

746 Iterative development of a novel exposure free Ifakara tent trap for monitoring malaria vectors population [MIM16757752]

Nicodem J. Govella, Prosper P. Chaki, Yvonne Geissbuhler, J.D. Charlwood, Robert A. Anderson, Gerry F. Killeen

Efficient and effective mosquito traps are important for monitoring and assessment of any malaria vector control programme. In the Dar es Salaam Urban Malaria Control Program (UMCP), human landing catch (HLC) is the only method currently sensitive enough for monitoring Anopheles gambiae. HLC is labour intensive, requires intense supervision and increases the participant’s exposure to the risk of malaria infection. Novel sampling tools were iteratively developed. The Ifakara A, B tent trap designs and the CDC Light Trap (CDC-LT) were compared with HLC in rural urban Tanzania, using Latin-square and cross over experimental designs, respectively, and analysed using generalized estimating equations. Ifakara B was then modified by changing the structure of netting panels and compared with Ifakara B in rural Tanzania using similar methodology. Ifakara B catches correlated far better to HLC ($r^2 = 0.73$, $P < 0.001$) than any other method with a sensitivity varying from 0.32 to 0.65 relative to HLC. Nonetheless it failed to reduce the proportion of fed mosquito relative to HLC ($P = 0.998$). The Ifakara C, however, reduced the proportion of fed mosquitoes (OR [95% CI] = 0.27 [0.12, 0.59], $P = 0.001$) and was twice as sensitive as design B. The Ifakara C trap has potential for monitoring and evaluation under programmatic conditions. Despite being exposure free, validation by comparing with HLC may be necessary to confirm that this design produces results which are epidemiologically meaningful.

Email address for correspondence: govella@ihi.or.tz

747 Impact of insecticide-treated bed nets (ITNs) distribution program on malaria vectors densities and parasite infection in western Kenya Highlands [MIM15047127]

Yousif E. Himeidan, Yaw A. Afrane, Harrysone Atieli, Peter Wamae, Andrew K. Githeko, Guiyun Yan

Malaria vectors larvae were found cluster around the main valleys of western Kenya highlands, but its occurrences in hilltop villages in a distance away from the main valleys where inhabitants are known vulnerable to malaria infection is remain mysterious. Understanding this occurrences in relation to topography and habitat stability during the potential epidemic season of El Niño promote to identify the limits of vector control in East African highlands. A total of 109 larval habitats were identified at two topographical level of uphill and stream valleys in two hilltop villages of Kakamega district. Water availability in the habitats was followed daily from August 3, 2006 to February 23, 2007. Habitat was defined stable when remains aquatic continuously for at least 12 days. Mosquito larvae were observed weekly. Frequencies of aquatic, stable and larvae positive habitats were compared among the different topography and seasons using $x^2$-test. Factors associated with occurrences (present/absent) of Anopheles gambiae larvae in the area were determined using multiple logistic regression analysis. In the uphill, neither the stream valley, the frequency of daily occurrence of aquatic habitats was low and restricted to the weekly cumulative rainfall with time lag effect of 2 weeks. The proportion of that a positive habitat can be occurred in the uphill was 0.01. In the stream valley, the frequency of aquatic habitat was remained the same during and after El Niño, but the mean length of days that habitat stayed aquatic was significantly high after El Niño whilst stable habitats occurred in high frequency. Both An. gambiae and An. funestus larvae were often occurred in the stream valley and more significant after El Niño season. Factors associated with An. gambiae larvae occurrence were topography, habitat surface area and number of days that habitat stayed aquatic. In hilltop areas during the potential malaria epidemic season of El Niño, malaria vectors larvae can breed locally at small valleys where streams are starting flow within to the main one. Larval control targeted at these valleys may be more cost-effective for malaria epidemic interventions in East African highlands.

Email address for correspondence: yosifhimeidan@hotmail.com

748 Community participation in decision making in mosquito control in Malindi District, Kenya [MIM15877389]

Lydia W. Kibe, Francis Kerre, Paul Achola, Sassy Molyneux, Peter Luethy, Anisa Omar, John I. Githure, Charles M. Mbogo

A qualitative study was conducted in Malindi District to document and highlight the process and challenges of community participation in decision making in mosquito control. Focus group discussions were conducted with members of organized community groups involved in mosquito control activities. Individual interviews were held with stakeholders and key individuals from organized community groups while participant observations were made during group meetings. Purposive and convenience sampling was used to select study participants. Results from the study indicate that PUMMA (Punguza Mbu Malindi), a voluntary community...
group acts as an umbrella body coordinating 10 voluntary community groups in mosquito control activities in Malindi. Major activities include observance of an annual mosquito field day event, buying and selling of insecticide treated bed nets, filling and draining of stagnant water and educating residents on mosquito control. Perceived social pressure, importance attached to mosquito control and perceived benefits were cited as individual motivations to join voluntary community groups. The study further shows that factors such as individual expectations, organizational support and community characteristics contribute to the success of community actions in mosquito control. Community groups were motivated by support from the stakeholders in form of training, resource mobilization, material support and expectations of "better things to come". Lack of tools, inadequate finances and lack of knowledge on mosquitoes hindered group decision making power. In conclusion, PUMMA a grass-root community group has the potential for influencing community participation in mosquito control in Malindi. However, additional technical guidance and financial support needs to be provided to the groups to facilitate their activities and enhance their interest in mosquito control.

Email addresses for correspondence: lkibe@kilifi.kemri-wellcome.org

749
Dynamic archiving system for tracking mosquito data
Zacharia Mtema, Liz Burd, Deo Maliti, Beatrice Chipwaza, Oscar Mukasa, Prosper Chaki, Alpha Maligania, Nicodemas Covella, Joseph Wmbura, Sarah Moore, Gerry Killeen, Tanya Russell

To solve today's malaria problems, scientists need new bioinformatics tools to synthesise and validate data across studies, space and time. Historically, medical entomologists have often stored data idiosyncratically and still face many problems when managing, archiving and searching datasets. We describe the development of a generic schema and database for collecting and managing ecological mosquito data. The database is web-based and was developed using PHP, MySQL and Java scripting languages. The use of a generic schema ensures that all essential information is collected by the researcher and also provides for controlled vocabulary and coding. The database allows the scientist to select which scientific observations will be recorded (e.g. household mosquito collections, ovary dissections, or CSP ELISA) and provides the template for data entry. The hidden information in the database includes data validation, the relationships between attributes, data encryption and the ontology. Scientists who adapt the generic schema and database will benefit from automated data integration, improve the quality of data archives, data validation, time management and provision for structural metadata. Because the database is web-based it allows researchers to share and monitor research projects from geographically remote locations. The archiving of samples and data will allow in future studies (i.e. new molecular analyses) or statistical analysis. Importantly, the database will help to drive entomology data synthesis and sharing across collaborative projects.

Email address for correspondence: zjohn@ihi.or.tz

750
Detection of fungal infections in malaria vectors population using molecular tools in Kilombero valley
Dickson Lweitojera

The study aims to determine entomopathogenic fungus (Beauveria bassiana) infections using quantitative PCR in An. gambiae s.l and An. funestus under field conditions in the Kilombero valley. Malaria is the mosquito borne disease causing the biggest public health burden worldwide. Vector control strategies, use of insecticides (ITNs and IRS), and larvicides (Bti), have been put into practices. However current existing multifaceted efforts need to be strengthened due challenges of insecticides resistances, mosquitoes behaviour changes, and operational costs. There is a need to develop entomopathogenic agent which has shown a potential in slow killing of malaria vectors as a novel biocontrol strategy that can complement on existing methods to sustain the pressure of the diseases and shift dynamics towards the eradication. The question will be answered by examining the impact of the fungus on mosquitoes using a before/after and control/impact experimental design. Mosquitoes will be collected simultaneously throughout the year to show the seasonality change with mosquito abundance. Up to 6 households will be sampled each night during the 3–5 days weekly sampling period for 3 weeks in a month, using one CDC light trap, cardboard box and four resting boxes for indoor and outdoor resting mosquitoes respectively. Fungal substrates will be collected from same houses. Therefore we aim to develop a multi-plex PCR that contain primers for B.bassiana and An. gambiae s.l. Data will be analysed to determine the status of infection by natural and specific B. bassiana strain, and the effect of seasonal variation on fungal.

Email address for correspondence: vmayagaya@ihi.or.tz

751
The impact of livestock availability on malaria vector abundance, species composition and feeding behaviour in an endemic region of Tanzania
Valeriana Mayagaya, Japhet Kihonda, Matthew Alexander, Tanya Russell, Heather Ferguson

We investigated the impact of livestock availability on the abundance and species composition of Anopheles malaria vectors in the Kilombero Valley, Tanzania, where malaria is endemic. In the wet season 2008 (February–June), mosquitoes were collected from 6 to 8 households in 10 villages (half where cattle were present, half not). Mosquitoes were sampled from houses, animal sheds, and outdoor resting boxes over 4 days. PCR was performed to identify the species of individuals in the An. gambiae s.l. species complex, as were ELISAs to identify the human blood index (HBI). 8543 An. gambiae s.l. and 418 An. funestus were collected during the study. The average number of An. gambiae s.l. captured in light traps varied substantially between households, but not between villages. The abundance of An. gambiae s.l. was approximately 1.3 times higher at households with livestock than without (p < 0.001). Overall, An. arabiensis was the most abundant species (74.4% of the An. gambiae s.l complex, n = 2735), and was more prevalent at households with livestock (92%) than without (60%). As expected, the human blood index of An. arabiensis was substantially lower (34.5%) than An. gambiae s.s. (94.5%). The presence of livestock at households reduced the HBI of An. arabiensis from 100% to 27.5%, but had no impact on the HBI of An. gambiae s.s. The substantial drop in human feeding observed in the presence of livestock suggests zooprophylaxis may be a viable malaria control option in areas such as Kilombero where An. arabiensis is the dominant vector.

Email address for correspondence: vmayagaya@ihi.or.tz
752 Development of entomopathogenic fungi for the control of adult African malaria vectors in Tanzania [MIM16668886]

Ladslaus L. Mnyonea, Matthew J. Kirby, Monika W. Mpingwa, Dickson W. Lwetoijera, Bart G.J. Knols, Willem Takken

Biological control using fungal entomopathogens has great potential as a vector control strategy. It has been demonstrated that the fungi *Metarhizium anisopliae* and *Beauveria bassiana* can infect and kill adult malaria vectors, reduce their blood feeding propensity and fecundity, and impair the development of malaria parasites inside the mosquito. Methods: To produce an efficacious fungal agent for use in a village-scale trial, a series of laboratory investigations and small-scale field trials were conducted. A series of variables purported to affect the efficacy and persistence of *B. bassiana* ICIPE-30 and *B. bassiana* 193-825 against adult *Anopheles gambiae* sensu stricto were assessed. These were spore concentration (1 × 10^7 to 4 × 10^10 spores/m^2), carrier oils, exposure time (15 min to 6 h), delivery surface (netting, cotton cloth and mud wall), mosquito age (2–12 days) and time since bloodmeal (3–72 h). Co-formulations of the two fungal species in ratios of 4:1, 2:1 and 1:1 were also tested. Mosquitoes were exposed to fungal formulations applied to paper inside holding tubes, except when different delivery surfaces were assessed. The most efficacious formulations in the laboratory tests were assayed in experimental hut trials in a rural village setting. Spore-treated netting of a range of mesh sizes was fitted over the eaves of the huts, and all other routes of entry closed, to determine whether wild *Anopheles* mosquitoes could be infected during passage through the netting into the huts. Mosquitoes were caught leaving the huts in exit traps. Results and discussion: The results and discussion of these laboratory and field-based experiments will be presented. Email address for correspondence: laurent@ihi.or.tz

753 Evaluation of long-lasting organophosphate indoor residual spray (IRS) formulations for control of susceptible and pyrethroid resistant *Anopheles gambiae* and *Culex quinquefasciatus* in Benin and Tanzania [MIM16692016]


The Pan-African Malaria Vector Research Consortium (PAMVERC) is a partnership of three African vector control research sites specializing in development and evaluation of malaria vector control products and interventions. PAMVERC sites, Moshi, Tanzania and Cotonou, Benin, are evaluating long-lasting IRS formulations of an existing WHOPES-approved organophosphate (OP) developed by Syngenta. The aim is to develop an OP with effective residual lifespan of 6 months or more. Blocks of concrete, dried mud, and wood were sprayed using Potter Tower precision spray equipment. WHO cone bioassay of pyrethroid susceptible and resistant *Anopheles gambiae* and *Culex quinquefasciatus* are tested every 4 weeks to evaluate the effective lifespan of these products. Lambda-cyhalothrin and the existing OP formulation are being used as controls. Field trials using experimental huts are running concurrently to compare residual efficacy against wild mosquitoes. Initial results are encouraging and show the new formulations are killing a high proportion of all species and strains tested. There are early signs that at least one of the formulations has a longer residual lifespan than the existing formulation. This research is a prime example of successful collaboration between research sites in Africa, northern institutions, and the chemical industry to develop new insecticide products in the fight against malaria and dengue. Early results show promise and if they continue to be positive a valuable long-lasting insecticide showing no cross-resistance to pyrethroids will be added to the limited arsenal for use against mosquitoes. Email address for correspondence: fmosha@hotmail.com

754 Insecticide selection decisions for IRS in the US Government President’s Malaria Initiative: The contribution of entomological evidence [MIM16650134]

Rodaly Muthoni, John Morgan, Hilary Ranson, Eve Worrrall, John Chimumbwa

RTI is supporting the President’s Malaria Initiative (PMI) to carry out Indoor Residual Spraying (IRS) in a number of African countries. LATH and LSTM support these efforts by providing technical assistance to strengthen in-country capacity to make evidence based decisions. Decisions on insecticide selection must take into account levels of vector susceptibility to insecticides and their residual efficacy under local conditions. This paper reports on how entomological data informs programmatic IRS decision making in PMI countries. Before the insecticide class for the next round of IRS in a country is selected the susceptibility of the local malaria vectors must be established. WHO susceptibility tests should be used to monitor resistance to DDT, pyrethroid and carbamate insecticides. After IRS is complete, WHO cone bioassays can then be employed to measure the residual efficacy of the insecticide on the sprayed surface. The above protocols were followed in three IRS districts in Northern Uganda. Two of these districts had previously sprayed with DDT and one with lambdacyhalothrin. High levels of DDT and pyrethroid resistance were detected in *Anopheles gambiae* from all three districts but the malaria vectors remain susceptible to carbamates. The cone bioassays were conducted six months post spraying by which time the residual activity of both insecticide classes was poor timely implementation of simple entomological monitoring can help improve the efficacy of IRS programme. Email address for correspondence: rmuthoni@nb.rti.org

755 A self-sustaining population for studying ecology and behaviour of African malaria mosquitoes [MIM16710785]

Kija Ng’habi

The prospects to use the sterile insect technique and genetically engineered insects to control malaria vectors are promising. However, their field application is mainly hampered by our limited knowledge of the biology and ecology of the malaria vectors. Ecological and behavioural studies of these vectors in their natural settings are difficult to undertake due to seasonal fluctuation in the vector population density and limitations in samplings techniques and tools. Also laboratory colonized strains have been reported to provide unrealistic representation of their field counterparts due to genetic bottlenecking. Thus, a near realistic, population establishment is required to address this gap. In this study we report, a successful establishment of a self sustaining An. arabiensis population in a near natural environment. This development is novel, as for the first time in the world a large An. arabiensis self-sustaining population is being established in near natural environment. This development is of practical implication not only that it will easy ecological and behavioural studies of this vector, but a potential intermediate testing ground for feasibility studies of SIT and GM vector control approaches. Email address for correspondence: kija@ihi.or.tz
756  
**Modeling the suitability of odor-baited mosquito traps for malaria control in Africa [MIM16030605]**
Fredros Okumu, Sarah J. Moore, Gerry F. Killeen

Synthetic human odors have been proposed as potential elements of future technologies against malaria vectors. However, it is not clear whether such technologies would be appropriate, especially where Insecticide Treated Nets (ITNs) are increasingly being adopted. Using a deterministic mathematical model, we investigated the suitability of odor-baited mosquito traps for malaria control in Africa, paying attention to the trends of ITNs use. We simulated scenarios with different vector species, ITN properties and presence or absence of cattle. In each scenario we examined effects of the traps by varying the degree of bait attractiveness to malaria vectors and the method of spatially allocating those traps.

The number of odor-baited traps required in any simulated scenario was significantly lower when ITNs had diversionary characteristics as opposed to when the nets were mainly toxic to mosquitoes \( (P < 0.001) \). Similarly, we project that fewer traps would be required where residents do not keep cattle than where cattle are introduced and also where the main vector is *An. gambiae* s.s. than where it is *An. arabiensis* \( (P < 0.001) \). The critical number of traps required to provide 80% protection to persons not using ITNs can be decreased by using more attractive odor baits as well as by locating the traps in malaria transmission ‘hotspots’. In malaria endemic Africa where ITN coverage is increasing, odor-baited traps can substantially increase protection against malaria. Most of this additional protection would go to people who do not already use nets. We recommend that trap developers should focus on super-attractive baits as well as on cheap and small traps which can be easily deployed even in large communities.

Email addresses for correspondence: fredros@ihi.or.tz

757  
**Insecticides susceptibility status of the malaria vector Anopheles arabiensis Patton at the African continental fringe in Sudan [MIM16093275]**
Osama M.E. Seidahmed, Hamooda T. Kafy, Mustafa Y.H. Dakeen

The northern continental fringe for the distribution of *Anopheles arabiensis* in Africa resides in Sudan, particularly at the northern region. In this work, current status of insecticide susceptibility at this area is reported. Susceptibility tests were carried out on six sentinel sites. All tests were performed according to WHO protocols for eight types of insecticides: deltamethrin 5%, permethrin 0.75%, lambdacyhalothrin 0.05%, DDT 4%, malathion 5%, fenitrothion 1%; bendiocarb 1% and propoxur 0.1%. The total number of all tested specimens is 3438 composing 155 exposure replicates. Overall, *An. arabiensis* is susceptible to all types of insecticides except being tolerant to Malathion. Mortality rates were 100% to the susceptible ones except DDT (98.42%). However, *An. arabiensis* was tolerant to Malathion at three sites averaging in a mortality rate of 97.53%. Knockdown times revealed Deltamethrin has a fastest time \( (KDT50 = 8.44 \text{ and } 9.14, \text{ females and males, respectively}) \). The highest % respondents knocked down after 30 min was by permethrin \( (99.11\% \text{ and } 98.67\%, \text{ females and males, respectively}) \). In respect to malathion, a significant difference on KDT50 \( (97.53\%) \). Knockdown times revealed Deltamethrin has a fastest time \( (KDT50 = 8.44 \text{ and } 9.14, \text{ females and males, respectively}) \).

Even so, IRS benefits are temporary because treatments need to be reapplied. New technologies, such as Durable Residual Wall Lining (DL) may eliminate the need for a re-spray program in rural communities. DL is a dual purpose tool for traditional house improvement and malaria prevention in development since 2004.

758  
**Laboratory and semi field efficacy of PermaNet 3.0 a bite-treated mosquito net for combating insecticide resistance in mosquitoes [MIM16394444]**
Patrick Tungu, Robert Malima, Frank Magogo, Wema Sudi, Joseph Myamba, Stephen Magesa, Caroline Maxwell, Mark Rowland

Insecticide-treated bed nets (ITN) are undoubtedly among the proven effective malaria control tools. However, the commonly observed low re-treatment rate and emerging pyrethroid resistance threaten the usefulness of the ITNs for malaria control. In the context of fighting insecticide resistance and low re-treatment rate, Vestergaard Fransden has developed PermaNet® 3.0; a long lasting (LN) insecticide combination treated net, bi-treated with deltamethrin and the oxidase synergist piperonyl butoxide (PBO).

We evaluated PermaNet® 3.0 for its efficacy and wash fastness in semi field condition against wild free flying mosquitoes. The evaluation of efficacy of PermaNet® 3.0 was conducted in laboratory and in experimental huts following the standard WHOPEs Protocol (WHO 2006, WHO/CONTD/WHOEPS/GCDPP/2006.3). The main evaluation criteria were based on mortality score and blood feeding inhibition effects. We evaluated and compared the following treatment arms: Unwashed PermaNet® 3.0; Unwashed PermaNet® 2.0; PermaNet® 3.0 washed 20 times; PermaNet® 2.0 washed 20 times; Deltamethrin 25 mg/m² treated net (ITN) washed 3 times (positive control) and an untreated net (negative control). Unwashed PermaNet® 3.0; PermaNet® 3.0 washed 20 times and PermaNet® 2.0 treatments were more effective in reducing blood feeding success of both *Anopheles gambiae* and *Culex quinquefasciatus* (pyrethroid resistant strain) than the untreated control net. However, this success was statistically similar to that scored with ITN washed 3 times. The unwashed PermaNet® 3.0 outperformed the unwashed PermaNet® 2.0 in terms of mortality inflicted against *C. quinquefasciatus*. However, mortalities scored for both net types after 20 washes were found to be statistically similar. Similar results were recorded laboratory tunnel tests. This observed higher efficacy of the PermaNet® 3.0 against pyrethroid resistant mosquitoes raised the prospect of it being a more effective malaria control tool even in areas with pyrethroid resistant mosquitoes.

Email address for correspondence: PATRICKKIJATUNGU@HOTMAIL.COM

759  
**Durable Residual Wall Lining (DL) as a replacement for IRS in malaria vector control [MIM16669401]**
Richard Allan, Mikkel Vestergaard, Omer Imtiazuddin

Community protection requires high coverage for maximum impact on malaria transmission (>85% households). Indoor residual spraying (IRS) provides protection without significant behavioral change by the family. Continued coverage is only achieved with significant and sustained levels of political commitment, leadership, and management of planning, organization, and implementation. Even so, IRS benefits are temporary because treatments need to be reapplied. New technologies, such as Durable Residual Wall Lining (DL) may eliminate the need for a re-spray program in rural communities. DL is a dual purpose tool for traditional house improvement and malaria prevention in development since 2004.
DL material combines aesthetic decorative values with vector control performance based on IRS principles. The concept is an adaptation of WHO-approved technology developed for other materials including LNs using deltamethrin. Field trials were established in Benin, Equatorial Guinea, Kenya, Angola, Mali, Ghana, South Africa, Vietnam, and India in 2008 to evaluate installation, durability and household acceptability of DL. Each trial had baseline survey prior to installation, 1-month opinion survey combined with inspection and photo documentation, and periodic entomological and residual analyses during 12 months post-installation. Preliminary results showed a high user acceptance at all locations. Country-specific reports provided feedback on durability and efficacy under conditions where DL was installed on mud, concrete, and wood walls. Results from these studies confirm the advantages of Durable Residual Wall Lining (DL) over traditional IRS programs across a variety of housing conditions. They also identified user preferences between these new tools and IRS.

Email address for correspondence: richard@mentor-initiative.net

760 Ecological Niche modeling and habitat suitability of major malaria vectors in Cameroon, Central Africa [MIM16600772]

Diego Ayala, Carlo Costantini, Kenji Ose, Guy Colince Kamdem, Christophe Antonio-Nkondjio, Jean-Pierre Agbor, Parfait Awono-Ambene, Didier Fontenille, Frederic Simard

Suitability of environmental settings determines species’ distribution in space and time. Modeling mosquitoes’ ecological niche can therefore reveal a powerful predictor of the risk of exposure to the diseases they transmit. In Africa, five anopheline mosquito species are responsible for more than 95% of total malaria transmission. However, current knowledge of these species’ distributions remains incomplete. Mosquitoes were sampled in 386 villages throughout Cameroon, Central Africa, across a wide range of ecological settings. Using a predictive species distribution model based on presence-only, habitat suitability was modeled for the five major malaria vectors in Cameroon: Anopheles gambiae, An. funestus, An. arabiensis, An. nili and An. moucheti. Ecological Niche Factor Analysis, habitat suitability modeling and Canonical Correspondence Analysis revealed marked differences among the five major malaria vector species, both in terms of ecological requirements and niche breadth. Geo-geographical variables (EGVs) related to human activities showed a major influence on the niche breadth of all afrotropical malaria vectors. Rainfall, evapotranspiration, sunlight exposure and wind speed were among the most discriminative EGVs separating “forest” and “savanna” species. Major malaria vector species distribution in Africa is prominently linked to the proximity of human settings. Overall, An. gambiae and An. funestus are present in a wide range of ecological settings compared to other major vectors, which are more ecologically specialized. Such approach should help improve malaria vector control implementation by targeting interventions to specific places and at specific times where the impact on vector populations and disease transmission intensity will be maximized.

Email address for correspondence: dayalag@yahoo.es

762 Fungal infection counters insecticide resistance in African malaria mosquitoes [MIM16691118]

Marit Farenhorst, Joel C. Mouatcho, Christophe K. Kikankie, Basil D. Brooke, Richard H. Hunt, Matthew B. Thomas, Lizette L. Koekemoer, Bart G.J. Knols, Maureen Coetzee

The evolution of insecticide resistance in mosquitoes is threatening the effectiveness and sustainability of malaria control programmes in various parts of the world. Use of entomopathogenic fungi as biopesticides provides a promising alternative to chemical control. We investigated the susceptibility of insecticide-resistant anophelines to fungal infection and potential interactions between fungal infection and insecticide resistance. Susceptibility of insecticide-resistant mosquitoes to Beauveria bassiana infection was tested using four Anopheles strains with high resistance levels against pyrethroids, organochlorines or carbamates. Survival after fungus exposure was measured in resistant mosquitoes and their baseline counterparts. Effects of fungal infection on the expression of permeability and DDT resistance were tested in three resistant Anopheles strains. Mortality after insecticide exposure of mosquitoes pre-infected with B. bassiana or Metarhizium anisopliae was compared with uninfected control mortality. Insecticide-resistant mosquito strains were equally susceptible to B. bassiana infection as their baseline counterparts. Survival analyses showed that fungus infection decreased mosquito longevity in all tested mosquito strains. Fungal infection reduced the expression of resistance to permethrin and DDT. Insecticide-resistant mosquitoes pre-infected with B. bassiana or M. anisopliae showed a significant increase in mortality after insecticide exposure compared with uninfected mosquitoes. Results show that entomopathogenic fungi can effectively infect and kill insecticide-resistant Anopheles mosquitoes and decrease the expression of insecticide resistance after fungus infection. Fungal biopesticides, therefore, show high potential to complement existing vector control measures and provide novel products for use in resistance management strategies.

Email address for correspondence: marit.farenhorst@wur.nl
Can synergists be used to enhance the efficacy of pyrethroids against pyrethroid resistant mosquitoes? [MIM16667003]

Helen Pates Jamet, Georgina Bingham, Linh Vu, Nam Le, Lien Tra

Pyrethroid resistance can be considered the main threat to the continued control of many mosquito vectors of disease. Piperonyl butoxide (PBO) has been used as a synergist for many years to help increase the efficacy of certain pesticides. This enhancement stems from its ability to inhibit two major metabolic enzyme systems, microsomal oxidases and non-specific esterases and enhance penetration of the insecticide. This study investigated the mortality rate of a characterized resistant mosquito strain using deltamethrin alone and in combination with PBO. The resistance mechanisms in a pyrethroid resistant strain of *Aedes aegypti* were characterized using standard PCR for *kdr* mutation and biochemical assays. The WHO cone bioassay was used to investigate the mortality rate with incorporated deltamethrin treated nets with and without PBO. PBO used in combination with deltamethrin resulted in a higher mortality rate than when deltamethrin was used alone. These results will be discussed in the context of the resistance mechanisms that were identified. The ability of PBO to enhance the efficacy of pyrethroids currently used in vector control products is determined by the resistance mechanisms present in the mosquito species. Synergists have an important role to play in the future design of vector control products in an era when alternatives to pyrethroids are very limited.

Email address for correspondence: hjp@vestergaard-frandsen.com

Simple tests for measuring insecticides in treated/sprayed surfaces [MIM16678583]

John Vontas, Andrew Dowd, Evangelia Morou, Hanafy Ismael, Andrew Stevens, Janet Hemingway, Mark J.I. Paine

Bednets and IRS are major control measures against malaria. The only way to check that protection is being provided by an intervention is to measure the amount of insecticide residue. Such information is important for local users for routine testing and QC, to provide early warning of population risk/control failure and support evidence-based policy. Detoxification enzymes with affinity for insecticides, isolated from resistant mosquitoes, have been employed to develop simple tests, for measuring insecticides. Here we have exploited GST/dehydrochlorinase (GSTe2) for measurement of DDT. The GSTe2 biosensor assay is based on the ability of the enzyme to metabolize DDT and proportionally release $H^+$ and Cl$^-$ ions, which can be easily detected. The system is specific for the insecticidal ppDDT. Simple strip and electrode formats suited to field conditions have been developed. The test involves extraction with sello-tape and measurement of DDT using Cl or pH indicators, which allows visual assessment of the insecticide levels. Validation against samples from the field demonstrates reproducibility and reliability compared with HPLC. Additional insecticide detection systems are under development. Simple tests are described which could aid the implementation of malaria control interventions.

Email address for correspondence: vontas@imbb.forth.gr

Factors affecting *Plasmodium falciparum* sporozoite production in *Anopheles* mosquitoes [MIM16566366]

G. Humphreys, L. Ranford-Cartwright

The malaria parasite is haploid for most of its life cycle, with a brief diploid stage when the zygote is formed after fertilisation between haploid gametes in the mosquito midgut. Fertilisation can take place between genetically identical gametes arising from human infections of a single parasite genotype (selfing) or between genetically dissimilar gametes arising from human infections of mixed parasite genotypes (crossing). The zygote develops into an oocyst on the mosquito midgut wall within which sporozoites subsequently form. Sporozoites then make their way to the salivary glands ready for inoculation into the next host. Relatively little is known about the genetic or environmental factors which influence sporozoite production within a single oocyst. Our aims were to study the variation in sporozoite numbers (i) from different clones (genotypes) and (ii) in oocysts derived from self-fertilisation events and cross-fertilisation events. In addition we have investigated the influence of environmental factors such as mosquito size and infection density on sporozoite numbers.

Email address for correspondence: g.humphreys.1@research.gla.ac.uk

Managing insecticide resistance in the formulation of vector control strategies [MIM16704095]

Zachary Brown, Katherine Dickinson, Randall Kramer

Anopheles populations in Africa have demonstrated varying abilities to evolve resistance to the major insecticides used in vector control (e.g. in indoor residual spraying, or IRS). We aim to identify decision rules, informed both by this resistance potential and by epidemiological conditions, which minimize the joint costs of malaria infections and insecticide-based vector control programs. We apply continuous time dynamic programming (CTDP) techniques (steady state analysis and numerical simulation) to a synthesized mathematical model of vector-borne disease transmission (Macdonald-Ross) and insecticide-induced mortality and selection for resistant genotypes among the vector population. Reasonable estimates of IRS program costs and conservative estimates of costs per malaria infection suggest in simulations that effective eradication of malaria using an insecticide-based control strategy is optimal if (a) there is a sufficiently high “fitness cost” in resistant genotypes within the vector population (so that eradication using such a strategy is feasible) and (b) initial insecticide-susceptibility is high enough. In general, simulations predict that the decision rules yielded by CTDP can save at least 10% of total costs relative to an otherwise cost-minimizing policy which ignores the “user costs” associated with insecticide resistance. When there is potential for insecticide resistance, simulation results indicate that cost-minimizing insecticide-based vector control programs are highly sensitive to the initial levels of insecticide susceptibility within the vector population. The predicted cost savings from implementing such programs represent the potential benefit to be gained from effective monitoring of insecticide resistance in vectors.

Email address for correspondence: zsb2@duke.edu

Induction and characterization of ion channels in *Anopheles gambiae* cells [MIM16702184]

Lacey J Jenson, Sally L. Paulson, Jeffrey R. Bloomquist

Due to the increasing resistance demonstrated by insects to conventional neurotoxins, the need for insecticides with novel modes of action are becoming increasingly important. The goal of this research is to express insecticides target sites from undifferentiated *Anopheles gambiae* cell lines. This procedure uses a culture of gambiae cells and a mixture of transformation agents
to initiate expression of neuronal proteins. The molting hormone 20-hydroxyecdysone will be added to induce or enhance cell process (axon-like growth). After application of agents such as 20-hydroxyecdysone, insulin, and caffeine have been run, characterization of ion channels and receptors within the An. gambiae cells will be performed. Veratridine, a sodium channel activator, has been shown to increase the number and/or survival of cells differentiated into presumptive neurons by ecdysone, an effect that was blocked by 1 μM tetrodotoxin (a specific sodium channel blocker) in Spodoptera frugiperda (SF21) cell culture studies. Characterization of differentiated cells with any ion channels or receptors that they may express will be accomplished by using inhibitors such as tetrodotoxin to suppress sodium channels and cobalt to block calcium. On-going studies will provide more data on An. gambiae cell differentiation and try to further characterize these cells. Future studies will label calcium channels with a florescent marker, perform an immunofluorescence assay to label sodium channels in these neuron-like cells. We hope to be able to develop these transformed cells for use in high throughput screening platforms in search of mosquitoe-selective insecticides.

Email address for correspondence: ljenson@vt.edu

768 Understanding the effects of climate on malaria transmission potential [MIM16700265]

Krijn P. Paaijmans, Simon Blanford, Andrew F. Read, Matthew B. Thomas

Climate change has the potential to affect the dynamics and distribution of malaria. However, our ability to quantify risk is limited due to the poorly specified relationship between transmission and environmental parameters. Here we show how the influence of temperature fluctuations and extreme events can be as, or more important than changes in mean conditions for malaria transmission. We investigated the effects of mean temperature and temperature fluctuation on key aspects of mosquito and parasite life history using a combination of empirical and theoretical approaches. We find that, in general, temperature fluctuation reduces the impact of increasing mean temperatures. Specifically, we show that diurnal temperature fluctuation around warmer mean temperatures slows processes such as larval development and parasite incubation, whereas fluctuation around cooler mean temperatures speeds up these processes, compared with constant temperatures. These effects suggest that by ignoring fluctuation, we may currently be overestimating malaria risk in warmer environments, and underestimating risk in cooler environments. We further show that the effects of temperature fluctuation are important for understanding the dynamics of seasonal malaria in areas such as the Kenyan Highlands. To better understand the ecology of mosquitoes and malaria we need to consider not just basic measures of ambient temperature, but the fine-scale thermal environment in which the vector–parasite interaction is actually played out. Effects of short-term temperature fluctuations are not widely considered but appear central to understanding current malaria transmission and for evaluating consequences of future climate change.

Email address for correspondence: kpp2@psu.edu

769 Prospects for sustainable malaria control using fungal biopesticides [MIM16700156]

Matthew B. Thomas, Simon Blanford, Andrew F. Read

There is an urgent need for alternative tools to reduce reliance on chemical insecticides in contemporary malaria control programmes. Here we present an overview of recent research demonstrating the potential for using biopesticides based on insect pathogenic fungi, in novel integrated strategies for sustainable control of malaria. A range of laboratory assays were conducted to determine the effects of different fungal isolates on the ability of mosquitoes to transmit malaria. These data were further evaluated using population dynamic and evolutionary models to identify the short- and long-term impacts of fungal biopesticides on malaria transmission. Numerous fungal isolates were shown to infect Anopheles mosquitoes via exposure to biopesticide-treated surfaces. Depending on fungal isolate, malaria transmission potential could be reduced through direct mortality (i.e. virulent isolates killing mosquitoes before they can transmit), conditional mortality (i.e. enhanced impact of fungal infection in mosquitoes carrying malaria) and/or transmission blocking (i.e. development of malaria parasites blocked in mosquitoes following fungal infection). Exploration of these diverse infection phenotypes using models revealed that fungal biopesticides have the potential to cause considerable reductions in the density of malaria-transmitting mosquitoes while potentially imposing minimal selection pressure for evolution of resistance. Our results point to the practical use of insect fungal pathogens within novel strategies of integrated vector management, with potential to both augment existing control measures and enhance long-term sustainability.

Email address for correspondence: mbt13@psu.edu

770 Evaluation de l’environnement sociologique des populations de l’Ouémé et de l’Atlantique, Rép. du Bénin, en prélude à la mise en place de deux outils de lutte antivectorielle: la MIILD et la PID. H. Nlouko; G. Padouon & M. Akogbeto [MIM15053908]

L’approche adoptée actuellement par le Bénin est basée sur la stratégie de Pulvérisation Intra Domiciliaire et l’usage des Moustiquaires de longue durée d’action. La présente étude a pour objectif de recueillir des informations sur la perception des communautés Goun, Toffin et Xla à l’égard du paludisme, sur les moyens que ces communautés utilisent pour se protéger contre les piqûres de moustiques, leurs points de vue sur les pulvérisations intra domiciliaires et les caractéristiques physiques des habitations à traiter à l’insecticide. Les données ont été recueillies auprès de 1201 ménages dans l’Ouémé et 403 dans l’Atlantique, par interview face à face à l’aide d’un questionnaire structuré administré en langues locales. Des focus group discussion avec des observations du cadre de vie interne sont venus complétés ces entretiens individuels. De l’analyse des résultats, il ressort que les principaux outils de prévention contre les piqûres de moustiques sont les moustiquaires (57%; 60%), les serpentins fumigènes (28%; 25%) et les bombes aérosols (7%; 4%) respectivement dans l’Ouémé et l’Atlantique. Plus de la moitié des unités de couchage ne disposaient pas de filet et la majorité (84%) des cases visitées ne révélait pas de plafond. Dans la portion congrue des habitations disposant de plafond, il existe des fissures au plafond (16%) et des plafonds en bambou (21%). Toutes ces caractéristiques sont mal appropriées pour les outils de lutte antivectorielle tels que les MIILD et la PID.

Email address for correspondence: hnoukpo@yahoo.fr
(species composition, susceptibility to insecticides, etc.); (2) efficient implementation of the control intervention; and (3) effective monitoring and evaluation to assess the impact of the interventions. There are, of course, numerous activities that contribute to each of these. This presentation looks at the complexities facing vector control programmes today and the new information that is coming through on vector biology and insecticide resistance. We ask the question “Can malaria be eliminated in southern Africa?” Email address for correspondence: maureen.coetzee@wits.ac.za

772
Entomological monitoring of an indoor-residual spray (IRS) pilot program in northern Nkhota kota, central Malawi [MIM16696716]

T. Mzilahowa, J. Hemingway, D. Mathanga, J. Chiphwanya, V. Uzalili, M. Coleman

A pilot indoor-residual spray (IRS) program using ICON 10 CS was initiated in northern Nkhota kota in the central region of Malawi covering a total of 25,000 households in November 2007. The district is characterised by intense and perennial malaria transmission (holoendemic). The actual spray area formed good boundaries with Lake Malawi to the east and a forest game reserve on the western edge. There were two rivers marking the northern and southern boarders which made the spray area an “island” of some sort. Six sentinel sites or villages were randomly selected for entomological monitoring. Four sites were located in the spray area and two sites were controls located outside the spray area. At each site six houses were selected for mosquito collection using window traps hung on the bedroom window. Each site had 6 window traps giving a total of 36 window traps in the whole area. Mosquitoes were collected daily. Data collection began 2 months before the commencement of the spray exercise. The principal anopheline mosquito species in the area were identified as *Anopheles gambiae* s.l. and *An. funestus* s.l. There was a marked decrease (>70%) in anopheline mosquito abundance in the IRS area compared to the un sprayed area. However, the number of culicines substantially increased in the spray area compared to the un sprayed area. Preliminary data showed that IRS using ICON 10 CS was effective at reducing anopheline density in the spray area. The insecticide was not effective against culicine mosquitoes. Both field and laboratory investigations are continuing looking at insecticide resistance and infectivity rates which will further confirm the effectiveness of the spray program.

Email address for correspondence: tmzilahowa@mlw.medcol.mw

773
Insecticide resistance and malaria vector status in *Anopheles arabiensis* from Gokwe, a malaria endemic area in Zimbabwe [MIM16091964]

Givemore Munhenga, Hieronymo T. Masendu, Basil D. Brooke, Richard H. Hunt, Lizette L. Koekemoer

Insecticide resistance dramatically reduces the effectiveness of malaria vector control programmes. Following the recent detection of DDT resistance in *Anopheles arabiensis* in Gokwe, Zimbabwe, the underlying resistance mechanisms in this population were studied. Standard WHO bioassays, using insecticides covering all the four classes currently available for use in public health were performed on wild-collected adult anophelines and F1 progeny of *An. arabiensis* from wild-caught females. Molecular assays were carried out to identify kdr and ace-1 mutations. Biochemical assays designed to quantify relative levels of non-specific esterases, monooxygenases, glutathione-S-transferases and as well as to detect the presence of an altered acetylcholinesterase (AChE) were performed. Wild-caught *An. arabiensis* were identified as such by PCR. Among the females, 0.5% (*n = 436*) were positive for *Plasmodium falciparum*. WHO diagnostic tests showed 47% and 68.2% mean mortality against 0.75% permethrin and 68.4% and 96% mortality against 4% DDT for collections done in 2006 and 2008, respectively. Insecticide susceptibility tests on F1 *An. arabiensis* families showed an average mortality of 87% (*n = 758*) after exposure to DDT and 65% (*n = 587*) after exposure to permethrin. Eight families showed resistance to both DDT and permethrin. Biochemical assays using samples from F1 *An. arabiensis* families revealed comparatively high levels of monooxygenase (48.5%, *n = 33*, *p < 0.05*) and glutathione S-transferase (25.8%, *n = 31*, *p < 0.05*) activity compared to a reference insecticide susceptible *An. arabiensis* colony. Knock-down resistance (kdr) and ace-IR mutations were not detected. The detection of permethrin and DDT resistances in *An. arabiensis* populations from Gwave has serious implications for malaria vector control in Zimbabwe, particularly since DDT has been reintroduced for indoor house spraying. Use of mosaic insecticide application or rotational use of insecticides and regular monitoring of insecticide resistance is recommended.

Email address for correspondence: givemorem@nicd.ac.za

774
In-silico studies of multi-drug resistance (MDR) genetic markers of *Plasmodium* species [MIM16234612] 

Yah Clarence Suh, Segun Fatumo

Multi-drug resistance malaria species has been and still is the cause of much morbidity and mortality of malaria throughout the tropics. This epidemic has devastated large populations likewise posed a serious barrier to economic growth in developing countries. The major obstacle however, is it prevention and treatment due to emerging multi-drug resistant (MDR) malaria species. Therefore, anti-malarial drug development needs to continue so that novel and highly effective anti-malarials can be plugged into recommended strategy of malaria therapies. The sequencing of various MDR genes of *Plasmodium* contributes substantially to our understanding of the multi-drug resistance that permits the identification of novel therapeutic strategies and new malaria parasites targets for drug and vaccine development. The current research therefore, engaged the use of an in-silico approach to seek new chemotherapeutic strategies in analyzing as well as offering some likely solutions to malaria therapies. Four *Plasmodium* species: two from rodents (*Plasmodium chabaudi* and *Plasmodium yoelii*) and two from human (*Plasmodium vivax* and *Plasmodium falciparum*) multi-drug resistance genes were compared using bioinformatics tools. The phylogenetic relationships and species identification of the MDR genes of the parasites were downloaded from web base resources and performed as confirmed by the BLAST and ClustalX programs. The results showed a variation in the up/down stream algorithms alignment of their phylogenetic relationships. This therefore, showed that some resistance genes within a population may vary within the same drug. The results showed a significant different of *p < 0.001* with a 95% CI. Through these efforts our goal is to better understand how drug resistance occurs and to develop new approaches to combat this global problem. This knowledge therefore, will facilitate the rationale to design new effective as well as check the emerging of multi-drug resistant *Plasmodium* strains.

Email address for correspondence: clarence.yah@wits.ac.za
775 Identification of novel color variant of Anopheles arabiensis Patton (Diptera: Culicidae) [MIM16696620]
Mulenga Musapa, Sungano Mharakurwa, Douglas E. Norris

Anopheles mosquitoes often undergo morphological mutation that affect eye and body color, wing shape or venation or seta form. These aberrations provide useful markers for genetic manipulation and genomic mapping. We have isolated color variants in Anopheles arabiensis Patton. A black-spot (Bs) phenotype is apparent on the thoracic dorsum of larvae and pupae and is variable in penetrance and expressivity. This trait is apparently female sex-limited. To determine inheritance pattern, we attempted to purify a stock homozygous for Bs by selection of spotted females and crossing them to sibling males. After 4 generations of purification inbreeding, black-spotted females were crossed to wild-type males from another stock in which Bs is absent. When we crossed Bs females to wild-type males, serendipitously, a surge of 10% males from another stock in which Bs is absent. When we crossed Bs females to wild-type males, serendipitously, a surge of 10% lethal mutation resulted from these crosses. Mutant larvae were characterized by black pigmentation of entire body and larval development progressed normally but died at larval–pupal ecdisis. Among the F1 progeny, melanotic lethal mutants were completely absent and penetrance of 44% was observed. Our preliminary data from the above crosses superficially indicates that Bs is dominant and the aberration is lethal when homozygous. Further investigation involving genetic crosses to describe genotypes of individuals and inheritance pattern are pending.

Email address for correspondence: musapa.mulenga@miamb.org.zm

776 A new species of the Anopheles funestus group from Malawi [MIM16597663]
Belinda Spillings, Lizette L. Koekemoer, Basil D. Brooke, Maureen Coetzez, Richard H. Hunt

Successful malaria vector control relies on the ability to accurately identify the mosquito species responsible for malaria transmission. In the past, there has been the general assumption that any mosquitoes caught inside houses were responsible for the malaria transmission taking place. Modern identification methods have proved this to be incorrect. This study shows how modern identification methods failed to identify indoor resting mosquitoes that appeared to be Anopheles funestus. Collections of indoor resting anopheline mosquitoes were carried out in Karonga, northern Malawi. Morphological, cytogenetic, cross-mating and molecular studies were carried out on iso-female lines reared from wild females. No distinct morphological characteristics were noted. All wild females tested negative for the presence of Plasmodium falciparum. Cytogenetic analysis revealed that the chromosomes were homozygous for the inverted 3a, 3b and 5a arrangements of An. funestus. With a unique polymorphic inversion on autosomal arm 2. Cross-mating between Malawi males and An. funestus colony females produced >900 eggs with a very low hatch rate (0.22%). The reciprocal cross produced hybrid males that appeared fertile. Hybrid chromosomes showed characteristic asynapsis indicative of inter-species crosses. PCR identification targeted at the ITS2 gene region repeatedly failed due to high levels of sequence variation in these specimens. Although these mosquitoes were found resting inside houses they could not be linked to malaria transmission. The specimens could not be identified using standard molecular techniques and further studies are required to elucidate their host preferences. At present we conclude that we are dealing with an undescribed member of the An. funestus subgroup.

Email address for correspondence: belindas@nicd.ac.za

777 The biological performance and home improvement value of Durable Residual Wall Lining (DL) when used in a rural village of Mali [MIM15058323]
M.B. Coulibaly, M.L. Larsen, B. Diallo, A.S. Traoré, M. Konaté, A. Guindo, S.F. Traore

Geographic coverage in remote rural settlements is often limited for indoor residual spraying (IRS) because getting to many villages for applications can be challenging. A trial was undertaken to evaluate acceptability and practicality of Durable Residual Wall Lining (DL) as an alternative vector control technology to IRS in village settings. In N’Galamadibi, a village 130 km northeast of Bamako, Mali, DL was installed in 24 houses based upon their representation of typical rural construction materials. The DL contained deltamethrin at 170 mg/m². At 3 weeks post-installation a survey was made of user impressions of the DL appeal and appearance, changes in indoor environment, and impact on perception of mosquito presence. At 3 months post installation entomological assessments of residual efficacy were conducted using WHO/ES cone tests. All participants liked the DL 3 weeks after installation. No major defects were noted. Occupants experienced no adverse reactions. No change in the odor was noticed by 62.5% (15/24) while 37.5% (9/24) thought there was a slight change. It was noticed in the first two days after installation, and caused no problem. 29.2% and 25% thought there was an increase in light and temperature, respectively, whereas 70.8% thought there were no changes. Susceptible mosquitoes were exposed to the DL 3 months after installation. There was an increase in light and temperature, respectively, whereas 70.8% thought there were no changes. Susceptible mosquitoes were exposed to the DL 3 months after installation. No major defects were noted. Occupants experienced no adverse reactions. No change in the odor was noticed by 62.5% (15/24) while 37.5% (9/24) thought there was a slight change. It was noticed in the first two days after installation, and caused no problem. 29.2% and 25% thought there was an increase in light and temperature, respectively, whereas 70.8% thought there were no changes. Susceptible mosquitoes were exposed to the DL 3 months later in 12 houses of different types with 80 specimens per wall. Mortality was 98.4–100%. This study reports on the acceptability to homeowners, durability of product, and efficacy/performance of DL as a potential replacement for IRS.

Email address for correspondence: doudou@mrtcbko.org

778 Comparaison de deux techniques d’évaluation de la sensibilité des vecteurs aux insecticides: La technique oms et la technique CDC [MIM16646315]
Alia Roland

En Afrique de l’Ouest et du Centre, seule la technique OMS est utilisée pour évaluer la sensibilité des moustiques aux insecticides. L’une des difficultés de cette technique repose sur le fait qu’elle exige des moustiques ayant le même état physiologique. Par ailleurs, après le test, une mise en observation de 24heures est exigée avant la lecture des résultats. Or, en milieu périphérique, ces conditions sont rarement remplies. Les techniciens entomologistes qui travaillent en milieu périphérique ont donc besoin d’une technique plus simple et plus rapide d’où la présente étude de comparer les protocoles OMS et CDC. L’ensemble kit OMS + papiers imprégnés et des flacons CDC wheaton de 250 mL ont été respectivement utilisés pour les tests OMS et CDC. Des souches locales d’An. gambiae, ont été exposées à différentes concentrations d’insecticide puis retirés après un certain temps de contact. Les données enregistrées ont été traitées à l’aide des logiciels Excel et Epi info. Les souches testées se sont comportées de façon similaire vis-à-vis des deux méthodes. Toutefois, une divergence de niveau de sensibilité à la Deltamétrine et à la Lambdaclathrothrine a été constatée avec la localité de Ladji. Bien qu’ayant chacune sa spécificité, les deux techniques ont globalement conduit aux mêmes résultats. Comparativement au test OMS, le test CDC présente cependant de nombreux avantages sur le plan de la rapidité et de la praticabilité sur le terrain.

Email address for correspondence: azoland@yahoo.fr
779

Entomological profile and cartography of malaria vector insecticide resistance in Benin [MIM16669973]

Roseric Azondekon

Anopheles gambiae populations transmitting malaria in Benin, its distribution and susceptibility to insecticides are the main focus of National Malaria Control Program. This study was carried out through a transect in various ecological areas from the southern to the northern Benin. Overall, more than 40 localities were surveyed to establish the distribution of An. gambiae species complex and to map its resistance. Anopheles breeding sites were identified in each locality where larvae was collected and reared and kept at the insectarium. Female aged 2–5 days old, were subjected to susceptibility tests through WHO method and the bottles for CDC protocol. Dead and alive mosquitoes were analysed with PCR techniques to identify the sub-species of An. gambiae, the molecular and Kdr mutation. Data were subjected to ArcView software to establish the resistance mapping and the distribution of An. gambiae species complex. Data from WHO and CDC methods were compared to conclude for the resistance status. Data obtained have shown an introduction of this species complex and to map its resistance. Anopheles gambiae species complex. Data from WHO and CDC methods revealed a non-significant drop ($p > 0.05$). Multiple resistance mechanisms were probably developed by An. gambiae in West Africa, but the main form is Kdr mutation found everywhere in Benin, in M and S form with a high level in urban and cotton-cultivated areas. Email address for correspondence: roseric.2000@yahoo.fr

780

Efficacite biologique de la moustiquaire olyset sur les vecteurs du paludisme au niger [MIM16315887]

Fouta Boubakar

La moustiquaire imprégnée d’insecticide (MII) est l’un des principaux outils de lutte contre le paludisme recommandés par l’OMS. Plusieurs études de terrain ont démontré l’efficacité de son utilisation à grande échelle. Les MII réduisent de 50% la mortalité et de 20 à 30% la mortalité globale chez les enfants de 0 à 5 ans. C’est pourquoi sa promotion au sein des populations est inscrite comme la composante essentielle du Programme national de lutte contre le paludisme au Niger. Seuls les pyrèthroides sont utilisés pour l’imprégnation des moustiquaires compte tenu de leur action rapide (effet Knockdown ou Kd), de leur fort effet excitoto-répulsif et de leur faible toxicité pour les mammifères. Plusieurs études ont montré que l’action combinée de ces effets constituait une barrière efficace contre les vecteurs du paludisme. L’utilisation à grande échelle des moustiquaires imprégnées de pyrèthroides étant fortement recommandée par les Programmes nationaux il est nécessaire de surveiller régulièrement leur efficacité sur les vecteurs incriminés dans la transmission du paludisme. Sites de l’Etude: L’étude a été réalisée dans 3 localités du Niger. Le choix de ces localités a tenu compte du degré d’urbanisation, des activités agricoles et du degré de transmission du paludisme. Ces sites sont: site sahéli-soudanien avec une transmission hyper-endémique, site sahélien transmission méso-endémique, site pré-désertique avec une transmission épidémique. Le site soudanien sahélien est caractérisé par la présence du fleuve Niger avec ses grands aménagements hydro-agricoles, ces champs de rizières avec de larges mailles de savane et de lambeaux de forêt. Le site sahélien caractérisé par des mares permanentes et semi permanentes où se font des cultures maraîchères, du mil, sorgho. Le site pré désertique à la limite de la zone sahélienne et saharienne avec des aménagements de cultures maraîchères (lac artificiel). Enquêtes CAP (connaissances, attitudes et pratiques) sur l’utilisation des insecticides Une enquête relative à l’utilisation des insecticides agricoles et domestiques a été réalisée auprès des habitants des sites d’étude. Ces personnes ont été interrogées sur la base d’un questionnaire relatif à la nature, la dose, le rythme, la concentration des insecticides agricoles et domestiques utilisés. Dans chaque site, le nombre des personnes interrogées dépendait du bon vouloir des populations. Cette enquête vise à connaître la nature et les modalités d’utilisation des insecticides par les populations afin d’établir éventuellement la relation entre l’agriculture et la pression de sélection sur An.gambiae. Notre étude a eu pour but d’examiner la situation de l’efficacité des moustiquaires imprégnées Olyset sur Anophelles gambiae-sl dans trois endroits du Niger, en fonction des différents faciès épidémiologiques. Tests de sensibilité: Les tests d’efficacité ont été effectués avec des moustiques femelles issues de l’émergence des larves d’anophèles gambiae récoltées sur le terrain (Novembre 2007 en fin de saison des pluies) dans les 3 sites de l’étude. Les larves ont été élevées dans des conditions approximatives de température (27 à 30°C) et d’humidité (70 à 80%) dans les locaux aménagés à cet effet sur le terrain. Les tests d’efficacité ont été réalisés selon la méthode Bio-essai OMS avec des cônes en plastique fixés sur les différentes faces de la moustiquaire Olyset avec des femelles non gorgées, âgée de 2 à 3 jours. Les moustiquaires imprégnées Olyset Net sont en polyéthylène 100% de haute densité, mélange à la perméthrine 2% (correspondant à environ 1000 mg de produit actif par m2 de tissu incorporée dans la fibre lors du traitement des filets). Pour chaque moustiquaire test, 4 lots de 25 moustiques ont été exposés pendant 3mn (contact forcé des moustiques dans le cône fixé à la face de la moustiquaire avec un lacer élastique), puis retirés et mis en observation dans des pots de yaourt fermés par une tulle moustiquaire non imprégnée retenue par un élastique. Les témoins comportant aussi 4 lots de 25 moustiques ont été réalisés avec des moustiquaires simples non imprégnées. Le nombre de moustiques assommés (effet Kd: Knock down) après exposition et mis en observation au bout de 5, 10, 15, 20, 30, 40, 50, 60 minutes a été enregistrée. Après les moustiques ont été mis en observation avec un accès libre au jus sucré. La mortalité au bout de 24 heures a été déterminée. Les tests n’ont été validés que lorsque la mortalité observée dans les témoins a été inférieure à 5%. Enquête CAP: Le nombre d’enquêtés a été de 35,26, et 25 respectivement dans le site soudano sahélien, le site sahélien le site pré-dessertique. Les insecticides domestiques utilisés dans les sites d’étude sont, par ordre de préférence, les serpentins fumigènes, puis les bombes aériosols à base de pyrèthroides (esbiothrine,cyfluthrine), de carbamates (propoxur) et organophosphorés (dichlorvos). Les préférences des paysans et les fréquences d’utilisation de ces insecticides sont ditionnées par leur pouvoir d’achat. Dans le site soudanais, les populations utilisent les serpentins fumigènes de manière permanente en raison de la forte pullulation des moustiques toute l’année. Les insecticides agricoles employés dans les sites sont des pyrèthroides (deltaméthrine 12,5 g/l), cyfluthrine, lambda-cyhalothrine 18 g/ha, cyperméthrine 36 g/l), des organophosphorés (triazophos, chlorpyriphos-éthyl, diazinon.), les organochlorés (endosulfan 350 et 500 g/l, lindane),et les carbamates (oxamyl, fénobucarb). Ces insecticides sont utilisés à des doses allant de 1 à 4 l/ha. Effet Knock down: Des tests d’efficacité biologique ont été réalisés avec les populations naturelles d’An-gambiae échantillonnées dans 3 écosystèmes différents du Niger. Nos résultats ont montré un effet Knock down (Kd) à des degrés variables à la perméthrine (insecticide utilisé en imprégnation des moustiquaires Olyset) dans tous
Anopheles gambiae, the main malaria vector in Benin has developed high resistance to pyrethroid insecticides, which is a serious concern to the use of ITNs and IRS for malaria. In this context, it is important to investigate other families of insecticides. The present study aims to verify whether, carbamate and organophosphates can be effective against pyrethroids resistant anophelines. Two pyrethroids (deltamethrin 0.025 g/m² and alpha-cypermethrin 0.03 g/m²), one organophosphate (fenitrothion 2 g/m²), one carbamate (Bendiocarb 200 mg/m²) and a mixture of chlorpyriphos (0.56 g/m²) + deltaméthrine (0.025 g/m²) + chloro(2) were tested in experimental huts as IRS treatment. The following parameters were measured: deterrence, exophily, blood feeding rate and mortality rates. Deltamethrin, alpha-cypermethrin and bendiocarb significantly reduced mosquitoes entry in the huts (p<0.05). Blood feeding in fenitrothion and chloro(2) were respectively 4.47% and 3.7% (third month) and 2.04% and 4.44% (fourth month). Exophily rate in deltamethrin, alpha-cypermethrin and chloro(2) was significantly higher than fenitrothion. Deltamethrin and alpha-cypermethrin have the lowest mortality rate while fenitrothion killed 100% (first month) and 77.8% (fourth month). Concerning bendiocarb and the chloro(2), mortality rates were respectively 97.9% and 100% (first month), 77.7% and 88% (third month). After 4 months evaluation, fenitrothion, chloro(2) and bendiocarb proved to be the most effective alternatives against pyrethroids resistant An. gambiae, but bendiocarb is the best because secondary effects of chloro(2) and fenitrothion are disapproved by the public.

Email address for correspondence: biobanganaa@yahoo.fr
et 10e lavages). Une baisse d’efficacité a été relevée pour la plupart des fibres après une série de 10 lavages. La fréquence de lavage, le trempe et le séchage au soleil ont été potentiellement identifiés comme les principales causes. Cependant, les moustiquaires en polyester apparaissent les meilleures. Elles donnent après 5 lavages, un taux de mortalité de 67% avec la souche Kisumu d’Anopheles gambiae. Une étude similaire réalisée sur les MIILD d’usine, rapporte avec la même souche et toujours après 5 lavages, 66% de mortalité avec Olyset contre 83% pour Permanet® (Akogbéto et al., 2006). Ainsi, dans les communautés béninoises, les MIILD manuelles de type K-O Tab1-2-3® se rapprochent de celles développées industriellement. Mots clés: K-O Tab1-2-3® - MIILD - Efficacité - Habitudes de lavage.

Email address for correspondence: renaud292@yahoo.fr

784 Distribution of S and M molecular forms of Anopheles gambiae s.s. and knockdown resistance in Côte d’Ivoire [MIM14867086]

M. Touré, F. Chandre, P. Carnevale

Anopheles gambiae s.s. is the major vector of malaria in Côte d’Ivoire in West Africa. This mosquito is represented by two molecular forms, S and M. The eco-climatic variability and agricultural activities in Côte d’Ivoire and the spread of knockdown mutation conferring cross-resistance to pyrethroids and DDT are seen as a threat for malaria vector control. Two large scale trials were implemented in villages near Korhogo located in Savannah area in the north, then in Danané, a forested area in the West in Côte d’Ivoire. Genotypes of individuals for M or S molecular forms of An. gambiae s.s. and knockdown mutation were detected using PCR. Both S and M molecular forms were found in sympathy in all study sites. The S form was 97% in the north, versus 26% in the West. No heterozygote [SM] of the molecular forms was found. The frequencies of knockdown mutation ranged from 0.82 to 0.96 in the north, and were less than 0.20 in the West. However, this mutation was only detected in An. gambiae s.s. belonging to the S form because there is an absence of gene flow between wild mosquitoes of the sympatric S and M molecular forms of An. gambiae s.s. Development and evolution of knockdown mutation essentially depends on the genetic of individuals than on external selection pressure exerted by regular use of insecticides in agriculture. Thus, it is now essential to characterize the genetic structure of An. gambiae s.s. for an effective use of the potential transgenetic mosquitoes preventing malaria transmission.

Email address for correspondence: mahamataoure@yahoo.fr

785 Malaria transmission in urban area and environment (Adzope, Côte d’Ivoire) [MIM15018685]


An entomological study was conducted in the city of Adzope; (6° 11′ N, 3° 42′ W) from forested area in Côte d’Ivoire. The impact of shallow management for agricultural activities on malaria transmission was investigated during 1 year. Two districts have been chosen in the town of Adzope to carry out this study: Tsasodji located in the city center in close proximity to a shallow, and Port Bouet located at the periphery of the city close to vegetable garden and rice field. Mosquitoes were captured when landing on human volunteers. Culex quinquefasciatus and Anopheles gambiae were the most abundant mosquitoes captured during the study, accounting for 87% (n = 2352) and 94% (n = 2157) of total mosquitoes caught in Tsasodji and Port-Bouet, respectively. The estimated overall biting rate for An. gambiae was 10.3 bites per person per night in Tsasodji and 77.6 in Port-Bouet. An. gambiae was the only Anopheles infected. The circumsorozoite rate was 5.1% in Tsasodji and 2.1% in Port-Bouet. The annual Plasmodium falciparum entomological inoculation rates measured by ELISA CSP were 190 infected bites per human for Tsasodji and 595 for Port-Bouet district. This study showed that the populations living in the peripheral districts from Adzope were three times more exposed to malaria risk than people living in the town centre. This high malaria transmission rate among the urban populations in Port Bouet was probably due to the presence of the shallow to produce the vegetable and rice offering ideal breeding sites for the main malaria vector An. gambiae.

Email address for correspondence: adjamaurice@yahoo.fr

786 Laboratory and field evaluation of Fendona 6SC®-treated bednets and Interceptor® long lasting nets in personal protection against Anopheles gambiae s.l. malaria vectors in Burkina Faso [MIM16699997]

A. Badolo, W.M. Guelbego, A. Traoré, A. Tiono, N.F. Sagnon, S.B. Sirima

The coverage rate of treated bednets among pregnant women and children under the age of five in Burkina Faso is still very low. The availability of home impregnation kits and long lasting nets can increase the bednet coverage rate in the target population. We compared, under laboratory and field conditions, the efficacy of two long lasting nets (PermaNet® versus Interceptor®) and two bednet treatment kits K-O TAB® (Deltamethrin) versus Fendona 6SC® (Alpha-cypermethrin) against the Anopheles gambiae s.l. malaria vector. Bednets effectiveness was assessed in the laboratory by contact bioassays using WHO cones. The knock-down and the after 24 h mortality rates were scored. In the field, the bednets were evaluated using experimental huts and sleepers. Results are expressed in terms of induced exophily, after 24 h mortality, and blood-feeding inhibition. Mortality after 24 h was similar for the Fendona 6SC®-treated bednets and the K-O TAB®-treated bednets [79.4% CL (73.9–84.6) and 74% CL (68.3–80.0), respectively]. However, the Fendona 6SC®-treated bednets were superior in 50% knock-down times to the K-O TAB®-treated bednets. The field trial showed the same blood-feeding reduction, induced exophily, and mortality after 24 h between Interceptor® and PermaNet®. The bednets treated with Fendona 6SC® and K-O TAB® exhibited the same effectiveness. The Fendona 6SC® kit and the Interceptor® bednets showed consistent comparable efficacy with the already-in-use K-O TAB® kit and PermaNet® bednets. Both products can help increase the availability of treated bednets and the coverage rate of nets among population.

Email address for correspondence: athanase_badolo@univ-ouaga.cf

787 Patterns and seasonality of malaria transmission in the forest-savannah transitional zones of Ghana [MIM16690254]

Dominic Dery, Charles Brown, Kwaku Poku Asante, Mohammed Adams, David Dosoo, Seeba Amenga-Etego, Mike Wilson, Daniel Chandramohan, Brian Greenwood, Seth Owusu-Agyei

The seasonality of malaria vector dynamics plays an important role in malaria transmission and needs to be studied prior to malaria clinical trials. Baseline entomological survey was carried out in the forest-Savannah transitional zone of Ghana. Mosquito collections by CDC light traps were performed in houses. Species identification was done using morphological identification keys with PCR.
confirmation. Molecular form differentiation and kdr genotyping were also performed in addition to CS-ELISA analysis. 23,406 mosquitoes were captured from 919 traps: 54.3% were Culicines, 36.2% Anopheles funestus, and 9.4% An. gambiae. The infectivity of Plasmodium falciparum was 1.5% and 4.7% for An. funestus and An. gambiae, respectively. ELRs were 269ib/p/y in 2004 and 318ib/p/y in 2005. PCR analysis indicated An. gambiae s.s species only. By PCR, in the wet season, 88.8% (N = 19) were S-molecular forms, 11.1% (N = 19) M-molecular forms and 5.56% (N = 19) hybrids (S/M). In the dry season, S-molecular form were 68.75% (N = 16), M-molecular 12.50% (N = 16) and hybrids 18.75% (N = 16). The frequency of kdr resistant genotypes F(R) was 0.60. Genotypes kdrRR were in M-molecular forms whereas kdrRS in hybrids (M/S). All susceptible genotypes kdrrss were in S-molecular forms. The dynamics and seasonal abundance of vectors was influenced by existing micro-ecology, rainfall and temperature. Though CDC light traps set up were reduced in 2005, transmission pattern did not differ significantly. An. gambiae and An. funestus were both effective vectors. Kdr genotype frequency F(R) 0.06 is relatively high and kdrRR genotypes in M and kdrRS genotypes in hybrids requires further investigation.

Email address for correspondence: dominic.dery@ghana-khrc.org

788
Etude de la transmission du paludisme en prélude a une campagne de pulvérisation intradomiciliaire dans les départements de l’ouemé et du littoral [MIM14878332]
Virgile Gnanguenon

Pour aider le Bénin à éradiquer le paludisme, le “Presidential Malaria Initiative” (PMI) du gouvernement américain a décidé d’apporter son appui à travers la mise en place des pulvérisations intradomiciliaires d’insecticide (IRS) qui débute en 2008 dans le département de l’Ouémé. En prélude aux opérations de pulvérisation, nous avons effectué une enquête sociologique, réalisé des tests de sensibilité sur Anopheles gambiae et effectué des captures nocturnes de moustiques sur appâts humains pour apprécier le comportement du vecteur. De l’analyse des résultats, il ressort que: le paludisme est considéré comme une maladie très grave par la population. Sur la base de l’expérience des uns et des autres, l’adhésion à la campagne de pulvérisation est presque totale (94,8%). De plus, les tests de sensibilités ont montré une nette résistance des anophèles au DDT et à la perméthrine. Ils sont par contre sensibles à la deltaméthrine et à lambdacyhalothrine avec une lenteur de l’effet knock-down. Le choix d’une molécule n’appartenant pas à ces catégories d’insecticide se trouve alors justifié. Des captures nocturnes, il ressort que Anopheles gambiae est moins dense en saison sèche qu’en saison pluvieuse. En effet son taux de piqûre est très faible en saison sèche (1 piqûre en moyenne) et augmente dès le début de la saison pluvieuse (11,15 piqûre moyenne). Le degré d’endophagie-exophagie montre qu’il pique beaucoup plus à l’intérieur des habitations qu’à l’extérieur. La pulvérisation sera donc certainement un succès. Mots clés: Sensibilité - Piqûre - Pulvérisation intradomiciliaire - Paludisme Email address for correspondence: juddyanne@yahoo.com

789
Distribution and insecticide resistance of Anopheles gambiae s.l. in Yame (Togo) [MIM16697919]
Guillaume Ketoh

The aim of the present study is to investigate on the anopheline population specific composition and to characterise the mechanisms involved in pesticide resistance in 2005 and 2008 in Togo. Frequency and abundance were used to investigate on anopheline population dynamics in Lome during a 1-year long survey. Anopheles gambiae s.l. sibling species were characterised by PCR-RAPD and genetic mutations by PCR-PASA. Two sibling species were characterised: An. gambiae s.s. (92.7%) and An. arabiensis (7.3%). The two molecular forms, M and S were found among An. gambiae s.s. population and represented 86.1% and 13.9%, respectively. Three years later, M form was always predominantly high (97%) with no S form detected. There was however one hybrid form detected (3%). Within the populations of An. gambiae s.s., 65% and 50% of the M and S forms, respectively, were homozygote resistant with the kdr mutation (kdr/kdr). The proportion of this mutation was 33% in An. arabiensis populations. The kdr resistant genotype frequency F(R) recorded after 3 years was 0.84 with approximately 70% homozygote kdrRR. Conversely, no individuals of An. gambiae were homozygote for ace1R gene but 23% of individuals of the S form and 13% of the M form were heterozygote (ace1R/ace1S). The ace1R allele was not observed within An. arabiensis population. The presence of two genetic mutations; kdr and ace1R, in the An. gambiae s.l. population was also reported in Benin and could affect the success of an integrated distribution campaign to provide individuals with ITNs in Togo.

Email address for correspondence: ketoh@hotmail.com

790
Insecticide resistance status of malaria vector Anopheles gambiae breeding in polluted water in urban Lagos [MIM16693208]
Judith Obansa

Insecticide resistance in the major Africa malaria vector Anopheles gambiae threatens the usefulness of insecticide treated bed net programmes. Although various factors contribute to resistance, the role of water pollution at the larval breeding sites has been poorly documented. Here we report the insecticide susceptibility status of An. gambiae from polluted water bodies at three permanent breeding sites in urban Lagos. Anopheline larvae sampled at Akoka, Idiaraba and Okobaba were raised to adulthood in the insectary. 2–3 days old adult female mosquitoes were exposed in batches to 0.75% permethrin and 0.05% lambdacyhalothrin using the WHO insecticide susceptibility test kits. All samples exposed were identified morphologically by PCR assay. Atomic absorption spectrophotometry was used to analyse the water samples. All samples tested belong to the An. gambiae complex. The PCR assay revealed a mixture of An. gambiae and An. arabiensis: (32–68%) at Akoka, (51–49%) at Idiaraba, and 100% An. arabiensis at Okobaba. The 24-h post exposure mortality rates in permethrin and lambdacyhalothrin were 60% and 100% at Akoka, 49% and 60% at Idiaraba, 69% and 71% at Okobaba. The result of water analysis was characterized by high concentration of heavy metals, low dissolved oxygen and high conductivity. An. gambiae is adapting to a variety of water habitat. Although the effect of water pollution on longevity of adult Anopheles mosquitoes is not clear, the level of insecticide resistance is a concern and could be of interest in the epidemiology of urban malaria.

Email address for correspondence: juddyanne@yahoo.com

791
Effects of environmental variables and male size on swarming and mating behaviour of natural populations of Anopheles gambiae s.s. in Burkina Faso [MIM16689693]

www.mimalaria.org
The progress in genomic have increased the prospects of using genetically modified mosquitoes or sterilised males in malaria vector control. This strategy requires a proper understanding of potential interactions with naturally occurring populations. 

Anopheles gambiae, the main malaria vector in Africa, mates in swarms. Factors that may be involved in the swarming and mating system are poorly documented. We characterized swarming behaviour and determined whether male size affects mating success of An. gambiae. Swarms of An. gambiae s.s. were followed up from July 2006 to December 2007 in Vallée du Kou and Soumouso situated in southwestern Burkina Faso. Environmental parameters such as light intensity, temperature, and relative humidity were recorded at the time of swarm formation. Wing lengths of 654 swarming males were measured and then compared to those of 152 males collected in copula to assess whether there was an allometric relationship with mating success. Males activity began generally 1–10 min after sunset. Swarms stopped with the onset of darkness, usually 9–35 min after their formation. Males swarmed 0.6–4 m above field markers constituted by stored wood, wells, heaps of refuse and open areas. The environmental parameters at swarm starting and ending showed substantial spatio-temporal variation. Copulating males were significantly larger than males sampled from the swarms ($F=63.98, p=0.001$). This study showed that the environmental parameters we measured were not correlated with the swarm formation, and that male size may play an important role in mating success of An. gambiae mosquitoes.

Email address for correspondence: sawsimp2005@yahoo.fr

---

793
Insecticide resistance network for African malaria vectors: Anopheles gambiae s.l. resistance to multiple insecticides in Burkina Faso [MIM16680110]

A. Badolo, B.V.E.J.T. Bazié, W.M. Guelbeogo, A. Traoré, A. Sanou, R. K. Dabiré, N.F. Sagnon, H. Ranson

In Burkina Faso, malaria vector control relies on LLIN distribution. However, insecticide resistance probably caused by agricultural insecticide usage may compromise bednet efficacy. This study aims to improve the monitoring of insecticide resistance, characterize the molecular basis of this resistance and assess the impact of resistance on control activities. Four sentinel sites throughout Burkina Faso were selected according to insecticide selection pressure (Soumouso, Kuinima, Koupel and Goundry). Larvae were collected during two seasons and reared in local insectaries. Insecticide susceptibility tests were performed on mosquitoes 3–5 days old according to WHO procedures. Indoor and outdoor adult mosquitoes were collected in order to assess the Plasmodium circumsporozoite rate. PCR was used for species and molecular form identification and to detect resistance alleles kdrW, kdrE and mAce. 8028 mosquitoes were exposed to permethrin, deltamethrin, DDT, bendiocarb and fenitrothion during two rounds. Resistance to all five insecticides was detected in Soumouso and Kuinima. Vector populations remained sensitive or partially sensitive to deltamethrin, bendiocarb and fenitrothion but were resistant to DDT and permethrin in Koupel and Goundry. 2242 indoor and 218 outdoor resting Anopheles gambiae s.l. mosquitoes were collected. The ELISA test for parasite infectivity and the PCR identification for species and resistance mutations are in progress. Resistance to the four classes of insecticides available for public health is present in malaria vectors in Burkina. News strategies for vector control and resistance management are needed.

Email address for correspondence: a.badolo@gmail.com

---

794
Insecticidal activity of Chenopodium ambrosioides L. (Chenopodiaceae) extracts against anopheles gambiae (Diptera: Culicidae) [MIM16730556]

Abiodun A. Denloye, Oluwakemi K. Ajelara, Rasaq A. Olowu, Adeolu O. Eshilokun, Winifred A. Makanjuola

Rural folks in Ipara, Badagy Local Government Area, Lagos State, Nigeria use Chenopodium ambrosioides L. (Chenopodiaceae) as worm expeller and repel mosquitoes with the leaves. Laboratory tests were therefore carried out to evaluate the toxicity of C. ambrosioides leaf petroleum ether extract and essential oil against the malaria vector, Anopheles gambiae (Diptera: Culicidae). Thirty first, second, third and fourth instar larvae of An. gambiae, respectively, at ambient laboratory temperature were exposed to 50, 100, 250, 500, 750 and 1000 ppm of extract and essential oil for 24 and 48 h, and mortality recorded. Thirty 0–2 day old adults were exposed to C. ambrosioides oil vapour in air-tight glass cages at 0.8, 1.6 and 2.4 µL/L and mortality recorded after 24 h of exposure. The results showed that the test extract and essential oil were toxic to all larval stages and adults of An. gambiae. The extract was most toxic to the first instar larvae (14.89 ppm) followed by the fourth instar (18.90 ppm) and least toxic to the third instar (183.77 ppm). The essential oil was most toxic to the fourth (36.62 ppm) followed by the first instar (90.75 ppm) larvae. The 24-h LC50 value of C. ambrosioides oil against adult An. gambiae was 1.01 µL/L. The results give empirical support to the traditional use of C. ambrosioides in keeping mosquitoes away from dwellings and highlights the potential of the plant species for the control An. gambiae.

Email address for correspondence: bio_denloye@yahoo.com

---

L’identification des espèces et la détermination de certains paramètres entomologiques de la transmission sont des informations importantes pouvant permettre de mettre en place et évaluer l’impact d’une stratégie de lutte antivectorielle. Une étude entomologique longitudinale de la transmission du paludisme en saison sèche a été réalisée de décembre 2007 à juin 2008 à Bancoumana (Mali). Les techniques d’échantillonnages ont été: la capture diurne par Spray Catch et la capture nocturne sur appât humains. La détermination du taux d’infection et la nature du repas sanguin ont été effectuées par ELISA, l’identification des espèces et des formes moléculaires par la PCR. La faune vectrice était composée d’An. gambiae s.l. (99,47%, n = 1708) et An. funestus (0,53%); An. gambiae s.l. était essentiellement composé d’An. gambiae s.s. (93,40%, n = 894) et An. arabiensis (6,60%); La forme M était prédominante (70,54% n = 835), suivie de la forme S (15,93%) et enfin de l’hybride (18,90 ppm) et le moins toxique à la troisième instar (183.77 ppm). Le test extract et essential oil vapour in air-tight glass cages at 0.8, 1.6 and 2.4 µL/L and mortality recorded after 24 h of exposure. The results showed that the test extract and essential oil were toxic to all larval stages and adults of An. gambiae. The extract was most toxic to the first instar larvae (14.89 ppm) followed by the fourth instar (18.90 ppm) and least toxic to the third instar (183.77 ppm). The essential oil was most toxic to the fourth (36.62 ppm) followed by the first instar (90.75 ppm) larvae. The 24-h LC50 value of C. ambrosioides oil against adult An. gambiae was 1.01 µL/L. The results give empirical support to the traditional use of C. ambrosioides in keeping mosquitoes away from dwellings and highlights the potential of the plant species for the control An. gambiae.

Email address for correspondence: bilkissou@yahoo.fr
Anopheles funestus is one of the most important vectors of malaria in sub-saharan Africa together with An. gambiae and An. arabiensis. Recent cytogenetic studies showed a genetic structure discriminating two chromosomal forms provisionally named Kiribina and Folonzo with limited gene flow and contrasting degrees of chromosomal polymorphism. No data is however available on the bionomics of these forms in sympatric area in Senegal. This context justified two consecutive years (in October and November 2006 and 2007) of entomological study in Kouvar village where the two forms are sympatric with the aim to assess their differences in behavior, occurrence and infection rates to further estimate their respective role in malaria transmission. Significant departures from Hardy–Weinberg equilibrium were observed across three of the four chromosomal arms (2R, 3Ra, and 3Rb) in the whole population. However, when each specimen is assigned to the corresponding chromosomal form, the Hardy–Weinberg equilibrium was restored in each form. However, no significant difference was observed either for the anthropophilic or for the infection rates for the two forms. The frequencies of the two forms were statistically different between the 2 years. The presence of the two chromosomal forms is confirmed in Kouvar village. Despite the splitting of the population into two chromosomal forms, the observed homogeneity of the anthropophilic and infection rates could be a result of the relative homogeneity of the ecological conditions in Kouvar village and the non-existence of alternative conditions for each chromosomal form. However, the differences observed between the 2 years probably expressed a differential adaptation to different erratically environments. Studies using microsatellites and mtDNA markers are in progress in order to confirm the genetic isolation of the two forms, their taxonomic status and bionomical characteristics.

Email address for correspondence: dia@pasteur.sn

Anopheles gambiae is divided into two incipient species known as the M and S molecular forms. The forms display partial habitat segregation and they are characterized by a systematic deficit of hybridization in the field. However, when they mate in the laboratory, segregation and they are characterized by a systematic deficit of Hardy–Weinberg equilibrium were observed across three of the four chromosomal arms (2R, 3Ra, and 3Rb) in the whole population. However, when each specimen is assigned to the corresponding chromosomal form, the Hardy–Weinberg equilibrium was restored in each form. However, no significant difference was observed either for the anthropophilic or for the infection rates for the two forms. The frequencies of the two forms were statistically different between the 2 years. The presence of the two chromosomal forms is confirmed in Kouvar village. Despite the splitting of the population into two chromosomal forms, the observed homogeneity of the anthropophilic and infection rates could be a result of the relative homogeneity of the ecological conditions in Kouvar village and the non-existence of alternative conditions for each chromosomal form. However, the differences observed between the 2 years probably expressed a differential adaptation to different erratically environments. Studies using microsatellites and mtDNA markers are in progress in order to confirm the genetic isolation of the two forms, their taxonomic status and bionomical characteristics.

Email address for correspondence: dia@pasteur.sn

Swarm segregation is the main mechanism that prevents mating between sympatric molecular forms of Anopheles gambiae [MIM15599914]

Diabate Abdoulaye, Dao Adama, Yaro Alpha, Alpha Adamou, Traore S. Cheick, Gonzalez Rodrigo, Gwadz Bob, Lehmann Tovi

Anopheles gambiae is divided into two incipient species known as the M and S molecular forms. The forms display partial habitat segregation and they are characterized by a systematic deficit of hybrids in the field. However, when they mate in the laboratory, their offspring are viable and fertile. Evidence for assortative mating in the field was recently reported but the underlying mechanisms awaited discovery. To understand the role of swarm segregation as mechanisms of premating barriers, we collected 1145 males from 68 swarms over 2 years in Doneguebougou, Mali—an area where the forms are sympatric. Identification of the males showed a remarkable spatial segregation in the mating swarms. The molecular forms cluster in distinct form-specific mating units within the village. Moreover, distinctive markers were associated with their swarming sites. Additionally, indoor collection was made in different houses near each swarming site to see if the specific composition of the swarms was a by-product of the spatial clustering of the molecular forms across the village. Both M and S forms of An. arabiensis were spread over the village and found cohabiting houses in the vicinity of the different swarms suggesting that the males and females use specific cues to locate swarms of their own. Our results therefore provide strong support that swarms segregation is the prime mechanism of mate isolation between the forms in Mali. However, this does not exclude the possibility that more than one mechanism of mate recognition operates across the range distribution of the molecular forms.

Email address for correspondence: a_diabate@hotmail.com

Optimising odour baited mosquito trap methods at an experimental field site in the Gambia [MIM16663846]

Musa Jawara

The African malaria mosquito, Anopheles gambiae is highly anthropophilic resulting in its efficiency as vector of the most deadly parasite: Plasmodium falciparum. Vector control is a major component of the global effort of reducing malaria related morbidity. However, insecticide resistance in mosquitoes and concern for environmental impact of pesticides necessitated the search for alternative mosquito control strategies. Host odours play a major role in the orientation of blood-seeking nocturnal female mosquitoes and skin residues are highly attractive for such vectors. Many chemicals present in human odour have been identified. This knowledge may help develop mosquito traps that can reduce the contacts between vectors and humans, or as a surveillance tool. Here we present results of field studies conducted using experimental huts to optimise the use of odour baited MM-X traps as sampling devices for trapping malaria vectors in the Gambia. Four series of experiments were carried out using a Latin square design in six experimental huts to determine the efficiency of traps placed indoors or outdoors, the best trap height for collecting mosquitoes when the best placement was found, the most effective height and distance from the hut for collecting mosquitoes, effect of multiple trapping and orientation for collecting mosquitoes around the huts. Results from these experiments showed that MM-X trap baited with CO2 and nylon sock worn by known volunteer over 12 h attract more mosquitoes than other distances. There was no significant effect on the number of mosquitoes collected inside the huts when multiple traps were used around the hut. MM-X trap set at 15 cm above the ground outdoor at 15 cm above the ground. Traps positioned (0 m from the hut) collected a significantly higher number of mosquito compared to other distances. There was no significant effect on the number of mosquitoes collected inside the huts when multiple traps were used around the hut. MM-X trap set at 15 cm above the ground outdoor and baited with worn nylon sock and CO2 is a positive tool against which Anopheles attraction to synthetic odour blends could be compared.

Email address for correspondence: mjawara@mrc.gm

Can petroleum products (petrol, kerosene and engine oil) be efficiently used for malaria vector control in areas of pyrethroid resistance? [MIM16696153]

Souradjou Mouinatou

The use of petroleum products (PP) against mosquito larvae had an immense success during early programmes of malaria control, but these compounds were abandoned and replaced in the 1950s by synthetic insecticides probably because of the high performances given by these new products. In the current context of vector resistance, it is important to elucidate the empirical use of PP by quantifying their efficiencies on resistant strains of Anopheles. Pyrethroid resistant larvae of Anopheles gambiae were exposed to increasing concentrations of various PP (kerosene, petrol and engine oils) for 24 h and their lethal activities recorded. The highest
concentration (HiC) having no lethal and the lowest concentration (LoC100) yielding 100% mortality were rated for each PP on the Ladiji strain. KAP studies were conducted in three traditional communities where insecticide resistance is clearly established to assess the potential use of PP against mosquitoes. Laboratory analysis of petrol, kerosene and engine oils, clearly established their lethal activities on resistant strains of Anopheles larvae. Contrary to existing references, this research revealed that exposed larvae of Anopheles were mostly killed by direct contact toxicity and not by suffocation as indicated in some earlier reports. This research could serve as scientific basis to backup the empirical utilization of PP on mosquito larvae and to envisage possibilities of using PP in some traditional settings where Anopheles have developed resistance to currently used insecticides.

Email address for correspondence: badmore2@yahoo.fr

A better understanding of the relationships between malaria vectors and their ecological requirements, in particular their trophic relationships not only with human host but also with plant sugar sources is essential for an effective anti-vectorial control strategy. From March to October 2007, sugar feeding behaviour of malaria vectors was monitored within two villages, Bama and Soumouso, Western Burkina Faso. Wild mosquitoes caught outside and inside human dwellings by various collection methods were identified by PCR while the presence of fructose in their crops was detected by cold Anthrone test. A total of 1600 mosquitoes were caught, among which Anopheles gambiae s.l. was the major vector species in the two localities. An unprecedented high sugar feeding rate was observed: 59% of M forms and 80% of S forms were positives for fructose in the semi-arid area (Bama) against 77.6% of M forms and 85.2% of S forms in the Savannah area (Soumouso). There was no significant seasonal variation in the proportion of sugar-fed mosquitoes in both localities. Equal proportions of sugar positive An. gambiae females were nulliparous or parous, inseminate or non-inseminate, indicating similar sugar feeding at any age and physiological status. Collectively, these results indicate that sugar feeding is an important aspect of malaria vector ecology in these localities.

Email address for correspondence: ouedrobert_k@yahoo.fr

**801**

**Simalikalactone D is responsible for the larvicidal properties of Quassia africana [MIM15676802]**

Woquan Sama, Edith O, Ajayeoba, Mohammed I, Choudhary

Botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented non-toxic effects on non-target organisms. The discovery of new insecticides is imperative because of the development of resistance by the mosquitoes to the readily available insecticides. The aim of this study was to isolate and characterize compounds from Quassia africana that are toxic to Anopheles gambiae. The methanol extracts of the leaf, stem and roots of Quassia africana were tested against the fourth instar larvae of An. gambiae. The root extract was partitioned into hexane, chloroform and ethylacetate and the resulting extracts screened for larvicidal properties. The most active extract was subjected to column chromatography and the fractions obtained were screened for larvicidal properties. The fraction with the highest bioactivity was subjected to repeated column chromatography and isolated compounds evaluated for potential toxicity to An. gambiae larvae. Experiments were accompanied by controls. The active compound structure was elucidated using spectroscopic techniques. The root extract showed strongest activity (LD50 = 75 µg/mL). The chloroform soluble fraction obtained after partitioning the crude extract into solvents based on polarities was the most toxic. Further bioactivity-guided chromatographic separation of the chloroform fraction of the root extract led to the identification and isolation of a simalikalactone D as the larvicidal compound in Q. africana (LD50 = 2 µg/mL). The larvicidal properties of Q. africana and simalikalactone D are reported for the first time. Results suggest that Q. africana may serve as a source for vector control agents for malaria.

Email address for correspondence: woquan@gmail.com

**M1**

**MalariaGEN: A global network for genomic epidemiology of malaria**

The MalariaGEN Consortium

MalariaGEN is working to overcome the scientific, ethical and practical challenges involved in performing large-scale studies of genome variation in developing countries by taking a consortial approach. Establishing MalariaGEN involved developing governance policies for a central resource of DNA and phenotypic data; defining a core scientific programme whose results belong to all communities.
MalariaGEN partners involved (Consortial Projects); and supporting partners in endemic countries to develop local studies relating to the core scientific programme. MalariaGEN’s principle scientific goal is to discover the mechanisms of protective immunity against malaria by combining analysis of human genome variation with large-scale epidemiological studies, by: (1) building a global data-sharing network for genomic epidemiology of malaria, (2) generating a DNA and clinical data collection from individuals with different malaria phenotypes, (3) characterising genetic variation in malaria-endemic populations, (4) identifying genetic variants that protect against severe malaria and (5) defining immunological mechanisms of genetic variants that protect against severe malaria. >50,000 human DNA samples and associated clinical data have been collected from 17 sites in 14 countries. This sample set underpins a consortial genome-wide association study (GWAS) of severe malaria, while local data subsets are being used to address unique questions at partner sites. Results from these studies will be presented. Multicentre GWAS of human resistance to malaria and other studies are in progress. However, learning to build and maintain the relationships, shared values, and best practices that underpin this type of scientific collaboration remains an ongoing process for MalariaGEN.

M3
Association of alpha(+)thalassemia and subpatent malaria parasite carriage at different transmission intensities in north-east Tanzania
Alphaxard Manjurano, Tedson Lukindo, Hugh Reyburn, Raimos Olomi, Cally Roper, Sarah Joseph, Eleanor Riley, Chris Drakeley, the MalariaGEN Consortium
We have previously described correlations between altitude and Plasmodium falciparum (Pf) and antibody rates, haemoglobin levels and alpha(+)thalassemia in children under five in the eastern arc mountains of north-east Tanzania. As part of MalariaGEN, we have expanded the original studies to assess the relationship between alpha(+)thalassemia and subpatent Pf carriage in all age groups. Samples were collected from individuals aged 1–45 years in 12 villages in north-east Tanzania where entomological inoculation rates range from <1 to >50 infectious bites per person per year. Genotyping for alpha(+)thalassemia and subpatent parasite carriage was undertaken and analysed by age and transmission intensity. The alpha(α3.7) deletion allele frequency was 16.9% (N = 2999); ranging from 6.6% in one highland village to 33.9% in a lowland village. Overall, ~28% and ~3% were heterozygous and homozygous for a alpha(+)thalassemia, respectively. Carriage of sub-microscopic parasites was higher in heterozygous and homozygous alpha(+)thalassemics individuals compared with normal individuals. There was a decrease in carriage of sub-microscopic parasites with increasing altitude. A higher prevalence of malaria parasites was observed by PCR compared to microscopy. Heterozygous and homozygous alpha(+)thalassemics are associated with subpatent Pf carriage but further studies are required to determine how this may protect from disease. The increased sensitivity of PCR over microscopy highlights the question of using it as a more sensitive diagnostic tool for malaria epidemiological studies to fully appreciate the reservoir of infection.

M4
Preliminary results of an immunogenetic investigation in Dielmo, Senegal
Previous studies have suggested that host genetic make-up is important for determining the level of antibody production during malaria infections. We have combined immunological and genetic studies to investigate this question. Ongoing longitudinal cohort studies are being conducted in two Senegalese villages, Dielmo (holoendemic area) and Ndopi (mesoendemic area). DNA and serum samples were collected for the period 2007–2008 for inclusion in MalariaGEN Consortial Project 2. Sera were assayed by ELISA with several malaria antigens by standardised protocols and DNA samples were genotyped by Sequenom iPLEX in candidate gene regions for antibody production and known malaria-associated genes. Among 518 subjects (mean age 13.9 years [7.2–30.9 years]), the total number of Plasmodium falciparum (Pf) attacks were 345 and the mean peak parasitaemia was 15,973.48 ± 31,247.98 during these attacks. Controlling for village and age, the antibody responses to AMA1 and to MSP1 were significantly associated with a higher (RR = 1.2 [1.0–1.2]) and a lower (RR = 0.7 [0.6–0.9]) number of Pf malaria attacks, respectively. MSP1 antibodies were significantly associated with a lower mean Pf parasitaemia during clinical malaria attacks (RR = 0.90 [0.81–0.99]). AMA1 antibodies were significantly associated to higher mean Pf parasitaemia during asymptomatic period, RR = 1.2 [1.1–1.4]. Further analysis of other immunological and genetic data will be performed since previous studies in Dielmo had found that other immune responses such as IgG3 antibodies directed to MSP3 were strongly associated with protection against clinical malaria.
A recent study from Kenya demonstrated that approximately 25% of the variation in response to malaria infection is due to host genetic factors. HBs in this setting for example only accounts for 2% of the total variation. Our aim is therefore to identify and investigate other variants associated with resistance or susceptibility to malaria. Cases (children suffering from strictly defined severe malaria) were recruited at the Kilifi District Hospital, Kenya through a system of routine ward surveillance. Standardised information was collected on all children presenting to the ward for admission. Children were examined according to a fixed protocol and samples collected for malaria microscopy, haematology, bacteriology and study-specific assays. Controls were healthy children recruited during their first 12 months of life. Approximately 2226 cases of severe malaria (mean age, 31 months) have been identified with their corresponding clinical data. 41.4% of the cases presented with coma, 35.6% with deep breathing and 34.3% with prostration and 21.3% with severe malaria anaemia. Overall mortality was 10.8%. A set of 4000 controls has been selected and matched to cases for the confounders’ ethnic group, sex and location of residence. The collection of cases and controls and validation of clinical and epidemiological data forms an essential part of a process for genome-wide studies. Samples then undergo a QC process involving quantification and genotyping for 65 SNPs for which data will be presented.

Host and parasite factors play an important role in determining the outcome of malaria parasite infections. This study was conducted within the MalariaGEN consortium to explore genetic determinants of immune responses to malaria. We conducted cross-sectional malaria surveys in eight villages (four from highland and four from lowland areas) with similar parasite rates (35.5% vs 33.6%; \( p = 0.40 \)) in Muheza, northeastern Tanzania. Plasma and DNA samples were collected from 768 individuals aged 0–45 years. DNA samples underwent initial assay for 65 SNPs across a range of malaria-associated genes and the plasma were assayed by ELISA for five antigens. After adjusting for age, ethnicity, parasite density and transmission intensity, eleven SNPs (HBs, IL4:rs2243250, IL22:rs2227478, IL10:rs1800896, IL4R:rs1805015, ADCY9:rs223073, GNAS:rs8386, NOS2A:rs2279518, TNF:rs3093662, CD40LG:rs17424229 and G6PD202:rs1050828) were significantly associated with antibody responses to one or more of the tested antigens (\( p < 0.05 \)). LTA and NOD1 were significantly associated with antibody response to CSP and AMA1, respectively, but only when unadjusted for other covariates. Only three SNPs (ADCY9:rs223073 and G6PD202) were associated with antibody response to CSP. HBs is involved in protection against erythrocyte invasion probably indicating why heterozygous individuals had low antibody levels. Individual heterozygous to IL4, IL10 and IL22 had high antibody levels suggesting the potential role of genetic mechanisms for enhancing antibody production.

Inter-ethnic differences in the susceptibility to malaria: A large-scale immunogenetic study from Burkina Faso

Previous investigations showed that the Fulani are less susceptible to malaria than sympatric populations. This study aims to identify protective immune responses and underlying genetic factors in the Fulani and sympatric ethnic groups from Burkina Faso. The study was carried out in four rural villages near Ouagadougou. It consisted of a combination of cross-sectional and longitudinal surveys conducted in children aged 0–5 years during the high transmission seasons. Data collected from the start of the study and at the beginning of the rainy season showed lower Plasmodium falciparum infection rates (\( P < 0.001 \)) and densities (\( P < 0.001 \)) in Fulani (35.9%, 170/473; 338 parasites/\( \mu l \)) compared with Mossi (56.4%, 224/397; 518 parasites/\( \mu l \)) and Rimaibé (50.2%, 254/506; 501 parasites/\( \mu l \)). At the end of that rainy season lower infection rates (\( P < 0.001 \)) but similar parasite densities (\( P = 0.87 \)) were observed in Fulani (25%, 185/741; 334 parasites/\( \mu l \)), compared with Mossi (51.6%, 297/576; 364 parasites/\( \mu l \)) and Rimaibé (50.8%, 216/425; 302 parasites/\( \mu l \)). During the longitudinal survey of morbidity, 235 Fulani, 221 Mossi and 198 Rimaibé children were followed up. No inter-ethnic differences were observed for the incidence of clinical episodes associated with \( P. falciparum \) density. These data confirm the observation that the Fulani are more resistant to malaria than either the Mossi or Rimaibé in Burkina Faso. We therefore believe that the design and sample size of the present study will prove powerful for the investigation of inter-ethnic differences in the susceptibility to malaria.

Ethical issues in genome-wide association studies in developing countries

With the proliferation of whole-genome association (WGA) studies there is increasing consideration of the ethical challenges that such research invokes. We discuss each of these challenges and their ethical implications, and suggest possible solutions or research avenues leading to a better understanding of how these issues play out in genomic research in resource-poor countries. We worked closely with the MalariaGEN Consortium which is applying whole genome analysis to malaria. In this capacity, we identified, discussed and provided ways of managing ethical challenges. We developed a consultative model in which we worked with various stakeholders to achieve this goal. We identified a number of fundamental ethical challenges in undertaking WGA studies in resource-poor countries. These include sample and data ownership, fair distribution of the risks and benefits of research, consent, and obtaining appropriate ethics approval. As a result we developed guidance on informed consent and policies on data release and confidentiality, and a research programme to investigate some of these challenges. In order for WGA methodology to be successful in resource-poor countries, attention needs to be paid to the ethical challenges that this invokes. Our model of close interaction with the scientific project offers an example of how this can be done.
M10
Sample Handling for undertaking genome-wide studies of severe malaria
Kirk Rockett on behalf of the MalariaGEN Consortium

One of the challenges in undertaking a study into the genetic basis of malaria is to achieve high enough power for robust analyses. MalariaGEN has created a network of researchers from 15 malaria-endemic countries to address this question. This has meant building systems to gather, store and share information/data as well as standardised sample-handling and processing procedures. The sample-handling pipeline takes advantage of the diverse expertise across the network. Sample collections are managed locally, while standardised methods for sample volume/concentration measurements, storage and genomewide genotyping are handled centrally. All samples are genotyped upon receipt for 65 malaria-associated SNPs plus gender on the Sequenom iPLEX platform. Across 15 malaria-endemic countries, the network has currently built a resource of >50,000 DNA samples. Greater than 95% of samples have >95% success rate in Sequenom genotyping with data immediately available to partners through a secure web interface. Samples passing these quality control are then prepared for genomewide and fine-mapping studies.

Collective experiences across sites have allowed not only method development appropriate to each setting but also capacity building for future projects. Analyses within and across sites for the QC polymorphisms may help clarify the published associations in the largest study of its kind. The amounts of DNA available from malaria settings have meant that further experiments will require whole-genome amplification, notwithstanding this, to date >5000 cases and >5000 controls have undergone whole-genome amplification and genomewide genotyping.

M11
Polymorphism in the inductive nitric oxide synthase reductase domain is associated with protection from severe malaria in Northern Ghana
Lucas N. Amenga-Etego, Anita K. Ghansah, Abraham R. Odouro, Kwadwo A. Koram, Abraham V.O. Hodgson, Michael D. Wilson, William O. Rogers, the MalariaGEN Consortium

Nitric oxide is an important mediator in the host defense against *Plasmodium falciparum* malaria. However, its role in clinical disease remains poorly understood. We investigated variations in the NOS2A gene and susceptibility to severe malaria in Ghana. Six NOS2A promoter single nucleotide polymorphisms (SNPs) and one exon SNP were tested in a case–control study among children with severe malaria, and healthy population controls from Kassena-Nankana district (KND) in Northern Ghana. A frequency-matched case–control study design was used with children aged 6–60 months matched on age, sex and location. SNP genotyping was performed using primer extension mass spectrometry (Sequenom iPLEX). We analyzed seven SNPs in the NOS2A gene in 792 cases and 805 controls. We report a novel association of the NOS2A gene at the rs2297518A/G polymorphism with severe malaria (*P* = 0.01). The homozygous mutant (rs2297518AA) confers protection from severe malaria (OR = 0.18 [0.04–0.08] adjusted *P* = 0.02). This mutation leads to a nonsynonymous substitution at amino acid position 608 (Ser → Leu). It has been reported that the Leu variant leads to a more activated NOS2A expression and elevated levels of NO in target cells. Therefore, the over representation of the rare Leu variant in controls in our study suggests that over production of NO may be beneficial during the acute phase of malaria disease and may be the source of directional selection pressure that drives this mutation.
M13
Antenatal versus during labour consenting in a semi-urban Gambian population

M. Jallow, J. de Vries, K. Bojang, D. Conway, the MalariaGEN Consortium

To investigate the genetic basis of resistance to malaria we aimed to collect 2000 population controls. Having carefully considered the ethical issues associated with collecting such samples from healthy children we decided to use cord bloods. This approach presents challenges regarding the timing of consent as there are concerns about the appropriateness of seeking consent during labour. An alternative is to consent during antenatal health checks but this presents challenges in settings where such checks are not routine. We piloted a study to evaluate the feasibility and appropriateness of both approaches. We obtained consent and enrolled 1055 women during labour and visited 100 of them one week after childbirth to seek their opinions on participating. Antenatal consent was obtained from 858 women in two health clinics in semi-urban Gambia. On the follow-up visit the women consented during labour seemed to have understood the study well and were happy to maintain their consent. However, only 155 (18.1%) of women consented during antenatal visits were traced at delivery. Of those women 115 (13.4%) enrolled into the study after confirming that they still maintained their consent and 40 (4.7%) were not enrolled for reasons other than withdrawal of consent. Only four (0.5%) of those who consented antenatally withdrew their consent. This study shows that while antenatal consenting is not an efficient method of collecting large numbers of controls in this setting, consenting during labour seems both appropriate and effective.

M14
Malaria-candidate SNPs in population and hospital-based studies: A role for sub-structuring?

Nahid A. Eid, Abier M. Elzein, Aymen Hussein, Hiba S. Mohammed, Muntaser E. Ibrahim, the MalariaGEN Consortium

The MalariaGEN Consortium is investigating the genetic basis of immunity to malaria across 15 countries with different malarial epidemiological settings. In eastern Sudan we study malaria in two populations of different ethnic origins (Hausa and Massalit) where endemicity is hypo to meso-endemic with clinical outcomes of mostly mild to subclinical disease. We contrasted this with a hospital-based study from the Sinnar area in eastern Sudan where malaria is mesoendemic but with more clinical disease. A total of 400 DNA samples were collected from the study populations and genotyped on the Sequenom iPLEX platform for a selection of SNPs defined in Hausa compared to Massalit. In Sinnar there was no significant association between cases and controls for several SNPs: IL4R-63011 (p = 0.001), CR rs17047661 (p = 0.02) and TNFSF5A (p = 0.01) in Sinnar; IL10-1082 and G6PD-376,202 in Massalit, and IL4-590 in Hausa. Significant associations were found in the hospital study in SNPs that have already been implicated in protection/susceptibility to severe malaria. We also noted an interesting distribution of malaria between the different sub-structure clusters in the population study which suggests a role for population stratification in resistance/susceptibility and warrant further investigation.

M15
Cytokine gene SNPs are associated with severe malaria in Vietnam

N.T.N. Quyen, N.H. Phu, C.Q. Thai, T.T. Hien, J.J. Farrar, S.J. Dunstan, the MalariaGEN Consortium

Although the incidence has decreased in the last decade, malaria still remains a serious public health problem in Vietnam. It has been suggested that in some populations genetic factors may contribute as much as 25% towards protection/susceptibility to malaria. We are undertaking studies to identify genes associated with protection from severe malaria in the Vietnamese. OUCRU Vietnam has contributed DNA samples from >1000 severe malaria cases and >2500 controls to MalariaGEN. Severe malaria patients were recruited between 1991 and 2008 and cases and controls are predominantly of the Vietnamese Khinh ethnicity. We have begun to prepare samples for high-density SNP typing by assaying 72 malaria-associated single nucleotide polymorphisms (SNPs) using the Sequenom iPLEX platform. Sixty-eight SNPs in 41 malarial candidate genes and four SNPs in the AMELX gene (gender confirmation) were genotyped in 942 cases and 2520 controls of the Vietnamese Khinh ethnicity. Three SNPs were associated with severe malaria; IL17RE rs708567, OR 1.24, 95% CI 1.05–1.47, p = 0.013; IL13 rs2054, OR 1.13, 95% CI 1.01–1.27, P = 0.029 and TGFalpha rs1799694, OR 1.16, 95% CI 1.02–1.31, P = 0.026. One SNP was marginally associated with severe malaria; IL1A rs17411697, OR 0.85, 95% CI 0.67–1.07, P = 0.0116. The SNP associations found within or near cytokine genes in Vietnam support those previously identified mostly in Africa. Finer-mapping will be required to further explore these regions of association to investigate any commonality with Africa. In addition genome-wide association studies will help identify other regions and genes in the search for new mechanisms of protective immunity against malaria.

M16
A sero-epidemiological study in a previously highly malaria endemic area in Sri Lanka

R.L. Dewasurendra, S.D. Fernando, R. Carter, N.D. Karunaweera, the MalariaGEN Consortium

Kataragama lies in the dry lowland coastal plains of southeast Sri Lanka. Though considered to be a malaria endemic area, the number of malaria cases has decreased dramatically over the past 5 years. This study investigates the immune status of residents from selected areas by measuring antibody levels against known malaria antigens. Blood was collected from 1011 individuals (50.8% male) and serum was separated. Antibody titres against antigens PfAMA1, PfMSP1, PfMSP2, PfNANP, IgE, PvAMA1, PvMSP1 and total IgE level were determined by ELISA and analyzed in relation to gender, age group, history and number of malaria attacks. IgE levels were highly significant in males (p < 10^{-3}). No other significant differences were found between sexes for the other antibodies. There were significant increases in antibody levels for PfMSP1 (p = 0.001) and PfAMA1 (p < 10^{-3}) in individuals between 45 and 59 years. Over the last 10 years 188/1011 individuals suffered one or more malaria attacks (Group A), 530 suffered none (Group B) and 293 could not remember (Group C). There were significant increases in antibody levels for PfAMA1 (p = 0.004), PfMSP2 (p = 0.027), PfNANP (p = 0.002) and PvMSP1 (p = 0.003) in Group A compared with Groups B and C. No significant correlations were identified between antibody titres and the number of malaria attacks. In this low malaria transmission area there appears to be age-acquired immunity up to 59 years old which is likely to be due to repeated exposure to malaria.
M17
Developing research capacity in genetic data analysis in malaria-endemic countries


The MalariaGEN Network is a collaborative data-sharing community where members (1) contribute to a central repository of DNA samples and core clinical data and (2) receive genotype and phenotypic data to enable local analyses. A key aim of the network is to develop research capacity across malaria-endemic countries in the new science of genomic epidemiology. To build a cohort of scientists with expertise in genetic data analysis a network of 14 Junior Fellows and three Senior Fellows was established across partner sites. These fellows have a wide range of backgrounds including clinicians, molecular biologists and informaticians. Support has been provided in the collection, management and analysis of linked genetic and phenotypic data through data analysis clinics (regional and global), training workshops, site visits and distance mentoring. Feedback suggests that the support has enabled Junior Fellows to understand basic concepts (genetic epidemiology, data management and statistical analysis), share experiences, understand the large-scale genome wide association analysis and carry out their own local genetic analyses. As a result all 14 fellows have been able to submitted abstracts to the 2009 MIM Conference based on their local studies and analysis. Due to its collaborative nature and the emphasis placed on building capacity the start up phase of MalariaGEN has taken time. What has been gained is a dynamic and mutually supportive group of scientists from malaria-endemic countries with expertise in genetic data analysis. This has laid the foundation for on-going locally generated research.

M18
A cost-effective method to purify Plasmodium DNA from clinical malaria samples for “next generation” whole genome sequencing

Sarah Auburn, Susana Campino, Abdoulaye A. Djimde, Jean Bosco Ouédraogo, Issaka Zongo, Taane G. Clark, Valentina Mangano, David Modiano, Robert Pinche, Ogobara K. Doumbo, Christopher I. Newbold, Dominic P. Kwiatkowski

“Next-generation” whole genome sequencing technologies are facilitating investigations of genetic diversity of Plasmodium falciiparum genomes within and between populations. To determine the diversity in “natural” parasite populations requires the ability to sequence clinical (non-cultured) malaria samples. A major hurdle is the abundance of human DNA in samples extracted directly from patient blood. The aim of this study was to identify a simple and cost-effective method to remove human white blood cells (WBCs) from clinical blood samples using standard laboratory facilities. A preliminary assessment of the WBC-depletion efficacy of combinations of lymphoprep density gradient centrifugation, Plasmodipur filtration and magnet-based anti-HLA1 dynabead separation were undertaken on clinical malaria samples in Mali. Further validation of a method using lymphoprep + Plasmodipur was sought in a larger set of clinical malaria samples from Burkina Faso. In the preliminary assessment, a combination of lymphoprep + Plasmodipur + dynabeads yielded the lowest average human contamination (~10%), followed by Plasmodipur + lymphoprep (~40%), lymphoprep + dynabeads (~62%) and Plasmodipur alone (~63%). Despite high WBC-depletion efficacy, the 3-method approach also proved to be the most laborious and costly. Large-scale assessment of Plasmodipur + lymphoprep in Burkina Faso yielded a lower average human contamination level (~20%). For large-scale genetic studies requiring near-pure Plasmodium DNA from clinical malaria samples, a combination of lymphoprep and Plasmodipur is time and cost-efficient. For more bespoke studies, a combination of lymphoprep, Plasmodipur and dynabeads is more effective.

M19
Challenges of acquiring patient consent for genetic studies in Cameroon

Eric Achidi, Tobias Apinjoh, Regina Mugri, Andre Ndi, the MalariaGEN Consortium

We are performing case–control and family trio studies to investigate the genetic basis of resistance against malaria. Here we describe the challenges of patient recruitment for this study and the approaches taken to overcome them. To obtain controls for our case–control study, informed consent was sought from parents of healthy children after authorisation by relevant school/health authorities. Establishing direct contact with parents was difficult so teachers were asked to forward information sheets and written informed consent forms. For family trio studies, relevant candidates were identified and approached through respected community leaders, who accompanied clinicians on house visits to discuss the project’s objectives, risks, and possible benefits. Using unsupported written consent with parents of school children we experienced >50% rejections, 25% indecisiveness/confusion and <25% acceptance. This could mainly be attributed to illiteracy, fear of contamination (HIV) and cultural bias associated with blood collection (e.g. witchcraft). With the enhanced trust that resulted from discussing doubts/questions directly with research team members and community leaders, >90% of cases, families, and blood donors agreed to participate. Improved participant recruitment was achieved through a combination of written and oral education approaches, and was particularly enhanced through personal contact using local languages. Engaging the participation of government authorities and teachers was instrumental in assuring parental consent for healthy children. Motivating factors included: contributing towards a lasting solution to malaria, receiving results of laboratory analyses and necessary malaria treatment and free transport.

M20
Haplotypic analysis of SNPs in candidate genes for severe malaria in Malawi

V.B. Nyirongo, T.E. Taylor, M.E. Molyneux, the MalariaGEN Consortium

We selected samples from an on-going study of severe malaria in Malawi to contribute to a genome-wide association study. We added information on malarial retinopathy in all patients with cerebral malaria (CM) to explore the impact of using a definition of cerebral malaria that includes retinopathy (CM-R). We recruited cases of cerebral malaria from 1997 to 2005. As controls we collected cord blood samples from babies born at the same hospital from 2005 to 2007. SNPs across a number of published malaria-associated genes were genotyped on the Sequenom platform. We analysed the association with cerebral malaria of 77 SNPs in candidate genes in 1383 cases and 3578 controls, and compared the findings of patients meeting the standard case definition of cerebral malaria (CM) with those from patients with CM-R. Where several SNPs were assayed in a single gene, haplotype analysis was
undertaken. Our single SNP analysis confirms the known protection afforded by the sickle-cell trait, our positive control in the experiment (CM, \( p = 1.5 \times 10^{-12} \), CM-R, \( p = 5.74 \times 10^{-12} \)). We also found strong associations between CM and haplotypes on ABO blood group and IL22 genes on chromosomes 9 and 12 and a mild association for haplotypes on the IL10 gene on chromosome 1 \( (p = 1.93 \times 10^{-6}, 7.00 \times 10^{-11} \text{ and } 7.00 \times 10^{-3}) \). We find suggestive evidence for IL22, ABO and IL10 associations in Malawian children with cerebral malaria. These, and the value of the more specific case-definition (CM-R), will require larger numbers for their confirmation.

**M21**

**Insights and challenges to large scale genetic epidemiology in Africa from a case study of severe malaria in the Gambia**

Yik Y. Teo on behalf of the MalariaGEN Consortium

Genetic epidemiology in Africa poses a different set of challenges to similar studies performed in European or Asian populations, in terms of both designing the experiment and analyzing the genetic data for phenotypic association. To investigate whether a genome-wide strategy is applicable in the context of African populations, we undertook a genome-wide survey of severe malaria in 2560 Gambian children, assaying across 500,000 SNPs. In addition to confirming the protective effect at the Hbs locus, we successfully identified 18 regions with putative association to malaria, and verified three of these regions in an independent cohort of 3463 Gambian children. This pilot study provided vital insights into the challenges of conducting large-scale genetic studies in Africa, verifying the use of sophisticated statistical strategies to handle the high genetic diversity from multiple sub-populations in a single African country. Imputation strategies that relied on leveraging off information obtained from the International HapMap Project have been shown to work well for European populations. This pilot study showed that the Yoruba dataset from the HapMap was of limited use for a different African population and instead required a population-specific reference panel. Understanding the analytical challenges is thus vital to the successful conduct of large-scale genetic epidemiological studies in Africa.

**A1**

**Integration of drug metabolism and pharmacokinetics (DMPK) in the chemistry and pharmacology of the discovery and development of antimalarials**

Collen Masimirembwa, Peter Smith, Kelly Chibale

In the pre-1990 era, poor pharmacokinetics (PK) accounted for more than 40% of the failure of new chemical entities (NCEs). Strengthening of DMPK research in the pharmaceutical industry over the past 15 years reduced attrition rates due to PK to below 10%. Our work therefore aimed at setting up industrial DMPK platforms to support drug discovery activities in Africa. Thirty (30) antiparasitic drugs and 30 NCEs with in vitro antimalarial activity were evaluated using lead discovery and optimisation strategies. During lead discovery, compound libraries from various series were screened for DMPK properties using in silico and in vitro systems. In silico approaches included evaluation of compounds for drugability properties. In vitro studies included metabolic studies using human and rat liver microsomes. During lead identification, computational and in vitro evaluations were done with respect to permeability, metabolic stability, enzyme identification, and metabolite identification with a view to identify ADMET issues and propose ways to solve them during lead optimisation. Results on the evaluations of 30 antiparasitic drugs in current use and 30 NCEs will be used to demonstrate this paradigm of drug discovery. Results on amodiazole DMPK/PD-Tox demonstrate the utility DMPK/PD-Tox in the safe clinical deployment of medicines. We have demonstrated the utility and feasibility of integrating industrial DMPK in the discovery and use of antiparasitic drugs. Researchers in Africa can now have access to these in silico and in vitro platforms at AiBST.

**A2**

**Novel functionalized benzimidines as potent antimalarial agents**

Stefano Pegoraro, Yulin Wang, Michael Lanzer, Christian Portaluppi, Alicia Moreno-Sabater, Henri Vial, Clemens Kocken

Within the EU financed AntiMal project (FP6–Malaria Drugs Initiative) we have been able to further develop a novel class of substances which showed high in vitro activity vs multidrug-resistant *Plasmodium falciparum* strains (Leban J. et al., Sulfonyl-phenyl-ureido benzimidines: a novel structural class of potent antimalaria agents. Bioorg. Med. Chem. Lett. 2004;14:1979–1982). With the goal to discover a potential new antimalaria drug, we started a lead optimization program to improve the ADMET/PK properties of these promising compounds and to prove antimalaria activity in suited in vivo malaria models. Several compounds were synthesized and tested vs *P. falciparum* in a cellular assay and a clear SAR was established. Further profiling of the most promising molecules includes full ADMET/PK characterization and antiparasite mode of action (intra-erythrocyte stage specificity and rate of parasite killing). Antimalaria efficacy in vivo was determined using different animal models. The most common rodent model (*P. berghei*) was not suitable to test the efficacy of this class of molecules, as *P. berghei* was inert both in vivo and ex vivo. Efficacy was demonstrated with an alternative rodent model (*P. vinckey*) although at relative high doses. Finally, we were able to obtain full parasite clearance at low doses using the humanized-mouse model with *P. falciparum*. A novel class of antimalaria compounds has been found. The synthesis of these compounds is relatively easy; they possess good physico-chemical properties and are not toxic. Antimalaria properties were confirmed in vivo in the humanized-mouse *P. falciparum* model. An early development candidate was selected and a primate model is in preparation.

**A3**

**New approaches for the treatment of severe malaria: Bis-thiazolium SAR97276**

Henri Vial, M. Calas, S. Peyrottes, F. Bressolle, S. Wein, C. Kocken, S. Herrera, L. Fraisse

An advanced pharmacological approach targeting parasite metabolism relates to inhibition of de novo *Plasmodium falciparum* phosphatidylcholine biosynthesis. We have identified choline analogs that inhibit *P. falciparum* asexual blood stages at single digit nanomolar concentrations. Proof of fully curative antimalarial activity with short course treatments has been obtained in rodents and non-human primates infected at high parasitemias. These compounds are thought to inhibit choline transport and also exert an effect on parasite phospholipid biosynthesis. Effect of the bithiazolium salts on the phosphatidylcholine biosynthesis has been studied in detail. We have provided experimental evidence that potency of the bis-ammoniums benefits from their mechanism of action. This includes what we called now a Trojan horse effect due to the drug accumulation inside the parasite. Indeed, the bithiazolium salts exert very rapid effect on parasite viability in the
infected erythrocyte even though the morphological alterations are delayed. Compounds also accumulate in vivo in parasitized erythrocyte of \textit{P. vinckei}-infected mice. PK/PD studies in \textit{P. vinckei}-infected mice show enhanced level of the lead compound T3 in infected mice. Human clinical trials with this exciting new class of compounds are currently under way for severe malaria operated by Sanofi-Aventis. Current goal is to succeed a formulation or a prodrug approach for an oral treatment of uncomplicated malaria.

A4 Assessment of \textit{Plasmodium falciparum} tyrosine kinase-like (TKL) protein kinases as potential anti-malarial drug targets

Abdirahman Abdi, Sylvain Eschenlauer, Christian Doerig

\textbf{Introduction:} Protein kinases are targets for cancer chemotherapy, and we propose that enzymes of this class might also be considered as targets in the context of malaria. The vast phylogenetic distance between \textit{Plasmodium} and human protein kinases may translate into selective inhibition of the former. In this study, we assessed the potential of a group of five parasite protein kinase that belong to tyrosine kinase-like kinases (TKLs) as anti-malarial drug target.

We expressed five \textit{Plasmodium} TKLs as recombinant proteins in \textit{E. coli} to gather information about their biochemical properties and used reverse genetics to assess their role in the parasite’s life cycle. We succeeded in producing three PTKLs as active recombinant proteins, the activity of one of these (PTKL3) is dependent on a regulatory domain called sterile alpha motif (SAM domain) that mediates oligomerisation of the kinase. Disrupting SAM domain-dependent protein–protein interaction represents a possible strategy for chemotherapy, in addition to the classical approach consisting of targeting the catalytic domain. We obtained reverse genetics evidence that PTKL3 and PTKL1 are essential for completion of the erythrocytic asexual cycle, while the other three PTKLs are dispensable for this stage. Through biochemical and functional analysis, we validated two members of \textit{P. falciparum} TKLs as potential schizonticidal targets, one of which is active as a recombinant enzyme that can be used for the screening of chemical libraries.

A5 \textit{In silico} and \textit{in vitro} ADMET screening for lead identification in the discovery of new chemical entities (NCEs) against malaria and other infectious diseases

Roslyn Thelingwani, Ismael Zamora, Peter Smith, Kelly Chibale, Collen Masimirembwa

There is widespread resistance to many of the antimalarials currently on the market. There is, therefore, a need to find drugs which have better efficacy and safety profiles. In this study, 30 new chemical entities (NCEs) that had \textit{in vitro} activity against malaria have been screened for ADMET properties using \textit{in silico} and \textit{in vitro} methods. The aim of the screen strategy was to define the chemical space of the NCEs with respect to ADMET properties. HPLC-UV methods were used to determine compound purity and estimate lipophilicity from $k'$ measurements. Compound solubility was determining using the turbidometric methods. GRID software was used to calculate the molecular interactions fields (MIF) of the compounds and VOLSURF® for the prediction of permeability. Metasite®, was used to predict sites of metabolism. NCEs were characterised with respect to metabolic stability in rat and human liver microsomes. Identification of the human CYP450s involved was done by screening against a panel of 7 rCYP450s. The inhibitory effects of the NCEs were evaluated on five major CYP450. Each compound violated at least two of the Lipinski’s rules for compound drug ability and lead-like properties with respect to Mwt, logP, PSA, solubility, metabolic stability, predicted permeability, metabolic clearance and CYP inhibition. The results from these ADMET evaluations are now being used to select a series with optimizable ADMET properties. Based on the SAR for the ADMET properties, \textit{in silico} methods are being used to virtually design molecules predicted to have favourable ADMET properties.

A6 Towards candidate selection of a novel 1,2,4,5-tetraoxane with antimalarial properties superior to the semi-synthetic artemisinins

R. Amewu, S.A. Ward, P.M. O’Neill

Currently the semi-synthetic artemisinin derivatives artesunate and artemether are the mainstay of malaria chemotherapy but their use is hampered by supply and cost. Most people needing treatment for malaria cannot afford drugs containing artemisinin; nevertheless, artemisinin and its derivatives remain the most effective antimalarials and are currently used in combination with other drugs as recommended by the World Health Organization. Interest in the antimalarial properties of tetraoxanes is growing. This stable class of endoperoxide is believed to possess a similar mode of action as Artemisinin and related compounds, however, since they are purely synthetic and made from readily available cheap materials they may offer a very cheap and effective solution for malaria chemotherapy. Here we report the synthesis of a library of 1,2,4,5-tetraoxanes and exploration of their antimalarial properties towards the selection of a potential clinical candidate. In parallel to antimalarial evaluation, by systematic evaluation of in vitro metabolic stability and pharmacokinetic properties in rodent models, we have discovered several novel, polar 1,2,4,5-tetraoxanes with superior pharmacokinetic and stability profiles to the semi-synthetic artemisinins and the 1,2,4-trioxolane drug candidate OZ277. Preliminary in vivo evaluation also demonstrates outstanding profiles for these analogues. The presentation will conclude with our most recent medicinal chemistry optimisation work that has led to a simple two-step procedure for the synthesis of novel analogues from cheap starting materials with excellent activity profiles.

A7 Anti-inflammatory and vasoactive properties of quinoline and artemisinins antimalarials: Modulation of endothelin-1 and cytokines production by microvascular endothelial cells

Nicoletta Basilico, Silvia Parapini, Sarah D’Alessandro, Yolanda Corbett, Sara Finaurini, Donatella Taramelli

Severe malaria pathogenesis involves both overproduction of inflammatory cytokines and cytoadherence of parasitized erythrocytes to the microvasculature, leading to local obstruction and hypoxia. Hypoxia and cytokines stimulate endothelial cells to produce endothelin-1 (ET-1), a vasoconstrictor peptide that, in turn, regulates the inflammatory response and, in pregnancy, is involved in pre-eclampsia. Active ET-1 derives from BigEndothelin-1 after processing by endothelin converting enzymes (ECE). To investigate whether, beyond parasite killing, antimalarial drugs could also play an anti-inflammatory role, the effects of quinolines and artemisinins antimalarials on the in vitro production of ET-1 and inflammatory cytokines was tested. Human microvascular endothelial cell line (HMEC) treated with quinolines or artemisinin derivatives under normoxia or hypoxia were analyzed for the
production of ET-1 and cytokines (IL-6, IL-8) by RT PCR and ELISA. The inhibition of constitutive or hypoxia-induced ET1 secretion by HMEC in the presence of quinolines or arteisinins was dose and time-dependent, and not due to direct toxicity. However, the mechanism of inhibition was different: quinolines, including chloroquine, inhibited the processing by ECE, but not the production, of the precursor Big-ET1 to active ET1. Activity was dependent upon the weak base properties of the drugs. On the contrary, arteisinins inhibited the synthesis of Big-ET1 indicating an effect on transcription. Modulation of cytokine secretion was also observed. These results may explain some of the pharmacological effects of antimalarial drugs such as hypotension after CQ treatment and suggest that their use in pregnancy could help preventing preeclampsia.

A8 Synthesis and metabolism studies of antimalarial biscationic drugs and their prodrugs
Olivier Berger, Françoise Bressolle, Yen Vo-Hoang, Roger Escale, Thierry Durand, Henri Vial

In the frame of the development of a new chemotherapy to fight against the emerging multi-drug resistance of Plasmodium falciparum malaria, we describe here the design of a new series of asymmetric bicationic compounds targeting plasmodial phospholipidic metabolism. Indeed, bis-alkylamidines are potent antimalarials in vitro and in vivo after ip administration.1,2 N-substituted C-alkylamidoximes were synthesized as prodrugs of these biscationic drugs. Subsequently, we established that the amidoxime prodrug strategy is less efficient at delivering oral antimalarial activity for alkylamide drugs than when this strategy is used for the benzamidine series. The aim of this study was to establish structure-bioconversion relationships applying to asymmetrical compounds possessing an aromatic ring. Since they can be detected by UV, we optimized conditions for HPLC-UV analysis. Before evaluating metabolic transformations, we studied the stability of drugs in plasma and blood. The incubation of pro-drugs in presence of hepatic microsomes led to the disappearance of amidoxime pro-drugs and the formation of alkylamide drugs.3–5 Thanks to these products, we showed that benzamidoxime function as well as alkylamidoxime moiety are efficiently converted into benzamidines or alkylamidines and this reduction is not altered by N-substitutions. These results suggest that other factors must be involved in adequately explaining the difference in antimalarial activity for the two sub-types of pro-drug following oral administration in rodent models of malaria.

A9 Role of heme in the antimalarial activity of peroxide-containing drugs
Fatima Bousejra-El Garah, Bernard Menunier, Paul M. O’Neill, Anne Robert

The 1,2,4-tioxane core structure of artemisinin is essential for its activity. In the 90s, it was shown that iron(II) catalyses the reductive cleavage of the peroxide bond of the drug, leading to the formation of oxy-radicals that rapidly rearrange to more stable C-centered radicals.2,3 These latter radicals were shown to react with heme, resulting from host cell hemoglobin digestion, and parasitic proteins. Today, many researches continue to investigate arteisinin’s mechanism of action. The involvement of iron(II)-heme in arteisinin antimalarial activity has been further investigated in vivo and covalent heme-artemisinin adducts were detected in the spleen and urine (as metabolized adducts) of P. vinckei infected mice, orally treated with arteisinin, whereas they were absent in healthy mice treated in the same condition. These alkylation processes are believed to induce parasite death. In this presentation we will report our investigation into the reactivity of iron(II)-heme towards highly active antimalarial peroxide-containing drugs including a novel class of antimalarial 1,2,4,5-tetraoxane.

A10 Development of a synthetic strategy to novel endoperoxides structurally related to Plakortin
Francisc Marti, Sandra Gemma, Giuseppe Campiani

Artemisinin is an endoperoxide derived from Artemisia annua and along with its derivatives has proved to be very potent against Pf. Its antimalarial activity is mainly due to its endoperoxide pharmacophore. Another natural source of stable cyclic endoperoxides is the marine organisms. One of these organisms, the Caribbean sponge Plakortis simplex, has turned out to be a source of several of these cycloperoxides. Some natural endoperoxides isolated in Plakortis species were investigated in vitro for antimalarial properties and the six-membered endoperoxides plakortin and dihydroplakortin proved to be the most potent against CQ-resistant Pf strains, although 50 times less active than arteisinin. Our goal was to create a synthetic strategy to plakortin derivatives and to the natural compound 9,10-dihydroplakortin. One of the key issues in our synthetic approach was the stereoselectivity of the reactions and the formation of the chiral 1,2-dioxane skeleton. By combining Sharpless asymmetric epoxidation to the Mukaiyama-lsayaama Co(II)-catalyzed regioselective hydroperoxysilylation of an alkene, the desired stereochemistry could be achieved in good yields with excellent stereochecmical control. We have also developed a synthetic strategy to plakortin-related derivatives with simplified structure by means of similar reactions in order to obtain the desired chiral products. The presentation will also include an approach to the total synthesis of the natural compound 9,10-dihydroplakortin.

A11 Novel 4-aminooquinoline compounds: Binding, internalization and effects on normal human RBC
L. Cortelezzì, N. Basilico, M. Casagrande, A. Sparatore, D. Taramelli, F. Omodeo Salè

A series of quinolizidine derivatives of 7-chloro-4-aminooquinolines have been synthesised and shown to be very active against P. falciparum in vitro and against P. berghei ANKA in vivo (Sparatore A., 2005). Binding to the corpuscular blood fraction has been observed after in vivo treatment of rodents. Therefore, we investigated the binding/internalization of two of these compounds, AM1 and AP4b, into normal human erythrocytes (RBC) and their effect on RBC membrane stability. AM1 and AP4b binding to RBC was estimated by a new fluorimetric method (Basilico, N., et al., 2008. Anal. Biochem.) using chloroquine as reference; the effect on RBC stability by measuring RBC lysis after incubation with the quinolines alone or in the presence of ferrirprotoporphyrin IX (FP). Binding of AP4b and AM1 to RBC was rapid, dose-dependent and linearly related to the amount of compound or the concentration of RBC. Total AP4b and AM-1 binding was higher compared to CQ, the majority of the compounds being in the cytosol and little amount trapped into the RBC membrane. FP interacts with the quinolines and increases the binding by 2–3-fold leading to
membrane destabilisation. However, haemolysis induced by AM1-FP or AP4b-FP complexes was lower compared to CQ. The new fluorimetric method appears sensitive and reproducible. AM1 and AP4b that are highly active in vitro against CQ resistant parasites, seem to be able to enter more freely inside RBC and be less toxic than CQ. They deserve further studies as potential new antimalaria.

A12 Modulation of the monocyte scavenger receptor CD36 expression and non-opsonic phagocytosis of Plasmodium falciparum infected erythrocytes by the natural product curcin


The class B scavenger receptor CD36 on monocytes/macrophages plays an important role in innate immunity through opsonin-independent phagocytosis of P. falciparum parasitized erythrocytes (PE). Up-regulation of CD36 expression by peroxisome proliferator activated receptor gamma-retinoic-X-receptor (PPARγ-RXR) agonists has been shown to enhance phagocytosis of PE. We explore for the first time the effect of curcin on CD36-mediated non-opsonic phagocytosis of PE by monocytes/macrophages. Cultured human THP1 monocytes and PBMC were exposed to curcin and CD36 and PPARγ expression evaluated by real-time PCR, flow cytometry and western blotting. Phagocytosis after curcin treatment was assessed by microscopy. Curcin increased CD36 expression in human monocytes at the mRNA and protein level and enhanced non-opsonic phagocytosis of PE. This increase in CD36 expression took place following the production of reactive oxygen intermediates (ROI) and could be inhibited by the antioxidant N-acetylcysteine. However, this effect was not abrogated by the PPARγ-antagonist, GW9662 indicating that CD36 expression on monocytes following curcin exposure, is independent of this nuclear transcription factor. We also demonstrate here that the nuclear related (erythroid-derived 2) factor 2 (Nrf2), a stress sensitive nuclear transcription factor, is an alternative PPARγ-independent pathway for CD36 induction by curcin. The increase in CD36 expression and non-opsonic phagocytosis of PE induced by curcin may play an important role in parasite clearance in vivo. This “host targeted approach” represents a novel strategy to complement the direct anti-parasitic effect of compounds with antimalarial activity and as such could be a valuable tool in limiting the emergence of drug-resistant parasites.

A13 Effect of dihydroartemisinin (DHA) on human erythroid cell differentiation: Implications for malaria treatment in pregnancy

Sara Finaurini, Luisa Ronzoni, Alessandra Colancecco, Maria Domenica Cappellini, Donatella Taramelli

Severe malaria in pregnancy causes anaemia, low birth weight and increased mortality of both mother and infants. WHO recommends few antimalarials due to safety problem. Artemisinin derivatives showed animal embryotoxicity with a reduction of embryonic erythrocytes when treatment is performed on certain days of gestation. Our aim was to study the effect of Dihydroartemisinin (DHA), the metabolite of artemisinins, on an in vitro model reproducing human erythropoiesis. CD34+ human stem cells differentiate towards orthochromatic erythroblasts in 14 days and into erythrocytes in 21 days under erythropoietin stimulus. DHA, 0.5 or 2 μM, was added on different stages of erythroid differentiation chain: on stem cells, on pro-erythroblasts or on basophilic erythroblasts and samples were collected at several time points to analyse: cell growth; CD34, CD45, CD71 and Glycophorin A expression by flow cytometry; morphological changes with Giemsa staining, mRNA globin expression with PCR-RT and erythroid colonies formation. DHA added on stem cells or on early progenitors caused a transient inhibition of both cell growth and erythroid differentiation (P<0.05), although at day 14, cell proliferation and differentiation completely restored. On the contrary, DHA added on basophilic erythroblasts reduced dose-dependently both proliferation and differentiation towards orthochromatic erythroblasts at day 14. Our results indicated that DHA’s toxicity could occur also in pregnant women during the secondary yolk sac erythropoiesis (weeks 4-8 of gestation), when foetal blood is mostly formed of primitive erythroblasts. For this reason further clinical investigations are needed.

A14 Antiparasitic activities and toxicities of individual enantiomers of 7-chloro-N-[(9α)-octahydro-2H-quinoxizin-1-yl]methyl)-4-quinolinamine

Donatella Taramelli, Anna Sparatore, Sergio Romeo, Nicoletta Basilico, Nadia Vaiana, Manolo Casagrande, Luca Rizzi, Sarah D’Alessandro, Silvia Parapini, Chiara Rusconi, Hollie Lander, Livia Vivas, Daniela Jubes

To overcome chloroquine (CQ) resistance, new octahydroquinolinizyl-alkyl derivatives of 4-aminoquinoline have been obtained by a semi-synthetic route starting from 1-lupinine (an alkaloid extracted from Lupinus luteus), leading to an optically active compound, 7-chloro-N-[(1S,9α)-octahydro-1H-quinoxizin-1-yl]methyl)-4-quinolinamine, named (-)AM-1, highly effective in vitro against multi-drug resistant strains of P. falciparum and not toxic (Sparatore, A., et al., 2005). In vivo (-)AM-1, similarly to CQ, inhibits parasitemia with ED50 of 5.1 mg/kg, per os against murine P. berghei ANKA. Since the present supply of (-)-lupinine is not sufficient for market purposes, we attempted the total synthesis and characterisation of racemic AM1 and isolated enantiomers. Racemic lupinine was synthesized and the enantiomers separated by enzyme-catalyzed kinetic resolution, with good yield and high purity. (r)AM1, (−)AM1, (+)AM1 were then obtained in a 4 steps synthesis, as described. They were assayed in vitro for activity against different strains of P. falciparum and for cytotoxicity in normal human cells and in vivo for efficacy in the murine P. berghei ANKA model. (r)AM1, (−)AM1 and (+)AM1 showed comparable activity in vitro against drug resistant strains of P. falciparum with IC50 ranging between 15 and 35 nM. The cytotoxicity index was 3000 compared to human fibroblasts. In vivo, at 30 mg/kg, per os, all three compounds inhibited P. berghei parasitaemia with ED50 of 1.50, 1.33, 1.98 mg/kg for (r)AM1, (−)AM1, (+)AM1, respectively. These encouraging preliminary results on rAM1, a lead candidate belonging to a new quinoline type antimalarial class, worth further investigation.

A15 Synthesis of fluorescent, biotinylated and radiolabelled antimalarial endoperoxides to determine mechanism of action and protein drug targets

Victoria E. Barton, Stephen A. Ward, Paul M. O’Neill

The discovery of artemisinin has provided an entirely new antimalarial structural prototype for the design of novel peroxide containing antimalarial drugs. An endoperoxide bridge is the key pharmacophore in the semi-synthetic (and synthetic) antimalarial
peroxide compounds however there is much controversy on how artemisinins exert their fatal effects. A strategy incorporating endoperoxide probe drugs with fluorescent, biotinylated or radiolabelled moieties is an effective way of detecting probe labelled targets, integrating the rapidly advancing field of proteomics. In this presentation, we will provide evidence by a combination of isobole analysis and confocal imaging in living parasites that fluorescent semi-synthetic analogues of artemisinin and fully synthetic ozonide antimalaria share a common, parasite-specific mechanism of action that involves iron-mediated bioactivation and irreversible alkylation of parasite targets. This work describes for the first time that the endoperoxide drugs as a class share the common phenotype of irreversible bioactivation and parasite labelling and that the iron chelators DFO and DFP can protect against this process. In addition, we will also describe the novel syntheses of biotinylated artemisinins and trioxolane probe drugs which, due to the strong affinity of biotin for streptavidin, may be used to identify drug-protein targets. For comparison, the first synthesis of artemisinin tagged chemically cleavable biotinylated probes will be presented; these probes incorporate disulphide and sulfonamide linkages which should allow for simpler purifications, reduced contamination and improved protein identification. For comparison we will also describe the synthesis of a radiolabelled artemisinin probe.

A16
Antimalaria activity and cytotoxicity of crude extracts from three plants used in traditional medicine for the treatment of fever/malaria in Burkina Faso
Gansané Adama, Sanon Souleymane, Nebié Issa, Traoré Abdoulaye, Hutter Sébastien, Ollivier Evelyne, Azas Nadine, Traore Alfred, Guissou I. Pierre, Sirima B. Sodionmon

We conducted an ethno-pharmacological study in Comoe, a wet area of Burkina Faso, where traditional medicine practices are developed. Based on questionnaire records, 17 practitioners were selected and interviewed. This allowed the identification of 31 plants commonly used to treat malaria/fever. Based on the literature, we selected three species not well studied and most cited for pharmacological and toxicological studies: Cassia sieberiana, Zanthoxylum zanthoxylaides and Combretum molle. Following desiccation and pulverization of different parts of plants cited, 20 crude extracts were prepared using solvents with different polarities. Antimalarial activity of these extracts was evaluated in vitro using Plasmodium falciparum multiresistant strain (W2) and flux cytometer. Antiproliferative activity was evaluated on human monocyte K562S cells and the selectivity index (SI) of each extract was calculated. The highest in vitro antimalarial activity was found in the alkaloid extract from the bark of the trunk of Zanthoxylum zanthoxylaides (IC50 = 1.16 µg/ml) with good selectivity index (20.70). Moderate activity were found with MeOH leaves extract (IC50 = 5.66 µg/ml) and MeOH/H2O leaves extract (IC50 = 7.89 µg/ml) of Combretum molle. No extracts from Cassia sieberiana showed any antimalarial activity in vitro at tested concentrations. All the extracts tested displayed a low cytotoxicity activity against K562S cells. Our in vitro results confirm the traditional use of bark from the trunk of Zanthoxylum zanthoxylaides and leaves from Combretum molle for the treatment of malaria in Comoe and further investigation will be conducted using bioassay-guided fractionation to isolate active antimalarial molecules.

A17
Mechanism(s) of action of bis-thiazolium salts
M. Maynadier, S. Wein, Y. Bordat, J. Perez, K. Le Roch, M. Calas, L. Fraisse, Henri Vial

Newly designed bis-thiazolium salts are choline analogs. They show outstanding antimalarial activities being active in the low nanomolar range in vitro against Plasmodium and lower than 1 µg/(kg/day) in vivo in the P. vinckei rodent model. Total cure without recrudescence is obtained at 2–4 times their ED50 in rodent malaria and in non-human primate model. They have an innovative mechanism of action, targeting de novo biosynthesis of phosphatidylcholine. The effect of the bis-thiazolium drug(s) was studied in vitro in P. falciparum on the individual steps of the de novo phosphatidylcholine biosynthesis pathway using radiolabelled precursors, in particular choline, and a radiolabelled drug ([14C]-T3). Experiments were done with purified infected red blood cells, free parasites or P. falciparum lysates. Bis-thiazolium accumulates into the infected erythrocytes and has been quantified along the blood stages of both P. falciparum and P. vinckei-infected erythrocytes using radiolabelled drugs. Mechanism of action was also studied using transcriptomic and proteomic analyses of P. falciparum blood stage parasites. Bis-thiazolium salts, and in particular T3, are very potent choline mimicking structures affecting phospholipid biosynthesis. They inhibit malarial de novo phosphatidylcholine biosynthesis at different steps along the metabolic pathway. Moreover, the compounds interact with malarial pigment, thus potentially further enhancing the antimalarial effect. These antiparasitic compounds are accumulated in hematozoan (Babesia and Plasmodium) infected erythrocytes, what ensures their potency and specificity. It is likely that these compounds are dual molecules, i.e. they exert their antimalarial activity via two simultaneous toxic effects on intraerythrocytic parasites.
extract), *E. Africana* (6.69 μg/ml for CH₂OH extract) shown a good antiplasmodial activity IC⁵₀-Plasmodium < 10 μg/ml. The others have IC⁵₀-Plasmodium between 10 and 50 μg/ml. No cytotoxicity effect has been noted with IS value comprised between 4 and 10 for all extracts. These results confirm that *Anogeissus leiocarpus* and *Entada africana* have antiplasmodial properties in vitro and will be used for further investigation within antimalarial drug development.

A19 Which in vitro test(s) for new antimalarials?
S. Wein, M. Maynadier, M. Calas, L. Fraisse, Henri Vial

Resistance to the existing antimalarial drugs is an increasing problem and new antimalarial drugs with new mechanisms of action are needed. Assessment of new molecules requires in vitro evaluation of their antimalarial activity against laboratory strains as well as, at a later stage, against field isolates including multi-drug resistant strains of *P. falciparum*. This requests reliable method of in vitro antimalarial activity test. The most widely used method for determination of antimalarial activity (IC⁵₀) is the hypoxanthine in vitro antimalarial activity test. The most active molecules demonstrated significant in vitro activity against drug resistant strains and normal human cells. Our results highlight that the most commonly used tests of in vitro antimalarial activity do not have the same potential. Some of them might not detect the antimalarial potential of classes of compounds which later could be promising new antimalarial drugs.

A20 Targeting mutant PfCRT in antimalarial drug development
Patrick Bray, Paul Stocks, Enrique Salcedo-Sora, Archana Kanit, Steve Ward

CQ resistance is mediated primarily by mutations in the digestive vacuole transmembrane protein: PfCRT. These PfCRT mutations lead to a reduced concentration of chloroquine in the digestive vacuole, which in turn reduces the binding of chloroquine to its target. The exact mechanism of CQ resistance is unknown, with popular theories suggesting that PfCRT acts either an energy-dependent efflux carrier or as a channel allowing a leak of protonated chloroquine. Here we show that CQ displays an inverse cross resistance pattern with bis-benzyl amine heme-binding drugs. Using allelically exchanged parasite lines we show that chloroquine resistant PfCRT mutant parasite lines are hypersensitive to several bis-benzyl amides, including some new drugs in development. PfCRT mutant parasite lines exhibit increased binding of bis-benzyl amides to heme a phenomenon underlying the increased sensitivity of these parasite lines to the drug class. Using heterologous expression we show that PfCRT mutations permit the transport of charged forms of chloroquine or bis-benzyl amides down a concentration gradient. In contrast, no drug transport was found using wild-type PfCRT. Taken together, our results suggest that mutant forms of PfCRT permit the direct transport of two classes of cationic antimalarial drugs across the digestive vacuole membrane. Drug transport of both drug classes modulates drug-heme binding and hence drug sensitivity. Drug transport appears to follow the direction of the drug concentration gradient across the digestive vacuole membrane. The implication that new heme binding drugs can be engineered to preferentially target quinoline resistant malaria will be discussed.

A21 Potential of methotrexate in the treatment of malaria
Alexis Nzila, John Okombo, Steven Murithi

Methotrexate (MTX) is used at high dose, up to 130–300 mg/kg (9–20 g per adult) for the treatment of cancer, and this use is associated with high toxicity. A low dose of MTX (LD-MTX), 0.1–0.35 mg/kg (7.5–25 mg per adult) weekly is used against arthritids in adults and children on a chronic basis. At this dose, MTX is safe and well-tolerated. We have provided evidence that MTX concentration <100 μM could inhibit parasites growth of laboratory reference strains, including multi-drug resistant parasites, and this concentration could be achieved in vivo when LD-MTX is used. We report on the antimalarial activity of fresh Kenyan field isolates, and on the combination of MTX with other antimalarials. We used the in vitro system, based on the hypoxantine incorporation, to establish MTX activity against field isolates, and data were presented as concentrations that kill 50% of parasitaemia (IC⁵₀). We also investigated the activity of MTX in the presence of commonly used antimalarials lumefantrine, piperazine, dihydroartemisinin. We tested MTX activity against 29 Kenyan fresh *P. falciparum* isolates. MTX is active against both pyrimethamine-sensitive and resistant parasites, with IC⁵₀ <75 nM. The activity of MTX in the presence of the tested antimalarial drug was additive, an indication that any of the tested drugs can be used in combination with MTX. MTX is potent against current field isolates and lumefantrine, piperazine and dihydroartemisinin do not antagonize its activity and could therefore be used in combination with these drugs.

A22 Optimization of double-drugs: Low molecular weight inhibitors with high antiplasmodial activity
Sergio Romeo, Nadia Vaiana, Nicoletta Basilico, Luca Rizzi, Yolanda Corbett, Silvia Parapini, Donatella Taramelli

Plasmepsins (PLMs) are a family of aspartic proteases involved in the degradation of haemoglobin by intraerythrocytic malaria parasites. Identification of protease inhibitors able to efficiently inhibit *P. falciparum* (PF) growth has been so far unsuccessful, probably due to the redundancy of PLMs. We developed double drugs using statine as PLMs inhibitor, bound to Primaquine or Atovaquone by means of different linkers (Romeo, S., et al., 2008). Some of these compounds showed IC⁵₀ <500 nM against drug resistant strains of PF in vitro, low toxicity and in vivo activity. However, their further development as drug candidate is hampered by their high molecular weight. By sequential modification of the molecules, we synthesized and tested a new series of low MW compounds. The new compounds were obtained in a 4–6 steps synthesis using straightforward low cost chemistry. They were assayed in vitro for cytotoxicity against different strains of PF and normal human cells. The most active molecules demonstrated significant in vitro activity against both chloroquine resistant and sensitive strains of PF with IC⁵₀ below 100 nM. The cytotoxicity index was greater than 1000 compared to human fibroblasts. In vivo activity is also under
investigation. By reducing the MW of the original double-drugs, new compounds were designed which follow two basic criteria: (1) affordable costs of goods and (2) easy industrial development. The promising antiplasmodial activity of these molecules is probably directed against molecular targets different from proteases that are currently under investigation.

A23
The development of effective novel 4-aminoquinolines
A.E. Shone, B.K. Park, P.M. O’Neill, S.A. Ward, J. Davies, P. Stocks

The 4-aminoquinolines were excellent antimalarials until resistance developed first for chloroquine (CQ) and then for amodiaquine (AQ), a drug which also has toxicological liabilities. We have been investigating a series of rationally designed 4-aminoquinoline analogues for development as part of the AntiMal EU initiative to overcome the pharmacological shortcomings of these earlier molecules. Analogues have been synthesised to provide candidate back-up 4-aminoquinolines for pre-clinical evaluation. The optimised chemistry has delivered these novel synthetic quinolines in two-step procedures from cheap and readily available starting materials on a multi-gram scale.

Employing CQ and AQ as comparator molecules, a pre-clinical dossier of pharmacokinetic and safety pharmacology has been initiated for three compounds, FAQ-4, C2TB and F2TB. All have shown excellent activity against a number of chloroquine-sensitive and chloroquine-resistant strains of malaria. Cytotoxicity studies in rat hepatocytes have shown that FAQ-4, C2TB and F2TB have LD50 values of 160, 220 and 130 μM (CQ 190 μM, AQ 60 μM) with no evidence of glutathione depletion. Initial metabolism studies indicate no sign of toxic metabolite formation with the main route of metabolism being N-oxidation at the quinoline nitrogen. The pharmacokinetics of C2TB and F2TB are currently being investigated in the rat and in vitro in the Caco-2 cell line (results pending). Both drugs appear to be well tolerated in single dose studies at doses 5–80 mg/kg. We have designed a series of novel 4-aminoquinolines which in early pre-clinical evaluation demonstrate potential as affordable, safe and effective molecules for further development.

A24
Multiple targets of antimalarial bis-cations and delayed parasite death
Archana Kaniti, Paul Stocks, Steve Ward, Patrick Bray, Henri Vial

The Vial group in Montpellier has developed several series of bis-cations with potent antimalarial activity, including quaternary ammonium molecules, alkyl amides and guanidines. All these compounds have been shown to inhibit the synthesis of phosphatidyl choline in the parasite. This prevents the synthesis of new membranes and kills the parasite. In Liverpool, we have developed a related range of compounds, based on the bis-benzyl amidine structure. Interestingly, these compounds appear to work in a very different way to the bis-cations developed by the Vial group. The bis-benzyl amidines act more like chloroquine: i.e. by binding to heme and interfering with the production of hemozoin crystals in the parasite. We have undertaken a study of the heme-binding properties of bis-quaternary ammonium compounds and alkylamidines from the Vial group. The strength of the interaction with heme and the ability of these compounds to inhibit the production of hemozoin crystals were measured. These results are compared to results obtained with the Liverpool compounds and indicate that the Vial compounds have a dual mode of action, hitting both hemozoin crystallization and phosphatidyl choline synthesis. As the membrane synthesis is inhibited at much lower concentrations than heme crystallization we propose that heme binding functions as a drug reservoir mechanism to drive and maintain drug uptake at very high levels in the parasite, possibly explaining the “delayed death” effects of these drugs.

A25
Genetic validation of the hexose transporter of Plasmodium falciparum as a drug target
Slavic Ksenija, Doerig Christian, Reininger Luc, Krishna Sanjeev

The developed resistance of malaria parasites against commonly used antimalarials is imposing a huge obstacle to the treatment of this deadly disease. The identification and assessment of possible novel drug targets is a crucial step in the rational development of new chemotherapeutic agents. Interference with the parasite’s uptake of essential nutrients can represent a new direction in the development of antimalarials. Plasmodium falciparum hexose transporter, PfHT, is the major route for the uptake of host glucose. PfHT was chemically validated as a novel drug target by using a glucose-derived inhibitor. Here, we employed a reverse genetics approach to investigate the essentiality of the PfHT at the erythrocytic stage. P. falciparum parasites were either transfected with a knock-out construct containing 1 kb of PfHT sequence to promote gene disruption by homologous recombination, or co-transfected with both a knock-out and complementation construct, the latter enabling episomal expression of PfHT. PCR and Southern blot data show that disruption of the endosomal PfHT occurred only when co-transfection was performed with both knock-out and complementation vectors. We demonstrated that the disruption of the PfHT gene does not occur in the absence of PfHT expression from the complementation episome. Our results provide evidence that PfHT is essential for the asexual stages of the P. falciparum and genetically validates PfHT as a novel drug target. Furthermore, we are currently assessing the effect of PfHT Q169N mutation (which mediates fructose/glucose discrimination) on the viability of the parasite supplied with either glucose or fructose as an energy source.

A26
Efficacy and toxicity studies of Methotrexate in the Olive baboon model of Plasmodium knowlesi malaria
Maina Ichagichu, Ozwara Hastings, Mwatha Joseph, Karanja Simon, Kokwaro Gilbert, Ngotho Maina, Nzila Alexis

High dose of Methotrexate (MTX), 9–20 g per adult, is used for the treatment of cancer, and this use is associated with life threatening toxicity. However the same drug is used at low and safe dose (LD-MTX), 7.5–25 mg per adult, for the treatment of arthritis. Our preliminary data indicate that LD-MTX could yield effective concentration that kill parasite in vivo, making MTX a good antimalarial. We report on the efficacy and toxicity of LD-MTX in the olive baboon (Papio anubis) infected with Plasmodium knowlesi. Twelve olive baboons were selected and allocated to 3 groups of 4 animals each. Group 3 (controls, non-infected) and were administered 1.0 mg/(kg day)/5 days. After infection with 1 × 10^6 Plasmodium knowlesi H strain, Groups 1 and 2 were administered MTX at doses of 0.35 and 1 mg/(kg day)/5 days, respectively, and were followed up for 42 days. Clinical biochemistry, post-mortem and histopathological assays were conducted. Group 3 animals remained healthy throughout the experiment. Their clinical chemistry parameters and blood profile fluctuated within the
normal range. Post-mortem and histological studies did not show any pathology. Group 1 had higher mean parasitemia and showed malarial symptoms earlier than Group 2. Two of animals in Group 2 survived 2 days longer. Clinical, toxicity, gross pathology and histological studies on infected animals revealed observations consistent with malarial infection in baboons. This finding indicates that MTX is safe up to 1.0 mg/kg and would be efficacious and safe if administered at a dose of ≥1.0 mg/kg body weight.

A27

Drug targets in nucleoside metabolism in *Plasmodium falciparum*

Huaqing Cui, Dolores Gonzalez-Pacanowska, Ian Gilbert

In malaria, de novo pyrimidine nucleotide biosynthesis is a promising route for therapeutic intervention since, unlike mammalian cells, the parasite lacks the enzymes required for pyrimidine base and nucleoside salvage and hence completely depends on de novo synthesis to meet its metabolic requirements. Indeed, dihydrofolate reductase is a clinically proven drug target in malaria. The aim of this project is to further explore nucleoside metabolism as a source of drug targets against *Plasmodium*. Various enzymes involved in pyrimidine metabolism were purified and assays set up. A fluorescence-based assay was used for *in vitro* anti-malarial drug screening. Various libraries of compounds were designed as potential inhibitors and then synthesised and evaluated. Assays have been developed for several enzymes involved in pyrimidine metabolism. Two enzymes have been selected for development of a medicinal chemistry programme. Compounds were designed as potential inhibitors, prepared and then assayed against both the enzyme and intact parasite. This has led to further rounds of design, synthesis and evaluation, optimising the compounds. The enzymes involved in nucleoside metabolism in *Plasmodium* are promising targets for anti-malarial chemotherapy. Progress on developing enzyme inhibitors is reported.