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Abstracts


Mothers knowledge on the cause, prevention and symptoms of malaria in a university staff clinic in an urban setting in Southwestern Nigeria.

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This prospective study was carried out at Jaja Clinic, University of Ibadan, Nigeria. The study documented the knowledge of mothers of children about the cause, prevention and symptoms of malaria. These were mothers of children between ages 1 month to 12 years that presented with fever at the clinic for the first time during the current illness. Data was collected with structured questionnaires administered to the mothers of the enrolled children. The children were clinically examined by clinicians and blood films for malaria parasites were taken and examined in the laboratory using Giemsa stain. The haematocrit level of each child was also determined. 60.4% of the children were 1 month–5 years (mean age 33.0 +/- 15.2 months) while 39.6% of them were over 5–12 years (mean 8.1 +/- 2.1 years). Most of the mothers (58.3%) had above secondary school education. Blood films for malaria parasites were positive in 76% of the children that presented with fever. 74.2% of the mothers knew mosquito bite as the cause of malaria while 13.2% of them were ignorant of the cause of malaria. The main protective measures practiced by the mothers against mosquito bites were netted windows (86.2%), use of aerosol insecticides (76.1%), and mosquito coil (17.0%). Most mothers were not knowledgeable about the use of insecticide treated nets (ITN) which is the most recently introduced protective measure against mosquito bite. Ninety percent of the mothers knew fever as the major symptom of malaria. The degree of parasitaemia affected the PCV level. The greater the parasite count, the lower the PCV level.


Effects of trimethoprim-sulfamethoxazole and insecticide-treated bednets on malaria among HIV-infected Ugandan children.


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BACKGROUND:: Trimethoprim-sulfamethoxazole (TMP/SMX) prophylaxis and insecticide-treated bednets reduce malaria risk among HIV-infected adults. The efficacy of TMP/SMX may be diminished where antifolate resistance to malaria is high. We evaluated the efficacy of these interventions for malaria prevention among Ugandan children. METHODS:: We concurrently followed 300 HIV-infected children aged 1-10 years and a community-based cohort of 561 healthy children aged 1-11 years over 11 months in Kampala, Uganda. The HIV-infected children received TMP/SMX prophylaxis and insecticide treated bednets. In the community cohort, insecticide-treated bednets were introduced during the observation period. Children from both cohorts were followed using a standardized protocol to measure the incidence of malaria. RESULTS:: Only nine episodes of malaria were diagnosed among HIV-infected children (incidence = 0.07/person-year) in comparison with 440 episodes among children from the community (incidence = 0.90/person-year; P < 0.0001). The use of insecticide-treated bednets was associated with a 43% reduction in malaria incidence (P < 0.001), and a combination of TMP/SMX and use of insecticide-treated bednets with a 97%
reduction in malaria incidence ($P < 0.001$). The prevalence of five mutations associated with antifolate resistance was high among malaria cases detected in both the HIV (100%) and community cohorts (75%). Malaria accounted for only 4% of febrile episodes in the HIV cohort in comparison with 33% in the community-based cohort ($P < 0.0001$). CONCLUSION: In a malaria endemic area with a high level of molecular markers of antifolate resistance, the combined use of TMP/SMX prophylaxis and insecticide-treated bednets was associated with a dramatic reduction in malaria incidence among HIV-infected children.


**Self-reported adverse events associated with antimalarial chemoprophylaxis in Peace Corps Volunteers.**

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OBJECTIVES: To determine adverse events associated with the use of antimalarial chemoprophylaxis in Peace Corps volunteers who have served in malaria-endemic countries 6 months or longer. METHODS: Survey of Peace Corps volunteers' adverse events associated with the use of antimalarial chemoprophylaxis between August 1, 2005 and July 31, 2006. Analyses were conducted in fall 2006. RESULTS: Two thousand seven hundred and one surveys were received (43% response rate) from volunteers in malaria-endemic countries, with 1,731 (64%) in country for 6 months or longer (average 19 months). Nearly two thirds (62%) reported at least one adverse event. Those on mefloquine reported the most neuropsychologic adverse events; those on doxycycline reported the most gastrointestinal as well as skin and vaginal adverse events. Nearly one quarter of respondents (23%) changed their chemoprophylaxis regimen due to adverse events. Severe adverse events were reported by 9% of respondents, and 1% reported being hospitalized. CONCLUSIONS: Adverse events were frequently reported among volunteers using antimalarial medications for more than 6 months, but very few were severe or required hospitalization. In light of the large number of adverse events, having multiple drug regimens available for the long-term traveler may be one method of maintaining adherence to antimalarial chemoprophylaxis.


**A New Robust Diagnostic Polymerase Chain Reaction for Determining the Mating Status of Female Anopheles gambiae Mosquitoes.**

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The principal malaria vector in Africa, Anopheles gambiae, contains two pairs of autosomes and one pair of sex chromosomes. The Y chromosome is only associated with males and other Y chromosome-specific DNA sequences, which are transferred to women during mating. A reliable tool to determine the mating status of dried wild An. gambiae females is currently lacking. DNA was extracted from dried virgin and mated females and used to test whether Y chromosome-specific polymerase chain reaction (PCR) markers can be successfully amplified and used as a predictor of mating. Here we report a new PCR-based method to determine the mating status among successfully inseminated and virgin wild An. gambiae females, using three male-specific primers. This dissection-free method has the potential to facilitate studies of both population demographics and gene flow from dried mosquito samples routinely collected in epidemiologic monitoring and aid existing and new malaria-vector control approaches.
Prevalence and Risk of \textit{Plasmodium falciparum} and \textit{P. vivax} Malaria among Pregnant Women Living in the Hypoendemic Communities of the Peruvian Amazon.

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The Amazon region of Iquitos, Peru is hypoendemic for \textit{Plasmodium vivax} and \textit{P. falciparum}. There is limited information regarding the epidemiology of malaria during pregnancy in this region. Passive surveillance for clinical malaria among pregnant women was conducted in eight health posts in 2004 and 2005. Community-based active surveillance was conducted to determine the incidence of malarial infection among pregnant women in the community of Zungarococha in 2004 and 2005. Passive surveillance demonstrated that pregnant women had a prevalence of clinical malaria of 7.5\% in 2004 and 6.6\% in 2005 compared with 20.6\% and 22.4\% of the total population. Active surveillance showed that pregnant women were 2.3 (95\% confidence interval = 1.32-3.95, \(P = 0.004\)) times more likely to have a \textit{P. falciparum} infection compared with non-pregnant women. This study demonstrated that because of detection bias, passive surveillance underestimates the burden of malarial infection during pregnancy, and that subclinical malarial infections may occur frequently among pregnant women in this region. Furthermore, pregnant women in this low-transmission and \textit{P. vivax}-dominant setting, experience an increased risk for \textit{P. falciparum} infection, but not \textit{P. vivax} infection.

Electrocardiographic Safety Evaluation of Dihydroartemisinin Piperaquine in the Treatment of Uncomplicated falciparum Malaria.

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Dihydroartemisinin-piperaquine (DP) could become a leading fixed combination malaria treatment worldwide. Although there is accumulating evidence of efficacy and safety from clinical trials, data on cardiotoxicity are limited. In two randomized controlled trials in Thailand, 56 patients had ECGs performed before treatment, 4 hours after the first dose, and 4 hours after the last dose. The mean (95\% CI) changes in QTc interval (Bazett's correction) were 2 (-6 to 9) ms and 14 (7 to 21) ms, respectively. These small changes on the third day of treatment are similar to those observed elsewhere in the convalescent phase following antimalarial treatment with drugs known to have no cardiac effects and are therefore likely to result from recovery from acute malaria and not the treatment given. At therapeutic doses, DP does not have clinically significant effects on the electrocardiogram.
Polymerase Chain Reaction Detection of Plasmodium vivax and Plasmodium falciparum DNA from Stored Serum Samples: Implications for Retrospective Diagnosis of Malaria.


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Polymerase chain reaction (PCR) detection of Plasmodium DNA is highly sensitive in diagnosing malaria. The specimen of choice for this assay has been whole blood samples from malaria patients. To retrospectively determine malaria infection rates in populations or cohorts for whom stored serum samples are available, we determined the ability of a nested PCR assay to detect Plasmodium DNA in stored serum samples. The PCR result was positive in 20 of 23 serum samples from patients with microscopy-confirmed malaria and negative in 8 of 8 healthy controls, resulting in a sensitivity of 87% and specificity of 100%. In all positive samples, species were correctly identified by PCR except for one case where a mixed infection was detected. The PCR is able to detect Plasmodium DNA in serum samples frozen up to 2.5 years and has the potential for the retrospective identification of malaria parasitemia in patient cohorts to determine potential interactions of malaria and other diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome.

Selection of Antifolate-Resistant Plasmodium falciparum by Sulfadoxine-Pyrimethamine Treatment and Infectivity to Anopheles Mosquitoes.

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Resistance-conferring mutations in dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) in Plasmodium falciparum are selected by treatment with sulfadoxine-pyrimethamine (SP). We assessed the association between these mutations and transmission capacity of parasites to Anopheles mosquitoes on the Pacific coast of Colombia. Patients with uncomplicated P. falciparum malaria received SP treatment and were followed-up to compare the prevalence of DHFR and DHPS mutations before and after SP treatment. Membrane feeding assays were used to measure infectivity to mosquitoes of post-treatment gametocytes with and without these mutations. Per-protocol treatment efficacy was 95.0% (132 of 139). Gametocytes carrying resistance-conferring mutations were selected after SP treatment and were infective to mosquitoes. Seven days after treatment, infections with two DHFR mutations had a 10-fold higher probability of infecting mosquitoes than infections with no DHFR mutations (odds ratio = 10.23, P < 0.05). Low-level drug resistance mutations have the potential to enhance transmission of P. falciparum and spread resistant parasites.


A total of 248 Plasmodium falciparum isolates were sampled in travelers with malaria who came to Marseille, France from Comoros to investigate in vitro activities of antimalarial drugs and molecular markers of drug resistance. Of the 248 isolates, 126 were maintained in culture. Of these, 53% were resistant to chloroquine, and 3% had reduced susceptibility to quinine, mefloquine, and atovaquone; 1% had reduced susceptibility to halofantrine and dihydroartemisinin; 7% had reduced susceptibility to monodesethylamodiaquine; 37% had reduced susceptibility to cycloguanil; and none had reduced susceptibility to lumefantrine. Resistance-associated point mutations were screened in 207 isolates. No mutations in the cytochrome b gene were found. Of the 207 isolates, 119 (58%) had a mutation in the P. falciparum dihydrofolate reductase (Pfdhfr) gene at codon 108, 6 (5%) had mutations in both Pfdhfr codon 108 and the P. falciparum dihydropteroate synthase codon 437, and 115 (56%) had the chloroquine resistance-associated K76T mutation in the P. falciparum chloroquine resistance transporter gene. This study represents a unique opportunity to improve surveillance of P. falciparum drug resistance in Comoros with consequences for treatment and chemoprophylaxis guidelines.

Comparison of Artemether-Lumefantrine with Sulfadoxine-Pyrimethamine for the Treatment of Uncomplicated Falciparum Malaria in Eastern Nepal.

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Because available data suggest that resistance of Plasmodium falciparum to sulfadoxine-pyrimethamine (SP) is increasing in Nepal, an open-label, parallel-group efficacy/safety study was conducted in 99 Nepalese patients with uncomplicated falciparum malaria randomized 2:1 to artemetherlumefantrine (AL) or SP. Efficacy was assessed from clinical and microscopic evidence of treatment failure. Four SP-treated patients (12.1%; 95% CI, 4.0-29.1%) redeveloped parasitemia during the 28-day follow-up versus 0% (95% CI, 0-6.9%) in the AL group (P = 0.011), a difference that was confirmed by polymerase chain reaction (PCR) analysis of parasite DNA. PCR detected an additional six patients (two SP and four AL) with sub-microscopic gametocytemia or breakthrough parasitemia between Days 14 and 28, suggesting that AL efficacy was lower than estimated by microscopy. Dhfr and dhps mutations were not associated with outcome. AL is more effective than SP for uncomplicated malaria in Nepal, but regular monitoring of its efficacy should be carried out if this combination therapy is introduced.

Malaria in Pregnancy Before and After the Implementation of a National IPTp Program in Gabon.


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Environmental Health at USAID – Malaria Bulletin, October 2007
Intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine has recently been adopted by many African countries to reduce maternal and neonatal morbidity and mortality associated with malaria in pregnancy. We assessed the impact of a newly established national IPTp program on maternal and neonatal health in Gabon. Data on prevalence of maternal Plasmodium falciparum infection, anemia, premature birth, and birth weight were collected in cross-sectional surveys in urban and rural regions of Gabon before and after the implementation of IPTp in a total of 1403 women and their offspring. After introduction of IPTp, the prevalence of maternal Plasmodium falciparum infection decreased dramatically (risk ratio 0.16, P < 0.001). Whereas only a modest effect on the rate of anemia in pregnant women was observed, there was a marked benefit on the prevalence of low birth weight and premature birth for women adhering to national recommendations. These effects were most pronounced in primi- and secundigravid women.


Impact of intermittent preventive anti-malarial treatment on the growth and nutritional status of preschool children in rural Senegal (West Africa).


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Negative consequences of malaria might account for seasonality in nutritional status in children in the Sahel. We report the impact of a randomized, double-blind, placebo-controlled trial of seasonal intermittent preventive anti-malarial treatment on growth and nutritional status in 1,063 Senegalese preschool children. A combination of artesunate and sulfadoxine-pyrimethamine was given monthly from September to November. In the intervention arm, mean weight gain was significantly greater (122.9 +/- 340 versus 42.9 +/- 344 [SD] g/mo, P < 0.0001) and losses in triceps and subscapular skinfold measurements were less (-0.39 +/- 1.01 versus -0.66 +/- 1.01 mm/mo, and -0.15 +/- 0.64 versus -0.36 +/- 0.62 mm/mo, respectively, P < 0.0001 for both). There was no difference in height increments. The prevalence of wasting increased significantly in the control arm (4.6% before versus 9.5% after, P < 0.0001), but remained constant in intervention children: 5.6% versus 7.0% (P = 0.62). The prevention of malaria would improve child nutritional status in areas with seasonal transmission.


Personal-protection measures against mosquitoes: a study of practices and costs in a district, in the Indian state of Orissa, where malaria and lymphatic filariasis are co-endemic.

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In a study undertaken among rural and urban communities in a district of Orissa,
India, the personal-protection measures used against mosquitoes, and the household costs of these measures, were investigated. Most people living in the study communities perceived mosquitoes as a problem, both as a biting nuisance and as vectors of human disease. Almost all (99%) of the urban households investigated and most (84%) of the rural each reported the use of at least one measure against mosquitoes. Most of the study households (92% of the urban and 64% of the rural) used a 'modern' chemical method (coils, vaporizing mats, liquid vaporizers or sprays), with mosquito coils used more frequently than any other personal-protection measure. Untreated bednets were also used by most of the households investigated (76% of the urban and 58% of the rural) and some households (about 10% of the urban and 8% of the rural) still used the more traditional method of burning dried dung or vegetation indoors, specifically to create smoke to drive away mosquitoes. Setting, house type, as indicated by the material used as roofing, and number of people in the household were each a significant predictor of the use of personal protection, with households in an urban setting, large households, and households occupying a concrete-roofed building relatively more likely to use some form of personal protection. Although 'modern', chemical-based methods were frequently employed, about one in every two interviewees (57% of the urban and 43% of the rural) considered the use of such methods to be harmful to their health. The mean monthly expenditures on personal-protection measures were 101 Indian rupees (U.S.$2.20)/urban household and 72 Indian rupees (U.S.$1.60)/rural household. Setting, family income, family size and number of sleeping rooms in the house each affected such expenditure significantly. As a proportion of household income, expenditure on controlling mosquitoes was surprisingly high.

14: Antimicrob Agents Chemother. 2007 Sep 10


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A series of novel 10-N-substituted acridones, bearing alkyl side chains with tertiary amine groups at the terminal position, were designed, synthesized, and evaluated for their ability to enhance the potency of quinoline drugs against multi-drug resistant (MDR) Plasmodium falciparum malaria parasites. A number of acridone derivatives, with side chains bridged three or more carbon atoms apart between the ring nitrogen and terminal nitrogen, demonstrate chloroquine (CQ) chemosensitizing activity against the MDR strain of P. falciparum (Dd2). Isobologram analysis revealed that selected candidates demonstrate significant synergy with CQ in chloroquine-resistant (CQR) parasite Dd2, but only additive (or indifferent) interaction in the chloroquine-sensitive (CQS) D6. These acridone derivatives also enhance the sensitivity of other quinoline antimalarials such as desethylchloroquine (DCQ) and quinine (QN) in Dd2. The pattern of chemosensitizing effects of selected acridone on CQ and QN are similar to those of verapamil against various parasite lines with mutations encoding amino acid 76 of the Plasmodium falciparum chloroquine resistance transporter (PfCRT). Unlike other known chemosensitizers with recognized psychotropic effects (e.g., desipramine, imipramine, and chlorpheniramine), these novel acridone derivatives exhibit no demonstrable effect on the uptake or binding of important biogenic amine neurotransmitters. The combined results indicate that 10-N-substituted acridones present a novel pharmacophore for the development of chemosensitizers against P. falciparum.
Clinical and pharmacological determinants of the therapeutic response to dihydroartemisinin piperaquine for drug resistant malaria.


Dihydroartemisinin-piperaquine (DHP) is an important new treatment for drug-resistant malaria, although pharmacokinetic studies on the combination are limited. In Papua, Indonesia we assessed determinants of the therapeutic efficacy of DHP for uncomplicated malaria. Plasma piperaquine levels were assessed on day 7 and day 28 and the cumulative risk of parasitological failure calculated at day 42 using survival analysis. Of the 598 patients in the evaluable population 342 had infections with P. falciparum, 83 with P. vivax and 173 with a mixture of both species. The unadjusted cumulative risk of recurrence was 7.0% [95%CI: 4.6-9.4%] for P. falciparum and 8.9% [95%CI: 6.0-12] for P. vivax. After correcting for reinfections the risk of recrudescence with P. falciparum was 1.1% [95%CI: 0.1-2.1]. The major determinant of parasitological failure was the plasma piperaquine concentration. A concentration below 30 ng/ml on day 7 was observed in 38% (21/56) of children less than 15 years and 22% (31/140) of adults (p=0.04), even though the overall mg per kg dose in children was 9% higher than that in adults (p<0.001). Patients with piperaquine levels below 30 ng/ml were more likely to have a recurrence with P. falciparum (Adjusted Hazard Ratio= 6.6 [95%CI: 1.9-23]; p=0.003) or P. vivax (AHR=9.0 [95%CI: 2.3-35] for P. vivax; p=0.001). The plasma concentration of piperaquine on day 7 was the major determinant of the therapeutic response to DHP. Lower plasma piperaquine concentrations and higher failure rates in children suggest that an increase in the dose maybe warranted in this age group.

Challenges in the prevention, diagnosis, and treatment of malaria in human immunodeficiency virus infected adults in sub-saharan Africa.

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BACKGROUND: Many countries in sub-Saharan Africa currently report high prevalences of both human immunodeficiency virus (HIV) and Plasmodium falciparum malaria. The likelihood of HIV-malaria coinfection may affect clinical management of patients. The extent to which standard clinical guidelines address HIV-malaria coinfection is unclear. METHODS: We reviewed standard World Health Organization and other guidelines for diagnosis and treatment of malaria and/or HIV-related illness. We also searched PubMed (1990 to present) for literature on HIV-malaria interactions and treatment of coinfection. We restricted our review to the situation of the nonpregnant HIV-infected adult. RESULTS: We found only 6 articles describing the clinical presentation of HIV-malaria coinfection in adults. We also identified 10 clinical or laboratory syndromes that are shared by malaria and AIDS-related conditions and that might provoke diagnostic confusion. We identified 12 antimalarial medications whose coadministration with antiretrovirals is known or suspected to result in drug-drug interactions or overlapping toxicities. CONCLUSIONS: Substantial overlap in the clinical and laboratory characteristics of malaria and HIV-related syndromes generates potential difficulties in AIDS staging and in diagnosis and management of patients at risk for coinfection. Significant drug-drug interactions and overlapping drug toxicity profiles further complicate concurrent management of malaria and HIV. Standard clinical guidelines do not reflect the full complexity
of the interactions and overlaps between the 2 infections. Clinicians who manage HIV-infected patients in malaria-affected regions should systematically consider malaria when evaluating patients with a broad spectrum of symptoms. Further research is urgently needed to define best practices for prevention, diagnosis, and management of HIV-malaria coinfection in this region.

17: Biochem J. 2007 Sep 17

Kinetic and biochemical characterization of Plasmodium falciparum guanosine 5'-monophosphate synthetase.

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Plasmodium falciparum the causative agent of the fatal form of malaria synthesizes GMP primarily from IMP and hence, needs active GMP synthetase (GMPS) for its survival. GMPS, a G-type amidotransferase catalyzes the amination of XMP to GMP with the reaction occurring in two domains, the glutamine amidotransferase (GAT) and ATP pyrophosphatase (ATPPase). The GAT domain hydrolyzes glutamine to glutamate and ammonia while the ATPPase domain catalyzes the formation of the intermediate AMP-XMP from ATP and XMP. Coordination of activity across the two domains, achieved through channeling of ammonia from GAT to the effector domain is the hallmark of amidotransferases. Our studies aimed at understanding the kinetic mechanism of Plasmodium falciparum GMPS (PfGMPS) indicated steady state ordered binding of ATP followed by XMP to the ATPPase domain with glutamine binding in a random manner to the GAT domain. We attribute the irreversible, ping-pong step seen in initial velocity kinetics to the release of glutamate prior to the attack of adenyl-XMP intermediate by ammonia. Specific aspects of the overall kinetic mechanism of PfGMPS are different from that reported for the human and E. coli enzymes. Unlike human GMPS, absence of tight coordination of activity across the two domains was evident in the parasite enzyme. Variations seen in the inhibition by nucleosides and nucleotide analogs between human and P. falciparum GMPS highlighted differences in ligand specificity that could serve as a basis for the design of specific inhibitors. This study forms the first report on recombinant His-tagged GMPS from parasitic protozoa.

18: Bioorg Med Chem Lett. 2007 Sep 15

Synthesis and evaluation of naphthyridine compounds as antimalarial agents.

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Primaquine is the drug of choice for the radical cure of Plasmodium vivax malaria, but possesses serious side effects. In this study novel primaquine analogues were designed and synthesized. Lower toxicity was achieved by reducing or eliminating the tendency of forming chemically reactive and toxic intermediates and metabolites. In vitro and in vivo studies found that synthesized compounds were less toxic than the parent compound primaquine, while preserving the desired antimalarial activity. Some of these compounds possess a therapeutical index over 10 times superior to that of the commonly used antimalarial drug chloroquine. These compounds, as well as the underlying design rationale, may find usefulness in the discovery and development of new antimalarial drugs.
Yield enhancement strategies for artemisinin production by suspension cultures of Artemisia annua.

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Artemisinin, isolated from the shrub-Artemisia annua, is a sesquiterpene lactone used to treat multi-drug resistant strains of falciparum malaria. It is also effective against a wide variety of cancers such as leukemia and colon cancer. To counter the present low content in leaves and uneconomical chemical synthesis, alternate ways to produce artemisinin have been sought. But this compound remains elusive in cell cultures of A. annua despite the extensive studies undertaken. This work reports the first successful approach for production of artemisinin by cell cultures of Indian variety of A. annua. In the present study, an integrated yield enhancement strategy, developed by addition of selected precursor (mevalonic acid lactone) and elicitor (methyl jasmonate) at optimized concentrations, resulted in 15.2g/l biomass and 110.2mg/l artemisinin, which was 5.93 times higher in productivity in comparison to control cultures.

Population's behaviour and expectations concerning malaria control in Ouidah (Benin) [Article in French]

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The aim of this study conducted in April 2006 in Ouidah (Benin), was to measure some results indicators of the implementation of "Roll Back Malaria" in Benin. Impregnated bed nets are available in 25.6% of the households and are used by 21.2% of children under five years old and 26.7% of pregnant women. The main reasons for this use are protection against harmful effect of mosquitoes (74%) and prevention of the malaria transmission (51%). The cost of impregnated bed net purchased is in average 2115 FCFA. Malaria morbidity in household is very high (48%) among children under five years old. The percentage of feverish children correctly treated in household according to the National Malaria Control Program's protocol is 1%. The major expectations of the households are supply of impregnated bed nets free of charge (33%), reduction of its delivery price (18%), its availability in health facilities (15%) and free treatment of malaria cases (12%).

Estimated global resources needed to attain international malaria control goals.


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OBJECTIVE: To provide the international community with an estimate of the amount of financial resources needed to scale up malaria control to reach international goals, including allocations by country, year and intervention as well as an indication of the current funding gap. METHODS: A costing model was used to estimate the total costs of scaling up a set of widely recommended interventions, supporting services and programme strengthening activities in each of the 81 most heavily affected malaria-endemic countries. Two scenarios were evaluated, using different assumptions about the effect of interventions on the needs for diagnosis and treatment. Current health expenditures and funding for malaria control were compared to estimated needs. FINDINGS: A total of US$ 38 to 45...
billion will be required from 2006 to 2015. The average cost during this period is US$ 3.8 to 4.5 billion per year. The average costs for Africa are US$ 1.7 billion and US$ 2.2 billion per year in the optimistic and pessimistic scenarios, respectively; outside Africa, the corresponding costs are US$ 2.1 billion and US$ 2.4 billion. **CONCLUSION:** While these estimates should not be used as a template for country-level planning, they provide an indication of the scale and scope of resources required and can help donors to collaborate towards meeting a global benchmark and targeting funding to countries in greatest need. The analysis highlights the need for much greater resources to achieve the goals and targets for malaria control set by the international community.


[Uncomplicated Plasmodium vivax and P. falciparum malaria in Brazil: evidence on single and combined drug treatments recommended by official guidelines.] [Article in Portuguese]

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Malaria is the most important endemic parasitic disease in the world. Conditions are favorable for transmission of the disease in 60% of Brazil's territory. Over 500,000 cases per year are recorded in the country. However, the geographic distribution is uneven, which may explain differences in the efficacy and effectiveness of antimalarial drugs. We conducted an extensive literature review of antimalarial treatment in Brazil from 1980 to 2005 in order to identify evidence that might have been available for the 2001 Edition of the Malaria Treatment Manual, the official Ministry of Health guidelines. Only a few studies, of low methodological quality, were identified by the search. None of the studies would have been capable of generating evidence-based guidelines according to the current classification of levels of pharmacological and clinical evidence. Studies published after 2001 drew on more evidence and are expected to provide the basis for the next edition of the manual, due in 2007. References in the 2001 Edition were outdated, possibly perceived as traditional references in the field, but lacking in specificity for region, population, and/or type of malaria.

23: ChemMedChem. 2007 Sep 4;2(10):1480-1497

**The Fe(2+)-Mediated Decomposition, PfATP6 Binding, and Antimalarial Activities of Artemisone and Other Artemisinins: The Unlikelihood of C-Centered Radicals as Bioactive Intermediates.**

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The results of Fe(2+)-induced decomposition of the clinically used artemisinins, artemisone, other aminoartemisinins, 10-deoxoartemisinin, and the 4-fluorophenyl derivative have been compared with their antimalarial activities and their ability to inhibit the parasite SERCA PfATP6. The clinical artemisinins and artemisone decompose under aqueous conditions to give mixtures of C radical marker products, carbonyl compounds, and reduction products. The 4-fluorophenyl derivative and aminoartemisinins tend to be inert to aqueous iron(II) sulfate and anhydrous iron(II) acetate. Anhydrous iron(II) bromide enhances formation of the carbonyl compounds and provides a deoxyglycal from DHA and enamines from the aminoartemisinins. Ascorbic acid (AA) accelerates the aqueous Fe(2+)-mediated decompositions, but does not alter product distribution. 4-Oxo-TEMPO intercepts C
radicals from a mixture of an antimalaria-active trioxolane, 10-deoxoartemisinin, and anhydrous iron(II) acetate to give trapped products in 73 % yield from the trioxolane, and 3 % from the artemisinin. Artemisinone provides a trapped product in 10 % yield. Thus, in line with its structural rigidity, only the trioxolane provides a C radical eminently suited for intermolecular trapping. In contrast, the structural flexibility of the C radicals from the artemisinins allows facile extrusion of Fe(2+) and collapse to benign isomerization products. The propensity towards the formation of radical marker products and intermolecular radical trapping have no relationship with the in vitro antimalarial activities of the artemisinins and trioxolane. Desferrioxamine (DFO) attenuates inhibition of PfATP6 by, and antagonizes antimalarial activity of, the aqueous Fe(2+)-susceptible artemisinins, but has no overt effect on the aqueous Fe(2+)-inert artemisinins. It is concluded that the C radicals cannot be responsible for antimalarial activity and that the Fe(2+)-susceptible artemisinins may be competitively decomposed in aqueous extra- and intracellular compartments by labile Fe(2+), resulting in some attenuation of their antimalarial activities. Interpretations of the roles of DFO and AA in modulating antimalarial activities of the artemisinins, and a comparison with antimalarial properties of simple hydroperoxides and their behavior towards thapsigargin-sensitive SERCA ATPases are presented. The general basis for the exceptional antimalarial activities of artemisinins in relation to the intrinsic activity of the peroxide within the uniquely stressed environment of the malaria parasite is thereby adumbrated.

24: ChemMedChem. 2007 Sep 4;2(10):1464-1479


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As the clinically used artemisinins do not withstand the thermal stress testing required to evaluate shelf life for storage in tropical countries where malaria is prevalent, there is a need to develop thermally more robust artemisinin derivatives. Herein we describe the attachment of electron-withdrawing arene- and alkanesulfonyl and -carbonyl groups to the nitrogen atom of the readily accessible Ziffer 11-azaartemisinin to provide the corresponding N-sulfonyl- and -carbonylazaartemisinins. Two acylurea analogues were also prepared by treatment of the 11-azaartemisinin with arylisocyanates. Several of the N-sulfonylazaartemisinins have melting points above 200 degrees C and possess substantially greater thermal stabilities than the artemisinins in current clinical use, with the antimalarial activities of several of the arylsulfonyl derivatives being similar to that of artesunate against the drug-sensitive 3D7 clone of the NF54 isolate and the multidrug-resistant K1 strain of P. falciparum. The compounds possess relatively low cytotoxicities. The carbonyl derivatives are less crystalline than the N-sulfonyl derivatives, but are generally more active as antimalarials. The N-nitroarylcarbonyl and arylurea derivatives possess sub-ng ml(-1) activities. Although several of the azaartemisinins possess log P values below 3.5, the compounds have poor aqueous solubility (<1 mg L(-1) at pH 7). The greatly enhanced thermal stability of our artemisinins suggests that strategic incorporation of electron-withdrawing polar groups into both new artemisinin derivatives and totally synthetic trioxanes or trioxolanes may assist in the generation of practical new antimalarial drugs which will be stable to storage conditions in the field, while retaining favorable physicochemical properties.
Malaria pharmacovigilance in Africa: lessons from a pilot project in Mpumalanga province, South Africa.

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BACKGROUND AND OBJECTIVES: Prior to the introduction of artemisinin-based combination antimalarial therapy in Mpumalanga province, South Africa, a pharmacovigilance strategy was developed to pilot locally relevant surveillance methods for detecting serious adverse drug reactions (ADRs) and signals related to artesunate plus sulfadoxine/pyrimethamine. STUDY DESIGN: From 1 March 2002 to 30 June 2004, five methods for detecting ADRs in patients receiving antimalarials were piloted in the rural communities of Mpumalanga province in South Africa: (i) home follow-up of patients by malaria control staff; (ii) enhanced spontaneous reporting of suspected ADRs by health professionals at clinics and hospitals; (iii) active hospital surveillance for malaria-related admissions and patients recently treated for malaria; (iv) a confidential enquiry into malaria-related deaths; and (v) adverse events monitoring during two therapeutic efficacy studies conducted in 2002 and 2004. RESULTS: During the study period, the malaria control programme was notified of 4778 cases of malaria while sulfadoxine/pyrimethamine monotherapy was the recommended treatment and 7692 cases after the introduction of artesunate plus sulfadoxine/pyrimethamine in January 2003. Of 2393 home follow-up visits of reported cases of malaria, three fatal adverse events were identified where recent use of artesunate plus sulfadoxine/pyrimethamine treatment was reported. Two cases were attributed to poor response to treatment, while one case was considered possibly related to artesunate plus sulfadoxine/pyrimethamine treatment. Clinic and hospital surveillance reported six ADRs in association with sulfadoxine/pyrimethamine treatment, five being treatment failures and one being a non-serious rash. During active hospital surveillance, 38 inpatients exposed to sulfadoxine/pyrimethamine were identified, including one child who experienced pancytopenia following treatment with sulfadoxine/pyrimethamine 11 days before admission; this adverse effect was considered to be possibly due to sulfadoxine/pyrimethamine treatment. The confidential enquiry into malaria-related deaths identified three adverse events, including a death where the contribution of treatment could not be excluded. A therapeutic efficacy study of 95 patients followed over 42 days identified one case of repeated vomiting possibly associated with artesunate plus sulfadoxine/pyrimethamine. CONCLUSION: Multifaceted monitoring throughout the malaria patient journey is necessary in developing countries implementing new treatments to safeguard against missing serious complications associated with malaria treatment.

Assessment of safety of the major antimalarial drugs.

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Antimalarial drugs remain the major intervention tool for the global malaria control efforts that save millions of lives. Nonetheless, emergence and spread of Plasmodium parasites resistant against chloroquine and other major antimalarial drugs has brought the urgency to develop a new generation of safe and effective drugs against malaria. In this article, the safety data for major antimalarial drugs is reviewed. Although an ample amount of clinical data regarding the safety and tolerability of several of these drugs in older children and adults is...
available, more critical safety and tolerability studies in pregnant women and young children is desirable. To offset the partial loss in efficacy due to drug resistance in malaria parasites acquired against specific drugs, treatment regimens often rely upon the combination of two or more drugs. However, combination therapy requires additional safety, toxicity and tolerability studies in all population groups where these drugs are administered. A uniform standard in assessing the safety and tolerability of antimalarial drugs will be useful in the formulation and implementation of malaria treatment policies that are based on the drug effectiveness, safety and tolerability.


Helicases - feasible antimalarial drug target for Plasmodium falciparum.

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Of the four Plasmodium species that cause human malaria, Plasmodium falciparum is responsible for the most severe form of the disease and this parasite is developing resistance to the major antimalarial drugs. Therefore, in order to control malaria it is necessary to identify new drug targets. One feasible target might be helicases, which are important unwinding enzymes and required for almost all the nucleic acid metabolism in the malaria parasite.


Drug-resistant malaria - an insight.

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Despite intensive research extending back to the 1930s, when the first synthetic antimalarial drugs made their appearance, the repertoire of clinically licensed formulations remains very limited. Moreover, widespread and increasing resistance to these drugs contributes enormously to the difficulties in controlling malaria, posing considerable intellectual, technical and humanitarian challenges. A detailed understanding of the molecular mechanisms underlying resistance to these agents is emerging that should permit new drugs to be rationally developed and older ones to be engineered to regain their efficacy. This review summarizes recent progress in analysing the causes of resistance to the major antimalarial drugs and its spread.


Vaccines against malaria - an update.

Matuschewski K, Mueller AK. Department of Parasitology, Heidelberg University School of Medicine, Germany.

Malaria vaccine discovery and development follow two principal strategies. Most subunit vaccines are designed to mimic naturally acquired immunity that develops over years upon continuous exposure to Plasmodium transmission. Experimental model vaccines, such as attenuated live parasites and transmission-blocking antigens, induce immune responses superior to naturally acquired immunity. The promises and hurdles of the different tracks towards an effective and affordable vaccine against malaria are discussed.
Larvicidal and growth inhibition of the malaria vector Anopheles stephensi by triterpenes from Dysoxylum malabaricum and Dysoxylum beddomei.

Nathan SS, Hisham A, Jayakumar G.

Secondary metabolites from Dysoxylum malabaricum and Dysoxylum beddomei were tested against mature and immature stage of the mosquito vector Anopheles stephensi under laboratory conditions. The triterpenes 3beta,24,25-trihydroxycycloartane and beddomeilactone from D. malabaricum and D. beddomei showed strong larvicidal, pupicidal and adulticidal activity. They also affected the reproductive potential of adults by acting as oviposition deterrents. The highest concentration tested (10 ppm) of both compounds evoked more than 90% mortality and oviposition deterrence.

Phagocytosis in mosquito immune responses.

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Anopheles mosquitoes are the only vectors of human malaria parasites. Mosquito-parasite interactions are critical for disease transmission and therefore are a potential target for malaria control strategies. Mosquitoes mount potent immune responses that efficiently limit proliferation of a variety of infectious agents, including microbial pathogens and malaria parasites. The recent completion of the Anopheles gambiae genome sequencing project combined with the development of the powerful RNA interference-based gene silencing helped to identify major players of the immune defenses and uncovered evolutionarily conserved mechanisms in the anti-bacterial and anti-Plasmodium responses. The anti-bacterial responses are based on phagocytosis at early steps of infections, followed, several hours later, by the synthesis of anti-microbial peptides. The principal regulators of anti-parasitic responses are predominantly synthesized by the mosquito blood cells; however, the exact molecular mechanisms of parasite killing remain unclear. Several regulators of phagocytosis are also required for efficient parasite killing. Here, we summarize our current knowledge of the anti-bacterial and anti-parasitic responses, with the particular emphasis on the role of phagocytosis in mosquito immunity.

Evidence for a common role for the serine-type Plasmodium falciparum SERA proteases: Implications for vaccine and drug design.

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Serine repeat antigens (SERAs) are a family of secreted 'cysteine-like' proteases of Plasmodium parasites. Several SERAs possess an atypical active site serine residue in place of the canonical cysteine. The human malaria parasite P. falciparum possesses 6 'serine-type' (SERA1-5 and SERA9) and 3 'cysteine-type' (SERA6-8) SERAs. Here, we investigated the importance of the serine-type SERAs to blood-stage parasite development and examine the extent of functional redundancy amongst this group. We attempted to knockout the 4 P. falciparum serine-type SERA genes that have not been disrupted previously. SERA1, SERA4 and SERA9 knockout lines were generated while only SERA5, the most strongly expressed member of the...
SERA family, remained refractory to genetic deletion. Interestingly, we discovered that whilst SERA4-null parasites completed the blood-stage cycle normally, they exhibited a two-fold increase in the level of SERA5 mRNA. The inability to disrupt SERA5 and the apparent compensatory increase in SERA5 expression in response to deletion of SERA4 provides evidence for an important blood-stage function for the serine-type SERAs and supports the notion of functional redundancy amongst this group. Such redundancy is consistent with our phylogenetic analysis, which reveals a monophyletic grouping of the serine-type SERAs across the Plasmodia and a predominance of post-speciation expansion. While SERA5 is to some extent further validated as a target for vaccine and drug development, our data suggests that the expression level of other serine-type SERAs is the only barrier to escape from anti-SERA5 specific interventions.

33: Infect Immun. 2007 Sep 4

Protective Properties and Surface Localization of Plasmodium falciparum Enolase.

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The enolase protein of the human malarial parasite Plasmodium falciparum has recently been characterized. Apart from its glycolytic function, enolase has also been shown to possess antigenic properties and documented to be present on the cell wall of certain invasive organisms, such as Candida albicans. In order to assess whether enolase of P. falciparum is also antigenic, sera from residents of a malarial endemic region of Eastern India were tested against the recombinant P. falciparum enolase protein (r-Pfen). About 96% of immune adult sera samples reacted with r-Pfen over and above the sero-negative controls. Rabbit anti-r-Pfen antibodies inhibited the growth of in vitro cultures of P. falciparum. Mice immunized with r-Pfen showed protection against a challenge with the 17XL lethal strain of the mouse malarial parasite Plasmodium yoelii. The antibodies raised against r-Pfen were specific for Plasmodium and did not react to the host tissues. Immunofluorescence as well as electron microscopic examinations revealed localization of the enolase protein on the merozoite cell surface. These observations establish malaria enolase to be a potential protective antigen.

34: Int J Health Geogr. 2007 Sep 24;6(1):44

Developing a spatial-statistical model and map of historical malaria prevalence in Botswana using a staged variable selection procedure.

Craig MH, Sharp BL, Mabaso ML, Kleinschmidt I.

ABSTRACT: BACKGROUND: Several malaria risk maps have been developed in recent years, many from the prevalence of infection data collated by the MARA (Mapping Malaria Risk in Africa) project, and using various environmental data sets as predictors. Variable selection is a major obstacle due to analytical problems caused by over-fitting, confounding and non-independence in the data. Testing and comparing every combination of explanatory variables in a Bayesian spatial framework remains unfeasible for most researchers. The aim of this study was to develop a malaria risk map using a systematic and practicable variable selection process for spatial analysis and mapping of historical malaria risk in Botswana. RESULTS: Of 50 potential explanatory variables from eight environmental data themes, 42 were significantly associated with malaria prevalence in univariate logistic regression and were ranked by the Akaike Information Criterion. Those correlated with higher-ranking relatives of the same environmental theme, were temporarily excluded. The remaining 14 candidates were ranked by selection frequency after running automated step-wise selection procedures on 1000
bootstrap samples drawn from the data. A non-spatial multiple-variable model was developed through step-wise inclusion in order of selection frequency. Previously excluded variables were then re-evaluated for inclusion, using further step-wise bootstrap procedures, resulting in the exclusion of another variable. Finally a Bayesian geo-statistical model using Markov Chain Monte Carlo simulation was fitted to the data, resulting in a final model of three predictor variables, namely summer rainfall, mean annual temperature and altitude. Each was independently and significantly associated with malaria prevalence after allowing for spatial correlation. This model was used to predict malaria prevalence at unobserved locations, producing a smooth risk map for the whole country.

CONCLUSIONS: We have produced a highly plausible and parsimonious model of historical malaria risk for Botswana from point-referenced data from a 1961/2 prevalence survey of malaria infection in 1-14 year old children. After starting with a list of 50 potential variables we ended with three highly plausible predictors, by applying a systematic and repeatable staged variable selection procedure that included a spatial analysis, which has application for other environmentally determined infectious diseases. All this was accomplished using general-purpose statistical software.

35: Int J Health Geogr. 2007 Sep 4;6(1):37

Participatory mapping of target areas to enable operational larval source management to suppress malaria vector mosquitoes in Dar es Salaam, Tanzania.

Dongus S, Nyika D, Kannady K, Mtasiwa D, Mshinda H, Fillinger U, Drescher AW, Tanner M, Castro MC, Killeen GF.

ABSTRACT: BACKGROUND: Half of the population of Africa will soon live in towns and cities where it can be protected from malaria by controlling aquatic stages of mosquitoes. Rigorous but affordable and scaleable methods for mapping and managing mosquito habitats are required to enable effective larval control in urban Africa. METHODS: A simple community-based mapping procedure that requires no electronic devices in the field was developed to facilitate routine larval surveillance in Dar es Salaam, Tanzania. The mapping procedure included (1) community-based development of sketch maps and (2) verification of sketch maps through technical teams using laminated aerial photographs in the field which were later digitized and analyzed using Geographical Information Systems (GIS). RESULTS: Three urban wards of Dar es Salaam were comprehensively mapped, covering an area of 16.8 sq km. Over thirty percent of this area were not included in preliminary community-based sketch mapping, mostly because they were areas that do not appear on local government residential lists. The use of aerial photographs and basic GIS allowed rapid identification and inclusion of these key areas, as well as more equal distribution of the workload of malaria control field staff. CONCLUSIONS: The procedure developed enables complete coverage of targeted areas with larval control through comprehensive spatial coverage with community-derived sketch maps. The procedure is practical, affordable, and requires minimal technical skills. This approach can be readily integrated into malaria vector control programmes, scaled up to towns and cities all over Tanzania and adapted to urban settings elsewhere in Africa.

36: Int J Parasitol. 2007 Jul 26

Natural regulatory (CD4(+)CD25(+)FOXP(+)) T cells control the production of pro-inflammatory cytokines during Plasmodium chabaudi adami infection and do not contribute to immune evasion.

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Different functions have been attributed to natural regulatory CD4(+)CD25(+)FOXP(+) (Treg) cells during malaria infection. Herein, we assessed the role for Treg cells during infections with lethal (DS) and non-lethal (DK) Plasmodium chabaudi adami parasites, comparing the levels of parasitemia, inflammation and anaemia. Independent of parasite virulence, the population of splenic Treg cells expanded during infection, and the absolute numbers of activated CD69(+) Treg cells were higher in DS-infected mice. In vivo depletion of CD25(+) T cells, which eliminated 80% of CD4(+)FOXP3(+)CD25(+) T cells and 60-70% of CD4(+)FOXP3(+) T cells, significantly decreased the number of CD69(+) Treg cells in mice with lethal malaria. As a result, higher parasite burden and morbidity were measured in the latter, whereas the kinetics of infection with non-lethal parasites remained unaffected. In the absence of Treg cells, parasite-specific IFN-gamma responses by CD4(+) T cells increased significantly, both in mice with lethal and non-lethal infections, whereas IL-2 production was only stimulated in mice with non-lethal malaria. Following the depletion of CD25(+) T cells, the production of IL-10 by CD90(-) cells was also enhanced in infected mice. Interestingly, a potent induction of TNF-alpha and IFN-gamma production by CD4(+) and CD90(-) lymphocytes was measured in DS-infected mice, which also suffered severe anaemia earlier than non-depleted infected controls. Taken together, our data suggest that the expansion and activation of natural Treg cells represent a counter-regulatory response to the overwhelming inflammation associated with lethal P.c. adami. This response to infection involves TH1 lymphocytes as well as cells from the innate immune system.


**Efficacy of PermaNet 2.0 against Anopheles culicifacies and Anopheles stephensi, malaria vectors in India.**

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Bioefficacy of PermaNet was evaluated in both the laboratory and field against Anopheles culicifacies and An. stephensi, major malaria vectors in India. Contact bioassays were carried out after repeated washings and ring net bioassays to determine the median knockdown time of mosquitoes. Three villages were selected for the field trial: in the 1st village PermaNets were distributed, in the 2nd village untreated nets were distributed, and the 3rd village was a control. Entomological data were collected using standard procedures. The PermaNet contact bioassays showed high mortality (>80%) even after 20 washes against both the vector species. The median knockdown time of An. culicifacies and An. stephensi was 392 and 480 sec when exposed to fresh PermaNets and 472 and 986 sec when exposed to PermaNets that had been washed 20 times, respectively. PermaNets showed high efficacy in reducing the person-vector contact as evidenced by reduced person-hour density in the PermaNet village. Long-term field trials are indicated to test the impact of use of PermaNets in controlling malaria.


**Prey-predator relationship between the cyclopoids Mesocyclops longisetus and Mesocyclops meridianus with Anopheles aquasalis larvae.**

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Copepods from the genus Mesocyclops are considered predators and potential biological control for mosquito larvae. Two copepod species M. meridianus and M. longisetus were found in natural developmental habitat for malaria vector...
Anopheles aquasalis in Paria, Venezuela. Predatory potential on 1st-stage mosquito larvae An. aquasalis was evaluated under laboratory conditions for the 2 species of copepod. Further records of both copepod life cycle and body size were taken. A 2 x 3 factorial design was used, consisting of 1:1 and 10:1 prey-predator ratios with and without interspecific interactions. Despite significant body-size differences, M. longisetus and M. meridianus reached maturity 17 days after hatching with no significant differences. Life cycle span of both copepod species are described for the first time. The 2 species showed the same predatory potential despite larval (prey) abundance variation.


Constant temperature and time period effects on Anopheles gambiae egg hatching.

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Anopheles gambiae Giles sensu stricto (Diptera: Culicidae) egg development and its relation to environmental parameters is an understudied aspect of vector biology. Although several studies have illustrated the dramatic effects of temperature on egg development, egg hatching dynamics remain unclear. The objective of this study was to expose An. gambiae eggs to various temperatures for different lengths of time and determine the impact on egg development and hatching count. Batches of mosquito eggs (n = 30 eggs/replicate) were incubated under moist conditions at temperatures of 12, 22, 27, 33, and 42 degrees C for intervals of 1, 3, 7, and 10 days. After that, they were flooded with distilled water at 27 degrees C, and hatching counts were observed for up to 7 days. Mosquito eggs held at 22 and 27 degrees C had the highest overall mean hatching count. During early incubation periods, eggs held at 33 degrees C had hatching counts comparable to 22 and 27 degrees C, but counts decreased drastically during later incubation periods. Temperatures of 12 and 42 degrees C reduced mosquito egg viability, because few eggs hatched in these temperature regimes. Other experiments revealed that during early embryonic development, temperature had a major effect on the developing embryo, while later in embryonic development it had no dramatic effect. Microscopic observation of the An. gambiae embryo showed that extreme low and high temperatures affected the normal development of the embryo. A regression model was developed to describe the effect of incubation temperature and incubation period on egg hatching counts, which demonstrated that the optimum temperature for egg hatching ranges from 24 to 30 degrees C, irrespective of incubation period. The interaction between temperature and time period may have implications for dry-season survival and climate-based models of malaria risk.

40: J Am Mosq Control Assoc. 2007;23(2 Suppl):164-75.

Developing recombinant bacteria for control of mosquito larvae.

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Genetic engineering techniques have been used to significantly improve mosquito larvicides based on the bacteria Bacillus thuringiensis (Bt) subsp. israelensis (Bti) and Bacillus sphaericus (Bs). These new larvicides hold excellent promise for providing better and more cost-effective control of nuisance mosquitoes and vectors of important diseases, including the anopheline vectors of malaria and culicine vectors responsible for filariasis and viral encephalitides. The toxicity of Bti and Bs is due primarily to endotoxin proteins produced during
sporulation. After ingestion by larvae, these are activated and destroy the larval stomach, quickly resulting in death. By cloning the genes encoding various endotoxins from Bt and Bs species, and engineering these for high levels of synthesis, we have been able to generate recombinant bacterial strains based on Bti that are more than 10 times as effective as the conventional strains of Bti or Bs that serve as the active ingredients of commercial bacterial larvicides currently used for mosquito control. The best of these recombinants contain all major Bti endotoxins, specifically, Cry4A, Cry4B, Cry11A, and Cyt1A, plus the binary (Bin) endotoxin of Bs, the principal mosquitocidal protein responsible for the activity of this species. The presence of Cyt1A in these recombinants, which synergizes Cry toxicity and delays resistance to these proteins and Bs Bin, should enable long term use of these recombinants with little if any development of resistance. In the field, these new recombinants should be particularly effective larvicides against most important vectors and nuisance species of the genus Culex, the malaria vectors Anopheles gambiae and An. arabiensis, and species of Aedes and Ochlerotatus sensitive to Bs.

41: J Antimicrob Chemother. 2007 Sep 19

Pharmacokinetics of two paediatric artesunate mefloquine drug formulations in the treatment of uncomplicated falciparum malaria in Gabon.


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Objectives Paediatric drug formulations of artemisinin combination therapies and pharmacokinetic data supporting their use in African children are urgently needed for the effective treatment of young children suffering from falciparum malaria in sub-Saharan Africa. Patients and methods In this study, the pharmacokinetic characteristics of a novel paediatric granule formulation of artesunate-mefloquine therapy were evaluated in comparison to the standard tablet formulation in the treatment of uncomplicated malaria in paediatric patients. Twenty-four patients were assigned to treatment according to body weight with either a fixed-dose paediatric granule co-formulation (10-20 kg body weight) or a free-dose co-blister tablet formulation of artesunate-mefloquine (>20-40 kg body weight). Results Median values for C(max) (861 and 930 ng/mL), T(max) (1.5 and 1.5 h) and AUC(0-)(t) (2050 and 2470 ng.h/mL) were comparable for dihydroartemisinin in the two groups. Exploratory analysis of mefloquine plasma levels revealed a trend towards higher concentrations in the younger age group during the absorption phase (2550 and 1815 ng/mL, 54 h after initiation of treatment, respectively). Median mefloquine concentrations at day 28 were 197 and 343 ng/mL, respectively. Conclusions The pharmacokinetic characteristics of the two paediatric dosage forms, i.e. the novel fixed-dose co-formulation and the standard co-blister of artesunate-mefloquine show comparable results in the two treatment groups. The novel fixed-dose paediatric formulation is an interesting option for outpatient treatment of uncomplicated malaria in African children.

42: J Antimicrob Chemother. 2007 Sep 10

A systematic review and meta-analysis of the effectiveness and safety of atovaquone proguanil (Malarone) for chemoprophylaxis against malaria.

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Objectives A systematic review and meta-analysis of the effectiveness of atovaquone-proguanil (Malarone) as a chemoprophylactic agent against malaria.

Methods The data sources searched for this study included Cochrane systematic
reviews (on infectious diseases), MEDLINE and EMBASE, Web of Knowledge and Annals of Tropical Medicine. All unconfounded randomized controlled trials assessing the chemoprophylaxis against malaria with atovaquone-proguanil were included in the review. Data on study design, study sample, inclusion and exclusion criteria, allocation, blinding, primary and secondary study end points were all extracted by one reviewer and independently rechecked by the second reviewer. Results In general, all 10 studies identified had excellent quality with total scores of $\geq 4$ using the Jadad criteria. Ten controlled trials comprising 4539 participants were included for this review. A meta-analysis of six of the ten studies found chemoprophylaxis with atovaquone-proguanil, with a prophylaxis efficacy of 95.8% (95% CI = 91.5-97.9), to be superior to placebo. It was also considered safe and better tolerated with fewer treatment-related adverse events that could lead to premature discontinuation of prophylaxis than in controls. Comparison with alternative chemoprophylaxis also showed atovaquone-proguanil to be better tolerated with fewer treatment-related self-reported adverse events (RR = 0.8234; 95% CI = 0.673164-1.01) or severe adverse events (RR = 0.6140; 95% CI = 0.420055-0.8975). Atovaquone-proguanil is well tolerated with no difference in non-compliance with placebo (RR = 0.8804; 95% CI = 0.6964-1.113; I(2) = 31.4%). Conclusions Evidence from this review shows that atovaquone-proguanil is highly efficacious as a prophylactic agent against malaria infection and is very well tolerated compared with other antimalarial agents.

43: J Ethnopharmacol. 2007 Aug 17

Are West African plants a source of future antimalarial drugs?

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Ethnopharmacology is a very interesting resource in which new therapies may be discovered. In the case of malaria, two major antimalarial drugs widely used today came originally from indigenous medical systems, that is quinine and artemisinin, from Peruvian and Chinese ancestral treatments, respectively. There is an urgent need for the discovery of new drugs due to the critical epidemiological situation of this disease. New inexpensive therapies that are simple to use and that will limit the cost of drug research are good justifications for this ethnopharmacological approach. Therefore, the aim of this review is to empirically analyse plants that are used for antimalarial treatment in West Africa, and to determine those with real promising antimalarial activity. The major leads such as those extracted from Cochlospermum, Cryptolepis, Guiera and Azadirachta have been highlighted. Indeed, some extracts seem to be promising in future research, but development of new isolation and characterization techniques, for designing new derivatives with improved properties need to be discussed.


A study on malarial infection in HIV-infected individuals.

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To find out whether malaria occurred at an increased frequency in HIV-infected individuals and to evaluate the clinical course and risk factors for malarial infection in HIV, a prospective study was carried out in a tertiary care centre from June, 1999 to December, 2000 among HIV-infected individuals with HIV-uninfected Individuals taken as control. In this study, out of 250 individuals, 152 were HIV-infected and the remaining were HIV-negative. The odd’s ratio (OR) for the occurrence of malaria in the HIV-infected population compared
with the HIV-uninfected population was 2.5 (95% confidence interval: 1.01, 6.4; p < 0.02). The prevalence of malaria in HIV infection was 20.4%. The same was 8.3% in asymptomatic stage, and 22.6% and 21.3% in the early and late symptomatic stages of HIV disease respectively. Among those who came for follow-up 44.4% of the HIV-infected individuals had recurrence of malarial infection. Contrary to what was thought before, malaria occurred at an increased frequency in HIV cases. The occurrence of malaria increased in the symptomatic stages of HIV disease compared to the asymptomatic stage. Recurrence was high in the HIV-infected population.


Crystal Structure of Plasmodium falciparum Spermidine Synthase in Complex with the Substrate Decarboxylated S-adenosylmethionine and the Potent Inhibitors 4MCHA and AdoDATO.

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Plasmodium falciparum is the causative agent of the most severe type of malaria, a life-threatening disease affecting the lives of over three billion people. Factors like widespread resistance against available drugs and absence of an effective vaccine are seriously compounding control of the malaria parasite. Thus, there is an urgent need for the identification and validation of new drug targets. The enzymes of the polyamine biosynthesis pathway have been suggested as possible targets for the treatment of malaria. One of these enzymes is spermidine synthase (SPDS, putrescine aminopropyltransferase), which catalyzes the transfer of an aminopropyl moiety from decarboxylated S-adenosylmethionine (dcAdoMet) to putrescine, leading to the formation of spermidine and 5'-methylthioadenosine. Here we present the three-dimensional structure of P. falciparum spermidine synthase (pfSPDS) in apo form, in complex with dcAdoMet and two inhibitors, S-adenosyl-1,8-diamino-3-thio-octane (AdoDATO) and trans-4-methylcyclohexylamine (4MCHA). The results show that binding of dcAdoMet to pfSPDS stabilizes the conformation of the flexible gatekeeper loop of the enzyme and affects the conformation of the active-site amino acid residues, preparing the protein for binding of the second substrate. The complexes of AdoDATO and 4MCHA with pfSPDS reveal the mode of interactions of these compounds with the enzyme. While AdoDATO essentially fills the entire active-site pocket, 4MCHA only occupies part of it, which suggests that simple modifications of this compound may yield more potent inhibitors of pfSPDS.

46: J Mol Biol. 2007 Aug 3

Bipolar, Dual Plasmodium falciparum Helicase 45 Expressed in the Intraerythrocytic Developmental Cycle Is Required for Parasite Growth.

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Helicases are ubiquitous molecular motor proteins that have an important role in the metabolism of nucleic acids. The gene encoding a helicase was cloned from the human malaria parasite Plasmodium falciparum. The polypeptide of 398 amino acid residues has a molecular mass of 45 kDa, contains striking homology to eukaryotic translation initiation factor 4A (eIF4A) and all the conserved domains of the DEAD-box family. The recombinantly expressed and homogeneous P. falciparum protein PfH45 is an ATP-dependent DNA and RNA helicase, with ATPase and ATP-binding activities. PfH45 is a unique bipolar helicase that contains both the
3' to 5' and 5' to 3' directional helicase activities and anti-PfH45 antibodies curtail all its activities. PfH45 is expressed in all the intraerythrocytic developmental stages of the parasite and has a role in translation. Parasite cultures treated with PfH45 double-stranded RNA or purified immunoglobulins against PfH45 exhibited approximately 60% and approximately 55% growth inhibition, respectively. This inhibitory effect was due to interference with expression of the cognate messenger and down-regulation of synthesis of PfH45 protein in the parasite culture and was associated with morphologic deformation of the parasite. These studies indicate that PfH45 is an indispensable enzyme that is essential for growth, and probably survival, of P. falciparum.

47: Lancet Infect Dis. 2007 Sep 18


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Drug-resistant malaria is a substantial problem throughout Africa and most countries must regularly adapt their antimalarial drug policies to ensure a continued coverage of effective antimalarial treatment. The timing of drug policy change can be guided by several sources of data: molecular markers of resistance, in-vitro parasite sensitivity, parasitological and clinical failure rates, and community morbidity and mortality rates. Through mathematical simulations of the spread of parasite mutations through a population exposed to high-endemic malaria, we explore the causal and chronological relations between these indicators and show which of them are obscured or confounded by other factors. Taking into account the logistical and practical advantages and disadvantages of each type of data collection, we critically appraise the value of each indicator. A major problem is shown to be that drug efficacy as perceived by people at risk will remain high even after drugs have become almost completely ineffective, resulting in a lack of community pressure for drug policy change. We show that parasitological failure is the most sensitive and timely indicator, which allows around 2-3 years for drug policy change to be implemented, so as to prevent the most rapid rise in malaria-related mortality.

48: Malar J. 2007 Sep 26;6(1):132

Varying efficacy of intermittent preventive treatment for malaria in infants in two similar trials: public health implications.


ABSTRACT: BACKGROUND: Intermittent preventive treatment (IPTi) with sulphadoxine-pyrimethamine (SP) in infants resulted in different estimates of clinical malaria protection in two trials that used the same protocol in Ifakara, Tanzania, and Manhica, Mozambique. Understanding the reasons for the discrepant results will help to elucidate the action mechanism of this intervention, which is essential for rational policy formulation. METHODS: A comparative analysis of two IPTi trials that used the same study design, follow-up, intervention, procedures and assessment of outcomes, in Tanzania and Mozambique was undertaken. Children were randomized to receive either SP or placebo administered three times alongside routine vaccinations delivered through the Expanded Programme on Immunization (EPI). Characteristics of the two areas and efficacy on clinical malaria after each dose were compared. RESULTS: The most relevant difference was in the use of insecticide-treated nets (ITNs); 68% in Ifakara and zero in Manhica. In Ifakara, IPTi was associated with a 53% (95% CI 14.0; 74.1) reduction in the risk of clinical malaria between the second and the third dose; during the same period, there was no significant effect in Manhica. Similarly, protection
against malaria episodes was maintained in Ifakara during six months after dose
3, but no effect of IPTi was observed in Manhica. CONCLUSIONS: The high ITN
coverage in Ifakara is the most likely explanation for the difference in IPTi
efficacy on clinical malaria. Combination of IPTi and ITNs may be the most
cost-effective tool for malaria control currently available, and needs to be
explored in current and future studies.

49: Malar J. 2007 Sep 25;6(1):131

Standardizing estimates of the Plasmodium falciparum parasite rate.

Smith DL, Guerra CA, Snow RW, Hay SI.

BACKGROUND: The Plasmodium falciparum parasite rate (PfPR) is a
commonly reported index of malaria transmission intensity. PfPR rises after birth
to a plateau before declining in older children and adults. Studies of
populations with different age ranges generally report average PfPR, so age is an
important source of heterogeneity in reported PfPR data. This confounds simple
comparisons of PfPR surveys conducted at different times or places. METHODS:
Several algorithms for standardizing PfPR were developed using 21 studies that
stratify in detail PfPR by age. An additional 121 studies were found that
recorded PfPR from the same population over at least two different age ranges;
these paired estimates were used to evaluate these algorithms. The best algorithm
was judged to be the one that described most of the variance when converting the
PfPR pairs from one age-range to another. RESULTS: The analysis suggests that the
relationship between PfPR and age is predictable across the observed range of
malaria endemicity. PfPR reaches a peak after about two years and remains fairly
constant in older children until age ten before declining throughout adolescence
and adulthood. The PfPR pairs were poorly correlated; using one to predict the
other would explain only 5% of the total variance. By contrast, the PfPR
predicted by the best algorithm explained 72% of the variance. CONCLUSION: The
PfPR in older children is useful for standardization because it has good
biological, epidemiological and statistical properties. It is also historically
consistent with the classical categories of hypoendemic, mesoendemic and
hyperendemic malaria. This algorithm provides a reliable method for standardizing
PfPR for the purposes of comparing studies and mapping malaria endemicity. The
scripts for doing so are freely available to all.

50: Malar J. 2007 Sep 24;6(1):130

The effect of parental rearing conditions on offspring life history in Anopheles
stephensi.

Grech K, Aye Maung L, Read AF.

ABSTRACT: BACKGROUND: The environmental conditions experienced by parents are
increasingly recognized to impact the success of offspring. Little is known on
the presence of such parental effects in Anopheles. If present, parental effects
could influence mosquito breeding programmes, some malaria control measures and
have epidemiological and evolutionary consequences. METHODS: The presence of
parental effects on offspring emergence time, size, survival, blood meal size and
fecundity in laboratory reared An. stephensi were tested. RESULTS: Parental
rearing conditions did not influence the time taken for offspring to emerge, or
their size or survival as adults. However, parental effects were influential in
determining the fecundity of daughters. Counter-intuitively, daughters of parents
reared in low food conditions produced larger egg clutches than daughters of
parents reared in high food conditions. Offspring reared in low food conditions
took larger blood meals if their parents had also experienced a low food
environment. CONCLUSION: So far as we are aware, this is the first evidence of
parental effects on progeny in Anopheles.
Forecasting malaria incidence based on monthly case reports and environmental factors in Karuzi, Burundi, 1997-2003.

Gomez-Elipe A, Otero A, van Herp M, Aguirre-Jaime A.

ABSTRACT: BACKGROUND: The objective of this work was to develop a model to predict malaria incidence in an area of unstable transmission by studying the association between environmental variables and disease dynamics. METHODS: The study was carried out in Karuzi, a province in the Burundi highlands, using time series of monthly notifications of malaria cases from local health facilities, data from rain and temperature records, and the normalized difference vegetation index (NDVI). Using autoregressive integrated moving average (ARIMA) methodology, a model showing the relation between monthly notifications of malaria cases and the environmental variables was developed. RESULTS: The best forecasting model (R2adj=82%, p<0.0001 and 93% forecasting accuracy in the range +/-4 cases per 100 inhabitants) included the NDVI, mean maximum temperature, rainfall and number of malaria cases in the preceding month. CONCLUSIONS: This model is a simple and useful tool for producing reasonably reliable forecasts of the malaria incidence rate in the study area.

Malaria-related mortality based on verbal autopsy in an area of low endemicity in a predominantly rural population in Ethiopia.

Deressa W, Fantahun M, Ali A.

ABSTRACT: BACKGROUND: Although malaria is one of the most important causes of death in Ethiopia, measuring the magnitude of malaria-attributed deaths at community level poses a considerable difficulty. Nevertheless, despite its low sensitivity and specificity, verbal autopsy (VA) has been the most important technique to determine malaria-specific cause of death for community-based studies. The present study was undertaken to assess the magnitude of malaria mortality in a predominantly rural population of Ethiopia using VA technique at Butajira Rural Health Programme (BRHP) Demographic Surveillance Site (DSS).

METHODS: A verbal autopsy was carried out for a year from August 2003 to July 2004 for all deaths identified at BRPH-DSS. Two trained physicians independently reviewed each VA questionnaire and indicated the most likely causes of death. Finally, all malaria related deaths were identified and used for analysis.

RESULTS: A verbal autopsy study was successfully conducted in 325 deaths, of which 42 (13%) were attributed to malaria. The majority of malaria deaths (47.6%) were from the rural lowlands compared to those that occurred in the rural highlands (31%) and urban (21.4%) areas. The proportional mortality attributable to malaria was not statistically significant among the specific age groups and ecological zones. Mortality from malaria was reckoned to be seasonal; 57% occurred during a three-month period at the end of the rainy season between September and November. About 71% of the deceased received some form of treatment before death, while 12 (28.6%) of those who died neither sought care from a traditional healer nor were taken to a conventional health facility before death. Of those who sought treatment, 53.3% were first taken to a private clinic, 40% sought care from public health facilities, and the remaining two (6.7%) received traditional medicine. Only 11.9% of the total malaria-related deaths received some sort of treatment within 24h after the onset of illness. CONCLUSION: The results of this study suggest that malaria plays a considerable role as a cause of death in the study area. Further data on malaria mortality with a relatively large sample size for at least two years will be needed to substantially describe the burden of malaria mortality in the area.
Kerteszia subgenus of Anopheles associated with the Brazilian Atlantic rainforest: current knowledge and future challenges.

Marrelli MT, Malafronte RS, Sallum MA, Natal D.

BACKGROUND: The Atlantic rainforest ecosystem, where bromeliads are abundant, provides an excellent environment for Kerteszia species, because these anophelines use the axils of those plants as larval habitat. Anopheles (K.) cruzii and Anopheles (K.) bellator are considered the primary vectors of malaria in the Atlantic forest. Although the incidence of malaria has declined in some areas of the Atlantic forest, autochthonous cases are still registered every year, with Anopheles cruzii being considered to be a primary vector of both human and simian Plasmodium. METHODS: Recent publications that addressed ecological aspects that are important for understanding the involvement of Kerteszia species in the epidemiology of malaria in the Atlantic forest in the Neotropical Region were analysed. CONCLUSION: The current state of knowledge about Kerteszia species in relation to the Atlantic rainforest ecosystem was discussed. Emphasis was placed on ecological characteristics related to epidemiological aspects of this group of mosquitoes. The main objective was to investigate biological aspects of the species that should be given priority in future studies.

Interdependence of domestic malaria prevention measures and mosquito-human interactions in urban Dar es Salaam, Tanzania.


BACKGROUND: Successful malaria vector control depends on understanding behavioural interactions between mosquitoes and humans, which are highly setting-specific and may have characteristic features in urban environments. Here mosquito biting patterns in Dar es Salaam, Tanzania are examined and the protection against exposure to malaria transmission that is afforded to residents by using an insecticide-treated net (ITN) is estimated. METHODS: Mosquito biting activity over the course of the night was estimated by human landing catch in 216 houses and 1,064 residents were interviewed to determine usage of protection measures and the proportion of each hour of the night spent sleeping indoors, awake indoors, and outdoors. RESULTS: Hourly variations in biting activity by members of the Anopheles gambiae complex were consistent with classical reports but the proportion of these vectors caught outdoors in Dar es Salaam was almost double that of rural Tanzania. Overall, ITNs confer less protection against exophagic vectors in Dar es Salaam than in rural southern Tanzania (59% versus 70%). More alarmingly, a biting activity maximum that precedes 10pm and much lower levels of ITN protection against exposure (38%) were observed for Anopheles arabiensis, a vector of modest importance locally, but which predominates transmission in large parts of Africa. CONCLUSIONS: In a situation of changing mosquito and human behaviour, ITNs may confer lower, but still useful, levels of personal protection which can be complemented by communal transmission suppression at high coverage. Mosquito-proofing houses appeared to be the intervention of choice amongst residents and further options for preventing outdoor transmission include larviciding and environmental management.
Ensuring sustained ACT production and reliable artemisinin supply.

Kindermans JM, Pilloy J, Olliaro P, Gomes M.

BACKGROUND: This paper reviews recent trends in the production, supply and price of the active ingredients as well as finished ACT products. Production and cost data provided in this paper are based on an ongoing project (Artepal). Stability data are derived from a development project on rectal artesunate.

DISCUSSION: The artemisinin raw material and its derivatives appear to be very stable compared to the finished products. Supply of artemisinin changed in May 2004 when the Global Fund shifted financial support to qualified countries from chloroquine or sulphadoxine-pyrimethamine to an ACT for treatment of malaria. First, there was a sudden shortage of the starting material, and short term scarcity led to a steep rise in API price: it increased dramatically in 2004, from $350 per kg to more than $1000. Second, there was a parallel increase in the number of companies extracting artemisinin from 10 to 80 between 2003 and 2005 in China, and from 3 to 20 in Vietnam. Commercial cultivation began also in East Africa and Madagascar. A steady and predictable demand for the crop can eliminate such wide fluctuations and indirectly contribute to price stability of the herb, the API and ACT. With appropriate mechanisms to reduce those fluctuations, the cost of artemisinin might decrease sustainably to US$ 250-300 per kg.

CONCLUSIONS: Today the global health community is facing the risk of another cyclical swing with lower demand feeding into reduced planting of A. annua and, thereafter, a new shortage of the raw material and higher API prices. International donors, the largest purchasers for ACTs could better coordinate their activities, in order to guarantee purchase of ACTs and consequently of API with manufacturers. In parallel, the base of quality producers of APIs and finished ACT products needs to be broadened. While the ACT programme is still in its early stages, the consequences of another wave of artemisinin and ACT shortages would permanently discredit it and impede any progress in rolling malaria back.

Comparison of three molecular methods for the detection and speciation of Plasmodium vivax and Plasmodium falciparum.

Boonma P, Christensen PR, Suwanarusk R, Price RN, Russell B, Lek-Uthai U.

ABSTRACT: BACKGROUND: Accurate diagnosis of Plasmodium spp. is essential for the rational treatment of malaria. Despite its many disadvantages, microscopic examination of blood smears remains the current "gold standard" for malaria detection and speciation. PCR assays offer an alternative to microscopy which has been shown to have superior sensitivity and specificity. Unfortunately few comparative studies have been done on the various molecular based speciation methods. METHODS: The sensitivity, specificity and cost effectiveness of three molecular techniques were compared for the detection and speciation of Plasmodium falciparum and Plasmodium vivax from dried blood spots collected from 136 patients in western Thailand. The results from the three molecular speciation techniques (nested PCR, multiplex PCR, and real-time PCR) were used to develop a molecular consensus (two or more identical PCR results) as an alternative gold standard. RESULTS: According to the molecular consensus, 9.6% (13/136) of microscopic diagnoses yielded false negative results. Multiplex PCR failed to detect P. vivax in three mixed isolates, and the nested PCR gave a false positive P. falciparum result in one case. Although the real-time PCR melting curve analysis was the most expensive method, it was 100% sensitive and specific and least time consuming of the three molecular techniques investigated. CONCLUSIONS: Although microscopy remains the most appropriate method for clinical diagnosis in a field setting, its use as a gold standard may result in apparent false positive
results by superior techniques. Future studies should consider using more than one established molecular methods as a new gold standard to assess novel malaria diagnostic kits and PCR assays.

57: Malar J. 2007 Sep 12;6(1):123

Age-structured gametocyte allocation links immunity to epidemiology in malaria parasites.

Paul RE, Bonnet S, Boudin C, Tchuinkam T, Robert V.

BACKGROUND: Despite a long history of attempts to model malaria epidemiology, the over-riding conclusion is that a detailed understanding of host-parasite interactions leading to immunity is required. It is still not known what governs the duration of an infection and how within-human parasite dynamics relate to malaria epidemiology. PRESENTATION OF THE HYPOTHESIS: Immunity to Plasmodium falciparum develops slowly and requires repeated exposure to the parasite, which thus generates age-structure in the host-parasite interaction. An age-structured degree of immunity would present the parasite with humans of highly variable quality. Evolutionary theory suggests that natural selection will mould adaptive phenotypes that are more precise (less variant) in "high quality" habitats, where lifetime reproductive success is best. Variability in malaria parasite gametocyte density is predicted to be less variable in those age groups who best infect mosquitoes. Thus, the extent to which variation in gametocyte density is a simple parasite phenotype reflecting the complex within-host parasite dynamics is addressed. TESTING THE HYPOTHESIS: Gametocyte densities and corresponding infectiousness to mosquitoes from published data sets and studies in both rural and urban Cameroon are analysed. The mean and variation in gametocyte density according to age group are considered and compared with transmission success (proportion of mosquitoes infected). Across a wide range of settings endemic for malaria, the age group that infected most mosquitoes had the least variation in gametocyte density, i.e. there was a significant relationship between the variance rather than the mean gametocyte density and age-specific parasite transmission success. In these settings, the acquisition of immunity over time was evident as a decrease in asexual parasite densities with age. By contrast, in an urban setting, there were no such age-structured relationships either with variation in gametocyte density or asexual parasite density. IMPLICATIONS OF THE HYPOTHESIS: Gametocyte production is seemingly predicted by evolutionary theory, insofar as a reproductive phenotype (gametocyte density) is most precisely expressed (i.e. is most invariant) in the most infectious human age group. This human age group would thus be expected to be the habitat most suitable for the parasite. Comprehension of the immuno-epidemiology of malaria, a requisite for any vaccine strategies, remains poor. Immunological characterization of the human population stratified by parasite gametocyte allocation would be a step forward in identifying the salient immunological pathways of what makes a human a good habitat.

58: Malar J. 2007 Sep 6;6(1):118

The rationale and plan for creating a World Antimalarial Resistance Drug Network (WARN).

Sibley CH, Barnes KI, Plowe CV.

ABSTRACT: Drug resistant malaria was a major factor contributing to the failure of a worldwide campaign to eradicate malaria in the last century, and now threatens the large investment being made by the global community in the rollout of effective new drug combinations to replace failed drugs. Four related papers in this issue of Malaria Journal make the case for creating the World Antimalarial Resistance Network (WARN), which will consist of four linked open-access global databases containing clinical, in vitro, molecular and pharmacological data, and networks of reference laboratories that will support
these databases and related surveillance activities. WARN will serve as a public resource to guide antimalarial drug treatment and prevention policies and to help confirm and characterize the new emergence of new resistance to antimalarial drugs and to contain its spread.

59: Malar J. 2007 Sep 1;6(1):117

Evaluation of antibody response to Plasmodium falciparum in children according to exposure of Anopheles gambiae or Anopheles funestus vectors.


ABSTRACT: BACKGROUND: In sub-Saharan areas, malaria transmission was mainly ensured by Anopheles gambiae s.l. and Anopheles funestus vectors. The immune response status to Plasmodium falciparum was evaluated in children living in two villages where malaria transmission was ensured by dissimilar species of Anopheles vectors (An. funestus vs An. gambiae s.l.). METHODS: A multi-disciplinary study was performed in villages located in Northern Senegal. Two villages were selected: Mboula village, where transmission is strictly ensured by An. gambiae s.l., and Gankette Balla village, which is exposed to several Anopheles species but where An. funestus is the only infected vector found. In each village, a cohort of 150 children aged from one to nine years was followed during one year and IgG response directed to schizont extract was determined by ELISA. RESULTS: Similar results of specific IgG responses according to age and P. falciparum infection were observed in both villages. Specific IgG response increased progressively from one-year to 5-year old children and then stayed high in children from five to nine years old. The children with P. falciparum infection had higher specific antibody responses compared to negative infection children, suggesting a strong relationship between production of specific antibodies and malaria transmission, rather than protective immunity. In contrast, higher variation of antibody levels according to malaria transmission periods were found in Mboula compared to Gankette Balla. In Mboula, the peak of malaria transmission was followed by a considerable increase in antibody levels, whereas low and constant anti-malaria IgG response was observed throughout the year in Gankette Balla. CONCLUSION: This study shows that the development of anti-malaria antibody response was profoundly different according to areas where malaria exposure is dependent with different Anopheles species. These results are discussed according to i) the use of immunological tool for the evaluation of malaria transmission and ii) the influence of Anopheles vectors species on the regulation of antibody responses to P. falciparum.

60: Med Hypotheses. 2007 Sep 20

Hygiene hypothesis: Innate immunity, malaria and multiple sclerosis.

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The establishment of new hygienic conditions plays a role in the appearance of autoimmunity in "westernised" countries. Consistently, but still unconvincingly, several epidemiological and immunogenetic evidences link the disappearance of malaria with the increase of multiple sclerosis (MS) in Sardinia, insular Italy. To this purpose, we have made an attempt to build a relationship between malaria disappearance and MS under the light of the hygiene hypothesis. This relationship has taken into account the MS frequency increase soon after malaria eradication in Sardinia, the present malaria endemism in Africa, the innate immune system activity here represented by Chitotriosidase (Chit), an hydrolytic enzyme produced by macrophages, and an unproductive
polymorphism of Chit gene (CHIT1) as a measure of the genetic weight of Plasmodium-related immunity in these populations. Data were derived from both experimental results specifically designed for this study and other data obtained from the available literature. The experimental and the historical-epidemiological findings concur to indicate that whilst in Africa CHIT1 mutation is rare and MS incidence is very low due to unmodified parasitic influence and hygienic conditions, in Sardinia a relationships between CHIT1 mutation, plasma Chit activity and MS prevalence rate is detected, even to a higher extent compared to Sicily, area at former lower rate of malaria endemy. Upon such a basis, we have found convincing arguments that, at least in part, MS has increased over the last four decades in Sardinia also because of the eradication of malaria, 50 years ago. This infectious disease that run for centuries in Sardinia, besides well documented enzyme deficiencies and red cell pathologies, have left an abnormal macrophage reactivity against Plasmodium falciparum. As a result, some Sardinian individuals secrete abnormally high levels of mediators of the innate immunity, relics of former protective anti-malaria infection, in response to new environmental factors. Therefore, MS, an immune-conditioned pathology of the central nervous system has been subject to an unexplained epidemiological increase in the last few decades in Sardinia because cells of the innate immune system, immuno-genetically selected over the centuries in response to widespread P. falciparum malaria, have kept the tendency to over-respond to triggering factors even after the disappearance of malaria. This hypothesis may have an influence in re-directing clinicians toward an innate immunity-based rather than an antigen specific-based new MS therapies.


Molecular genetic studies of Anopheles stephensi in Pakistan.

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Anopheles stephensi Liston s.l. (Diptera: Culicidae) is one of the major vectors of malaria in Pakistan, India, Iran and Afghanistan. In parts of its range this species has shown increases in both relative and absolute abundance in what is hypothesized to be a response to human-mediated environmental change resulting from extensive irrigation. We attempted to detect the molecular genetic signatures of this population instability based on three samples obtained from two villages (149/6R and 111/6R) within an irrigation zone in Punjab Province and from one village (Azakhel) outside the irrigation scheme in Northwest Frontier Province (NWFP), Pakistan, using seven microsatellite loci and 682 basepairs of the mitochondrial CO1 gene. For microsatellite loci, high levels of genetic diversity were observed within populations (mean alleles per locus 10.71-11.57; mean heterozygosity 0.703-0.733). Deviation from Hardy-Weinberg expectations was observed for only two microsatellite loci in 21 tests. No genetic differentiation was observed between populations and average pairwise F(ST) values did not differ significantly from zero for any population pair or either marker system. Tests of population expansion for both mitochondrial and microsatellite loci were inconclusive.


Limited genetic diversity of the Plasmodium falciparum aquaglyceroporin gene.

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In Plasmodium falciparum small solutes like water, ammonium, glycerol and others
are transported by a parasite-encoded channel into the parasite. The gene encoding this channel is termed P. falciparum aquaglyceroporin (PfAQP) and is a single-copy gene and highly homologous to other aquaporins from other protozoa. Aquaporins are considered to be attractive targets for drug treatment and more so since the human and parasite aquaporins show considerable sequence differences. To investigate whether PfAQP may be suitable as a conserved target for potential aquaporin blocking agents we determined the DNA sequences of PfAQP from 65 parasite strains, either from in vitro cultured laboratory strains or from parasites obtained in an malaria-endemic region of Gabon. Only two non-synonymous mutations were found and functionally tested by a methylamine efflux assay. The efflux activity of all variants tested was similar. The lack of functionally variability suggests an invariable protein core, which may restrict parasite populations from evading therapeutic pressure if PfAQP inhibitors will be found.


**Plasmodium falciparum gametocytes: still many secrets of a hidden life.**

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Sexual differentiation and parasite transmission are intimately linked in the life cycle of malaria parasites. The specialized cells providing this crucial link are the Plasmodium gametocytes. These are formed in the vertebrate host and are programmed to mature into gametes emerging from the erythrocytes in the midgut of a blood-feeding mosquito. The ensuing fusion into a zygote establishes parasite infection in the insect vector. Although key mechanisms of gametogenesis and fertilization are becoming progressively clear, the fundamental biology of gametocyte formation still presents open questions, some of which are specific to the human malaria parasite Plasmodium falciparum. Developmental commitment to sexual differentiation, regulation of stage-specific gene expression, the profound molecular and cellular changes accompanying gametocyte specialization, the requirement for tissue-specific sequestration in P. falciparum gametocytophogenesis are proposed here as areas for future investigation. The epidemiological relevance of parasite transmission from humans to mosquito in the spread of malaria and of Plasmodium drug resistance genes indicates that understanding molecular mechanisms of gametocyte formation is highly relevant to design strategies able to interfere with the transmission of this disease.

64: Parasitol Int. 2007 Aug 24

**Peroxiredoxins in malaria parasites: Parasitologic aspects.**

Kawazu SI, Komaki-Yasuda K, Oku H, Kano S.

Malaria is one of the most debilitating and life threatening diseases in tropical regions of the world. Over 500 million clinical cases occur, and 2-3 million people die of the disease each year. Because Plasmodium lacks genuine glutathione peroxidase and catalase, the two major antioxidant enzymes in the eukaryotic cell, malaria parasites are likely to utilize members of the peroxiredoxin (Prx) family as the principal enzymes to reduce peroxides, which increase in the parasite cell due to metabolism and parasitism during parasite development. In addition to its function of protecting macromolecules from H(2)O(2), Prx has also been reported to regulate H(2)O(2) as second messenger in transmission of redox signals, which mediate cell proliferation, differentiation, and apoptosis. In the malaria parasite, several lines of experimental data have suggested that the parasite uses Prxs as multifunctional molecules to adapt themselves to asexual and sexual development. In this review, we summarize the accumulated knowledge on the Prx family with respect to their functions in mammalian cells and their possible function(s) in malaria parasites.
Pathogenesis of anemia in malaria: a concise review.

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Anemia is a common complication in malarial infection, although the consequences are more pronounced with Plasmodium falciparum malaria (Ghosh, Indian J Hematol Blood Transfus 21(53):128-130, 2003). Anemia in this infection is caused by a variety of pathophysiologic mechanisms, and in areas where malaria infection is endemic, co-morbidities like other parasitic infestations, iron, folate and Vitamin B12 deficiency, deficiency of other nutrients, and anemia, which is aggravated by anti-malarial drugs both through immune and non-immune mechanisms, are important considerations. In different endemic areas, beta-thalassemia, alpha-thalassemia, Hb S, Hb E, G6PD deficiency, or ovalocytosis in different proportions interact with this infection. Finally, aberrant immune response to repeated or chronic falciparum malarial infection may produce tropical splenomegaly syndrome, a proportion of which show clonal proliferation of B lymphocytes. Cooperation between chronic malarial infection and infection with E-B virus infection in producing Burkitt's lymphoma is well known. In this review, the fascinating and multifaceted pathophysiologoy of malarial anemia has been discussed.

Linkage group selection: towards identifying genes controlling strain specific protective immunity in malaria.

Pattaradilokrat S, Cheesman SJ, Carter R.

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Protective immunity against blood infections of malaria is partly specific to the genotype, or strain, of the parasites. The target antigens of Strain Specific Protective Immunity are expected, therefore, to be antigenically and genetically distinct in different lines of parasite. Here we describe the use of a genetic approach, Linkage Group Selection, to locate the target(s) of Strain Specific Protective Immunity in the rodent malaria parasite Plasmodium chabaudi chabaudi. In a previous such analysis using the progeny of a genetic cross between P. c. chabaudi lines AS-pyr1 and CB, a location on P. c. chabaudi chromosome 8 containing the gene for merozoite surface protein-1, a known candidate antigen for Strain Specific Protective Immunity, was strongly selected. P. c. chabaudi apical membrane antigen-1, another candidate for Strain Specific Protective Immunity, could not have been evaluated in this cross as AS-pyr1 and CB are identical within the cell surface domain of this protein. Here we use Linkage Group Selection analysis of Strain Specific Protective Immunity in a cross between P. c. chabaudi lines CB-pyr10 and AJ, in which merozoite surface protein-1 and apical membrane antigen-1 are both genetically distinct. In this analysis strain specific immune selection acted strongly on the region of P. c. chabaudi chromosome 8 encoding merozoite surface protein-1 and, less strongly, on the P. c. chabaudi chromosome 9 region encoding apical membrane antigen-1. The evidence from these two independent studies indicates that Strain Specific Protective Immunity in P. c. chabaudi in mice is mainly determined by a narrow region of the P. c. chabaudi genome containing the gene for the P. c. chabaudi merozoite surface protein-1 protein. Other regions, including that containing the gene for P. c. chabaudi apical membrane antigen-1, may be more weakly associated with Strain Specific Protective Immunity in these parasites.
Segmental duplication implicated in the genesis of inversion 2Rj of Anopheles gambiae.

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The malaria vector Anopheles gambiae maintains high levels of inversion polymorphism that facilitate its exploitation of diverse ecological settings across tropical Africa. Molecular characterization of inversion breakpoints is a first step toward understanding the processes that generate and maintain inversions. Here we focused on inversion 2Rj because of its association with the assortatively mating Bamako chromosomal form of An. gambiae, whose distinctive breeding sites are rock pools beside the Niger River in Mali and Guinea. Sequence and computational analysis of 2Rj revealed the same 14.6 kb insertion between both breakpoints, which occurred near but not within predicted genes. Each insertion consists of 5.3 kb terminal inverted repeat arms separated by a 4 kb spacer. The insertions lack coding capacity, and are comprised of degraded remnants of repetitive sequences including class I and II transposable elements. Because of their large size and patchwork composition, and as no other instances of these insertions were identified in the An. gambiae genome, they do not appear to be transposable elements. The 14.6 kb modules inserted at both 2Rj breakpoint junctions represent low copy repeats (LCRs, also called segmental duplications) that are strongly implicated in the recent (approximately 0.4N(e) generations) origin of 2Rj. The LCRs contribute to further genome instability, as demonstrated by an imprecise excision event at the proximal breakpoint of 2Rj in field isolates.

Malaria in Africa: vector species' niche models and relative risk maps.

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A central theoretical goal of epidemiology is the construction of spatial models of disease prevalence and risk, including maps for the potential spread of infectious disease. We provide three continent-wide maps representing the relative risk of malaria in Africa based on ecological niche models of vector species and risk analysis at a spatial resolution of 1 arc-minute (9 185 275 cells of approximately 4 sq km). Using a maximum entropy method we construct niche models for 10 malaria vector species based on species occurrence records since 1980, 19 climatic variables, altitude, and land cover data (in 14 classes). For seven vectors (Anopheles coustani, A. funestus, A. melas, A. merus, A. moucheti, A. nili, and A. paludis) these are the first published niche models. We predict that Central Africa has poor habitat for both A. arabiensis and A. gambiae, and that A. quadriannulatus and A. arabiensis have restricted habitats in Southern Africa as claimed by field experts in criticism of previous models. The results of the niche models are incorporated into three relative risk models which assume different ecological interactions between vector species. The "additive" model assumes no interaction; the "minimax" model assumes maximum relative risk due to any vector in a cell; and the "competitive exclusion" model assumes the relative risk that arises from the most suitable vector for a cell. All models include variable anthropophilicity of vectors and spatial variation in
human population density. Relative risk maps are produced from these models. All models predict that human population density is the critical factor determining malaria risk. Our method of constructing relative risk maps is equally general. We discuss the limits of the relative risk maps reported here, and the additional data that are required for their improvement. The protocol developed here can be used for any other vector-borne disease.

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**A genome-wide analysis in Anopheles gambiae mosquitoes reveals 46 male accessory gland genes, possible modulators of female behavior.**

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The male accessory glands (MAGs) of many insect species produce and secrete a number of reproductive proteins collectively named Acps. These proteins, many of which are rapidly evolving, are essential for male fertility and represent formidable modulators of female postmating behavior. Upon copulation, the transfer of Acps has been shown in Drosophila and other insects to trigger profound physiological and behavioral changes in females, including enhanced ovulation/oviposition and reduced mating receptivity. In Anopheles gambiae mosquitoes, the principal vectors of human malaria, experimental evidence clearly demonstrates a key role of MAG products in inducing female responses. However, no Acp has been experimentally identified to date in this or in any other mosquito species. In this study we report on the identification of 46 MAG genes from An. gambiae, 25 of which are male reproductive tract-specific. This was achieved through a combination of bioinformatics searches and manual annotation confirmed by transcriptional profiling. Among these genes are the homologues of 40% of the Drosophila Acps analyzed, including Acp70A, or sex peptide, which in the fruit fly is the principal modulator of female postmating behavior. Although many Anopheles Acps belong to the same functional classes reported for Drosophila, suggesting a conserved role for these proteins in mosquitoes, some represent novel lineage-specific Acps that may have evolved to perform functions relevant to Anopheles reproductive behavior. Our findings imply that the molecular basis of Anopheles female postmating responses can now be studied, opening novel avenues for the field control of these important vectors of human disease.

**70: Proc Natl Acad Sci U S A. 2007 Sep 14**

**Plasmodium falciparum ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion.**

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Malaria transmission entails development of the Plasmodium parasite in its insect vector, the Anopheles mosquito. Parasite invasion of the mosquito midgut is the critical first step and involves adhesion to host epithelial cell ligands. Partial evidence suggests that midgut oligosaccharides are important ligands for parasite adhesion; however, the identity of these glycans remains unknown. We have identified a population of chondroitin glycosaminoglycans along the apical midgut microvilli of Anopheles gambiae and further demonstrated ookinete recognition of these glycans in vitro. By repressing the expression of the peptide-O-xylosyltransferase homolog of An. gambiae by means of RNA interference, we blocked glycosaminoglycan chain biosynthesis, diminished chondroitin sulfate
levels in the adult midgut, and substantially inhibited parasite development. We provide evidence for the in vivo role of chondroitin sulfate proteoglycans in Plasmodium falciparum invasion of the midgut and insight into the molecular mechanisms mediating parasite-mosquito interactions.


The salivary glands and saliva of Anopheles gambiae as an essential step in the Plasmodium life cycle: A global proteomic study.


Proteins synthesized in the salivary glands of the Anopheles gambiae mosquito are thought to be important in the life cycle of the malaria parasite Plasmodium. To describe A. gambiae salivary gland and saliva contents, we combined several techniques: 1-DE, 2-DE and LC MS/MS. This study has identified five saliva proteins and 122 more proteins from the salivary glands, including the first proteomic description for 89 of these salivary gland proteins. Since the invasion and sporozoite maturation take place during the process of salivary glands ageing, the effect of salivary gland age on salivary component composition was examined. LC MS/MS profiling of young versus old salivary gland proteomes suggests that there is an over-representation of proteins involved in signaling and proteins related to the immune response in the proteins from older mosquitoes. The iTRAQ labeling was used for a comparative proteomic analysis of salivary gland samples from infected or Plasmodium berghei-free mosquitoes. The expression levels of five secreted proteins were altered when the parasite was present. These observations will serve as a basis for future work concerning the possible role of these proteins in the interaction between A. gambiae, Plasmodium and the mammalian host.


[Sulfadoxine-pyrimethamine resistance in Maputo, Mozambique: presence of mutations in the dhfr and dhps genes of Plasmodium falciparum.] [Article in Portuguese]

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The frequency and distribution of mutations in Plasmodium falciparum, dihydrofolate reductase and dihydropteroate synthase genes were analyzed, using the polymerase chain reaction and restriction fragment length polymorphism methodology, in infected blood samples from Mozambican children living in Maputo, before and seven days after treatment with sulfadoxine/pyrimethamine (S/P). The results showed the occurrence of point mutations in the genes studied and the presence of combinations of three alleles in dhfr (51Ile, 59Arg and 108Asn) and "quintuple" mutant (dhfr 51Ile, 59Arg, 108Asn and dhps 437Gly, 540Glu). Both of these situations were associated with seven-day therapeutic failure, following treatment with S/P. These findings show the importance of studying S/P resistance in Mozambique, and how molecular markers for antimalarial resistance can provide important data for national malaria control policy.
A longitudinal investigation of Plasmodium falciparum malaria in children in northern India.

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A group comprising 27 young children (1-4 y of age) suffering from uncomplicated falciparum malaria were studied to characterize the isolates and to measure humoral immune responses during acute infection and after recovery. Finger prick blood from each individual was collected on d 1. After treatment with chloroquine, a further blood sample was collected from each child on d 7, 30, 90 and 180 for assay of antibody responses to P. falciparum antigens. Isolates from individual patients were incubated in vitro for demonstration of rosette formation, assay of plasmodial growth rate and analysis of Pfcrf gene polymorphism. Out of 27 isolates of P. falciparum, 20 showed formation of rosettes in vitro. The growth rate at 96 h varied widely among the isolates. In Pfcrf gene analysis at 76-codon site, 14 showed wild-type Lys 76, 7 showed mutant type Thr 76 and 6 had mixed type. 14 children, all with anaemia on d 7, showed a positive direct antiglobulin test (DAT). Sera positive by ELISA IgG on d 90 also showed parasite growth inhibitory activity in vitro. Significant levels of IgG, IgG1 and IgG3 subclass antibodies against MSP1 were detected in 14 sera collected on d 90. On d 180, there was a decline in IgG and its subtypes. These findings suggest that a variability in isolates may occur in one and the same seasonal area, making children prone to infection. As a consequence, they develop antibodies during recovery phase from an acute attack, which remain in circulation for a period of 4-5 months. After that, a decline in antibody level may again make them susceptible to the disease. Prevalence of different serotypes in a small area may suggest the complexity of malaria transmission.

Ecology of vector mosquitoes in Sri Lanka--suggestions for future mosquito control in rice ecosystems.

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Mosquito-borne diseases are a major public health threat in Asia. To explore effective mosquito control strategies in rice ecosystems from the ecological point of view, we carried out ecological analyses of vector mosquitoes in Sri Lanka. During the 18-month study period, 14 Anopheles, 11 Culex, 5 Aedes, 2 Mansonia, and 1 Armigeres species were collected, most of which are disease vectors for malaria, filariasis, Japanese encephalitis, or dengue in Sri Lanka and elsewhere in Asia. The density and occurrence of Anopheles and Culex species were the highest in seepage pools and paddy fields, where the majority of niche overlaps between larval mosquito and aquatic insect species were observed. All 7 aquatic insect species, which are larval mosquito predators, overlapped their niche with both Anopheles and Culex larvae. This suggests that conserving these aquatic insect species could be effective in controlling mosquito vectors in the study site. Correlations between several climatic factors and mosquito density were also analyzed, and weather conditions, including higher temperature, lower relative humidity, and higher wind velocity, were found to affect mosquito oviposition, propagation, and survival. These findings deepen our understanding of mosquito ecology and will strengthen future mosquito control strategies in rice ecosystems in Asia.
In vitro and in vivo antiplasmodial activity and cytotoxicity of extracts of Phyllanthus niruri L. herbs traditionally used to treat malaria in Indonesia.

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In endemic areas where malaria is prevalent, medicinal plants are often used to treat malaria. This study was conducted to evaluate the in vitro and in vivo antiplasmodial activity and cytotoxicity of extracts of meniran (Phyllanthus niruri L.) herb traditionally used to treat malaria in Indonesia. Three extracts viz aqueous, methanolic and chloroformic extracts were obtained by maceration of the herbs. A radioactive method was used to evaluate the in vitro antiplasmodial activity of the extracts on chloroquine-resistant (FCR-3) and chloroquine-sensitive (D-10) strains of Plasmodium falciparum. In vitro antiplasmodial activity was expressed by the concentration inhibiting 50% of parasite growth (IC50). Cytotoxicity was estimated on Hela cells and the Cytotoxicity Index (CI = IC50 on HeLa cells/IC50 on FCR-3 strain) was calculated to evaluate the safety of tested extracts. A standard 4-day test on P. berghei infected mice was used to evaluate the in vivo antiplasmodial activity of the extracts showing strong in vitro antiplasmodial activity, for both the methanolic and aqueous extracts. The in vivo antiplasmodial activity was expressed by the dose inhibiting 50% of parasite growth (ED50). The IC50 values obtained for these extracts against P. falciparum ranged from 2.3 to 202.4 microg/ml. The methanolic extract was the most active in vitro extract with an IC50 that ranged from 2.3 to 3.9 microg/ml and a CI that ranged from 41.3 to 57.5. This was also the most in vivo active extract with an ED50 of 9.1 mg/kg/d. Further study will be conducted to isolate and purify active compounds presented in the methanolic extract.

An empowerment program to enhance women's ability to prevent and control malaria in the community, Chiang Mai Province, Thailand.

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Paulo Freire's theory was modified to empower a women's group in Chiang Mai Province, Thailand, to prevent and control malaria. This study conducted an intervention in Mueang Na Wan Village, Mueang Na Sub-district, Chiang Dao District, Chiang Mai Province, where 45 women were systematically recruited into the study cohort. Navail Village was selected as a control village because it resembled the intervention village. Navail Village was selected as a control village because it resembled the intervention village. The empowerment program emphasized enhancement of malaria preventive levels, using insecticide-treated bed nets, self-esteem, and self confidence expectation to prevent and control malaria. Intensive training was conducted and activities performed among the women's group, with 10 participatory meetings in all. Data collection was conducted for the pre-test in month 1, and post-intervention in months 3, 6, 9, and 12. The qualitative methods used were focus-group discussions, non-participant observations, and in-depth interviews with housewives, their husbands, and youths at risk for malaria. The results showed that, post-intervention, there were significantly increased levels for malaria preventive behaviors, behaviors of using insecticide-treated nets, self-esteem, and self confidence expectations, in the intervention village compared with the control village. Insecticide-treated net usage and insecticide-treated net usage behaviors increased in the intervention village more than before and more than that in the control village. The women's group in the intervention village created the following plans, which were crucial to malaria prevention: (1) a family protection plan, (2) providing
malaria education to community members, (3) a mosquito-control campaign, (4) scaling-up insecticide-impregnated bed nets, and (5) malaria control among foreign laborers. Finally, the empowered women's group performed sustainable activities. Between malaria-prevention activities, they conducted a joint program to raise income for their families.

**Differential effects on angiogenesis of two antimalarial compounds, dihydroartemisinin and artemisone: Implications for embryotoxicity.**

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Artemisinin derivatives are highly effective and well-tolerated antimalarial drugs that now form the basis of antimalarial combination therapies recommended by the World Health Organization. Although not yet reported to be a problem in clinical use, neurotoxicity and embryotoxicity are displayed by the compound class in in vitro and in vivo experimental models, in particular by dihydroartemisinin, the main metabolite of all current clinical artemisinins. Embryotoxicity appears to be connected with defective angiogenesis and vasculogenesis in certain stages of embryo development. This may prevent the use of artemisinin derivatives in malaria during pregnancy, when both mother and fetus are at high risk of death. Artemisone is a novel 10-alkylamino derivative which is not metabolised to dihydroartemisinin. It was selected as a clinical drug candidate on the basis of its high efficacy against Plasmodium falciparum in vitro and its lack of detectable neurotoxicity in both in vitro and in vivo screens. Here we describe the results of a comparative study of the anti-angiogenic properties of both artemisone and dihydroartemisinin in different model systems. We evaluated the proliferation of human endothelial cells and their migration on a fibronectin matrix, the sprouting of new vessels from rat aorta sections grown in collagen and the production of pro-angiogenic cytokines such as vascular endothelial growth factor (VEGF) and interleukin-8 (CXCL-8). The data show that artemisone is significantly less anti-angiogenic than dihydroartemisinin in all the experimental models, suggesting that it will be safer to use than the current clinical artemisinins during pregnancy.

**Smoke and malaria: are interventions to reduce exposure to indoor air pollution likely to increase exposure to mosquitoes?**

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Indoor air pollution from the domestic use of biomass fuels by poor households in developing countries is known to be harmful to health, and efforts are being made to address this problem by changes in fuel type, stove technology, house design and fuel-use practices. However, anecdotal evidence suggests that smoke may play an important role by providing protection from biting insects and that efforts to reduce smoke may increase exposure, particularly to mosquitoes and malaria. This paper reviews the literature relating to the repellent effect of smoke on mosquitoes and finds that there is currently no evidence that smoke from domestic fuel use provides effective protection from mosquitoes and malaria. Given the limited number and quality of studies, this finding cannot be interpreted as conclusive. The literature relating to house ventilation and mosquito entry was also reviewed, and an association between eaves spaces and increased indoor mosquito density was noted. Additionally, literature on the effect of soot on the efficacy of insecticide-treated bed nets was considered, but no direct impact was
shown. Efforts to reduce indoor air pollution remain desirable even in areas of malaria transmission.


A community-based delivery system of intermittent preventive treatment of malaria in pregnancy and its effect on use of essential maternity care at health units in Uganda.

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Community delivery of intermittent preventive treatment of malaria in pregnancy (IPTp) is one potential option that could mitigate malaria in pregnancy. However, there is concern that this approach may lead to complacency among women with low access to essential care at health units. A non-randomised community trial assessed a new delivery system of IPTp through traditional birth attendants, drug shop vendors, community reproductive health workers and adolescent peer mobilisers (the intervention) compared with IPTp at health units (control). The study enrolled a total of 2081 pregnant women with the new approaches. Data on care-seeking practices before and after the intervention were collected. The majority of women with the new approaches accessed IPTp in the second trimester and adhered to two doses of sulfadoxine/pyrimethamine (SP) (1404/2081; 67.5%). Antenatal care (four recommended visits) increased from 3.4% (27/805) to 56.8% (558/983) (P<0.001). The proportion of women delivering at health units increased from 34.3% (276/805) to 41.5% (434/1045) (P=0.02), whilst the proportion of women seeking care for malaria at health units increased from 16.7% (128/767) to 36.0% (146/405) (P<0.001). Similarly, use of insecticide-treated nets increased from 7.7% (160/2081) to 22.4% (236/1055) (P<0.001). In conclusion, the community-based system was effective in delivering IPTp, whilst women still accessed and benefited from essential care at health units.


Larvivorous fish against malaria vectors: a new outlook.

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The use of larvivorous fish in malaria control is not new but a half-forgotten strategy. It has been shown to be effective and sustainable in many circumstances. A strategic action plan targeting relevant sibling species of the vectors as well as application of global positioning system technology to facilitate rapid re-checking of sites for the continuing presence of fish are important new features of this strategy.

81: Trends Parasitol. 2007 Sep 6

Modeling the molecular basis of atovaquone resistance in parasites and pathogenic fungi.

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Atovaquone is a substituted hydroxynaphthoquinone that is used therapeutically for treating Plasmodium falciparum malaria, Pneumocystis jirovecii pneumonia and Toxoplasma gondii toxoplasmosis. It is thought to act on these organisms by inhibiting parasite and fungal respiration by binding to the cytochrome bc(1) complex. The recent, growing failure of atovaquone treatment and increased mortality of patients with malaria or Pneumocystis pneumonia has been linked to
the appearance of mutations in the cytochrome b gene. To better understand the molecular basis of drug resistance, we have developed the yeast and bovine bc(1) complexes as surrogates to model the molecular interaction of atovaquone with human and resistant pathogen enzymes.

82: Trends Parasitol. 2007 Sep 4

Malaria and HIV: a silent alliance.

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HIV and malaria are leading causes of morbidity in sub-Saharan Africa. Recently, Abu-Raddad and colleagues explored the synergy between these diseases through a mathematical model that included all documented interactions. It emerges from the model parameter inputs that concomitant infection of both HIV and malaria fuels the spread of both diseases. For the first time, it is shown that, according to the model, transient but repeatedly elevated HIV viral loads due to recurrent co-infections, such as malaria, can also influence and increase HIV prevalence. Probably, these results are conservative and the true impact of the interaction could be even more important.


Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use.


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Objective To measure pyrethroid susceptibility in populations of malaria vectors and nuisance-biting mosquitoes in Tanzania and to test the biological efficacy of current insecticide formulations used for net treatment. Methods Anopheles gambiae Giles s.l., An. funestus Giles s.l. and Culex quinquefasciatus Say were collected during three national surveys and two insecticide-treated net (ITN) studies in Tanzania. Knockdown effect and mortality were measured in standard WHO susceptibility tests and ball-frame bio-efficacy tests. Test results from 1999 to 2004 were compared to determine trends in resistance development. Results Anopheles gambiae s.l. and An. funestus s.l. were highly susceptible to permethrin (range 87-100%) and deltamethrin (consistently 100%) in WHO tests in 1999 and 2004, while Culex quinquefasciatus susceptibility to these pyrethroids was much lower (range 7-100% and 0-84% respectively). Efficacy of pyrethroid-treated nets was similarly high against An. gambiae s.l. and An. funestus s.l. (range 82-100%) while efficacy against Cx. quinquefasciatus was considerably lower (range 2-100%). There was no indication of development of resistance in populations of An. gambiae s.l. or An. funestus s.l. where ITNs have been extensively used; however, susceptibility of nuisance-biting Cx. quinquefasciatus mosquitoes declined in some areas between 1999 and 2004. Conclusion The sustained pyrethroid susceptibility of malaria vectors in Tanzania is encouraging for successful malaria control with ITNs. Continued monitoring is essential to ensure early resistance detection, particularly in areas with heavy agricultural or public health use of insecticides where resistance is likely to develop. Widespread low susceptibility of nuisance-biting Culex mosquitoes to ITNs raises concern for user acceptance of nets.
Molecular analysis of chloroquine resistance in Plasmodium falciparum in Yunnan Province, China.


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Resistance of Plasmodium falciparum to chloroquine (CQ) is determined by the mutation at K76T of the P. falciparum chloroquine resistance transporter (pfcrt) gene and modified by other mutations in this gene and in the P. falciparum multidrug resistance 1 (pfmdr1) gene. To determine the extent of polymorphisms in these genes in field P. falciparum isolates from Yunnan province of China, we genotyped the pfcrt codon 76, pfmdr1 codons 86 and 1246. Our results showed that although CQ has been withdrawn from treating falciparum malaria for over two decades, 90.3% of the parasites still carried the pfcrt K76T mutation. In contrast, mutations at pfmdr1 codons 86 and 1246 were rare. Sequencing analysis of the pfcrt gene in 34 parasite field isolates revealed CVIET at positions 72-76 as the major type, consistent with the theory of Southeast Asian origin of CQ resistance in the parasite. In addition, two novel pfcrt haplotypes (75D/144Y/220A and 75E/144Y/220A) were identified. Real-time polymerase chain reaction was used to determine pfmdr1 gene amplification, which is associated with mefloquine resistance. Our result indicated that in agreement with that mefloquine has not been used in this area, most (>90%) of the parasites had one pfmdr1 copy. Genotyping at two hypervariable loci showed relatively low levels of genetic diversity of the parasite population. Meanwhile, 28.4% of cases were found to contain mixed clones, which favour genetic recombination. Furthermore, despite a unique history of antimalarial drugs in Yunnan, its geographical connections with three malarious countries facilitate gene flow among parasite populations and evolution of novel drug-resistant genotypes. Therefore, continuous surveillance of drug resistance in this area is necessary for timely adjustment of local drug policies and more effective malaria control.

A quantitative ultrastructural study of renal pathology in fatal Plasmodium falciparum malaria.

Nguansangiam S, Day NP, Hien TT, Mai NT, Chaisri U, Riganti M, Dondorp AM, Lee SJ, Phu NH, Turner GD, White NJ, Ferguson DJ, Pongponratn E.

Objective To use electron microscopy to examine the role of parasitized red blood cell (PRBC) sequestration in the pathogenesis of acute renal failure in severe falciparum malaria. Methods Ultrastructural pathological examination of renal tissues from Southeast Asian adults (n = 63) who died from severe falciparum malaria. Qualitative and quantitative determination of the major pathological features of disease, including PRBC and leukocyte sequestration. Clinico-pathological correlation with the pre-mortem clinical picture and peripheral parasite count. Results There was a high incidence of malaria-associated renal failure in this population (> 40%) and a correlation between this incidence, severe malarial anaemia and shock. Pathological features included PRBC sequestration in glomerular and tubulo-interstitial vessels, acute tubular damage and mild glomerular hypercellularity resulting from the accumulation of host monocytes within glomerular capillaries. No evidence for an immune complex mediated glomerulonephritis was found. There was a correlation between parasite sequestration in the kidney and pre-mortem renal failure, although overall levels of sequestration were relatively low. Levels of sequestration (Knob+ PRBC) were significantly higher in malaria-associated renal
failure than in fatal cases without renal failure (P = 0.005). Conclusion Malaria-associated renal failure is a common and serious complication of severe Plasmodium falciparum malaria in this population, associated with acute tubular injury rather than glomerulonephritis, and linked to localization of host monocytes in the kidney as well as sequestration of PRBCs.