The February 2009 issue contains citations and abstracts to 70+ recently published malaria studies. Author email addresses are included when available and the entries are arranged by journal title.

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- Statistical model to evaluate in vivo activities of antimalarial drugs in a Plasmodium cynomolgi-macaque model.

BMC Genomics. 2009 Feb 7;10(1):68.

- Analysis of small nucleolar RNAs reveals unique genetic features in malaria parasites.
- The salivary gland transcriptome of the neotropical malaria vector Anopheles darlingi reveals new insights into malaria biology.


- Cryptic species within Anopheles longipalpis from southern Africa and phylogenetic comparison with members of the An. funestus group.

Epidemiol Infect. 2009 Feb;137(2):294-304.

- Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo.


- The effect of weekly iron and vitamin A supplementation on hemoglobin levels and iron status in adolescent schoolgirls in western Kenya.

- Immunity to febrile malaria in children: an analysis that distinguishes immunity from lack of exposure.
- Baculovirus Dual Expression System in vaccine development: A baculovirus-based vaccine induces
- Polymorphisms in the erythrocyte binding antigens-140 and 181 affect function and binding
- Enhanced immunogenicity of Plasmodium falciparum peptide vaccines using a topical adjuvant
- Effect of Plasmodium yoelii exposure on vaccination with the 19-kilodalton carboxyl terminus of
- Dendritic cell and NK cell reciprocal cross talk promotes gamma interferon-dependent immunity to


- Spatial analysis of malaria incidence at the village level in areas with unstable transmission in Ethiopia.


- Effect of indoleamine dioxygenase-1 deficiency and kynurenine pathway inhibition on murine cerebrall
- Reticulocyte-binding protein homologue 5 - an essential adhesin involved in invasion of human
- A study on pathogenicity and mosquito transmission success in the rodent malaria parasite


- Impact of HIV-1 infection on the hematological recovery after clinical malaria.

J Ethnopharmacol. 2009 Jan 30;121(3):400-4.

- Screening of antiplasmodial properties among some traditionally used Iranian plants.

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- Emergence of an Unusual Sulfadoxine-Pyrimethamine Resistance Pattern and a Novel K540N Mutation
- High deformability of Plasmodium vivax-infected red blood cells under microfluidic conditions.


- Development of Nutritionally At-Risk Young Children Is Predicted by Malaria, Anemia, and Stunting in
  Pemba, Zanzibar.


- Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled

Malar J. 2009 Feb 20;8(1)

- The last man standing is the most resistant: eliminating artemisinin-resistant malaria in Cambodia.
- Household possession, use and non-use of treated or untreated mosquito nets in two ecologically
- Community knowledge, attitudes and practices (KAP) on malaria in Swaziland: A country earmarked for
- Polymorphism of PfATPase in Niger: detection of three new point mutations.
- Micro-geographic risk factors for malarial infection.
- Rural Gambian women’s reliance on health workers to deliver sulphadoxine-pyrimethamine as
- Estimating the burden of malaria in pregnancy: a case study from rural Madhya Pradesh, India.
- Quality of anti-malarial drugs provided by public and private healthcare providers in south-east Nigeria.
- Malaria and water resource development: the case of Gilgel-Gibe hydroelectric dam in Ethiopia.
- Molecular characterization of antifolates resistance-associated genes (dhfr and dhps) in Plasmodium

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- Histopathological changes in adult Schistosoma japonicum harbored in mice treated with a single dose
- Effect of dequalinium on the oxidative stress in Plasmodium berghei-infected erythrocytes.
• Reduced CD3/TCR complex expression leads to immunosuppression during Plasmodium falciparum
• In silico comparative genome analysis of malaria parasite Plasmodium falciparum and Plasmodium
• Initial characterization of Pf62, a novel protein of Plasmodium falciparum identified by


• High-level production of amorpha-4,11-diene, a precursor of the antimalarial agent artemisinin, in
• Loss of population levels of immunity to malaria as a result of exposure-reducing interventions:
• Poisoning pyridoxal 5-phosphate-dependent enzymes: a new strategy to target the malaria parasite
• Submicroscopic gametocytes and the transmission of antifolate-resistant Plasmodium falciparum in
• Retinal pathology of pediatric cerebral malaria in Malawi.
• Rapid changes in transcription profiles of the Plasmodium yoelii yir multigene family in clonal

**Proc Natl Acad Sci U S A. 2009 Feb**

• The Indian Ocean Dipole and malaria risk in the highlands of western Kenya.
• Structural basis for the inhibition of the essential Plasmodium falciparum M1 neutral aminopeptidase.

**Science. 2009 Feb 6;323(5915):797-800.**

• Platelets kill intraerythrocytic malarial parasites and mediate survival to infection.

**Trans R Soc Trop Med Hyg. 2009 Feb 9.**

• Toxoplasmosis screening and risk factors amongst pregnant females in Natal, northeastern Brazil.
• Agent-based modelling of mosquito foraging behaviour for malaria control.

**Trop Med Int Health. 2009 Feb 17.**

• Elimination of lymphatic filariasis in the Republic of Korea: an epidemiological survey of formerly
• Wash resistance and efficacy of three long-lasting insecticidal nets assessed from bioassays on
• Rapid malaria diagnostic tests vs. clinical management of malaria in rural Burkina Faso: safety and
• Evolutionary lability of odour-mediated host preference by the malaria vector Anopheles gambiae.
• In-vivo efficacy of amodiaquine-artesunate in children with uncomplicated Plasmodium falciparum
• Cost-effectiveness of artesunate for the treatment of severe malaria.
• Performance of OptiMAL-IT(R) compared to microscopy, for malaria detection in Burkina Faso.

**Vaccine. 2009 Feb 18;27(8):1266-71**

• Intranasal administration of the synthetic polypeptide from the C-terminus of the circumsporozoite
  protein of Plasmodium berghei with the modified heat-labile toxin of Escherichia coli…

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**Environmental Health at USAID – Malaria Bulletin, March 2009**
Plasmodium falciparum gametocyte sex ratios in symptomatic children treated with antimalarial drugs.

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The sex ratios of Plasmodium falciparum gametocytes, defined as the proportion of gametocytes in peripheral blood that were male, were evaluated in 1609 children with acute, symptomatic, uncomplicated malaria, pre- and post-treatment with various antimalarial drugs, over an 8-year period (1999-2006) in an endemic area of southwest Nigeria. Gametocyte carriage on presentation was 10% (162 children). In 162 children in whom 5797 gametocytes were sexed on presentation, the weighted mean sex ratio was 0.18 (95% confidence interval 0.13-0.25). Following therapy, in 446 children in whom 38,519 gametocytes were sexed, the weighted mean sex ratio was 0.38 (95% CI 0.33-0.43) on day 3 and 0.70 (95% CI 0.63-0.75) (P<0.000001) by day 7 after therapy commenced. Non-artemisinin monotherapy significantly increased sex ratio producing a male-biased ratio, but artemisinin combination therapy significantly reduced the sex ratio producing a female biased ratio. Pre-treatment sex ratio correlated negatively with haematocrit (r=-0.229, P=0.003) or gametocytaemia (r=-0.435, P<0.0001) but not with other clinical or parasitological parameters. The ratio of the sex-specific half lives male:female, the 'gametocyte maleness index' (GMI), was >1 with non-artemisinin monotherapy but <1 with artemesunate and artemisinin-based combinations. In a multiple regression model, anaemia, low gametocytaemia, and enrolment before 2004 were independent predictors of a male-biased sex ratio pre-treatment. A pre-treatment haematocrit <25%, enrolment before 2004 and treatment with non-artemisinin monotherapy were independent predictors of a male-biased sex ratio 7 days postinitiation of therapy. These findings may have implications for malaria management efforts in sub-Saharan Africa.

Efficacy of monotherapies and artesunate-based combination therapies in children with uncomplicated malaria in Somalia.

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In order to guide the antimalarial treatment policy of Somalia, we conducted therapeutic efficacy studies of routinely used antimalarial monotherapies as well as artemisinin-based combination therapies (ACTs) for uncomplicated malaria in three sentinel sites during 2003-2006. Therapeutic efficacy of chloroquine (CQ), amodiaquine (AQ) and sulfadoxine/pyrimethamine (SP) monotherapies, and artesunate plus SP (AS+SP) or AQ (AS+AQ) were evaluated in children 6 months to 10 years old with uncomplicated malaria. For the assessment of the monotherapies, 2003 WHO protocol with 14-day follow-up was used while the 2005 WHO protocol with 28-day follow-up was used for testing the ACTs. Of the monotherapies, CQ performed very
poorly with treatment failures varying from 76.5% to 88% between the sites. AQ treatment failure was low except for Janale site with treatment failure of 23.4% compared to 2.8% and 8% in Jamame and Jowhar, respectively. For SP, treatment failures from 7.8% to 12.2% were observed. A 28-day test of artemisinin-based combinations, AS+SP and AS+AQ, proved to be highly efficacious with cure rates of 98-100% supporting the choice of AS+SP combination as first line treatment for uncomplicated malaria for Somalia.


**Effect of temperature and inter-specific competition on the development and survival of Anopheles gambiae sensu stricto and An. arabiensis larvae.**

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The two major African malaria vectors Anopheles gambiae sensu stricto and An. arabiensis are sibling species that occupy different climatic niches but are frequently found in the same larval habitats. Differences in survival and development of the aquatic larval stages of these species at different temperatures may help explain adult distribution. The development time from first instar larva to adult at constant water temperatures (25, 30 and 35 degrees C) was measured in these two species when reared together in the same container (ratio 1:1) and separately. Survival to adult was highest in both species reared at 25 degrees C and decreased with increasing temperature. More adult An. gambiae s.s. were produced at 25 degrees C than An. arabiensis (80% interquartile range (78-88) versus 68% (63-78)) but this situation was reversed at 35 degrees C (7% (3-17) versus 33% (27-32)). The survival of An. gambiae s.s. when reared alone was similar to that when reared in the presence of An. arabiensis. In marked contrast An. arabiensis suffered reduced survival when raised with An. gambiae s.s. at 30 degrees C (20% (7-57)) than when reared independently (57% (45-72)). Mean age at eclosion and adult size decreased for both species with increasing water temperature, however An. arabiensis larvae developed at a slower rate and resulted in larger adults than An. gambiae s.s. throughout. The apparent greater production of An. arabiensis at high water temperatures and An. gambiae s.s. at lower water temperatures may in part explain the spatial and temporal distribution of the two species.


**The major insect lipoprotein is a lipid source to mosquito stages of malaria parasite.**

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Once mosquito midgut barrier was crossed malaria parasite faces a extensive metabolic developmental program in order to ensure its transmission. In the hemolymph of the mosquito the dynamics of lipid metabolism is conducted by a major lipoprotein, lipophorin (Lp). It was recently shown that Lp is engaged in the mosquito immune response to parasite infection. However, it is not clear if Lp is uptaken by the parasite. Here, we show that oocysts are able to uptake
mosquito Lp. The uptake of FITC-labeled Lp was demonstrated in midgut-associated oocysts. Alternatively, to confirm Lp incorporation by oocysts we have conducted another set of experiments with iodinated Lp ((125)I-Lp). Oocysts were able to incorporate (125)I-Lp and the process is both time and temperature dependent. This set of results indicated that no matter oocysts are attached to mosquito midgut wall they bear a lipid sequestering machinery from its surroundings. Phospholipid transfer to sporozoites was also demonstrated. In conclusion, these results demonstrate for the first time that malaria parasite undergoes lipid uptake while in the invertebrate host.


**A review on Anopheles culicifacies: from bionomics to control with special reference to Indian subcontinent.**

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Anopheles culicifacies, is a complex of five isomorphic sibling species A, B, C, D and E and is considered to be the major malaria vector in the Indian subcontinent. Despite numerous studies, it is difficult to have a global view of the ecological and bionomical characteristics of the individual sibling species, as different identification methods have been used. Major biological and ecological trends such as the high plasticity of behaviour and the sympatry of species are addressed. In spite of the availability of rapid molecular identification tools, we still lack important information concerning the biological characteristics of each sibling species. Resistance to insecticide is alarming as it has developed quadruple resistance in two states of India. An intensified and appropriate intervention measure to interrupt transmission is the call of the day. The authors focus on (1) reviewing the vectorial aspects of An. culicifacies (2) discussing recently published data on bionomics of each sibling species, (3) identifying lacunae in the understanding of the Culicifacies complex, and (4) exploring the possibility of proper control measures. Our understanding of the bionomics of all the five sibling species would certainly help, keeping in mind the climatic changes we are to face in the next few years.


**Competitive interactions between larvae of the malaria mosquitoes Anopheles arabiensis and Anopheles gambiae under semi-field conditions in western Kenya.**

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The present paper reports the occurrence of competition between larvae of the malaria mosquito sibling species Anopheles arabiensis and An. gambiae under ambient conditions in western Kenya. Larvae of both species were reared at the same density and under the same food conditions outdoors in single-species and mixed-species populations (species ratio 1:1) in transparent cups that floated in small and large semi-natural pools, which experienced different diurnal variations in water temperature. In a second experiment, both species were reared at similar densities and under the same food conditions in trays in either single-species or mixed-species populations at different proportions (species

Environmental Health at USAID – Malaria Bulletin, March 2009
ratio 1:1, 1:3 or 3:1). Competition affected the development rate of both species in an opposite way: the development time of larvae of An. arabiensis increased whereas the development time of larvae of An. gambiae decreased in the presence of its sibling species. In small pools larvae developing in mixed-species populations experienced a higher mortality than larvae reared in single-species populations, whereas no such effect was observed in the large pools. In both species the time to pupation was longer and emerging females were larger in the small pools. Larval mortality of An. arabiensis was lower in the small pools compared to the large pools, whereas An. gambiae showed the opposite trend. Overall An. arabiensis showed reduced development rates, higher mortality rates and emerged with a larger body size compared to An. gambiae. The implication of these competitive interactions between larvae of An. arabiensis and An. gambiae under semi-filed conditions needs to be considered in the design and implementation of programmes that aim to reduce malaria transmission as competition may alter the species composition in the field.


Short report: a multiplex PCR assay for simultaneous genotyping of kdr and ace-1 loci in Anopheles gambiae.

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The selection of insecticide-resistant genotypes in Anopheles gambiae, the most important malaria vector in Africa, makes disease control problematic in several endemic areas. The early detection and monitoring of resistance associated mutations in field mosquito populations is essential for the application of successful insecticide-based control interventions. Currently, the surveillance of these mutations is performed using individual assays, some of which require sophisticated and expensive equipment. Here we describe a novel multiplex polymerase chain reaction-based assay for detecting simultaneously the five single nucleotide polymorphisms in the voltage-gated sodium channel and the ace-1 genes, which have been associated with the mosquito response to most commonly used insecticides.


Studies on the Salvador I strain of Plasmodium vivax in non-human primates and anopheline mosquitoes.

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A review is presented on studies conducted in New World monkeys and chimpanzees with the Salvador I strain of Plasmodium vivax. This isolate has been adapted to Aotus and Saimiri (squirrel) monkeys and developed as a model for the testing of antimalarial vaccines. After the injection of 10,000 sporozoites, the median prepatent period in S. boliviensis monkeys was 21.5 days. In 103
sporozoite-induced infections in splenectomized monkeys, the median maximum parasite count ranged from 2,139 to 202,368/microL, with a median maximum parasite count of 48,174/microL. Median maximum parasite counts in Aotus lemurinus griseimembra, A. nancymaae, A. azarae boliviensis, and A. vociferans monkeys were 19,902, 18,390, 21,420, and 18,210/microL, respectively and ranged from 124 to 156,000/microL. Mosquito infections were readily obtained in different species of Anopheles mosquitoes. The S. boliviensis monkey and Salvador I strain seems suitable for the testing of sporozoite and liver stage vaccines but not for blood-stage vaccines against P. vivax unless adapted further in spleen-intact Saimiri boliviensis monkeys.


Genetic variation among Plasmodium vivax isolates adapted to non-human primates and the implication for vaccine development.

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Plasmodium vivax Duffy binding protein (DBP) is vital for parasite development, thereby making this molecule a good vaccine candidate. Preclinical development of a P. vivax vaccine often involves use of primate models prior to testing efficacy in humans, but primate isolates are poorly characterized. We analyzed the complete gene coding for the DBP in several P. vivax isolates that are used for experimental primate infections and compared these sequences with the Salvador I DBP isolate, which is being used for vaccine development. Our results affirm that primate-adapted isolates are genetically similar to P. vivax circulating in humans, but variability is greatest in the putative target of protective antibodies. In addition, some P. vivax isolates contain multiple genetically different clones. Testing a DBP vaccine may therefore be complicated by heterogeneity and diversity of the P. vivax isolates available for in vivo challenge.


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The number of Plasmodium vivax malaria patients in the Republic of Korea and North Korea since the re-emergence of malaria in 1993 is estimated to be approximately one million. To cope with this situation, the Army of the Republic of Korea has performed chemoprophylaxis with hydroxychloroquine and primaquine since 1997. The cumulative number of soldiers in the Army of the Republic of Korea given chemoprophylaxis exceeded 1.4 million by 2007. Extensive chemoprophylaxis contributed to preventing a rapid increase of malaria patients in the Army of the Republic of Korea, but increased the possibility of the occurrence of chloroquine (CQ)-resistant P. vivax strains. In this study, treatment responses of P. vivax malaria patients in the Republic of Korea monitored during 2003-2007, and CQ resistance was confirmed in 2 of 484 enrolled patients. Our results are the first report of CQ-resistant P. vivax in a
temperate region of Asia. Continuous surveillance is warranted to monitor the change in CQ resistance frequency of *P. vivax* in the Republic of Korea.


**Assessment of insecticide-treated bednet use among children and pregnant women across 15 countries using standardized national surveys.**

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Impact of insecticide-treated bednets (ITNs) on preventing malaria may be minimized if they are not used by vulnerable populations. Among ITN-owning households from 15 standardized national surveys from 2003 to 2006, we identify factors associated with ITN use among children younger than 5 years of age and make comparisons of ITN use among children and pregnant women across countries. Within ITN-owning households, many children and pregnant women are still not using them. Between-country analysis with linear regression showed child ITN use increases as intra-household access to ITNs increases (*P* = 0.020, R² = 0.404), after controlling for season and survey year. Results from within-country logistic regression analyses were consistent with between-country analysis showing intra-household access to ITNs is the strongest and most consistent determinant of use among children. The gaps in ITN use and possession will likely persist in the absence of achieving a ratio of no more than two people per ITN.


**Influence of wasting and stunting at the onset of the rainy season on subsequent malaria morbidity among rural preschool children in Senegal.**


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In sub-Saharan Africa, malaria and malnutrition are major causes of morbidity and mortality in children less than five years of age. To explore the impact of malnutrition on subsequent susceptibility to malaria, a cohort of 874 rural preschool children in Senegal was followed-up during one malaria transmission season from July through December. Data on nutritional status and *Plasmodium falciparum* parasitemia were collected at baseline. Malaria morbidity was monitored through weekly home visits. Wasted children (weight-for-height z-score < -2) were at lower risk of having at least one subsequent clinical malaria attack (odds ratio = 0.33; 95% confidence interval = 0.13-0.81, *P* = 0.02), whereas stunting (height-for-age z-score < -2) or being underweight (weight-for-age z-score < -2) was not associated with clinical malaria. Although non-biological explanations such as overprotection of wasted children by their mothers should be considered, immunomodulation according to nutritional status could explain the lower risk of malaria attack among wasted children.
**Short report: comparison of chlorproguanil-dapsone with a combination of sulfadoxine-pyrimethamine and chloroquine in children with malaria in northcentral Nigeria.**

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Effective and affordable treatment of malaria is critical in the face of resistance of Plasmodium falciparum to chloroquine (CQ) and sulfadoxine-pyrimethamine (SP). We conducted a randomized controlled trial comparing the efficacy of chlorproguanil-dapsone (CD) with a combination SP plus CQ in children in Nigeria less than five years of age with malaria. Of 264 children enrolled, 122 (89.7%) and 118 (92.2%) completed the study in the SP + CQ and CD groups, respectively. By day 3, 96 (78.7%) and 94 (79.7%) had cleared their parasitemia (P = 0.79), and 107 (87.7%) and 109 (92.4%) were symptom free (P = 0.32) in the SP + CQ and CD groups, respectively. Adequate clinical and parasitologic response at day 14 occurred in 111 (94.1%; 95% confidence interval [CI] = 91.6-95.7%) in the CD group and 113 (92.6%; 95% CI = 89.9-94.3%) in the SP + CQ group (P = 0.85). SP + CQ and CD had similar antimalarial efficacy and still provide affordable treatment of uncomplicated malaria in northcentral Nigeria.

**Severe Plasmodium vivax malaria: a report on serial cases from Bikaner in northwestern India.**


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Epidemiologic studies and clinical description of severe Plasmodium vivax malaria in adults living in malaria-endemic areas are rare and more attention is needed to understand the dynamics and its interaction with the immune system. This observational study included 1,091 adult patients admitted to medical wards of S. P. Medical College and associated group of hospitals in Bikaner, India from September 2003 through December 2005. The diagnosis of P. vivax malaria was established by peripheral blood film (PBF), rapid diagnostic test (RDT), and polymerase chain reaction (PCR), and severe malaria was categorized as per World Health Organization guidelines. Of 1,091 patients with malaria, 635 had P. falciparum malaria and 456 had P. vivax malaria. Among patients with severe manifestations, 40 had evidence of monoinfection of P. vivax malaria diagnosed by PBF, RDT, and PCR. Complications observed were hepatic dysfunction and jaundice in 23 (57.5%) patients, renal failure in 18 (45%) patients, severe anemia in 13 (32.5%) patients, cerebral malaria in 5 patients (12.5%), acute respiratory distress syndrome in 4 patients (10%), shock in 3 patients (7.5%), and hypoglycemia in 1 (2.5%) patient. Thrombocytopenia was observed in 5 (12.5%) patients, and multi-organ dysfunction was detected in 19 (47.5%) patients. Further large-scale multicentric epidemiologic studies are needed to define the basic pathology of this less known entity.
Methemoglobinemia and adverse events in Plasmodium vivax malaria patients associated with high doses of primaquine treatment.

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Primaquine (PQ) is recommended to prevent relapses in patients with Plasmodium vivax malaria infection. However, treatment with PQ causes methemoglobinemia. In this study, we measured the methemoglobin (MetHB) levels in three groups of subjects who received PQ treatment at 0.58, 0.83, or 1.17 mg/kg/d. A total of 112 subjects were studied. MetHB levels were detected at > or = 4% in 46-50% 1 day after PQ treatment in all three groups and 4-9% of subjects had MetHB levels > or = 4% 15 days after treatment. Only subjects receiving the highest doses of PQ had mild and brief adverse events, and 17% of them were associated with treatment. We conclude that when PQ is administered under certain conditions (i.e., normal glucose-6-phosphate dehydrogenase activity, in non-pregnant subjects and with a light meal), daily doses as high as 1.17 mg/kg do not represent a serious risk of high MetHB levels to patients.

Reduced efficacy of intermittent preventive treatment of malaria in malnourished children.

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Intermittent preventive treatment in infants with sulfadoxine-pyrimethamine (IPTi-SP) reduces malaria episodes by 20-59% across Africa. This protective efficacy, however, may be affected by the high frequency of malnutrition in African infants. We analyzed the impact of malnutrition as defined by anthropometry on the incidence of malaria and on the protective efficacy of IPTi in a cohort of 1200 children in hyperendemic northern Ghana. These children received IPTi-SP or placebo at 3, 9, and 15 months of age and were followed-up until 24 months of age. Malnutrition was present in 32%, 40%, and 50% of children at ages 3, 9, and 15 months, respectively. It was associated with increased risks of severe anemia and death but not of malaria. Although malaria slightly contributed to chronic malnutrition, IPTi did not substantially improve child growth. Importantly, the protective efficacies of IPTi in malnourished children were roughly half or even less of those observed in non-malnourished children. In the first year of life, IPTi reduced the incidence of malaria to a significantly lesser extent in infants who received both doses in a malnourished condition (25%; 95%CI, -7-48%) as compared to non-malnourished children (46%; 95%CI, 30-58%; P = 0.049). Moreover, in contrast to nutritionally advantaged children, the rate of severe malaria appeared to be increased in malnourished children who took IPTi. IPTi might exhibit reduced efficacy in regions of abundant malnutrition. There, concomitant nutrition programs may be needed to achieve the
Hydroxychloroquine (HCQ) is an anti-malarial drug used as chemoprophylaxis against vivax malaria in the Republic of Korea Army (ROKA). In this study, we evaluated the pharmacokinetics (PK) of HCQ and its metabolites, and the relationship between HCQ PK and treatment effect of HCQ on vivax malaria in Koreans. Three PK studies for HCQ were conducted in 91 healthy subjects and patients with vivax malaria. Plasma concentrations were analyzed by non-compartmental and mixed effect modeling approaches. Two-compartment model with first order absorption described the data best. Clearance, central and peripheral volume of distribution was 15.5 L/h, 733 L and 1,630 L, respectively. We measured the plasma concentrations of HCQ in patients with prophylactic failure of HCQ and compared them with prediction intervals of simulated concentrations for HCQ from the final PK model built in this study. In 71% of patients with prophylactic failure, plasma concentrations of HCQ were below the lower bounds of a 95% prediction interval, while only 8% of them showed higher levels than the upper bounds of 95% prediction interval. We report that a significant cause of prophylactic failure among the individuals of ROKA was ascribed to lower plasma concentrations of HCQ than that predicted by the PK model. However, prophylactic failure despite sufficient plasma concentrations of HCQ was confirmed in several individuals, warranting continued surveillance to monitor changes of HCQ-susceptibility of Plasmodium vivax in the ROK.


Pharmacokinetics and bioequivalence evaluation of two fixed-dose tablet formulations of dihydroartemisinin and piperaquine in Vietnamese subjects.

Chinh NT, Quang NN, Thanh NX, Dai B, Geue JP, Addison RS, Travers T, Edstein MD.

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The two fixed-dose combinations of dihydroartemisinin and piperaquine (Artekin and Arterakine) were found to be bioinequivalent in healthy Vietnamese subjects. However, because the peak plasma concentrations and areas under the concentration-time curves of dihydroartemisinin and piperaquine were only marginally different between the two formulations, similar therapeutic efficacies...
are expected in the treatment of malaria infections.


Multinormal in vitro distribution model suitable for the distribution of Plasmodium falciparum chemosusceptibility to doxycycline.


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The distribution and range of 50% inhibitory concentrations (IC(50)s) of doxycycline were determined for 747 isolates obtained between 1997 and 2006 from patients living in Senegal, Republic of the Congo, and Gabon and patients hospitalized in France for imported malaria. The statistical analysis was designed to answer the specific question of whether Plasmodium falciparum has different phenotypes of susceptibility to doxycycline. A triple normal distribution was fitted to the data using a Bayesian mixture modeling approach. The IC(50) geometric mean ranged from 6.2 microM to 11.1 microM according to the geographical origin, with a mean of 9.3 microM for all 747 parasites. The values for all 747 isolates were classified into three components: component A, with an IC(50) mean of 4.9 microM (+/-2.1 microM [standard deviation]); component B, with an IC(50) mean of 7.7 microM (+/-1.2 microM); and component C, with an IC(50) mean of 17.9 microM (+/-1.4 microM). According to the origin of the P. falciparum isolates, the triple normal distribution was found in each subgroup. However, the proportion of isolates predicted to belong to component B was most important in isolates from Gabon and Congo and in isolates imported from Africa (from 46 to 56%). In Senegal, 55% of the P. falciparum isolates were predicted to be classified as component C. The cutoff of reduced susceptibility to doxycycline in vitro was estimated to be 35 microM.


Statistical model to evaluate in vivo activities of antimalarial drugs in a Plasmodium cynomolgi-macaque model for Plasmodium vivax malaria.

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Preclinical animal models informing antimalarial drug development are scarce. We have used asexual erythrocytic Plasmodium cynomolgi infections of rhesus macaques to model Plasmodium vivax during preclinical development of compounds targeting parasite phospholipid synthesis. Using this malaria model, we accumulated data confirming highly reproducible infection patterns, with self-curing parasite peaks reproducibly preceding recrudescence peaks. We applied nonlinear mixed-effect (NLME) models, estimating treatment effects in three drug studies: G25 (injected) and the bisthiazolium prodrugs TE4gt and TE3 (oral). All compounds fully cured P. cynomolgi-infected macaques, with significant effects on parasitemia height and time of peak. Although all three TE3 doses tested were fully curative, NLME models discriminated dose-dependent differential
pharmacological antimalarial activity. By applying NLME modeling treatment effects are readily quantified. Such drug development studies are more informative and contribute to reduction and refinement in animal experimentation.

*BMC Genomics. 2009 Feb 7;10(1):68.*

**Analysis of small nucleolar RNAs reveals unique genetic features in malaria parasites.**

Mishra PC, Kumar A, Sharma A.

**ABSTRACT:** **BACKGROUND:** Ribosome biogenesis is an energy consuming and stringently controlled process that involves hundreds of trans-acting factors. Small nucleolar RNAs (snoRNAs), important components of ribosome biogenesis are non-coding guide RNAs involved in rRNA processing, nucleotide modifications like 2'-O-ribose methylation, pseudouridylation and possibly gene regulation. snoRNAs are ubiquitous and are diverse in their genomic organization, mechanism of transcription and process of maturation. In vertebrates, most snoRNAs are present in introns of protein coding genes and are processed by exonucleolytic cleavage, while in plants they are transcribed as polycistronic transcripts. **RESULTS:** This is a comprehensive analysis of malaria parasite snoRNA genes and proteins that have a role in ribosomal biogenesis. Computational and experimental approaches have been used to identify several box C/D snoRNAs from different species of Plasmodium and confirm their expression. Our analyses reveal that the gene for endoribonuclease Rnt1 is absent from Plasmodium falciparum genome, which indicates the existence of alternative pre-rRNA processing pathways. The structural features of box C/D snoRNAs are highly conserved in Plasmodium genus; however, unlike other organisms most parasite snoRNAs are present in single copy. The genomic localization of parasite snoRNAs shows mixed patterns of those observed in plants, yeast and vertebrates. We have localized parasite snoRNAs in untranslated regions (UTR) of mRNAs, and this is an unprecedented and novel genetic feature. Akin to mammalian snoRNAs, those in Plasmodium may also behave as mobile genetic elements. **CONCLUSIONS:** This study provides a comprehensive overview on trans-acting genes involved in ribosome biogenesis and also a genetic insight into malaria parasite snoRNA genes.

*BMC Genomics. 2009 Jan 29;10:57.*

**The salivary gland transcriptome of the neotropical malaria vector Anopheles darlingi reveals accelerated evolution of genes relevant to hematophagy.**

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**BACKGROUND:** Mosquito saliva, consisting of a mixture of dozens of proteins affecting vertebrate hemostasis and having sugar digestive and antimicrobial properties, helps both blood and sugar meal feeding. Culicine and anopheline mosquitoes diverged ~150 MYA, and within the anophelines, the New World species diverged from those of the Old World ~95 MYA. While the sialotranscriptome (from the Greek sialo, saliva) of several species of the Cellia subgenus of Anopheles has been described thoroughly, no detailed analysis of any New World anopheline has been done to date. Here we present and analyze data from a comprehensive salivary gland (SG) transcriptome of the neotropical malaria vector Anopheles
darlingi (subgenus Nyssorhynchus). RESULTS: A total of 2,371 clones randomly selected from an adult female An. darlingi SG cDNA library were sequenced and used to assemble a database that yielded 966 clusters of related sequences, 739 of which were singletons. Primer extension experiments were performed in selected clones to further extend sequence coverage, allowing for the identification of 183 protein sequences, 114 of which code for putative secreted proteins. CONCLUSION: Comparative analysis of sialotranscriptomes of An. darlingi and An. gambiae reveals significant divergence of salivary proteins. On average, salivary proteins are only 53% identical, while housekeeping proteins are 86% identical between the two species. Furthermore, An. darlingi proteins were found that match culicine but not anopheline proteins, indicating loss or rapid evolution of these proteins in the old world Cellia subgenus. On the other hand, several well represented salivary protein families in old world anophelines are not expressed in An. darlingi.


Cryptic species within Anopheles longipalpis from southern Africa and phylogenetic comparison with members of the An. funestus group.

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House-resting Anopheles mosquitoes are targeted for vector control interventions; however, without proper species identification, the importance of these Anopheles to malaria transmission is unknown. Anopheles longipalpis, a non-vector species, has been found in significant numbers resting indoors in houses in southern Zambia, potentially impacting on the utilization of scarce resources for vector control. The identification of An. longipalpis is currently based on classical morphology using minor characteristics in the adult stage and major ones in the larval stage. The close similarity to the major malaria vector An. funestus led to investigations into the development of a molecular assay for identification of An. longipalpis. Molecular analysis of An. longipalpis from South Africa and Zambia revealed marked differences in size and nucleotide sequence in the second internal transcribed spacer (ITS2) region of ribosomal DNA between these two populations, leading to the conclusion that more than one species was being analysed. Phylogenetic analysis showed the Zambian samples aligned with An. funestus, An. vaneedeni and An. parensis, whereas the South African sample aligned with An. leesoni, a species that is considered to be more closely related to the Asian An. minimus subgroup than to the African An. funestus subgroup. Species-specific primers were designed to be used in a multiplex PCR assay to distinguish between these two cryptic species and members of the An. funestus subgroup for which there is already a multiplex PCR assay.

Epidemiol Infect. 2009 Feb;137(2):294-304.

Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo.


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Maternal malaria and under-nutrition are established risk factors for small-for-gestational-age (SGA) births; however, whether malaria is associated with intrauterine growth restriction (IUGR) is unknown. We investigated IUGR risk among 177 HIV-negative pregnant women enrolled in a longitudinal ultrasound study conducted in Democratic Republic of Congo from May 2005 to May 2006. Malaria infection, maternal anthropometrics, and ultrasound estimated fetal weight were measured monthly. All positive malaria cases were treated and intermittent presumptive therapy (IPTp) provided. Log-binomial regression models for IUGR were fitted using generalized estimating equations to account for statistical clustering of repeat IUGR measurements. Twenty-nine percent of fetuses experienced an episode of IUGR with the majority occurring in the third trimester. The risk of IUGR associated with malaria was greatest after three or more cumulative infections (RR 3.3, 95% CI 1.3-8.2) and was two- to eight-fold higher among women with evidence of under-nutrition. Receiving antimalarial treatment in the previous month (for IPTp or treatment) was significantly protective against IUGR (RR 0.5, 95% CI 0.3-0.7). The interaction observed between malaria and under-nutrition suggests that antenatal programmes in malaria endemic areas should incorporate nutritional screening and supplementation in addition to IPTp.


**The effect of weekly iron and vitamin A supplementation on hemoglobin levels and iron status in adolescent schoolgirls in western Kenya.**

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BACKGROUND/OBJECTIVES: Iron deficiency anemia is a major public health problem in developing countries and may affect school performance and physical work capacity in nonpregnant adolescents, and may increase the risk of anemia during subsequent teenage pregnancies. We assessed the effect of weekly iron (120 mg elemental iron) and vitamin A (25 000 IU) supplementation on hemoglobin, iron status and malaria and nonmalaria morbidity in adolescent schoolgirls. SUBJECTS/METHODS: A total of 279 schoolgirls aged 12-18 years from public primary schools in Kisumu, western Kenya. Double-blind randomized placebo-controlled trial using a factorial design. RESULTS: Five months of iron supplementation was associated with a 0.52 g dl(-1) (0.21, 0.82) greater increase in hemoglobin relative to iron placebo. The effect was only observed in girls with iron deficiency on enrollment (1.34 g dl(-1) (0.79, 1.88)), but not in iron-replete girls (-0.20 g dl(-1) (-0.59, 0.18)). Similar differences in treatment effect were seen between menstruating and nonmenstruating girls. The effect of iron was independent of vitamin A. The baseline prevalence of vitamin A deficiency was low (6.7%) and no sustained increase in hemoglobin was seen with weekly vitamin A (-0.07 g dl(-1) (-0.38, 0.25)). Incidence of malaria parasitemia was higher in the iron than iron-placebo groups (Rate ratio 1.33 (0.94, 1.88)). Conclusions: Weekly iron supplementation results in substantial increases in hemoglobin concentration in adolescent schoolgirls in western Kenya, which may outweigh possible risks caused by malaria, but only in iron-deficient or menstruating girls and not in iron-replete and nonmenstruating girls.

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Immunity to febrile malaria in children: an analysis that distinguishes immunity from lack of exposure.


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In studies of immunity to malaria, the absence of febrile malaria is commonly considered 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 episodes 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 the protective antibody response. We show that removing these less exposed 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.

Baculovirus Dual Expression System in vaccine development: A baculovirus-based vaccine induces strong protection against Plasmodium berghei sporozoite challenge.

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We have previously shown that a recombinant baculovirus that displays Plasmodium berghei circumsporozoite protein (PbCSP), the homolog of the leading human malaria vaccine candidate, on the viral envelope protected 60% of mice against P. berghei infection. Here, we describe a second-generation baculovirus vaccine based on the "Baculovirus Dual Expression System", which drives PbCSP expression by a dual promoter that consists of tandemly arranged baculovirus-derived polyhedrin and mammalian-derived CMV promoters. The baculovirus-based PbCSP vaccine not only displayed PbCSP on the viral envelope but also expressed PbCSP upon transduction of mammalian cells. Immunization with the baculovirus-based PbCSP vaccine elicited high PbCSP-specific antibody titers (predominantly IgG1 and IgG2a) and PbCSP-specific CD8(+) T-cell responses without extraneous immunological adjuvants in mice, indicating induction of both Th1 and Th2
responses (a mixed Th1/Th2 response). Importantly, upon intramuscular inoculation, the baculovirus-based PbCSP vaccine conferred complete protection against sporozoite challenge. Thus, the baculovirus-based PbCSP vaccine induced strong protective immune responses against pre-erythrocytic parasites. These results introduce a novel concept of the Baculovirus Dual Expression System that functions as both a subunit and DNA vaccine, and offer a promising new alternative to current human vaccine delivery platforms.


**Polymorphisms in the erythrocyte binding antigens-140 and 181 affect function and binding but not receptor specificity in Plasmodium falciparum.**

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Invasion of human erythrocytes by the malaria parasite, Plasmodium falciparum, utilises multiple ligand-receptor interactions involving erythrocyte receptors and parasite erythrocyte binding proteins of the Duffy-binding-like (DBL) family. Erythrocyte binding antigen (EBA)-175 binds to glycophorin A, the most abundant protein on the human erythrocyte surface, EBA-140 (also known as BAEBL) binds to glycophorin C, whilst the receptor for EBA-181 (also known as JESEBL) remains unknown. EBA binding is mediated via region II, a highly structured extracellular domain that shows a degree of sequence variability between different laboratory strains/isolates. Here, we determined the influence of region II polymorphisms on host-cell receptor binding and overall function during invasion of EBA-140, EBA-175 and EBA-181. Polymorphisms in the binding domain of EBA-140 and EBA-181 have been suggested previously to alter their respective receptor specificity. In our hands, these polymorphisms affect the level of EBA-140 and EBA-181 binding to receptor but critically not their receptor specificity. The degree of EBA-140 binding to glycophorin C correlates with the level of function for this ligand-receptor interaction in merozoite invasion. In contrast, EBA-175, which is highly polymorphic in region II, shows no variability in its ability to bind its receptor, glycophorin A. Combined this data highlights the importance of sequence variability in EBAs as driven by immune selection but not by receptor specificity.


**Enhanced immunogenicity of Plasmodium falciparum peptide vaccines using a topical adjuvant containing a potent synthetic Toll-like receptor 7 agonist, imiquimod.**


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Plasmodium sporozoites injected into the skin by malaria-infected mosquitoes can be effectively targeted by antibodies that block parasite invasion of host hepatocytes and thus prevent the subsequent development of blood stage infections responsible for clinical disease. Malaria subunit vaccines require potent adjuvants, as they lack known pathogen-associated molecular patterns found in attenuated viral or bacterial vaccines that function as Toll-like receptor (TLR)
agonists to stimulate dendritic cells and initiate strong adaptive immune responses. A synthetic TLR7 agonist, imiquimod, which is FDA approved for topical treatment of various skin conditions, can function as a potent adjuvant for eliciting T-cell responses to intracellular pathogens and model protein antigens. In the current studies, the topical application of imiquimod at the site of subcutaneously injected Plasmodium falciparum circumsporozoite (CS) peptides elicited strong parasite-specific humoral immunity that protected against challenge with transgenic rodent parasites that express P. falciparum CS repeats. In addition, injection of a simple linear peptide followed by topical imiquimod elicited strong Th1 CD4(+) T-cell responses, as well as high antibody titers. The correlation of high anti-repeat antibody titers with resistance to sporozoite challenge in vivo and in vitro supports use of this topical TLR7 agonist adjuvant to elicit protective humoral immunity. The safety, simplicity, and economic advantages of a topical synthetic TLR7 agonist adjuvant also apply to other vaccines requiring high antibody titers, such as malaria asexual or sexual blood stage antigens to prevent red blood cell invasion and block transmission to the mosquito vector, and to vaccines to other extracellular pathogens.


Effect of Plasmodium yoelii exposure on vaccination with the 19-kilodalton carboxyl terminus of merozoite surface protein 1 and vice versa and implications for the application of a human malaria vaccine.


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It is well known that exposure to one antigen can modulate the immune responses that develop following exposure to closely related antigens. It is also known that the composition of the repertoire can be skewed to favor epitopes shared between a current infection and a preceding one, a phenomenon referred to as "original antigenic sin." It was of interest, therefore, to investigate the antibody response that develops following exposure to the malaria vaccine candidate homologue Plasmodium yoelii MSP1(19) in mice that had previously experienced malaria infection and vice versa. In this study, preexposure of mice to Plasmodium yoelii elicited native anti-MSP1(19) antibody responses, which could be boosted by vaccination with recombinant MSP1(19). Likewise, infection of MSP1(19)-primed mice with P. yoelii led to an increase of anti-MSP1(19) antibodies. However, this increase was at the expense of antibodies to parasite determinants other than MSP1(19). This change in the balance of antibody specificities significantly affected the ability of mice to withstand a subsequent infection. These data have particular relevance to the possible outcome of malaria vaccination for those situations where the vaccine response is suboptimal and suggest that suboptimal vaccination may in fact render the ultimate acquisition of natural immunity more difficult.


Dendritic cell and NK cell reciprocal cross talk promotes gamma interferon-dependent immunity to blood-stage Plasmodium chabaudi AS infection in mice.

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Dendritic cells (DCs) are important accessory cells for promoting NK cell gamma interferon (IFN-gamma) production in vitro in response to Plasmodium falciparum-infected red blood cells (iRBC). We investigated the requirements for reciprocal activation of DCs and NK cells leading to Th1-type innate and adaptive immunity to P. chabaudi AS infection. During the first week of infection, the uptake of iRBC by splenic CD11c(+) DCs in resistant wild-type (WT) C57BL/6 mice was similar to that in interleukin 15(-/-) (IL-15(-/-)) and IL-12p40(-/-) mice, which differ in the severity of P. chabaudi AS infection. DCs from infected IL-15(-/-) mice expressed costimulatory molecules, produced IL-12, and promoted IFN-gamma secretion by WT NK cells in vitro as efficiently as WT DCs. In contrast, DCs from infected IL-12p40(-/-) mice exhibited alterations in maturation and cytokine production and were unable to induce NK cell IFN-gamma production. Coculture of DCs and NK cells demonstrated that DC-mediated NK cell activation required IL-12 and, to a lesser extent, IL-2, as well as cell-cell contact. In turn, NK cells from infected WT mice enhanced DC maturation, IL-12 production, and priming of CD4(+) T-cell proliferation and IFN-gamma secretion. Infected WT mice depleted of NK cells, which exhibit increased parasitemia, had impaired DC maturation and DC-induced CD4(+) Th1 cell priming. These findings indicate that DC-NK cell reciprocal cross talk is critical for control and rapid resolution of P. chabaudi AS infection and provide in vivo evidence for the importance of this interaction in IFN-gamma-dependent immunity to malaria.


Spatial analysis of malaria incidence at the village level in areas with unstable transmission in Ethiopia.

Yeshiwondim AK, Gopal S, Hailemariam AT, Dengela DO, Patel HP.

ABSTRACT: BACKGROUND: Malaria is the leading cause of morbidity and mortality in Ethiopia, accounting for over five million cases and thousands of deaths annually. The risks of morbidity and mortality associated with malaria are characterized by spatial and temporal variation across the country. This study examines the spatial and temporal patterns of malaria transmission at the local level and implements a risk mapping tool to aid in monitoring and disease control activities. METHODS: In this study, we examine the global and local patterns of malaria distribution in 543 villages in East Shoa, central Ethiopia using individual-level morbidity data collected from six laboratory and treatment centers between September 2002 and August 2006. RESULTS: Statistical analysis of malaria incidence by sex, age, and village through time reveal the presence of significant spatio-temporal variations. Poisson regression analysis shows a decrease in malaria incidence with increasing age. A significant difference in the malaria incidence density ratio (IDRs) is detected in males but not in females. A significant decrease in the malaria IDRs with increasing age is captured by a quadratic model. Local spatial statistics reveals clustering or hot spots within a 5 and 10 km distance of most villages in the study area. In addition, there are temporal variations in malaria incidence. CONCLUSIONS: Malaria incidence varies according to gender and age, with males age 5 and above showing a statistically higher incidence. Significant local clustering of malaria incidence occurs between pairs of villages within 1-10 km distance lags. Malaria incidence was higher in 2002-2003 than in other periods of observation. Malaria hot spots are displayed as risk maps that are useful for monitoring and spatial targeting of prevention and control measures against the disease.
Effect of indoleamine dioxygenase-1 deficiency and kynurenine pathway inhibition on murine cerebral malaria.

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Cerebral malaria (CM) can be a fatal manifestation of Plasmodium falciparum infection. In this study, two different approaches were used to examine the role of indoleamine 2,3-dioxygenase-1 (IDO-1) and its metabolites in the development of murine CM. Mice genetically deficient in IDO-1 were not protected against CM, but partial protection was observed in C57BL/6 mice treated with Ro 61-8048, an inhibitor of kynurenine-3-hydroxylase. This protection was associated with suppressed levels of picolinic acid (PA) within the brain, but not with changes in the levels of kynurenic acid (KA) or quinolinic acid (QA). These data suggest that although IDO-1 is not directly involved in the pathogenesis of CM in C57BL/6 mice, the production of the kynurenine pathway metabolite PA may contribute to the development of murine CM.


Baum J, Chen L, Healer J, Lopaticki S, Boyle M, Triglia T, Ehlgen F, Ralph SA, Beeson JG, Cowman AF.

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Invasion of erythrocytes is a prerequisite in the life history of the malaria parasite. Members of the reticulocyte-binding homologue family (PfRh) have been implicated in the invasion process and in some cases have been shown to act as adhesins, binding to specific receptors on the erythrocyte surface. We have identified a further, putatively essential, PfRh family member in the most virulent human malaria Plasmodium falciparum, called PfRh5, which binds to an unknown class of glycosylated receptors on the erythrocyte surface. This protein is an atypical PfRh family member, being much smaller than others and lacking a transmembrane and cytosolic region at the C-terminus. This suggests it may be part of a functional protein complex. PfRh5 localises to the rhoptries in merozoites and follows the tight junction during the process of erythrocyte invasion. Furthermore, rabbit immune serum raised against a portion of the ecto-domain, inhibits parasite invasion in vitro. We hypothesise an essential role for the PfRh5 adhesin in erythrocyte selection and commitment to invasion. Given its small size, we believe PfRh5 may prove to be a valuable candidate for inclusion in a multi-component anti-malarial vaccine.

A study on pathogenicity and mosquito transmission success in the rodent malaria parasite Plasmodium chabaudi adami.

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We investigated the parasitology, pathogenicity (virulence) and infectivity to mosquitoes of blood infections in mice, of two strains, DS and DK, of the rodent malaria parasite Plasmodium chabaudi adami. Blood infections of DS were found to be highly pathogenic; the asexual parasites in these infections were fast-growing and showed no evidence of selectivity in their infection of host erythrocytes. In contrast to DS, blood infections of DK were much less pathogenic; the asexual parasites were slower-growing and showed a moderate degree of selectivity to a subset of erythrocytes which were not reticulocytes. In both DS and DK infections, infectivity to mosquitoes was highest before the peak of asexual parasitaemia had occurred; usually this did not coincide with the time when gametocyte numbers in the blood were highest. Infections with the pathogenic DS strain in CBA mice produced fewer gametocytes than did the less pathogenic DK strain. The DS strain infections in both CBA and C57 mice were also significantly much less infective to mosquitoes than the DK strain. Investigations by others on the related rodent malaria parasite subspecies, Plasmodium chabaudi chabaudi, have indicated that the mosquito infectivity of blood infections in mice tended to be higher in the more pathogenic (virulent) and lower in the less pathogenic strains of this parasite subspecies. This is the converse of the finding of the present investigation of blood infections of P. c. adami in mice in which a more pathogenic, or virulent, strain (DS) of these parasites was significantly much less infective to mosquitoes than was a less pathogenic strain (DK).


Impact of HIV-1 infection on the hematological recovery after clinical malaria.


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BACKGROUND: Anemia is the most frequent cytopenia in HIV-infected individuals and is often associated with malaria. OBJECTIVE: To assess the impact of HIV-1 on the hematological recovery after a clinical malaria episode. METHODS: In Ndola, Zambia, a region with high malaria and HIV prevalence, hemoglobin (Hb) was measured in 634 malaria patients 14 and 45 days after antimalarial treatment. Risk factors for hematological recovery were analyzed in a multivariate linear regression model. RESULTS: At enrollment, HIV-1-infected malaria patients had lower Hb compared with HIV-1 uninfected (122.7 vs 136.0 g/L; P < 0.001). In both groups, mean Hb was significantly lower at day 14 posttreatment than day 0 (P < 0.0001) and significantly higher at day 45 than at day 14 (HIV-1 negative: P = 0.0001; HIV-1 infected: P = 0.005). HIV-1 was a risk factor for a larger Hb decrease until day 14 (P < 0.001) and slower recovery until day 45 (P = 0.048). When considering the whole 45-day follow-up period, mean Hb increased in the HIV-1-negative group (+3.54 g/L; 95% confidence interval: 1.37 to 5.70; P = 0.001) but not in the HIV-1-infected group (-0.72 g/L; 95% confidence interval: -3.85 to +2.40; P = 0.64). HIV-1 infection as such (P < 0.0001), not CD4 cell count (P = 0.46), was an independent risk factor for a slower hematological recovery. CONCLUSIONS: HIV-1-infected malaria patients had a slower hematological recovery after successful parasite clearance. Malaria preventive measures should be targeted to this high-risk group.
Screening of antiplasmodial properties among some traditionally used Iranian plants.

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ETHNOPHARMACOLOGICAL RELEVANCE: An investigation of plants was undertaken through interviews and literature surveys on plants used to treat malaria or cancer or microbial diseases in Iran. AIM OF STUDY: In vitro and in vivo antiplasmodial tests were carried out on selected plants traditionally used in Iran. MATERIALS AND METHODS: Thirty-two plants were extracted with methanol and tested for their in vitro (pLDH assay) activity against Plasmodium falciparum, in vivo activity against Plasmodium berghei and assessed for any cytotoxicity against the human cancer cell line MCF7 and the normal cell MDBK. RESULTS: Extracts from four plants, Buxus hyrcana Pojark. (Buxaceae), Erodium oxyrrhinchum M. Bieb. (Geraniaceae), Glycyrrhiza glabra L. (Fabaceae) and Ferula oopoda (Boiss and Bushe) Boiss. (Apoaceae) were found to have significant antiplasmodial activity (IC(50) ranging from 4.7 to 26.6μg/ml). These findings lend support to the use of Buxus hyrcana and Glycyrrhiza glabra in traditional medicine. The chloroformic fraction also was active against K1 and 3D7 strains. The chloroformic fraction was studied at 10mg per kg body weight mouse per day. This fraction reduced parasitaemia by 86.1% compared to untreated control mice. CONCLUSION: Glycyrrhiza glabra showed antiplasmodial activity and has selectivity for Plasmodium falciparum and Plasmodium berghei when tested on mammalian cells. This is the first report that mentioned in vitro and in vivo antiplasmodial activity of Glycyrrhiza glabra.

Emergence of an Unusual Sulfadoxine-Pyrimethamine Resistance Pattern and a Novel K540N Mutation in Dihydropteroate Synthetase in Plasmodium falciparum Isolates Obtained from Car Nicobar Island, India, after the 2004 Tsunami.


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Background. Enormous amounts of drugs were used to contain the outbreak of infectious diseases in areas of India affected by the tsunami in December 2004. The impact of this drug use on the Plasmodium falciparum population needs to be investigated. Methods. The nucleotide sequence of the pfcr, pf dhps, and pf dhfr genes was determined for 229 clinical P. falciparum isolates collected from patients on Car Nicobar Island at 6 different time points between May 2004 and May 2008. Results. Over time, there was an increase in the proportion of the P. falciparum population that had mutations in the pf cr, pf dhps, and pf dhfr genes associated with higher levels of chloroquine, sulfadoxine, and pyrimethamine resistance, respectively. However, the parasites collected during October 2005 had mutations associated with a lower level of pyrimethamine resistance and a higher level of sulfadoxine resistance (a rare combination), as well as a novel
K540N mutation in P. falciparum dihydropteroate synthetase (PfDHPS). The emergence of this parasite population coincided with the widespread use of an additional antifolate drug, trimethoprim-sulfamethoxazole, to treat other infections during January-March 2005. Molecular modeling revealed that the sulfadoxine binding affinity of the new PfDHPS triple mutant A(436)G(437)N(540)A(581)A(613) was similar to that of A(436)G(437)E(540)A(581)A(613) (bold type indicates mutated amino acids).

Conclusions. The use of 2 antifolate drugs in combination should be avoided to prevent the selection of parasites with newer mutations and altered drug susceptibilities.


**Vaccine-Like Immunity against Malaria by Repeated Causal-Prophylactic Treatment of Liver-Stage Plasmodium Parasites.**

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Liver-stage development of Plasmodium parasites represents a dramatic expansion phase for the malarial parasite between vector transmission and onset of the pathogenic blood-stage cycle. Here, we report that repeated causal-prophylactic primaquine treatment of liver-stage Plasmodium parasites in rodents elicits vaccine-like protective immunity against sporozoite-induced malaria. This regimen differs fundamentally from those involving radiation- or genetically attenuated parasites, in which long-lasting immune responses are dependent on persistence of metabolically active parasites. Pharmacological inhibition of liver-stage parasites in the rodent malaria model offers a potential fast track toward development of a vaccine that targets parasites in preerythrocytic stages.


**High deformability of Plasmodium vivax-infected red blood cells under microfluidic conditions.**

Handayani S, Chiu DT, Tjitra E, Kuo JS, Lampah D, Kenangalem E, Renia L, Snounou G, Price RN, Anstey NM, Russell B.


Maturation of Plasmodium falciparum decreases the deformability of infected red blood cells (RBCs), increasing their clearance as they attempt to pass through endothelial slits of the splenic sinus. Previous studies of Plasmodium vivax-infected RBCs led to opposite conclusions with respect to cellular deformability. To resolve this controversy, P. vivax-infected RBCs were passed through a 2-microm microfluidic channel. In contrast to P. falciparum-infected RBCs, mature P. vivax-infected RBCs readily became deformed through 2-microm constrictions. After this extreme deformation, 67% of P. vivax-infected RBCs recovered a normal appearance; however, 15% of uninfected RBCs were destroyed. Results suggest mechanisms for both avoidance of splenic clearance and anemia in vivax malaria.
Development of Nutritionally At-Risk Young Children Is Predicted by Malaria, Anemia, and Stunting in Pemba, Zanzibar.

Olney DK, Kariger PK, Stoltzfus RJ, Khalfan SS, Ali NS, Tielsch JM, Sazawal S, Black R, Allen LH, Pollitt E.

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Nutritionally at-risk children suffer delays in physical growth and motor and language development. Infectious diseases such as malaria pose an additional risk. We examined the cross-sectional relationships among malaria infection, hemoglobin (Hb) concentration, length-for-age Z-scores (LAZ), motor activity, behavior, and motor and language development in 841 Zanzibari children 5-19 mo old. We used structural equation modeling to test the fit of the data to a theoretical model and to examine the relationships among the variables in 3 age groups (5-9, 10-14, and 15-19 mo). The model fit the data for all age groups. In the youngest and oldest groups, children with higher malaria parasite densities had significantly lower Hb and LAZ. Higher LAZ significantly predicted higher total motor activity, and motor and language development scores in all age groups. In the oldest group, children who had higher Hb had higher motor development and activity scores. Malaria was directly and indirectly related to motor activity in the 10- to 14-mo-old group [standardized total effects, -0.14; direct, -0.10 (P = 0.015); and indirect, -0.038]. The significant fit of the models to the data and the statistical significance of many of the specific pathways highlight the complexities of the relationships between health and nutrition and child development outcomes in this population. In addition, the results suggest that multiple interventions are likely necessary to improve child development outcomes in this population of nutritionally at-risk children and that the potential effectiveness of interventions may differ according to age (i.e. prevention and treatment of anemia, stunting, and malaria).

Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial.


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BACKGROUND: Most malaria deaths occur in rural areas. Rapid progression from illness to death can be interrupted by prompt, effective medication. Antimalarial treatment cannot rescue terminally ill patients but could be effective if given earlier. If patients who cannot be treated orally are several hours from facilities for injections, rectal artesunate can be given before referral and acts rapidly on parasites. We investigated whether this intervention reduced mortality and permanent disability. METHODS: In Bangladesh, Ghana, and Tanzania, patients with suspected severe malaria who could not be treated orally were allocated randomly to a single artesunate (n=8954) or placebo (n=8872)
suppository by taking the next numbered box, then referred to clinics at which
injections could be given. Those with antimalarial injections or negative blood
smears before randomisation were excluded, leaving 12 068 patients (6072
artesunate, 5996 placebo) for analysis. Primary endpoints were mortality,
assessed 7–30 days later, and permanent disability, reassessed periodically. All
investigators were masked to group assignment. Analysis was by intention to
treat. This study is registered in all three countries, numbers ISRCTN83979018,
46343627, and 76987662. RESULTS: Mortality was 154 of 6072 artesunate versus 177
of 5996 placebo (2.5% vs 3.0%, p=0.1). Two versus 13 (0.03% vs 0.22%, p=0.0020)
were permanently disabled; total dead or disabled: 156 versus 190 (2.6% vs 3.2%,
p=0.0484). There was no reduction in early mortality (56 vs 51 deaths within 6 h;
median 2 h). In patients reaching clinic within 6 h (median 3 h), pre-referral
artesunate had no significant effect on death after 6 h or permanent disability
(71/4450 [1.6%] vs 82/4426 [1.9%], risk ratio 0.86 [95% CI 0.63–1.18], p=0.35).
In patients still not in clinic after more than 6 h, however, half were still not
there after more than 15 h, and pre-referral rectal artesunate significantly
reduced death or permanent disability (29/1566 [1.9%] vs 57/1519 [3.8%], risk
ratio 0.49 [95% CI 0.32–0.77], p=0.0013). INTERPRETATION: If patients with severe
malaria cannot be treated orally and access to injections will take several
hours, a single inexpensive artesunate suppository at the time of referral
substantially reduces the risk of death or permanent disability. FUNDING:
UNICEF/UNDP/World Bank Special Programme for Research and Training in Tropical
Diseases (WHO/TDR); WHO Global Malaria Programme (WHO/GMP); Sall Family
Foundation; the European Union (QLRT-2000-01430); the UK Medical Research
Council; USAID; Irish Aid; the Karolinska Institute; and the University of Oxford
Clinical Trial Service Unit (CTSU).


The last man standing is the most resistant: eliminating artemisinin-resistant
malaria in Cambodia.

Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp AM, Day NP,
White NJ, White LJ.

ABSTRACT: BACKGROUND: Artemisinin combination therapy (ACT) is now the
recommended first-line treatment for falciparum malaria throughout the world.
Initiatives to eliminate malaria are critically dependent on its efficacy. There
is recent worrying evidence that artemisinin resistance has arisen on the
Thai-Cambodian border. Urgent containment interventions are planned and about to
be executed. Mathematical modeling approaches to intervention design are now
integrated into the field of malaria epidemiology and control. The use of such an
approach to investigate the likely effectiveness of different containment
measures with the ultimate aim of eliminating artemisinin-resistant malaria is
described. METHODS: A population dynamic mathematical modeling framework was
developed to explore the relative effectiveness of a variety of containment
interventions in eliminating artemisinin-resistant malaria in western Cambodia.
RESULTS: The most effective intervention to eliminate artemisinin-resistant
malaria was a switch of treatment from artemisinin monotherapy to ACT (mean time
to elimination 3.42 years (95% CI 3.32–3.60 years). However, with this approach
it is predicted that elimination of artemisinin-resistant malaria using ACT can
be achieved only by elimination of all malaria. This is because the various forms
of ACT are more effective against infections with artemisinin-sensitive
parasites, leaving the more resistant infections as an increasing proportion of
the dwindling parasite population. CONCLUSIONS: Containment of
artemisinin-resistant malaria can be achieved by elimination of malaria from
western Cambodia using ACT. The "last man standing" is the most resistant and thus this strategy must be sustained until elimination is truly achieved.

*Malar J. 2009 Feb 19;8(1):30.*

**Household possession, use and non-use of treated or untreated mosquito nets in two ecologically diverse regions of Nigeria - Niger Delta and Sahel Savannah.**

Afolabi BM, Sofola OT, Fatunmbi BS, Komakech W, Okoh F, Saliu O, Otsemobor P, Oresanya OB, Amajoh CN, Fasiku D, Jalingo I.

ABSTRACT: **BACKGROUND:** Current use of treated mosquito nets for the prevention of malaria falls short of what is expected in sub-Saharan Africa (SSA), though research within the continent has indicated that the use of these commodities can reduce malaria morbidity by 50% and malaria mortality by 20%. Governments in sub-Saharan Africa are investing substantially in scaling-up treated mosquito net coverage for impact. However, certain significant factors still prevent the use of the treated mosquito nets, even among those who possess them. This survey examines household ownership as well as use and non-use of treated mosquito nets in Sahel Savannah and Niger Delta regions of Nigeria. **Methodology** This survey employed cross-sectional survey to collect data from households on coverage and use of mosquito nets, whether treated or not. Fever episodes in previous two weeks among children under the age of five were also recorded. The study took place in August 1 - 14 2007, just five months after the March distribution of treated mosquito nets, coinciding with the second raining period of the year and a time of high malaria transmission during the wet season. EPI INFO version 2003 was used in data analysis. **RESULTS:** The survey covered 439 households with 2,521 persons including 739 under-fives, 585 women in reproductive age and 78 pregnant women in Niger Delta Region and Sahel Savannah Region. Of the 439 HHs, 232 had any mosquito nets. Significantly higher proportion of households in the Niger Delta Region had any treated or untreated mosquito nets than those in the Sahel Savannah Region. In the Niger Delta Region, the proportion of under-fives that had slept under treated nets the night before the survey exceeded those that slept under treated nets in the Sahel Savannah Region. Children under the age of five years in the Niger Delta Region were four times more likely to sleep under treated nets than those in the Sahel Savannah Region. **CONCLUSION:** This study found that despite the fact that treated nets were distributed widely across Nigeria, the use of this commodity was still very low in the Sahel Savanna region. Future campaigns should include more purposeful social and health education on the importance and advantages of the use of treated nets to save lives in the Sahel Savannah region of Nigeria.


**Community knowledge, attitudes and practices (KAP) on malaria in Swaziland: A country earmarked for malaria elimination.**

Hlongwana KW, Mabaso ML, Kunene S, Govender D, Maharaj R.

ABSTRACT: **BACKGROUND:** The potential contribution of knowledge, attitudes and practices (KAP) studies to malaria research and control has not received much attention in most southern African countries. This study investigated the local communities' understanding of malaria transmission, recognition of signs and symptoms, perceptions of cause, treatment-seeking patterns, preventive measures and practices in order to inform the country's proposed malaria elimination programme in Swaziland. **METHODS:** A descriptive cross-sectional survey was
undertaken in four Lubombo Spatial Development Initiative (LSDI) sentinel sites in Swaziland. These sentinel sites share borders with Mozambique. A structured questionnaire was administered to 320 randomly selected households. Only one adult person was interviewed per household. The interviewees were the heads of households and in the absence of the heads of households responsible adults above 18 years were interviewed. RESULTS: A substantial number of research participants showed reasonable knowledge of malaria, including correct association between malaria and mosquito bites, its potential fatal consequences and correct treatment practices. Almost 90% (n=320) of the respondents stated that they would seek treatment within 24 hours of onset of malaria symptoms, with health facilities as their first treatment option. Most people (78%) perceived clinics and vector control practices as central to treating and preventing malaria disease. Indoor residual spraying (IRS) coverage and bed net ownership were 87.2% and 38.8%, respectively. IRS coverage was in agreement with the World Health Organization's (WHO) recommendation of more than 80% within the targeted communities. CONCLUSION: Despite fair knowledge of malaria in Swaziland, there is a need for improving the availability of information through the preferred community channels, such as tinkhundlas (districts), as well as professional health routes. This recommendation emerges along with the documented evidence suggesting that as the level transmission and disease decreases so does the perception about the importance of malaria control activities. Finally, given the relatively moderate ownership of bed net there is a need for future studies to evaluate the distribution of insecticide-treated nets (ITNs) compared with IRS.


Polymorphism of PfATPase in Niger: detection of three new point mutations.

Ibrahim ML, Khim N, Adam HH, Ariey F, Duchemin JB.

ABSTRACT: BACKGROUND: Plasmodium falciparum resistance to drugs remains a major public health issue in Niger. The therapeutic failure index for chloroquine and sulphadoxine-pyrimethamine are, respectively 20% and 21.9%. In December 2005, the National Malaria Control Programme promoted the use of artemisinin combination therapy (ACT) as first-line treatment of the uncomplicated malaria cases. Recently, studies have shown a relationship between the SERCA PfATPase6 gene and artemisinin efficacy, and pointed it out as a potential molecular marker for resistance. The goal of this work was to describe the baseline polymorphism of PfATPase6 gene in Niger, at a time when the national implementation of the ACT policy had just begun. Materials and methods The DNA polymorphism of the PfATPase6 gene of 87 P. falciparum samples from Niger was analysed by sequencing. The links between the mutation occurrence and environment and human host factors were tested by bivariate analysis. RESULTS: The P. falciparum PfATPase6 gene presented polymorphisms at codons 537, 561, 569, 630, 639, 716 levels. All the mutations found were rare, except the PfATPaseN569K found in 17.2% of samples. No associated factor has been observed. CONCLUSION: The P. falciparum PfATPase gene is polymorphic at the 569 codon. As ACT is getting more and more used, the PfATPase6 gene polymorphism needs to be monitored in association with phenotypic - in vivo and/or in vitro - drug efficacy tests.


Micro-geographic risk factors for malarial infection.

Myers WP, Myers AP, Cox-Singh J, Lau HC, Mokuai B, Malley R.
ABSTRACT: BACKGROUND: Knowledge of geography is integral to the study of insect-borne infectious disease such as malaria. This study was designed to evaluate whether geographic parameters are associated with malarial infection in the East Sepik province of Papua New Guinea (PNG), a remote area where malaria is a major cause of morbidity and mortality. METHODS: A global positioning system (GPS) unit was used at each village to collect elevation, latitude and longitude data. Concurrently, a sketch map of each village was generated and the villages were sub-divided into regions of roughly equal populations. Blood samples were taken from subjects in each region using filter paper collection. The samples were later processed using nested PCR for qualitative determination of malarial infection. The area was mapped using the GPS-information and overlaid with prevalence data. Data tables were examined using traditional chi square statistical techniques. A logistic regression analysis was then used to determine the significance of geographic risk factors including, elevation, distance from administrative centre and village of residence. RESULTS: Three hundred and thirty-two samples were included (24% of the total estimated population). Ninety-six were positive, yielding a prevalence of 29%. Chi square testing within each village found a non-random distribution of cases across sub-regions (p<0.05). Multivariate logistic regression techniques suggested malarial infection changed with elevation (OR=0.64 per 10m, p<0.05) and distance from administrative centre (OR=1.3 per 100m, p<0.05). CONCLUSIONS: These results suggest that malarial infection is significantly and independently associated with lower elevation and greater distance from administrative centre in a rural area in PNG. This type of analysis can provide information that may be used to target specific areas in developing countries for malaria prevention and treatment.


Rural Gambian women's reliance on health workers to deliver sulphadoxine-pyrimethamine as recommended intermittent preventive treatment for malaria in pregnancy.

Brabin L, Stokes E, Dumbaya I, Owens S.

ABSTRACT: BACKGROUND: The use of most anti-malarial medications is restricted during pregnancy, but two doses of sulphadoxine-pyrimethamine are recommended after the first trimester as intermittent preventive treatment in pregnancy (IPTp). In The Gambia, only 32% of women receive two doses and very little research has been conducted on women's awareness of drug safety during pregnancy. The objective of this paper was to assess whether rural Gambian women were aware of the importance of the timing of the two-dose IPT dose schedule and its relevance to drug safety. METHODS: This was a qualitative study in which 41 interviews and 16 focus group discussions with women, adolescents, men and traditional birth attendants were conducted. A generic qualitative approach was used to generate a theory as to why women might not participate in IPTp as recommended. RESULTS: Although most women used calendar months to count their stage of pregnancy, these months did not correlate with their concept of foetal development. Foetal growth was described following Islamic tradition as water, clot, piece of meat and human being, although there was little consensus about the order or timing in which these stages occurred. Common signs and conditions of malaria were known. Women were anxious about miscarriage and recognized that some medicines should not be taken in the first trimester, but were urged by men and traditional birth attendants to attend for antenatal care in the first trimester to "start treatment." General knowledge about the purpose of pregnancy medications and when they should be taken was poor among both men and women. One
important result was that women relied entirely on health workers to provide safe
drugs, at the correct time. CONCLUSIONS: Women did not have relevant information
to judge the safety and appropriate timing of pregnancy drugs, which made them
over-reliant on health workers. They should be encouraged to date their own
pregnancies in culturally relevant terms and to anticipate when and which
medications they should receive.

*Estimating the burden of malaria in pregnancy: a case study from rural Madhya Pradesh, India.*

Diamond-Smith N, Singh N, Gupta RD, Dash A, Thimasarn K, Campbell OM, Chandramohan D.

ABSTRACT: BACKGROUND: Malaria in pregnancy (MiP) is inadequately researched in
India, and the burden is probably much higher than current estimates suggest.
This paper models the burden of MiP and associated foetal losses and maternal
deaths, in rural Madhya Pradesh, India. METHODS: Number of pregnancies per year
was estimated from the number of births and an estimate of pregnancies that end
in foetal loss. The prevalence of MiP, risk of foetal loss attributable to MiP
and case fatality rate of MiP were obtained from the literature. The estimated
total number of pregnancies was multiplied by the appropriate parameter to
estimate the number of MiP cases, and foetal loss and maternal deaths
attributable to MiP per year. A Monte Carlo simulation sensitivity analysis was
done to assess plausibility of various estimates obtained from the literature.
The burden of MiP in tribal women was explored by incorporating the variable
prevalence of malaria in tribal and non-tribal populations and in forested and
non-forested regions within Madhya Pradesh. RESULTS: Estimates of MiP cases in
rural Madhya Pradesh based on the model parameter values found in the literature
ranged from 183,000-1.5 million per year, with 73,000-629,000 lost foetuses and
1,500-12,600 maternal deaths attributable to MiP. The Monte Carlo simulation gave
a more plausible estimate of 220,000 MiP cases per year (inter-quartile range
(IQR): 136,000-305,000), 95,800 lost foetuses (IQR: 56,800-147,600) and 1,000
maternal deaths (IQR: 650-1,600). Tribal women living in forested areas bore 30%
of the burden of MiP in Madhya Pradesh, while constituting 18% of the population.
CONCLUSIONS: Although the estimates are uncertain, they suggest MiP is a
significant public health problem in rural Madhya Pradesh, affecting many
thousands of women and that reducing the MiP burden should be a priority.

*Clinical trials to estimate the efficacy of preventive interventions against malaria in paediatric populations: a methodological review.*

Moorthy VS, Reed Z, Smith PG.

ABSTRACT: BACKGROUND: Recent years have seen publication of a considerable number
of clinical trials of preventive interventions against clinical malaria in
children. There has been variability in the specification of end-points, case
definitions, analysis methods and reporting and the relative lack of
standardization complicates the ability to make comparative evaluations between
trials. METHODS: To prepare for a WHO consultation on design issues in malaria
vaccine trials, controlled trials of preventive interventions against malaria in
children in endemic countries were identified in which clinical malaria, or
death, had been one of the main end-points. Trials were included that evaluated
the impact of vaccines, insecticide-treated bed nets (ITN), intermittent presumptive or preventive therapy in infants (IPTi) or, in one instance, vitamin A supplementation. Methods that had been used in these trials were summarized and compared in order to identify issues that were directly relevant to the design of malaria vaccine trials. RESULTS: 29 controlled trials of preventive malaria interventions were identified, of which eight were vaccine trials. Vaccine trials that were designed to detect an effect on clinical malaria all reported the incidence rate of first episodes of clinical malaria as their primary endpoint. Only one trial of a preventive intervention (of ITN) was identified that was designed to detect an effect on severe malaria. A group of larger trials were designed to detect an effect of impregnated bed nets or curtains on all-cause mortality as the primary end-point. Key methodological and reporting differences between trials are noted in the text. Two issues have been identified that are of some concern. Firstly, the choice of primary endpoint is not stated in the reports of a number of the trials and, secondly, the relationship between pre-specified analysis plans and trial reports is rarely made clear. CONCLUSIONS: This article reports an investigation into the ways in which trial design and reporting could be improved and standardized to enable comparative evaluation of the relative merits of malaria control measures, and specifically with respect to the design of malaria vaccine trials. The need for standardization of clinical trial design, conduct, analysis and reporting has been also affirmed as a priority area by the Malaria Vaccine Technology Roadmap.


Quality of anti-malarial drugs provided by public and private healthcare providers in south-east Nigeria.

Onwujekwe O, Kaur H, Dike N, Shu E, Uzochukwu B, Hanson K, Okoye V, Okonkwo P.

ABSTRACT: BACKGROUND: There is little existing knowledge about actual quality of drugs provided by different providers in Nigeria and in many sub-Saharan African countries. Such information is important for improving malaria treatment that will help in the development and implementation of actions designed to improve the quality of treatment. The objective of the study was to determine the quality of drugs used for the treatment of malaria in a broad spectrum of public and private healthcare providers. METHODS: The study was undertaken in six towns (three urban and three rural) in Anambra state, south-east Nigeria. Anti-malarials (225 samples), which included artemunate, dihydroartemisinin, sulphadoxine-pyrimethamine (SP), quinine, and chloroquine, were either purchased or collected from randomly selected providers. The quality of these drugs was assessed by laboratory analysis of the dissolution profile using published pharmacopoeial monograms and measuring the amount of active ingredient using high performance liquid chromatography (HPLC). FINDINGS: It was found that 60 (37%) of the anti-malarials tested did not meet the United States Pharmacopoeia (USP) specifications for the amount of active ingredients, with the suspect drugs either lacking the active ingredients or containing suboptimal quantities of the active ingredients. Quinine (46%) and SP formulations (39%) were among drugs that did not satisfy the tolerance limits published in USP monograms. A total of 78% of the suspect drugs were from private facilities, mostly low-level providers, such as patent medicine dealers (vendors). CONCLUSION: This study found that there was a high prevalence of poor quality drugs. The findings provide areas for public intervention to improve the quality of malaria treatment services. There should be enforced checks and regulation of drug supply management as well as stiffer penalties for people stocking substandard and counterfeit drugs.
Malaria and water resource development: the case of Gilgel-Gibe hydroelectric dam in Ethiopia.


ABSTRACT: BACKGROUND: Ethiopia plans to increase its electricity power supply by five-fold over the next five years to fulfill the needs of its people and support the economic growth based on large hydropower dams. Building large dams for hydropower generation may increase the transmission of malaria since they transform ecosystems and create new vector breeding habitats. The aim of this study was to assess the effects of Gilgel-Gibe hydroelectric dam in Ethiopia on malaria transmission and changing levels of prevalence in children. METHODS: A cross-sectional, community-based study was carried out between October and December 2005 in Jimma Zone, south-western Ethiopia, among children under 10 years of age living in three 'at-risk' villages (within 3 km from dam) and three 'control' villages (5 to 8 km from dam). The man-made Gilgel-Gibe dam is operating since 2004. Households with children less than 10 years of age were selected and children from the selected households were sampled from all the six villages. This included 1,081 children from 'at-risk' villages and 774 children from 'control' villages. Blood samples collected from children using finger prick were examined microscopically to determine malaria prevalence, density of parasitaemia and identify malarial parasite species. RESULTS: Overall 1,855 children (905 girls and 950 boys) were surveyed. A total of 194 (10.5%) children were positive for malaria, of which, 117 (60.3%) for Plasmodium vivax, 76 (39.2%) for Plasmodium falciparum and one (0.5%) for both P. vivax and P. falciparum. A multivariate design-based analysis indicated that, while controlling for age, sex and time of data collection, children who resided in 'at-risk' villages close to the dam were more likely to have P. vivax infection than children who resided farther away (odds ratio (OR) = 1.63, 95% CI = 1.15, 2.32) and showed a higher OR to have P. falciparum infection than children who resided in 'control' villages, but this was not significant (OR = 2.40, 95% CI = 0.84, 6.88). A classification tree revealed insights in the importance of the dam as a risk factor for malaria. Assuming that the relationship between the dam and malaria is causal, 43% of the malaria occurring in children was due to living in close proximity to the dam. CONCLUSION: This study indicates that children living in close proximity to a man-made reservoir in Ethiopia are at higher risk of malaria compared to those living farther away. It is recommended that sound prevention and control programme be designed and implemented around the reservoir to reduce the prevalence of malaria. In this respect, in localities near large dams, health impact assessment through periodic survey of potential vectors and periodic medical screening is warranted. Moreover, strategies to mitigate predicted negative health outcomes should be integral parts in the preparation, construction and operational phases of future water resource development and management projects.

Molecular characterization of antifolates resistance-associated genes (dhfr and dhps) in Plasmodium vivax isolates from the Middle East.

Zakeri S, Motmaen SR, Afsharpad M, Djadid ND.

ABSTRACT: BACKGROUND: In Iran, co-infections of Plasmodium vivax and Plasmodium
falciparum are common and P. vivax infections are often exposed to sulphadoxine-pyrimethamine (SP). In the present study, the frequency distribution of mutations associated to SP resistance was investigated in pvdhfr and pvdhps genes from field isolates. METHODS: Clinical isolates of P. vivax were collected in two different malaria endemic regions in northern and south-eastern Iran, between 2001 and 2006. All 189 collected isolates were analysed for SNP/haplotypes at positions 13, 33, 57, 58, 61, 117 and 173 of the pvdhfr and 383 and 553 of pvdhps genes using nested PCR-RFLP methods RESULTS: All 189 examined isolates were found to carry wild-type amino acids at positions 13, 33, 61 and 173, while 57L and 58R and 117N mutations in pure form was detected among 1.1%, 17.5% and 26% examined samples, respectively, with no polymorphisms in different loci of dhps genes. Based on size polymorphism of pvdhfr genes at repeat region, among northern isolates, the frequency distribution for type A and B were 2.2% and 97.8% respectively. However, in southern samples the prevalence of type A, B and C were 7%, 89.5% and 7.7%, respectively. Mixed genotype infections (type B and C) were detected in only 4.2% (6/143) of southern, but in none of the northern isolates. The combination of pvdhfr and pvdhps haplotypes among all 189 samples demonstrated six distinct haplotypes. The two most prevalent haplotypes among all examined samples were I13P33F57S58T61S117I173/A383A553 (65.6%) and I13P33F57S58T61N117I173 (16.4%). Two other alleles with one point mutation I13P33F57R58T61S117I173/A383A553 and two mutations I13P33F57R58T61N117I173/A383A553 accounted for 7.4% and 9.5% of the total isolates. CONCLUSION: The present molecular data provide important information for making decisions on population based drug use in Iran. In addition, since October 2005, with more availability of SP as first-line treatment, P. vivax isolates are more exposed to SP and the selection or spread of resistant pvdhfr and pvdhps alleles might increase in the near future in this region.

Parasitol Res. 2009 Feb 17.

Histopathological changes in adult Schistosoma japonicum harbored in mice treated with a single dose of mefloquine.

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New research has shown that mefloquine, an arylaminoalcohol used against malaria, is active against Schistosoma japonicum and Schistosoma mansoni in vivo. To enhance our understanding of the potential mechanism of action of mefloquine against schistosomiasis, we examined the dynamics of histopathological changes in adult S. japonicum. Mice infected with S. japonicum for 35 days were treated intragastrically with a single dose of mefloquine (400 mg/kg). One to 35 days after mefloquine administration, drug-induced histopathological alterations were studied. Twenty-four hours after treatment, S. japonicum showed signs of degeneration, including focal roughing and swelling of the tegument and/or muscles, dilatation of the gut, focal desquamation of gut epithelial cells, and a decrease in pigment particles. There was extensive degeneration of vitelline cells and appearance of pigment particles visible in the cytoplasm in female worms. The extent and severity of histopathological changes increased over time; 48 h posttreatment, two thirds of female worms and a quarter of male worms were classified as dead. Three to 14 days posttreatment, typical histological changes observed in surviving male worms were vesiculation, swelling of parenchymal tissues, and dilatation of gut. In females, there was disintegration and
infiltration of inflammatory cells, forming dead worm abscesses and early stage of dead worm granuloma. Finally, 35 days posttreatment, only dead male and female worm granuloma were found. Our results provide further evidence of in vivo activity of mefloquine against adult schistosomes.

*Parasitol Res.* 2009 Feb 11.

**Effect of dequalinium on the oxidative stress in Plasmodium berghei-infected erythrocytes.**

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The bisquinoline drug dequalinium (DQ) has demonstrated remarkable activity against some infection diseases, including malaria. Oxidative stress represents a biochemical target for potential antimalarials. In this work, we have tested the ability of this compound to modify the oxidative status in Plasmodium berghei-infected erythrocytes. After hemolysis, activities of superoxide dismutase (SOD), catalase (CAT), glutathione cycle, and dehydrogenase enzymes were investigated. The activity of glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGLD) in infected cells were diminished by this drug compared to controls (300% and 80% approximately, respectively), while glutathione peroxidase (GPx), glutathione transferase (GST), and glutathione levels were also lowered. As a compensatory response, we could appreciate an increase of SOD activity (20% approximately) in infected cells treated with DQ; however, catalase was not affected by the compound. Lipid peroxidation was also decreased by this drug, protecting the cells from the hemolysis caused by the infection. In conclusion, oxidative stress represents a biochemical event which is modulated by DQ, interfering with the antioxidant regular activities in *P. berghei* infection.

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

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

*Parasitol Res. 2009 Jan 29.*

**In silico comparative genome analysis of malaria parasite Plasmodium falciparum and Plasmodium vivax chromosome 4.**

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Malarial parasite has long been a subject of research for a large community of scientists and has yet to be conquered. One of the main obstacles to effectively control this disease is rapidly evolving genetic structure of Plasmodium parasite itself. In this study, we focused on chromosome 4 of the Plasmodium falciparum and Plasmodium vivax species and carried out comparative studies of genes that are responsible for antigenic variation in respective species. Comparative analysis of genes responsible for antigenic variation (var and vir genes in P. falciparum and P. vivax, respectively) showed significant difference in their respective nucleotide sequence lengths as well as amino acid composition. The possible association of exon's length on pathogenicity of respective Plasmodium species was also investigated, and analysis of gene structure showed that on the whole, exon lengths in P. falciparum are larger compared to P. vivax. Analysis of tandem repeats across the genome has shown that the size of repetitive sequences has a direct effect on chromosomes length, which can also be a potential reason for P. falciparum's greater variability and hence pathogenecity than P. vivax.

*Parasitol Res. 2009 Jan 27.*

**Initial characterization of Pf62, a novel protein of Plasmodium falciparum identified by immunoscreening.**

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In order to find new antigens from Plasmodium falciparum, a complementary DNA (cDNA) library was constructed and screened. The study of expression library of P. falciparum was performed in an attempt to identify new antigens that could have potential relevance for the falciparum-malaria diagnosis and/or protection. Between the positive clones detected (ring erythrocyte surface antigen, merozoite erythrocyte surface antigen, RHOP H3, CSP, LSA), a new gene that correspond to a new protein (Pf62) was isolated and characterized. This antigen was useful for the diagnosis of malaria in enzyme-linked immunosorbent assay tests. The cDNA corresponding to this antigen and structure of the gene were characterized. Pf62 is a single copy gene that contains one exon. The Pf62 cDNA has an open reading frame of 1,599 nucleotides that code for a putative protein of 532 amino acids with a predicted molecular mass of 62 kDa. The polypeptide contains in the central section two regions of repeats of 21 and 19 amino acids, respectively.
The localization of the Pf62 protein was performed by immunoblot, indirect immunofluorescence assay and immunoelectron microscopy. Pf62 is localized in the cytoplasm of the parasite and also on the surface of the infected erythrocyte. Serologic assays by using synthetic peptides designed from different antigenic regions of the Pf62 protein resulted in acceptable data of sensitivity and specificity in symptomatic malaria patients.


High-level production of amorpha-4,11-diene, a precursor of the antimalarial agent artemisinin, in Escherichia coli.


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BACKGROUND: Artemisinin derivatives are the key active ingredients in Artemisinin combination therapies (ACTs), the most effective therapies available for treatment of malaria. Because the raw material is extracted from plants with long growing seasons, artemisinin is often in short supply, and fermentation would be an attractive alternative production method to supplement the plant source. Previous work showed that high levels of amorpha-4,11-diene, an artemisinin precursor, can be made in Escherichia coli using a heterologous mevalonate pathway derived from yeast (Saccharomyces cerevisiae), though the reconstructed mevalonate pathway was limited at a particular enzymatic step.

METHODOLOGY/PRINCIPAL FINDINGS: By combining improvements in the heterologous mevalonate pathway with a superior fermentation process, commercially relevant titers were achieved in fed-batch fermentations. Yeast genes for HMG-CoA synthase and HMG-CoA reductase (the second and third enzymes in the pathway) were replaced with equivalent genes from Staphylococcus aureus, more than doubling production. Amorpha-4,11-diene titers were further increased by optimizing nitrogen delivery in the fermentation process. Successful cultivation of the improved strain under carbon and nitrogen restriction consistently yielded 90 g/L dry cell weight and an average titer of 27.4 g/L amorpha-4,11-diene.

CONCLUSIONS/SIGNIFICANCE: Production of >25 g/L amorpha-4,11-diene by fermentation followed by chemical conversion to artemisinin may allow for development of a process to provide an alternative source of artemisinin to be incorporated into ACTs.


Loss of population levels of immunity to malaria as a result of exposure-reducing interventions: consequences for interpretation of disease trends.

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BACKGROUND: The persistence of malaria as an endemic infection and one of the major causes of childhood death in most parts of Africa has lead to a radical new call for a global effort towards eradication. With the deployment of a highly effective vaccine still some years away, there has been an increased focus on interventions which reduce exposure to infection in the individual and -by
reducing onward transmission—at the population level. The development of appropriate monitoring of these interventions requires an understanding of the timescales of their effect. METHODS & FINDINGS: Using a mathematical model for malaria transmission which incorporates the acquisition and loss of both clinical and parasite immunity, we explore the impact of the trade-off between reduction in exposure and decreased development of immunity on the dynamics of disease following a transmission-reducing intervention such as insecticide-treated nets. Our model predicts that initially rapid reductions in clinical disease incidence will be observed as transmission is reduced in a highly immune population. However, these benefits in the first 5-10 years after the intervention may be offset by a greater burden of disease decades later as immunity at the population level is gradually lost. The negative impact of having fewer immune individuals in the population can be counterbalanced either by the implementation of highly-effective transmission-reducing interventions (such as the combined use of insecticide-treated nets and insecticide residual sprays) for an indefinite period or the concurrent use of a pre-erythrocytic stage vaccine or prophylactic therapy in children to protect those at risk from disease as immunity is lost in the population. CONCLUSIONS: Effective interventions will result in rapid decreases in clinical disease across all transmission settings while population-level immunity is maintained but may subsequently result in increases in clinical disease many years later as population-level immunity is lost. A dynamic, evolving intervention programme will therefore be necessary to secure substantial, stable reductions in malaria transmission.


**Poisoning pyridoxal 5-phosphate-dependent enzymes: a new strategy to target the malaria parasite Plasmodium falciparum.**


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The human malaria parasite Plasmodium falciparum is able to synthesize de novo pyridoxal 5-phosphate (PLP), a crucial cofactor, during erythrocytic schizogony. However, the parasite possesses additionally a pyridoxine/pyridoxal kinase (PdxK) to activate B6 vitamers salvaged from the host. We describe a strategy whereby synthetic pyridoxyl-amino acid adducts are channelled into the parasite. Trapped upon phosphorylation by the plasmodial PdxK, these compounds block PLP-dependent enzymes and thus impair the growth of *P. falciparum*. The novel compound PT3, a cyclic pyridoxyl-tryptophan methyl ester, inhibited the proliferation of *Plasmodium* very efficiently (IC(50)-value of 14 microM) without harming human cells. The non-cyclic pyridoxyl-tryptophan methyl ester PT5 and the pyridoxyl-histidine methyl ester PHME were at least one order of magnitude less effective or completely ineffective in the case of the latter. Modeling in silico indicates that the phosphorylated forms of PT3 and PT5 fit well into the PLP-binding site of plasmodial ornithine decarboxylase (PFODC), the key enzyme of polyamine synthesis, consistent with the ability to abolish ODC activity in vitro. Furthermore, the antiplasmodial effect of PT3 is directly linked to the capability of *Plasmodium* to trap this pyridoxyl analog, as shown by an increased sensitivity of parasites overexpressing PfPdxK in their cytosol, as visualized by GFP fluorescence.
Submicroscopic gametocytes and the transmission of antifolate-resistant Plasmodium falciparum in Western Kenya.


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BACKGROUND: Single nucleotide polymorphisms (SNPs) in the dhfr and dhps genes are associated with sulphadoxine-pyrimethamine (SP) treatment failure and gametocyte carriage. This may result in enhanced transmission of mutant malaria parasites, as previously shown for chloroquine resistant parasites. In the present study, we determine the association between parasite mutations, submicroscopic P. falciparum gametocytemia and malaria transmission to mosquitoes.

METHODOLOGY/PRINCIPAL FINDINGS: Samples from children treated with SP alone or in combination with artesunate (AS) or amodiaquine were genotyped for SNPs in the dhfr and dhps genes. Gametocytemia was determined by microscopy and Pfs25 RNA-based quantitative nucleic acid sequence-based amplification (Pfs25 QT-NASBA). Transmission was determined by membrane-feeding assays. We observed no wild type infections, 66.5% (127/191) of the infections expressed mutations at all three dhfr codons prior to treatment. The presence of all three mutations was not related to higher Pfs25 QT-NASBA gametocyte prevalence or density during follow-up, compared to double mutant infections. The proportion of infected mosquitoes or oocyst burden was also not related to the number of mutations. Addition of AS to SP reduced gametocytemia and malaria transmission during follow-up. CONCLUSIONS/SIGNIFICANCE: In our study population where all infections had at least a double mutation in the dhfr gene, additional mutations were not related to increased submicroscopic gametocytemia or enhanced malaria transmission. The absence of wild-type infections is likely to have reduced our power to detect differences. Our data further support the use of ACT to reduce the transmission of drug-resistant malaria parasites.
height, weight, MUAC, clinical signs on admission including edema, and type of discharge (recovery, death, and default/loss to follow up). Additional data included results of a malaria rapid diagnostic test due to Plasmodium falciparum (Paracheck) and whether the child was a resident of the region of Maradi or came from bordering Nigeria to seek treatment. Multivariate logistic regression was performed on a subset of 27,687 children meeting the new WHO growth standards criteria for severe malnutrition (weight-for-height<-3 Z score, mid-upper arm circumference<110 mm for children taller than 65 cm or presence of bipedal edema). We explored two different models: one with only basic anthropometric data and a second model that included perfunctory clinical signs. PRINCIPAL FINDINGS: In the first model including only weight, height, sex and presence of edema, the risk factors retained were the weight/height(1.84) ratio (OR: 5.774; 95% CI: [2.284; 14.594]) and presence of edema (7.51 [5.12; 11.0]). A second model, taking into account supplementary data from perfunctory clinical examination, identified other risk factors for death: apathy (9.71 [6.92; 13.6]), pallor (2.25 [1.25; 4.05]), anorexia (1.89 [1.35; 2.66]), fever>38.5 degrees C (1.83 [1.25; 2.69]), and age below 1 year (1.42 [1.01; 1.99]). CONCLUSIONS: Although clinicians will continue to perform screening using clinical signs and anthropometry, these risk indicators may provide additional criteria for the assessment of absolute and relative risk of death. Better appraisal of the child's risk of death may help orientate the child towards either hospitalization or ambulatory care. As the transition from the NCHS growth reference to the WHO standards will increase the number of children classified as severely malnourished, further studies should explore means to identify children at highest risk of death within this group using simple and standardized indicators.


Retinal pathology of pediatric cerebral malaria in Malawi.

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INTRODUCTION: The causes of coma and death in cerebral malaria remain unknown. Malarial retinopathy has been identified as an important clinical sign in the diagnosis and prognosis of cerebral malaria. As part of a larger autopsy study to determine causes of death in children with coma presenting to hospital in Blantyre, Malawi, who were fully evaluated clinically prior to death, we examined the histopathology of eyes of patients who died and underwent autopsy. METHODOLOGY/PRINCIPAL FINDINGS: Children with coma were admitted to the pediatric research ward, classified according to clinical definitions as having cerebral malaria or another cause of coma, evaluated and treated. The eyes were examined by direct and indirect ophthalmoscopy. If a child died and permission was given, a standardized autopsy was carried out. The patient was then assigned an actual cause of death according to the autopsy findings. The eyes were examined pathologically for hemorrhages, cystoid macular edema, parasite sequestration and thrombi. They were stained immunohistochemically for fibrin and CD61 to identify the components of thrombi, beta-amyloid precursor protein to detect axonal damage, for fibrinogen to identify vascular leakage and for glial fibrillary acidic protein to detect gliosis. Sixty-four eyes from 64 patients were examined: 35 with cerebral malaria and 29 with comas of other causes. Cerebral malaria was distinguished by sequestration of parasitized erythrocytes, the presence and severity of retinal hemorrhages, the presence of cystoid macular edema, the occurrence and number of fibrin-platelet thrombi, the presence and amount of
axonal damage and vascular leakage. CONCLUSIONS/SIGNIFICANCE: We found significant differences in retinal histopathology between patients who died of cerebral malaria and those with other diagnoses. These histopathological findings offer insights into the etiology of malarial retinopathy and provide a pathological basis for recently described retinal capillary non-perfusion in children with malarial retinopathy. Because of the similarities between the retina and the brain it also suggests mechanisms that may contribute to coma and death in cerebral malaria.


Rapid changes in transcription profiles of the Plasmodium yoelii yir multigene family in clonal populations: lack of epigenetic memory?

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The pir multigene family, found in the genomes of Plasmodium vivax, P. knowlesi and the rodent malaria species, encode variant antigens that could be targets of the immune response. Individual parasites of the rodent malaria Plasmodium yoelii, selected by micromanipulation, transcribe only 1 to 3 different pir (yir) suggesting tight transcriptional control at the level of individual cells. Using microarray and quantitative RT-PCR, we show that despite this very restricted transcription in a single cell, many yir genes are transcribed throughout the intra-erythrocytic asexual cycle. The timing and level of transcription differs between genes, with some being more highly transcribed in ring and trophozoite stages, whereas others are more highly transcribed in schizonts. Infection of immunodeficient mice with single infected erythrocytes results in populations of parasites each with transcriptional profiles different from that of the parent parasite population and from each other. This drift away from the original 'set' of transcribed genes does not appear to follow a preset pattern and "epigenetic memory" of the yir transcribed in the parent parasite can be rapidly lost. Thus, regulation of pir gene transcription may be different from that of the well-characterised multigene family, var, of Plasmodium falciparum.


The Indian Ocean Dipole and malaria risk in the highlands of western Kenya.

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Epidemics of malaria in the East African highlands in the last 2 decades have often been associated with climate variability, particularly the El Niño-Southern Oscillation (ENSO). However, there are other factors associated with malaria risk and there is increased interest in the influences of the Indian Ocean Dipole (IOD), a climate mode of coupled ocean-atmosphere variability, on East African rainfall. This study explores the relationship between IOD and the number of malaria patients in 7 hospitals from 2 districts in the western Kenyan highlands, controlling for the effects of ENSO. We examined temporal patterns (1982-2001) in the number of malaria cases in relation to the dipole mode index (DMI), defined as the difference in sea surface temperature anomaly between the western (10
degrees S-10 degrees N, 50 degrees-70 degrees E) and eastern (10 degrees S-0 degrees, 90 degrees-110 degrees E) tropical Indian Ocean. We used Poisson regression models, adjusted for ENSO index Niño 3 region (NINO3), seasonal and interannual variations. The number of malaria patients per month increased by 3.4%-17.9% for each 0.1 increase above a DMI threshold (3-4 months lag). Malaria cases increased by 1.4%-10.7% per month, for each 10 mm increase in monthly rainfall (2-3 months lag). In 6 of 7 places, there was no evidence of an association between NINO3 and the number of malaria cases after adjusting for the effect of DMI. This study suggests that the number of malaria cases in the western Kenyan highlands increases with high DMI in the months preceding hospital visits.

Proc Natl Acad Sci U S A. 2009 Feb 5.

Structural basis for the inhibition of the essential Plasmodium falciparum M1 neutral aminopeptidase.


Department of Biochemistry and Molecular Biology and.

Plasmodium falciparum parasites are responsible for the major global disease malaria, which results in >2 million deaths each year. With the rise of drug-resistant malarial parasites, novel drug targets and lead compounds are urgently required for the development of new therapeutic strategies. Here, we address this important problem by targeting the malarial neutral aminopeptidases that are involved in the terminal stages of hemoglobin digestion and essential for the provision of amino acids used for parasite growth and development within the erythrocyte. We characterize the structure and substrate specificity of one such aminopeptidase, PfA-M1, a validated drug target. The X-ray crystal structure of PfA-M1 alone and in complex with the generic inhibitor, bestatin, and a phosphinate dipeptide analogue with potent in vitro and in vivo antimalarial activity, hPheP[CH(2)]Phe, reveals features within the protease active site that are critical to its function as an aminopeptidase and can be exploited for drug development. These results set the groundwork for the development of antimalarial therapeutics that target the neutral aminopeptidases of the parasite.

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Platelets kill intraerythrocytic malarial parasites and mediate survival to infection.


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Platelets play a critical role in the pathogenesis of malarial infections by encouraging the sequestration of infected red blood cells within the cerebral vasculature. But platelets also have well-established roles in innate protection against microbial infections. We found that purified human platelets killed Plasmodium falciparum parasites cultured in red blood cells. Inhibition of platelet function by aspirin and other platelet inhibitors abrogated the lethal
effect human platelets exert on P. falciparum parasites. Likewise, platelet-deficient and aspirin-treated mice were more susceptible to death during erythrocytic infection with Plasmodium chabaudi. Both mouse and human platelets bind malarial-infected red cells and kill the parasite within. These results indicate a protective function for platelets in the early stages of erythrocytic infection distinct from their role in cerebral malaria.


Toxoplasmosis screening and risk factors amongst pregnant females in Natal, northeastern Brazil.

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Toxoplasmosis results in systemic disease, and if the mother is infected for the first time during gestation, the fetus may suffer substantial damage. Relatively little is known about the epidemiology of toxoplasmosis in pregnancy in most states of northeastern Brazil. Seroprevalence of toxoplasmosis among pregnant women was studied in Natal, capital of Rio Grande do Norte State, in northeastern Brazil, from March to December 2007. The sera were tested for IgM and avidity of IgG antibodies to Toxoplasma by a microparticle enzyme immunoassay. The overall seroprevalence was high [126/190 (66.3%)]; prevalence increased with age indicating that in this setting most infections occur in adulthood (third decade of life). Only one pregnant woman was IgM positive and had high-avidity antibodies. The high percentage of pregnant women who are vulnerable to this parasite (33.1%) favors primary infection during pregnancy. Our studies show that direct contact with cats or dogs was highly associated with toxoplasmosis (odds ratio 2.72, P<0.001, 95% CI 1.46-5.02). The number of years in school (P<0.001), precarious socioeconomic status and limited knowledge about the disease (P</=0.05 for both) were also associated with toxoplasmosis infection. The pattern of risk factors for infection corroborate other studies in Brazil.


Agent-based modelling of mosquito foraging behaviour for malaria control.

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Traditional environmental management programmes require extensive coverage of larval habitats to reduce drastically the emergence of adult mosquitoes. Recent studies have highlighted the impact of reduced availability of aquatic habitats on mosquito foraging for hosts and oviposition sites. In this study, we developed an agent-based model to track the status and movement of mosquitoes individually. Mosquito foraging was represented as a two-stage process: random flight when the resource was not within the mosquito's perception range and directional flight to the resource when it was detected. Three scenarios of targeted source reduction were devised to eliminate all aquatic habitats within certain distances of human habitations. For comparison, three non-targeted source reductions randomly eliminated the same numbers of aquatic habitats as their corresponding targeted
scenarios. Our results show that the elimination of habitats within 100m, 200m and 300m of surrounding houses resulted in 13%, 91% and 94% reductions in malaria incidence, respectively; compared with -3%, 19% and 44%, respectively, for the corresponding conventional interventions. These findings indicate that source reduction might not require coverage of extensive areas, as previously thought, and that the distance to human habitations can be used for habitat targeting.

*Trop Med Int Health. 2009 Feb 17.*

**Elimination of lymphatic filariasis in the Republic of Korea: an epidemiological survey of formerly endemic areas, 2002-2006.**

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Summary Objectives To determine the current status of lymphatic filariasis (LF) in Korea. Methods Epidemiological surveys between 2002 and 2006 in areas where LF was previously endemic: remote and coastal areas Jeollanam-do, Gyeongsangnam-do, and Jeju-do, and inland Gyeongsangbuk-do. We took night blood smears from 9426 people for microfilaria testing and assayed samples from 3049 children (10- to 13-year-olds) and 1526 adults for Brugia malayi antibodies. Results We found two cases (0.01%) with low microfilaria density in their peripheral blood (1-2/20 mul) on the remote island of Jeollanam-do in the southern part of the Korean peninsula. These patients, males over 60-years old, were treated with diethylcarbamazine (DEC). None of the 4575 people surveyed tested positive for specific B. malayi antibodies. Conclusion Lymphatic filariasis appears to have been eliminated in Korea.

*Trop Med Int Health. 2009 Feb 17.*

**Wash resistance and efficacy of three long-lasting insecticidal nets assessed from bioassays on Anopheles culicifacies and Anopheles stephensi.**

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Summary Objective To test the wash resistance and efficacy of long-lasting insecticidal nets (LLINs), namely Olyset((R)) Net and PermaNet((R)) 2.0; and a long-lasting treatment kit, K-O Tab((R))1-2-3, on Anopheles culicifacies and An. stephensi, major malaria vectors in India, by bioassays. Conventionally treated deltamethrin net (CTDN with K-O Tab) was used for comparison. Method Mortality and median time for knockdown (MTKD) of mosquitoes were determined using contact bioassays and ball frame bioassays respectively. Hand washing and machine washing were used. Results LLINs showed good bio-efficacy against An. culicifacies and An. stephensi. The mortality of mosquitoes remained >80% after up to 20 hand washes and up to 15 machine washes on all LLINs tested. No significant differences were observed in mortalities between the An. culicifacies and An. stephensi in cone bioassays (P > 0.05). MTKD increased progressively with successive washes and there was a significant difference in median time for knockdown of test mosquitoes and between hand-washed and machine-washed nets (P < 0.05). Conclusion LLINs are more efficacious and last longer when washed by hand than by machine.
Rapid malaria diagnostic tests vs. clinical management of malaria in rural Burkina Faso: safety and effect on clinical decisions. A randomized trial.


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Summary Objectives To assess if the clinical outcome of patients treated after performing a Rapid Diagnostic Test for malaria (RDT) is at least equivalent to that of controls (treated presumptively without test) and to determine the impact of the introduction of a malaria RDT on clinical decisions. Methods Randomized, multi-centre, open clinical trial in two arms in 2006 at the end of the dry and of the rainy season in 10 peripheral health centres in Burkina Faso: one arm with use of RDT before treatment decision, one arm managed clinically. Primary endpoint: persistence of fever at day 4. Secondary endpoints: frequency of malaria treatment and of antibiotic treatment. Results A total of 852 febrile patients were recruited in the dry season and 1317 febrile patients in the rainy season, and randomized either to be submitted to RDT (P_RTD) or to be managed presumptively (P_CLIN). In both seasons, no significant difference was found between the two randomized groups in the frequency of antimalarial treatment, nor of antibiotic prescription. In the dry season, 80.8% and 79.8% of patients with a negative RDT were nevertheless diagnosed and treated for malaria, and so were 85.0% and 82.6% negative patients in the rainy season. In the rainy season only, both diagnosis and treatment of other conditions were significantly less frequent in RDT positive vs. negative patients (48.3%vs. 61.4% and 46.2%vs. 59.9%, P = 0.00 and 0.00, respectively). Conclusion Our study was inconclusive on RDT safety (clinical outcome in the two randomized groups), because of an exceedingly and unexpectedly low compliance with the negative test result. Further research is needed on best strategies to promote adherence and on the safety of a test based strategy compared with the current, presumptive treatment strategy.

Evolutionary lability of odour-mediated host preference by the malaria vector Anopheles gambiae.

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Summary Many species of disease-vector mosquitoes display vertebrate host specificity. Despite considerable progress in recent years in understanding the proximate and ultimate factors related to non-random host selection at the interspecific level, the basis of this selection remains only partially understood. Anopheles gambiae sensu stricto, the main malaria vector in Africa, is considered a highly anthropophilic mosquito, and host odours have been shown to play a major role in the host-seeking process of this species. Studies on host preference of An. gambiae have been either conducted in controlled conditions using laboratory reared mosquitoes and worn stockings as host-related stimuli, or have been done in the field with methods that do not account for internal (e.g. age of sampled mosquitoes) and/or environmental effects. We explored differential behavioural responses to host odours between two populations of the same sibling.
species, An. gambiae in semi-field conditions in Burkina Faso. The behavioural responses (i.e. degree of activation and strength of anemotaxis) were investigated using a Y-olfactometer designed to accommodate whole hosts as a source of odour stimuli. Two strains of An. gambiae (3 to 4-day-old female) from laboratory Kisumu strain, and from field-collected individuals were confronted to combinations of stimuli comprising calf odour, human odour and outdoor air. In dual-choice tests, field mosquitoes chose human odour over calf odour, outdoor air over calf odour and responded equally to human and outdoor air, while laboratory mosquitoes responded equally to human and calf odour, human odour over outdoor air and calf odour over outdoor air. Overall, no effect of CO(2) exhaled by humans and calves neither on the proportion of activated mosquitoes nor on the relative attractiveness to odour stimuli was found. We report for the first time an intraspecific variation in host-odour responses. This study clearly suggests that there may be genetic polymorphism underlying host preference and emphasizes that the highly anthropophilic label given to An. gambiae s.s. must be carefully interpreted and refer to populations rather than the whole sibling species.


**In-vivo efficacy of amodiaquine-артесунате in children with uncomplicated Plasmodium falciparum malaria in western Kenya.**


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Summary Objectives To assess the efficacy of amodiaquine-артесунате in an area with high chloroquine resistance in western Kenya. Methods Twenty-eight day in-vivo efficacy trial of amodiaquine-артесунате in 103 children aged 6-59 months in western Kenya with smear-confirmed uncomplicated Plasmodium falciparum malaria. Results The 28-day uncorrected adequate clinical and parasitological response (ACPR) was 69.0%, with 15.5% Late Clinical Failure and 15.5% Late Parasitologic Failure rates. The PCR-corrected 28-day ACPR was 90.2%. Clinical risk factors for recurrent infection (recrudescences and reinfections) were lower axillary temperature at enrolment and low weight-for-age Z-score. The presence of single nucleotide polymorphisms pfcr7 76T and pfmdr1 86Y at baseline was associated with increased risk of recurrent infections, both reinfections and recrudescences. Conclusion Although artemether-lumefantrine (Coartem(R)) is the first line ACT in Kenya, amodiaquine-артесунате is registered as an option for treatment of uncomplicated P. falciparum and remains an effective alternative to Coartem((R)) in western Kenya. Continued amodiaquine monotherapy in the private sector may jeopardise the future use of amodiaquine-артесунате as an alternative artemisinin-based combination therapy.


**Cost-effectiveness of artemunate for the treatment of severe malaria.**

Lubell Y, Yeung S, Dondorp AM, Day NP, Nosten F, Tjitra E, Abul Faiz M, Bin Yunus E, Anstey NM, Mishra SK, Mohanty S, White NJ, Mills AJ.

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Summary Objective To explore the cost-effectiveness of artemunate against quinine based principally on the findings of a large multi-centre trial carried out in
Southeast Asia. Methods Trial data were used to compare mortality of patients with severe malaria, treated with either artesunate or quinine. This was combined with retrospectively collected cost data to estimate the incremental cost per death averted with the use of artesunate instead of quinine. Results The incremental cost per death averted using artesunate was approximately 140 USD. Artesunate maintained this high level of cost-effectiveness also when allowing for the uncertainty surrounding the cost and effectiveness assessments. Conclusion This analysis confirms the vast superiority of artesunate for treatment of severe malaria from an economic as well as a clinical perspective.


**Performance of OptiMAL-IT(R) compared to microscopy, for malaria detection in Burkina Faso.**

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Summary Objective To compare the performance of OptiMAL-IT((R)), a rapid diagnostic test for malaria, with that of microscopy in Burkina Faso. Method Finger-prick blood samples of 464 children attending hospital for suspected malaria were tested for malaria by microscopy and OptiMAL-IT((R)). Results The sensitivity and specificity of OptiMAL-IT((R)) were 98.7% (CI 95% = 97.6-99.8) and 96.2% (CI 95% = 94.3-98.1) respectively, with a high positive likelihood ratio (25.97). Conclusion OptiMAL-IT((R)) can be considered a good method to diagnose malaria in Burkina Faso, particularly in remote areas with little or no access to microscopy services.


**Intranasal administration of the synthetic polypeptide from the C-terminus of the circumsporozoite protein of Plasmodium berghei with the modified heat-labile toxin of Escherichia coli (LTK63) induces a complete protection against malaria challenge.**


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Needle-free procedures are very attractive ways to deliver vaccines because they diminish the risk of contamination and may reduce local reactions, pain or pain fear especially in young children with a consequence of increasing the vaccination coverage for the whole population. For this purpose, the possible development of a mucosal malaria vaccine was investigated. Intranasal immunization was performed in BALB/c mice using a well-studied Plasmodium berghei model antigen derived from the circumsporozoite protein with the modified heat-labile toxin of Escherichia coli (LTK63), which is devoid of any enzymatic activity compared to the wild type form. Here, we show that intranasal administration of the two compounds activates the T and B cell immune response locally and systemically. In addition, a total protection of mice is obtained upon a challenge with live sporozoites.