This issue contains citations and abstracts to approximately 60 malaria studies published in August 2009.

### Journal/Title Index

**Acta Trop. 2009 Aug 13.**
- Public and private sector treatment of malaria in Lao PDR.
- Plasmodium falciparum parasite infection prevalence from a household survey in Zambia using
distribution and larval habitat characterization of Anopheles moucheti, Anopheles nili, and other malaria
- Species B of Anopheles culicifacies (Diptera: Culicidae) is reproductively less fit than species A and C
- Fertilization is a novel attacking site for the transmission blocking of malaria parasites.
- Pro-apoptotic effects of antimalarial drugs do not affect mature human erythrocytes.
- Efficacy of permethrin treated long-lasting insecticidal nets on malaria transmission and observations
- Prevention of Plasmodium vivax malaria recurrence: Efficacy of the standard total dose of primaquine.
- The suitability of clay pots for indoor sampling of mosquitoes in an arid area in northern Tanzania.
- Epidemiological situation of malaria in Madagascar: baseline data for monitoring the impact of malaria

**AIDS. 2009 Aug 1.**
- Impact of HIV infection on severity of imported malaria is restricted to patients with CD4 cell counts

- Quantitative determination of Plasmodium vivax gametocytes by real-time quantitative nucleic acid
- Common genotypic polymorphisms in glutathione S-transferases in mild and severe falciparum malaria
- Use of a histidine-rich protein 2-based rapid diagnostic test for malaria by health personnel during
- Resistance to chloroquine by Plasmodium vivax at Alor in the Lesser Sundas Archipelago in eastern
- Indonesia.

**Ann Bot (Lond). 2009 Aug;104(2):315-23.**
- Enhancement of artemisinin concentration and yield in response to optimization of nitrogen and
- potassium supply to Artemisia annua.

**Interdiscip Perspect Infect Dis. 2009;2009:617954.**

- Malaria prevention in Sub-Saharan Africa: a field study in rural Uganda.

**J Ethnopharmacol. 2009 Aug 7.**
- Quassinoid constituents of Quassia amara L. leaf herbal tea. Impact on its antimalarial activity and
- cytotoxicity.

**Malar J. 2009 Aug 23;8(1):203.**
- Efficacy of artemunate-amodiaquine for treating uncomplicated P. falciparum malaria in Sub-Saharan
- Atypical aetiology of a conjugal fever - Autochthonous airport malaria between Paris and French
- Implementation of an insecticide-treated net subsidy scheme under a public-private partnership for
- Application of the indirect fluorescent antibody assay in the study of malaria infection in the Yangtze

- Human IgG response to a salivary peptide, gSG6-P1, as a new immuno-epidemiological tool for
- Comparative evaluation of the Ifakara tent trap-B, the standardized resting boxes and the human
- Artemisinin-naphthoquine combination (ARCOTM) therapy for uncomplicated falciparum malaria in
- Differential gene expression mediated by 15-hydroxyeicosatetraenoic acid in LPS-stimulated RAW
- Malaria has no effect on birth weight in Rwanda.
- Coquillettidia (Culicidae, Diptera) mosquitoes are natural vectors of avian malaria in Africa.
- Varying efficacy of artesunate+amodiaquine and artesunate+sulphadoxine-pyrimethamine for the
- Community response to intermittent preventive treatment of malaria in infants (IPTi) delivered through
- Longitudinal survey of malaria morbidity over 10 years in Saharevo (Madagascar): further lessons for
- Epileptogenic potential of mefloquine chemoprophylaxis: a pathogenic hypothesis.
- Status of insecticide susceptibility in Anopheles gambiae s.l. from malaria surveillance sites in The
- Defining the relationship between Plasmodium falciparum parasite rate and clinical disease: statistical
- Spatial malaria epidemiology in Bangladeshi highlands.
- Sequence variation of PFEmp1-DBLalpha in association with rosette formation in Plasmodium
- MSP-1p42-specific antibodies affect growth and development of intra-erythrocytic parasites of
- Implementation of a novel PCR based method for detecting malaria parasites from naturally infected
- Space-time variation of malaria incidence in Yunnan province, China.
- Plasmodium falciparum enolase: stage-specific expression and sub-cellular localization.
- Discovery: an interactive resource for the rational selection and comparison of putative drug target
- Chloroquine-resistant Plasmodium vivax malaria in Serbo town, Jimma zone, south-west Ethiopia.
- Treatment of malaria from monotherapy to artemisinin-based combination therapy by health
- Decreased motivation in the use of insecticide-treated nets in a malaria endemic area in Burkina Faso.
- Treatment of malaria from monotherapy to artemisinin-based combination therapy by Knowledge on the
- A stratified random survey of the proportion of poor quality oral artesunate sold at medicine outlets in
- Major variations in malaria exposure of travellers in rural areas: an entomological cohort study in


- Artemisinin resistance in Plasmodium falciparum malaria.


_Proc Natl Acad Sci U S A. 2009 Aug 4;106(31):13004-9._

- Preerythrocytic, live-attenuated Plasmodium falciparum vaccine candidates by design.
- Understanding the link between malaria risk and climate.
- The origin of malignant malaria.


- Malaria and HIV co-infection in pregnancy in sub-Saharan Africa: impact of treatment using antimalarial and antiretroviral agents.

_Vaccine. 2009 Aug 20;27(38):5187-94._

- Plasmodium berghei HAP2 induces strong malaria transmission-blocking immunity in vivo and in vitro.
- Plasmodium falciparum apical membrane antigen 1 vaccine elicits multifunctional CD4 cytokine-
- Age-dependent systemic antibody responses and immunisation-associated changes in mice orally and nasally immunised with Lactococcus lactis expressing a malaria
Public and private sector treatment of malaria in Lao PDR.

Nonaka D, Vongseththa K, Kobayashi J, Bounyadeth S, Kano S, Phompida S, Jimba M.

Department of International Community Health, Graduate School of Medicine, The University of Tokyo, postal code: 113-0033, Hongou 7-3-1, Bunkyo-ku, Tokyo, Japan.

This study aimed to examine the care-seeking choices for treatment of a febrile illness compatible with malaria in the public and private sectors in Lao PDR. We conducted interviews with 745 heads of household in 14 villages in the Sekong province, using a structured-questionnaire. We asked each about who the care-providers were for febrile illness episodes affecting their household members during the past year. If patients used more than one care-provider for a single episode over a period of time, we identified patterns of the care-sequences for the initial and subsequent care choices. Then, we analyzed the relationship between the initial care choices and secondary care choices for care-providers by Chi-square test, categorizing care-providers into public (hospital, health centre, and village health volunteer) and private care-providers (private pharmacy, informal retailer, faith healing and herbs). As a result, we found that 624 patients sought care at least once, 255 (40.9%) twice, and 66 (10.6%) three times or more during a single episode. Of 138 patients who started with a public care-provider and then sought a secondary care, 71 (51.4%) switched to a private care-provider. In contrast, of 117 patients who started with a private care-provider and then sought a secondary care, 82 (70.1%) switched to a public care-provider (p<0.001). In conclusion, although most patients who failed being treated by a private care-provider switched to a public one, some exclusively relied on care within the private sector. An intervention is necessary to make the private sector an integral component of malaria treatment in Lao PDR.


Plasmodium falciparum parasite infection prevalence from a household survey in Zambia using microscopy and a rapid diagnostic test: implications for monitoring and evaluation.

Keating J, Miller JM, Bennett A, Moonga HB, Eisele TP.

Department of International Health and Development, Tulane University School of Public Health and Tropical Medicine, 1440 Canal Street, Suite 2200, New Orleans, LA, 70112, USA.

This paper presents estimates of P. falciparum infection prevalence in children under 5 years old in the context of a population-based household survey in Luangwa District (Lusaka Province), Zambia, an area where greater than 75% of households possess at least one insecticide-treated mosquito net (ITN).
sensitivity and specificity of an HRP-2 rapid diagnostic test (RDT) (ICT Malaria Pf ((R))) compared to microscopy, as well as factors associated with discordant diagnostic results are also presented. P. falciparum infection prevalence was estimated at 7.0% (95% CI 4.9 - 9.0%) using microscopy. Using microscopy as the gold standard, the sensitivity of the HRP-2 RDT was 100% and specificity was 91.5%; positive predictive value was estimated to be 46.7% (95% CI 36.3 - 57.4%). RDT discordance, or HRP-2 false positivity, was highest among older children, those in the northern part of Luangwa District, and those with a reported history of antimalarial treatment. These data suggest microscopy should remain the gold standard for estimating malaria parasite point prevalence from household surveys for monitoring and evaluation purposes.


Distribution and larval habitat characterization of Anopheles moucheti, Anopheles nili, and other malaria vectors in river networks of southern Cameroon.


Laboratoire de Recherche sur le Paludisme, Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale (OCEAC), P.O. Box 288, Yaoundé, Cameroon.

Despite their importance as malaria vectors, little is known of the bionomic of An. nili and An. moucheti. Larval collections from 24 sites situated along the dense hydrographic network of south Cameroon were examined to assess key ecological factors associated with these mosquitoes distribution in river networks. Morphological identification of the III and IV instar larvae by the use of microscopy revealed that 47.6% of the larvae belong to An. nili and 22.6% to An. moucheti. Five variables were significantly involved with species distribution the pace of flow of the river (lotic, or lentic), the light exposure (sunny or shady), vegetation (presence or absence of vegetation) the temperature and the presence or absence of debris. Using Canonical Correspondance Analysis, it appeared that lotic rivers, exposed to light, with vegetation or debris were the best predictors of An. nili larval abundance. Whereas, An. moucheti and An. ovengensis were highly associated with lentic rivers, low temperature, having Pistia. An. nili and An. moucheti distribution along river systems across south Cameroon was highly correlated with environmental variables. The distribution of An. nili conforms to that of a generalist species which is adapted to exploiting a variety of environmental conditions, Whereas, An. moucheti, An. ovengensis and An. carnevalei appeared as specialist forest mosquitoes.


Species B of Anopheles culicifacies (Diptera: Culicidae) is reproductively less fit than species A and C of the complex.

Sharma A, Parasher H, Singh OP, Adak T.

National Institute of Malaria Research, Sector 8, Dwarka, Delhi 110077, India.

Anopheles culicifacies, the most important malaria vector of peninsular India exist as a complex of five sibling species. The member species of the complex have various biological differences including their susceptibility to malaria parasites. The present attempt is made to study and compare the fecundity of the differentially susceptible members of the An. culicifacies complex. Gravid female
mosquitoes of species A, B and C were allowed to lay their eggs individually during first and second gonotrophic cycle. The eggs were counted after hatching and categorized as 'hatched eggs', 'unhatched eggs', 'embryonated eggs', 'unembryonated eggs' and 'non/partially melanized eggs'. The data was analyzed using Student's t test, ANOVA, Chi-square and Pearson's correlation analysis. All females that were visually categorized as 'gravid' did not lay eggs. Species C laid maximum number of eggs per female. The eggs laid per female mosquito of each species were found to be significantly higher during second gonotrophic cycle as compared with the first gonotrophic cycle. The eggs hatched per female in species C were found to be significantly higher than that of species A and B. The poor-vector species B mosquitoes were found to be the least fecund among the members of the species complex. The unembryonated eggs constitute the biggest proportion of the unhatched eggs in species A, B and C of the species complex.


**Fertilization is a novel attacking site for the transmission blocking of malaria parasites.**

Hirai M, Mori T.

Division of Medical Zoology, Department of Infection and Immunity, Jichi Medical University, School of Medicine, Shimotsuke City, Tochigi 329-0498, Japan.

Malaria parasites perform sexual reproduction in mosquitoes where a pair of gametes fertilizes and differentiates into zygotes, and a single zygote produces several thousands of progeny infectious to next vertebrates. Although the parasite fertilization step has been considered as Achilles' heel of parasite life cycle and thus a critical target for blocking malaria transmission in the mosquito, its molecular mechanisms are largely unknown. Previously, we identified that GENERATIVE CELL SPECIFIC 1 (GCS1) is a reproduction factor in angiosperm. Subsequently, it was found that rodent malaria parasite, Plasmodium berghei and green algae, Chlamydomonas reinhardtii possess GCS1 homologues which also play essential roles in gamete interaction. Moreover, intensive database mining revealed that GCS1-like gene homologues exist in the genomes of various organisms. Thus, it appears that GCS1 is an ancient and highly conserved molecule functioning at gamete interaction. In this mini-review, we describe the mechanisms of gametogenesis and fertilization in malaria parasites, comparing with other eukaryotic reproduction, and also speculate GCS1 functions in gamete interaction. We discuss the possibility of whether malaria GCS1 is a novel type of transmission blocking vaccine, by which anti-malaria GCS1 antibody may halt parasite fertilization and subsequent developments in the mosquitoes.


**Pro-apoptotic effects of antimalarial drugs do not affect mature human erythrocytes.**

Totino PR, Daniel-Ribeiro CT, Ferreira-da-Cruz MD.

Laboratory of Malaria Research, Instituto Oswaldo Cruz, Fiocruz, Avenue Brasil, 4365, Manguinhos, 21045-900 Rio de Janeiro, RJ, Brazil.

Malaria is an important public health problem worldwide, representing also an obstacle for the development of the countries, mainly in the African continent. Since no effective vaccine has been developed yet, early diagnosis and prompt
treatment are the main strategy to control malaria transmission. Many of the
drugs used for malaria treatment have the ability to induce apoptosis in
different cell types. In addition, apoptosis has also been identified in
enucleated cells. The present work is aimed, therefore, to evaluate the
pro-apoptotic aptness of chloroquine, quinine, artemisinin and mefloquine on
mature erythrocytes by flow cytometry through the detection of cell shrinkage and
phosphatidylserine exposure at the cell surface-hallmarks of apoptosis. Although
we observed that known apoptosis inducer, such as ionomycin, had led to
erthrocyte apoptosis, we were not able to detect any pro-apoptotic effect of the
studied antimalarial drugs on these cells. We conclude that chloroquine, quinine,
artemisinin and mefloquine may not be able to induce apoptosis in erythrocytes
and, therefore, do not seem to contribute to malaria associated erythrocyte
destruction and anemia.


Efficacy of permethrin treated long-lasting insecticidal nets on malaria
transmission and observations on the perceived side effects, collateral benefits
and human safety in a hyperendemic tribal area of Orissa, India.

Sharma SK, Tyagi PK, Upadhyay AK, Haque MA, Mohanty SS, Raghavendra K, Dash AP.
National Institute of Malaria Research (NIMR), Field Station, Sector-5, Rourkela
769002, Orissa, India.

Studies were conducted on the efficacy of Olyset nets-a long-lasting insecticidal
net (LLIN) factory treated with 2% (w/w) permethrin on malaria transmission in an
area under the influence of pyrethroid susceptible vector species Anopheles
culicifacies and A. fluviatilis in Sundargarh District, Orissa, India. The study
area comprised 22 villages that were randomized into three clusters and
designated as Olyset net, untreated net, and no net area. Malaria incidence in
the study population was measured through longitudinal active surveillance at
fortnightly intervals. There was a reduction of 65-70% in malaria incidence in
Olyset net area as compared to the control areas. The attack rate of Plasmodium
falciparum or number of episodes per person per year in different age groups also
showed significant reduction in Olyset net area as compared to untreated net and
no net areas. Cross-sectional point prevalence surveys showed 45.7% reduction of
malaria prevalence in Olyset net users, whereas there was an increase of 33.3%
and 51% in untreated net and no net villages respectively. The compliance rate of
Olyset net usage in the study population was 80-98% during different months,
whereas it was between 70% and 90% for untreated nets. There were minimal
complains of skin irritation (4%), itching (8%) and eye irritation (1.2%).
However, these effects were only transitory in nature lasting for few hours of
the first usage. Olyset nets also provided collateral benefits in terms of relief
not only from mosquitoes and malaria but also from other household pests such as
head lice, bed bugs, cockroaches, ants and houseflies. The Olyset nets were found
to be safe to humans as no adverse event was recorded in the net users that can
be attributed to the use of net. The study showed that Olyset nets are effective
personal protection tool that can be used in a community based intervention
programme.


Prevention of Plasmodium vivax malaria recurrence: Efficacy of the standard total
dose of primaquine administered over 3 days.
Carmona-Fonseca J, Maestre A.

Grupo Salud y Comunidad, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.

BACKGROUND: The standard total dose (STD) of primaquine to prevent Plasmodium vivax recurrence is 0.25mg/kg/day administered over 14 days (STD-14). We evaluated, in an endemic zone of Colombia, the anti-recurrence efficacy of the STD dose administered over 3 and 14 days, and of sub-STD dose administered over 3 days (71%STD-3, 50%STD-3). METHODS: A controlled clinical trial was carried out with 188 subjects allocated into one of four treatment groups: STD-14, STD-3, 71%STD-3, 50%STD-3. RESULTS: Recurrences during the 120 days of follow-up were 15% in STD-14, and 57% in STD-3. Treatment with 71%STD-3 and 50%STD-3 resulted in recurrence in >48% subjects within 120 days after the primary episode. High daily doses (1.17mg/kg/day) were well tolerated. CONCLUSIONS: (a) The standard dose and regimen (STD-14) of primaquine to prevent P. vivax relapse is recommended. The administration of the same dose over 3 days (STD-3) should be avoided; (b) doses lower than the STD doses administered over 3 days are ineffective in preventing relapse.


The suitability of clay pots for indoor sampling of mosquitoes in an arid area in northern Tanzania.


Department of Medical Microbiology 268, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. wvandenbijl@hotmail.com

Water storage clay pots have been recently explored as method for outdoor mosquito sampling and as novel device for administrating insect-pathogenic fungi to mosquitoes. Their suitability for indoor mosquito sampling in natural conditions is unknown. We tested clay pots as indoor resting sites alongside catches by CDC light trap in an area of low malaria endemicity in northern Tanzania. Mosquitoes were caught by clay pots although the rate of female Anopheles mosquito catches was 22.64 (95% CI 11.26-45.52) times greater for CDC light traps. The proportion of fed female Anopheles was significantly higher for clay pots compared to CDC light trap (p<0.001), indicating these methods sample different populations of mosquitoes. Although we were able to identify households with a consistently higher exposure to mosquitoes by CDC light trap, there was no apparent heterogeneity in mosquito catches by clay pots. We conclude that clay pots are not a reliable tool to sample mosquitoes in the dry season in an area of low transmission intensity with Anopheles arabiensis as principle vector.


Epidemiological situation of malaria in Madagascar: baseline data for monitoring the impact of malaria control programmes using serological markers.

The aim of this study was to provide baseline information of the epidemiological situation of malaria in Madagascar using serological markers. We carried out cross-sectional studies in schoolchildren from eight sites in the four different malarious epidemiological strata of Madagascar. We studied the prevalence of anti-MSP1 antibodies to assess the burden, and anti-CSP antibodies to estimate the transmission intensity, of malaria. The overall prevalence of each antibody tested was 46.1% for anti-PfMSP-1, 15.2% for anti-PvMSP-1, 14.9% for anti-PfCSP, 4.9% for anti-PvCSP and 2.4% for anti-PmCSP. The prevalence of the five antibodies varied significantly between the sites (P<10(-6)). We also found significant effects of ethnic origin on the prevalence of anti-PfMSP1 antibodies. With regular testing in the same target populations, this data will be particularly useful for managing the elimination strategy supported by the Malagasy Government.

AIDS. 2009 Aug 1. [Epub ahead of print]

Impact of HIV infection on severity of imported malaria is restricted to patients with CD4 cell counts < 350 cells/μl.


aINSERM U943, France bUPMC Univ Paris 06, UMR S943, France cHôpitaux de Paris (AP-HP), Groupe hospitalier Bichat Claude Bernard, Service de parasitologie, France dAP-HP, Groupe hospitalier Bichat Claude Bernard, Service de virologie, France eAP-HP, Groupe hospitalier Tenon, Service de maladies infectieuses et tropicales, France fCentre Hospitalier de Tourcoing, Service universitaire des maladies infectieuses et du voyage, Tourcoing, France gAP-HP, Groupe hospitalier Pitié-Salpêtrière, Service de maladies infectieuses et tropicales, France hAP-HP, Groupe hospitalier Bichat Claude Bernard, Service de maladies infectieuses et tropicales, Paris, France. * For the members of FHDH-ANRS CO4 Clinical Epidemiology Group, see acknowledgement.

OBJECTIVES:: To study the relative impact of HIV-1 infection and associated immunodepression on the severity of Plasmodium falciparum malaria in adults returning from areas of endemic malaria. METHODS:: We conducted a cross-sectional study, based on data from 104 HIV-infected patients from the French Hospital Database on HIV cohort (FHDH-ANRS CO4) and 161 HIV-negative patients from Bichat hospital, with a diagnosis of imported P. falciparum malaria between 2000 and 2003. The severity of falciparum malaria episode was graded with World Health Organization (WHO) criteria 2000 or on 2007 French recommendations. RESULT:: Depending on criteria used, 40% (WHO) and 28% (2007 French recommendations) of episodes of imported P. falciparum malaria in HIV-infected patients were classified as severe, compared with 21% (WHO) and 11% (2007 French recommendations) of episodes among HIV-negative patients. Among HIV-infected patients, the episodes were severe in between 22 (CD4 cell counts >/=350/μl) and 51% (CD4 cell counts <350/μl) of cases using WHO criteria, and between 12 (CD4 cell counts >/=350/μl) and 41% (CD4 cell counts <350/μl) of cases using 2007 French recommendations criteria. Relative to HIV-negative patients, after adjusting for confounding factors, HIV-infected patients with severe immunodepression (CD4 cell counts <350/μl) were at a significantly higher risk of severe malaria than HIV-negative patients (odds ratio 3.2-4.7, depending on the criteria) contrary to HIV-infected patients with CD4 cell counts more than 350/μl (odds ratio 0.7-0.9). CONCLUSION:: The association between HIV infection...
and severity of imported P. falciparum malaria is only observed for HIV-infected patients with severe immunodepression (CD4 cell counts <350/mul).


**Quantitative determination of Plasmodium vivax gametocytes by real-time quantitative nucleic acid sequence-based amplification in clinical samples.**


Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. martijnbeurskens@gmail.com

Microscopic detection of Plasmodium vivax gametocytes, the sexual life stage of this malaria parasite, is insensitive because P. vivax parasitaemia is low. To detect and quantify gametocytes a more sensitive, quantitative real-time Pvs25-QT-NASBA based on Pvs25 mRNA was developed and tested in two clinical sample sets from three different continents. Pvs25-QT-NASBA is highly reproducible with low inter-assay variation and reaches sensitivity approximately 800 times higher than conventional microscopic gametocyte detection. Specificity was tested in 104 samples from P. vivax-, P. falciparum-, P. malariae-, and P. ovale-infected patients. All non-vivax samples were negative in the Pvs25-QT-NASBA; out of 74 PvS18-QT-NASBA positive samples 69% were positive in the Pvs25-QT-NASBA. In a second set of 136 P. vivax microscopically confirmed samples, gametocyte prevalence was 8%, whereas in contrast 66% were positive by Pvs25-QT-NASBA. The data suggest that the human P. vivax gametocyte reservoir is much larger when assessed by Pvs25-QT-NASBA than by microscopy.


**Common genotypic polymorphisms in glutathione S-transferases in mild and severe falciparum malaria in Tanzanian children.**

Kavishe RA, Bousema T, Shekalaghe SA, Sauerwein RW, Mosha FW, van der Ven AJ, Russel FG, Koenderink JB.

Department of Pharmacology and Toxicology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Malaria infection induces oxidative stress in the host cells. Antioxidant enzymes such as glutathione S-transferases (GSTs) are responsible for fighting reactive oxygen species and reduction of oxidative stress. Common GST polymorphisms have been associated with susceptibility to different diseases whose pathologies involve oxidative stress. In this study, we tested the hypothesis that GST polymorphisms that lead to reduced or lack of enzyme activity are associated with severe Plasmodium falciparum malarial anemia. We studied the genotypic distribution of GSTM1, GSTT1, and GSTP1 polymorphisms between mild malaria (N = 107) and severe malarial anemia (N = 50) in Tanzanian children. We did not find a significant relationship with the GSTT1 polymorphism. GSTM1-null was higher in the severe malaria anemia group but the difference was not significant (P = 0.08). However, a significant association of GSTP1 I105V genotype with severe malarial anemia was discovered (26.0% against 10.3% mild malaria, P = 0.004). We concluded that GSTP1 and possibly GSTM1 may protect against severe falciparum malaria in children.

Use of a histidine-rich protein 2-based rapid diagnostic test for malaria by health personnel during routine consultation of febrile outpatients in a peripheral health facility in Yaounde, Cameroon.


Centre de Formation et Recherche en Médecine et Santé Tropicale, Faculté de Médecine Nord, Boulevard Dramard 13015 Marseille, France. sayangcollins@gmail.com

The role of a rapid diagnostic test (RDT) in the case management of Plasmodium falciparum malaria infections has not been determined in Africa. Our study was conducted during November 2007-January 2008 to assess test accuracy of an RDT in the management of febrile outpatients in a peripheral urban health facility in Cameroon. We found the overall sensitivity to be 71.4% and a specificity of 82.2%; the positive predictive value and negative predictive value were 73.8% and 80.4%, respectively. False-negative and false-positive cases represented 11.8% and 10.5% of all febrile patients. Malaria alone (31.3%) was the first cause of fever; 33.5% of fever cases were of unknown origin. Acute respiratory infections were common among children 0-2 years of age (25.5%) and decreased with age. The risk of having a clinical failure with the presumptive treatment of febrile children was seven times greater than that of the RDT-oriented management (relative risk = 6.8, 95% confidence interval = 0.88-53.4, P = 0.03) because of the delay of appropriate treatment of non-malarial febrile illness. Our results suggest that the RDT may be of limited utility for children greater than five years of age and adults and that diagnosis based on microscopic examination of blood smears should be recommended for these patient populations, as well as in areas of low transmission.


Resistance to chloroquine by Plasmodium vivax at Alor in the Lesser Sundas Archipelago in eastern Indonesia.

Sutanto I, Suprijanto S, Nurhayati, Manoempil P, Baird JK.

Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia. sutanto.inge@yahoo.com

The therapeutic response to standard chloroquine therapy against Plasmodium vivax was evaluated in 36 subjects living at Alor in the Lesser Sundas Archipelago of eastern Indonesia. Chloroquine level were measured on 32 individuals, and showed evidence of adequate absorption of standard chloroquine therapy. Three subjects failed treatment by Day 2 or 3, with evidence of rising asexual parasitemia, and two others had stable parasitemia to Day 7. Ten more subjects had recurrent parasitemia by Day 14, two by Day 21, and another one by Day 28. Three subjects had recurrent parasitemia on Days 14 and 28, but with chloroquine < 100 ng/mL. Eleven subjects cleared parasitemia by Day 3 and had no recurrences up to Day 28. In summary, 28-day cumulative incidence of confirmed resistance to chloroquine was 56% of infections evaluated. Chloroquine should not be considered adequate for treatment of acute vivax malaria acquired in this region.


Enhancement of artemisinin concentration and yield in response to optimization of nitrogen and potassium supply to Artemisia annua.
BACKGROUND AND AIMS: The resurgence of malaria, particularly in the developing world, is considerable and exacerbated by the development of single-gene multi-drug resistances to chemicals such as chloroquinone. Drug therapies, as recommended by the World Health Organization, now include the use of antimalarial compounds derived from Artemisia annua—in particular, the use of artemisinin-based ingredients. Despite our limited knowledge of its mode of action or biosynthesis there is a need to secure a supply and enhance yields of artemisinin. The present study aims to determine how plant biomass can be enhanced while maximizing artemisinin concentration by understanding the plant's nutritional requirements for nitrogen and potassium.

METHODS: Experiments were carried out, the first with differing concentrations of nitrogen, at 6, 31, 56, 106, 206 or 306 mg L(-1) being applied, while the other differing in potassium concentration (51, 153 or 301 mg L(-1)). Nutrients were supplied in irrigation water to plants in pots and after a growth period biomass production and leaf artemisinin concentration were measured. These data were used to determine optimal nutrient requirements for artemisinin yield.

KEY RESULTS: Nitrogen nutrition enhanced plant nitrogen concentration and biomass production successively up to 106 mg N L(-1) for biomass and 206 mg N L(-1) for leaf nitrogen; further increases in nitrogen had no influence. Artemisinin concentration in dried leaf material, measured by HPLC mass spectroscopy, was maximal at a nitrogen application of 106 mg L(-1), but declined at higher concentrations. Increasing potassium application from 51 to 153 mg L(-1) increased total plant biomass, but not at higher applications. Potassium application enhanced leaf potassium concentration, but there was no effect on leaf artemisinin concentration or leaf artemisinin yield.

CONCLUSIONS: Artemisinin concentration declined beyond an optimal point with increasing plant nitrogen concentration. Maximization of artemisinin yield (amount per plant) requires optimization of plant biomass via control of nitrogen nutrition.


HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria.


Centre for Geographic Medicine Research, Kilifi, Kenya.

jberkley@kilifi.kemri-wellcome.org

BACKGROUND: Human immunodeficiency virus (HIV) infection, malnutrition, and invasive bacterial infection (IBI) are reported among children with severe malaria. However, it is unclear whether their cooccurrence with falciparum parasitization and severe disease happens by chance or by association among children in areas where malaria is endemic. METHODS: We examined 3068 consecutive children admitted to a Kenyan district hospital with clinical features of severe malaria and 592 control subjects from the community. We performed multivariable regression analysis, with each case weighted for its probability of being due to falciparum malaria, using estimates of the fraction of severe disease attributable to malaria at different parasite densities derived from
cross-sectional parasitological surveys of healthy children from the same community. RESULTS: HIV infection was present in 133 (12%) of 1071 consecutive parasitemic admitted children (95% confidence interval [CI], 11%-15%). Parasite densities were higher in HIV-infected children. The odds ratio for admission associated with HIV infection for admission with true severe falciparum malaria was 9.6 (95% CI, 4.9-19); however, this effect was restricted to children aged 1 year. Malnutrition was present in 507 (25%) of 2048 consecutive parasitemic admitted children (95% CI, 23%-27%). The odd ratio associated with malnutrition for admission with true severe falciparum malaria was 4.0 (95% CI, 2.9-5.5). IBI was detected in 127 (6%) of 2048 consecutive parasitemic admitted children (95% CI, 5.2%-7.3%). All 3 comorbidities were associated with increased case fatality.

CONCLUSIONS: HIV, malnutrition and IBI are biologically associated with severe disease due to falciparum malaria rather than being simply alternative diagnoses in co-incidentally parasitized children in an endemic area.


Hochman S, Kim K.

Department of Medicine, Division of Infectious Diseases, Montefiore Medical Center, Albert Einstein College of Medicine, Ullmann 1205, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

HIV and malaria have similar global distributions. Annually, 500 million are infected and 1 million die because of malaria. 33 million have HIV and 2 million die from it each year. Minor effects of one infection on the disease course or outcome for the other would significantly impact public health because of the sheer number of people at risk for coinfection. While early population-based studies showed no difference in outcomes between HIV-positive and HIV-negative individuals with malaria, more recent work suggests that those with HIV have more frequent episodes of symptomatic malaria and that malaria increases HIV plasma viral load and decreases CD4+ T cells. HIV and malaria each interact with the host's immune system, resulting in a complex activation of immune cells, and subsequent dysregulated production of cytokines and antibodies. Further investigation of these interactions is needed to better define effects of coinfection.


Malaria prevention in Sub-Saharan Africa: a field study in rural Uganda.

Williams PC, Martina A, Cumming RG, Hall J.

St Cross College, Oxford, UK. phoebe.williams@dphpc.ox.ac.uk

Malaria, a completely preventable and treatable disease, remains one of the biggest killers in Sub-Saharan Africa today. The objectives of this study were to describe the impact of malaria on a small rural community in Uganda (Bufuula) and to implement and evaluate a malaria prevention program (subsidised insecticide treated nets with an accompanying education session). In January 2006, a survey of 202 households (100% response rate) was conducted, and meetings held with the Village Council, which revealed that malaria was the community's major cause of morbidity and mortality, and showed there was a lack of access to preventative
measures. Furthermore, 34% of each household's income was allocated to the burden of malaria. A malaria education and mosquito net distribution session was held in January 2006, which was attended by over 500 villagers who purchased 480 heavily-subsidised long lasting insecticide treated nets (LLINs). Home visits were conducted 1 week later to ensure the LLINs were hung correctly. A follow-up survey was conducted in January 2007. There was a rise in net ownership following the program (18% to 51%, P < 0.0001) and lower rates of childhood malaria prevalence (14%) than reported in Ugandan national statistics (40%). However, only half the nets owned were being used correctly by those most vulnerable to the illness. The findings suggest that mosquito nets must be provided with an effective education program and may be more successful if conducted in whole districts simultaneously rather than on a per-community basis. The evidence for super-targeting strategies for those most vulnerable is also considered. These findings provide important lessons and considerations for other wide-scale malaria prevention programs.


Quassinoid constituents of Quassia amara L. leaf herbal tea. Impact on its antimalarial activity and cytotoxicity.

Houël E, Bertani S, Bourdy G, Deharo E, Jullian V, Valentin A, Chevalley S, Stien D.

CNRS, UMR Ecofog, Université des Antilles et de la Guyane, Cayenne, France.

We discovered in this work that antimalarial Quassia amara young leaf tea contains several quassinoids: simalikalactone D (SkD, 1), picrasin B (2), picrasin H (3), neoquassin (4), quassin (5), picrasin I (6) and picrasin J (7). These last two compounds are new. In addition, our experiments demonstrate that both biological activity and cytotoxicity of the remedy may be attributed solely to the presence of SkD. Therefore, this preparation should not be recommended for treatment of malaria.


ABSTRACT: BACKGROUND: Artesunate and amodiaquine (AS&AQ) is at present the world's second most widely used artemisinin-based combination therapies (ACT). Evaluating the efficacy of ACT recently adopted by the World Health Organisation (WHO) and deployed over 80 countries was necessary to make evidence base drug policy. METHODS: An individual patient data (IPD) analysis was conducted on efficacy outcomes in 26 clinical studies in sub-Saharan Africa using the WHO protocol with similar primary and secondary endpoints. RESULTS: A total of 11,700 patients (75% under 5 years old), from 33 different sites in 16 countries were followed for 28 days. Loss to follow-up was 4.9% (575/11,700). AS&AQ was given to 5,897 patients. Of these, 82% (4,826/5,897) were included in randomized comparative trials with polymerase chain reaction (PCR) genotyping results and compared to 5,413 patients (half receiving an ACT). AS&AQ and other ACT
comparators resulted in rapid clearance of fever and parasitaemia, superior to non-ACT. Using survival analysis on a modified intent-to-treat population, the Day 28 PCR-adjusted efficacy of AS&AQ was greater than 90% (the WHO cut-off) in 11/16 countries. In randomised comparative trials (n=22), the crude efficacy of AS&AQ was 75.9% (95% CI 74.6-77.1) and the PCR-adjusted efficacy was 93.9% (95% CI 93.2-94.5). The risk (weighted by site) of failure PCR-adjusted of AS&AQ was significantly inferior to non-ACT, superior to dihydroartemisinin-piperaquine (DP, in one Ugandan site), and not different from AS+SP or AL (artemether-lumefantrine). The risk of gametocyte appearance and the carriage rate of AS&AQ was only greater in one Ugandan site compared to AL and DP, and lower compared to non-ACT (p=0.001, for all comparisons). Anaemia recovery was not different than comparator groups, except in one site in Rwanda where the patients in the DP group had a slower recovery. CONCLUSIONS: AS&AQ compares well to other treatments and meets the WHO efficacy criteria for use against falciparum malaria in many but not all the Sub-Saharan African countries where it was studied. Efficacy varies between and within countries. IPD analysis can inform general and local treatment policies. Ongoing monitoring evaluation is required.


Atypical aetiology of a conjugal fever - Autochthonous airport malaria between Paris and French Riviera: a case report.


ABSTRACT: Endemic malaria has been eradicated from France, but some falciparum malaria cases have been described in patients who have never travelled outside the country. Ms. V. 21 year-old and Mr. M. 23 year-old living together in Paris were on holiday in Saint-Raphael (on the French Riviera). They presented with fever, vertigo and nausea. A blood smear made to control thrombocytopaenia revealed intra-erythrocytic forms of Plasmodium falciparum. The parasitaemia level was 0.15 % for Ms. V and 3.2 % for Mr. M. This couple had no history of blood transfusion or intravenous drug use. They had never travelled outside metropolitan France, but had recently travelled around France: to Saint-Mard (close to Paris Charles de Gaulle (CdG) airport), to Barneville Plage (in Normandy) and finally to Saint-Raphael. The most probable hypothesis is an infection transmitted in Saint-Mard by an imported anopheline mosquito at CdG airport. The DNA analysis of parasites from Ms. V.'s and Mr. M.'s blood revealed identical genotypes. Because it is unlikely that two different anopheline mosquitoes would be infected by exactly the same clones, the two infections must have been caused by the infective bites of the same infected mosquito.


Implementation of an insecticide-treated net subsidy scheme under a public-private partnership for malaria control in Tanzania - challenges in implementation.

Njau RJ, de Savigny D, Gilson L, Mwageni E, Mosha FW.

ABSTRACT: BACKGROUND: In the past decade there has been increasing visibility of malaria control efforts at the national and international levels. The factors that have enhanced this scenario are the availability of proven interventions
such as artemisinin-based combination therapy, the wide scale use of insecticide-treated nets (ITNs) and a renewed emphasis in indoor residual house-spraying. Concurrently, there has been a window of opportunity of financial commitments from organizations such as the Global Fund for HIV/AIDS, Tuberculosis and Malaria (GFATM), the President's Malaria Initiative and the World Bank Booster programme. METHOD: The case study uses the health policy analysis framework to analyse the implementation of a public-private partnership approach embarked upon by the government of Tanzania in malaria control - 'The Tanzania National Voucher Scheme'- and in this synthesis, emphasis is on the challenges faced by the scheme during the pre-implementation (2001 - 2004) and implementation phases (2004 - 2005). Qualitative research tools used include: document review, interview with key informants, stakeholder's analysis, force-field analysis, time line of events, policy characteristic analysis and focus group discussions. The study is also complemented by a cross-sectional survey, which was conducted at the Rufiji Health Demographic Surveillance Site, where a cohort of women of child-bearing age were followed up regarding access and use of ITNs. RESULTS: The major challenges observed include: the re-introduction of taxes on mosquito nets and related products, procurement and tendering procedures in the implementation of the GFATM, and organizational arrangements and free delivery of mosquito nets through a Presidential initiative. CONCLUSION: The lessons gleaned from this synthesis include: (a) the consistency of the stakeholders with a common vision, was an important strength in overcoming obstacles, (b) senior politicians often steered the policy agenda when the policy in question was a 'crisis event', the stakes and the visibility were high, (c) national stakeholders in policy making have an advantage in strengthening alliances with international organizations, where the latter can become extremely influential in solving bottlenecks as the need arises, and (d) conflict can be turned into an opportunity, for example the Presidential initiative has inadvertently provided Tanzania with important lessons in the organization of 'catch-up' campaigns.


**Population pharmacokinetics of artesunate and amodiaquine in African children.**


**ABSTRACT:** BACKGROUND: Pharmacokinetic (PK) data on amodiaquine (AQ) and artesunate (AS) are limited in children, an important risk group for malaria. The aim of this study was to evaluate the PK properties of a newly developed and registered fixed dose combination (FDC) of artesunate and amodiaquine. METHODS: A prospective population pharmacokinetic study of AS and AQ was conducted in children aged six months to five years. Participants were randomized to receive the new artesunate and amodiaquine FDC or the same drugs given in separate tablets. Children were divided into two groups of 70 (35 in each treatment arm) to evaluate the pharmacokinetic properties of AS and AQ, respectively. Population pharmacokinetic models for dihydroartemisinin (DHA) and desethylamodiaquine (DeAq), the principal pharmacologically active metabolites of AS and AQ, respectively, and total artemisinin anti-malarial activity, defined as the sum of the molar equivalent plasma concentrations of DHA and artesunate, were constructed using the non-linear mixed effects approach. Relative bioavailability between products was compared by estimating the ratios (and 95% CI) between the areas under the plasma concentration-time curves (AUC). RESULTS: The two regimens had similar PK properties in young children with acute malaria. The ratio of loose formulation to fixed co-formulation AUCs, was estimated as 1.043 (95% CI:
0.956 to 1.138) for DeAq. For DHA and total anti-malarial activity AUCs were estimated to be the same. Artesunate was rapidly absorbed, hydrolysed to DHA, and eliminated. Plasma concentrations were significantly higher following the first dose, when patients were acutely ill, than after subsequent doses when patients were usually afebrile and clinically improved. Amodiaquine was converted rapidly to DeAq, which was then eliminated with an estimated median (range) elimination half-life of 9 (7 to 12) days. Efficacy was similar in the two treatments groups, with cure rates of 0.946 (95% CI: 0.840- 0.982) in the AS+AQ group and 0.892 (95% CI: 0.787 - 0.947) in the AS/AQ group. Four out of five patients with PCR confirmed recrudescences received AQ doses < 10 mg/kg. Both regimens were well tolerated. No child developed severe, post treatment neutropaenia (<1,000/muL). There was no evidence of AQ dose related hepatotoxicity, but one patient developed an asymptomatic rise in liver enzymes that was resolving by Day-28.

CONCLUSION: The bioavailability of the co-formulated AS-AQ FDC was similar to that of the separate tablets for desethylamodiaquine, DHA and the total anti-malarial activity. These data support the use this new AS-AQ FDC in children with acute uncomplicated falciparum malaria.


Application of the indirect fluorescent antibody assay in the study of malaria infection in the Yangtze River Three Gorges Reservoir, China.

Duo-Quan W, Lin-Hua T, Zhen-Cheng G, Xiang Z, Man-Ni Y.

ABSTRACT: BACKGROUND: China Yangtze Three Gorges Project (TGP) is one of the biggest construction projects in the world. The areas around the Three Gorge Dam has a history of tertian malaria and subtertian malaria epidemic, but there are no overall data about malaria epidemics before the completion of the project. The objective of this study was to get a reliable baseline on malaria infection in the Yangtze River Three Gorges reservoir area and to provide reference data for future studies about the impact of the project on malaria epidemics. METHODS: Two surveys of malaria infection were carried out in area, at six-month intervals in May and October 2008. About 3,600 dual specimens blood film samples for parasite diagnosis and filter paper blood spots for serology (using the immunofluorescence antibody test) were collected from the general population, including school populations, whenever possible. RESULTS: The overall percentage of positive response of the same population during post-transmission periods was about twice (1.40/0.72) of that in pre-transmission. Positive individuals under 15 years of age were detected in all the localities. CONCLUSION: A certain extent of malaria infection existed in this area. Additional studies are needed to determine the length of malaria experience, and chemotherapeutic intervention as well as the distribution of main vectors for transmission in this area.


Human IgG response to a salivary peptide, gSG6-P1, as a new immuno-epidemiological tool for evaluating low-level exposure to Anopheles bites.


ABSTRACT: BACKGROUND: Human populations exposed to low malaria transmission present particular severe risks of malaria morbidity and mortality. In addition, in a context of low-level exposure to Anopheles vector, conventional entomological methods used for sampling Anopheles populations are insufficiently

Environmental Health at USAID – Malaria Bulletin, August 2009
sensitive and probably under-estimate the real risk of malaria transmission. The evaluation of antibody (Ab) responses to arthropod salivary proteins constitutes a novel tool for estimating exposure level to insect bites. In the case of malaria, a recent study has shown that human IgG responses to the gSG6-P1 peptide represented a specific biomarker of exposure to Anopheles gambiae bites. The objective of this study was to investigate if this biomarker can be used to estimate low-level exposure of individuals to Anopheles vector. METHODS: The IgG Ab level to gSG6-P1 was evaluated at the peak and at the end of the An. gambiae exposure season in children living in Senegalese villages, where the Anopheles density was estimated to be very low by classical entomological trapping but where malaria transmission occurred during the studied season. RESULTS: Specific IgG responses to gSG6-P1 were observed in children exposed to very low-level of Anopheles bites. In addition, a significant increase in the specific IgG Ab level was observed during the Anopheles exposure season whereas classical entomological data have reported very few or no Anopheles during the studied period. Furthermore, this biomarker may also be applicable to evaluate the heterogeneity of individual exposure. CONCLUSIONS: The results strengthen the hypothesis that the evaluation of IgG responses to gSG6-P1 during the season of exposure could reflect the real human contact with anthropophilic Anopheles and suggest that this biomarker of low exposure could be used at the individual level. This promising immuno-epidemiological marker could represent a useful tool to assess the risk to very low exposure to malaria vectors as observed in seasonal, urban, altitude or travellers contexts. In addition, this biomarker could be used for the surveillance survey after applying anti-vector strategy.

Malar J. 2009 Aug 12;8(1):197. [Epub ahead of print]

Comparative evaluation of the Ifakara tent trap-B, the standardized resting boxes and the human landing catch for sampling malaria vectors and other mosquitoes in urban Dar es Salaam, Tanzania.

Sikulu M, Govella NJ, Ogoma SB, Mpangile J, Kambi SH, Kannady K, Chaki PC, Mukabana WR, Killeen GF.

ABSTRACT: BACKGROUND: Frequent, sensitive and accurate sampling of Anopheles mosquitoes is a prerequisite for effective management of malaria vector control programmes. The most reliable existing means to measure mosquito density is the human landing catch (HLC). However, the HLC technique raises major ethical concerns because of the necessity to expose humans to vectors of malaria and a variety of other pathogens. Furthermore, it is a very arduous undertaking that requires intense supervision, which is severely limiting in terms of affordability and sustainability. METHODS: A community-based, mosquito sampling protocol, using the Ifakara tent trap-B (ITT-B) and standardized resting boxes (SRB), was developed and evaluated in terms of the number and sample composition of mosquitoes caught by each, compared to rigorously controlled HLC. Mosquitoes were collected once and three times every week by the HLC and the alternative methods, respectively, in the same time and location. RESULTS: Overall, the three traps caught 44,848 mosquitoes. The ITT-B, HLC and SRB caught 168, 143 and 46 Anopheles gambiae s.l. as well as 26,315, 13,258 and 4,791 Culex species respectively. The ITT-B was three and five-times cheaper than the HLC per mosquito caught for An. gambiae and Cx. Species, respectively. Significant correlation between the numbers caught by the HLC and the ITT-B were observed for both An. gambiae s.l. (P<0.001) and Cx. species (P=0.003). Correlation between the catches with HLC and SRB were observed for Cx. species (P<0.001) but not for An. gambiae s.l. (P=0.195), presumably because of the low density of the latter. Neither ITT-B nor SRB exhibited any obvious density dependence for sampling the
two species. CONCLUSIONS: SRBs exhibited poor sensitivity for both mosquito taxa and are not recommended in this setting. However, this protocol is affordable and effective for routine use of the ITT-B under programmatic conditions. Nevertheless, it is recommended that the trap and the protocol be evaluated further at full programmatic scales to establish effectiveness under fully representative conditions of routine practice.


ABSTRACT: BACKGROUND: The use of anti-malarial drug combinations with artemisinin or with one of its derivatives is now widely recommended to overcome drug resistance in falciparum as well as in vivax malaria. The fixed oral dose artemisinin-naphthoquine combination (ANQ, ARCOTM) is a newer artemisinin-based combination (ACT) therapy undergoing clinical assessment. A study was undertaken to assess the safety, efficacy and tolerability of ANQ combination in areas of multi-drug resistance to generate preliminary baseline data in adult population of Papua New Guinea. METHODS: The clinical assessment was an open-labeled, two-arm, randomized study comparing ANQ combination as a single dose regimen and three days regimen (10mg/kg/day) of chloroquine plus single dose sulphadoxine-pyrimethamine (CQ+SP) for the treatment of uncomplicated falciparum malaria with 28 days follow-up in an adult population. The primary outcome measures for efficacy were day 1, 2, 3 7, 14 and 28-day cure rates. Secondary outcomes included parasite clearance time, fever clearance time, and gametocyte carriage. The main outcome measures for safety were incidences of post-treatment clinical and laboratory adverse events. RESULTS: Between June 2005 and July 2006, 130 patients with confirmed uncomplicated P. falciparum were randomly assigned to receive ANQ and CQ+SP, only 100 patients (51 in ANQ group and 49 in CQ+SP group) were evaluated for clinical and parasitological outcomes. All the patients treated with ANQ and CQ+SP showed adequate clinical and parasitological response with 28 days follow-up. The cure rate for ANQ on day 1, 2, 3, 7, 14, and 28 was 47%, 86%, 92%, 94%, 94% and 94%, respectively. Recrudescence account for 6%; all were cleared on day 21. For CQ+SP treated group the cure rates were 24%, 67%, 82%, 82%, 84% and 88%, respectively. Recrudescence accounted for 10%; all were cleared on day 28 except for one patient. Both regimens were well tolerated with no serious adverse events. The proportion of gametocyte carriers was higher in CQ+SP treated group than ANQ treatment (41% versus 12%; p<0.05). CONCLUSION: While these data are not themselves sufficient, it strongly suggests that the ANQ combination as a single dose administration is safe and effective for the treatment of uncomplicated P. falciparum malaria in the adult population of Papua New Guinea and deserves further clinical evaluation.


Differential gene expression mediated by 15-hydroxyeicosatetraenoic acid in LPS-stimulated RAW 264.7 cells.

Schrimpe AC, Wright DW.

ABSTRACT: BACKGROUND: Given the immuno-modulatory activity of native haemozoin...
(Hz), the effects of constitutive Hz components on immune response are of interest. Recently, gene expression changes mediated by HNE and the synthetic analogue of Hz, beta-haematin (BH), were identified and implicated a significant role for lipid peroxidation products in Hz's activity. The study presented herein examines gene expression changes in response to 15(S)-hydroxyeicosatetraenoic acid (HETE) in a model macrophage cell line. METHODS: LPS-stimulated RAW 264.7 macrophage-like cells were treated with 40 micromolar 15(S)-HETE for 24 h, and microarray analysis was used to identify global gene expression alterations. Fold changes were calculated relative to LPS-stimulated cells and those genes altered at least 1.8-fold (p value ≤ 0.025) were considered to be differentially expressed. Expression levels of a subset of genes were assessed by qRT-PCR and used to confirm the microarray results. RESULTS: Network analysis revealed that altered genes were primarily associated with "lipid metabolism" and "small molecule biochemistry". While several genes associated with PPAR-gamma receptor-mediated signaling were differentially expressed, a number of genes indicated the activation of secondary signaling cascades. Genes related to cytoadherence (cell-cell and cell-matrix), leukocyte extravasation, and inflammatory response were also differentially regulated by treatment, supporting a potential role for 15(S)-HETE in malaria pathogenesis. CONCLUSIONS: These results add insight and detail to 15-HETE's effects on gene expression in macrophage-like cells. Data indicate that while 15-HETE exerts biological activity and may participate in Hz-mediated immuno-modulation, the gene expression changes are modest relative to those altered by the lipid peroxidation product HNE.

Malar J. 2009 Aug 10;8:194.

Malaria has no effect on birth weight in Rwanda.

Rulisa S, Mens PF, Karema C, Schallig HD, Kaligirwa N, Vyankandondera J, de Vries PJ.

National University of Rwanda, Kigali University Teaching Hospital, BP 655, Kigali, Rwanda. stevenruse@yahoo.com

BACKGROUND: Malaria has a negative effect on pregnancy outcome, causing low birth weight, premature birth and stillbirths, particularly in areas with high malaria transmission. In Rwanda, malaria transmission intensity ranges from high to nil, probably associated with variable altitudes. Overall, the incidence decreased over the last six years (2002-2007). Therefore, the impact of malaria on birth outcomes is also expected to vary over time and space. METHODS: Obstetric indicators (birth weight and pregnancy outcome) and malaria incidence were compared and analyzed to their association over time (2002-2007) and space. Birth data from 12,526 deliveries were collected from maternity registers of 11 different primary health centers located in different malaria endemic areas. Malaria data for the same communities were collected from the National Malaria Control Programme. Associations were sought with mixed effects models and logistic regression. RESULTS: In all health centres, a significant increase of birth weight over the years was observed (p < 0.001) with a significant seasonal fluctuation. Malaria incidence had no significant effect on birth weight. There was a slight but significant decreasing effect of malaria incidence on the occurrence of premature delivery (p-value 0.045) and still birth (p-value 0.009). Altitude showed a slight but significant negative correlation with birth weight. Overall, a decrease over the years of premature delivery (p = 0.010) and still birth (p = 0.036) was observed. CONCLUSION: In Rwanda, birth weight and pregnancy outcome are not directly influenced by malaria, which is in contrast to many.
other studied areas. Although malaria incidence overall has declined and mean birth weight increased over the studied period, no direct association was found between the two. Socio-economic factors and improved nutrition could be 
Malar J. 2009 Aug 10;8:193.

Coquillettidia (Culicidae, Diptera) mosquitoes are natural vectors of avian malaria in Africa.


Center for Tropical Research, UCLA Institute of the Environment, Los Angeles, California, USA. kynjabo@hotmail.com

BACKGROUND: The mosquito vectors of Plasmodium spp. have largely been overlooked in studies of ecology and evolution of avian malaria and other vertebrates in wildlife. METHODS: Plasmodium DNA from wild-caught Coquillettidia spp. collected from lowland forests in Cameroon was isolated and sequenced using nested PCR. Female Coquillettidia aurites were also dissected and salivary glands were isolated and microscopically examined for the presence of sporozoites. RESULTS: In total, 33% (85/256) of mosquito pools tested positive for avian Plasmodium spp., harbouring at least eight distinct parasite lineages. Sporozoites of Plasmodium spp. were recorded in salivary glands of C. aurites supporting the PCR data that the parasites complete development in these mosquitoes. Results suggest C. aurites, Coquillettidia pseudoconopas and Coquillettidia metallica as new and important vectors of avian malaria in Africa. All parasite lineages recovered clustered with parasites formerly identified from several bird species and suggest the vectors capability of infecting birds from different families. CONCLUSION: Identifying the major vectors of avian Plasmodium spp. will assist in understanding the epizootiology of avian malaria, including differences in this disease distribution between pristine and disturbed landscapes.


ABSTRACT: BACKGROUND: Very few data on anti-malarial efficacy are available from the Democratic Republic of Congo (DRC). DRC changed its anti-malarial treatment policy to amodiaquine (AQ) and artesunate (AS) in 2005. METHODS: The results of two in vivo efficacy studies, which tested AQ and sulphadoxine-pyrimethamine (SP) monotherapies and AS+SP and AS+AQ combinations in Boende (Equatorial province), and AS+SP, AS+AQ and SP in Kabalo (Katanga province), between 2003 and 2004 are presented. The methodology followed the WHO 2003 protocol for assessing the efficacy of anti-malarials in areas of high transmission. RESULTS: Out of 394 included patients in Boende, the failure rates on day 28 after PCR-genotyping adjustment of AS+SP and AS+AQ were estimated as 24.6% [95% CI: 16.6-35.5] and 15.1% [95% CI: 8.6-25.7], respectively. For the monotherapies, failure rates were 35.9% [95% CI: 27.0-46.7] for SP and 18.3% [95% CI: 11.6-28.1] for AQ. Out of 207 patients enrolled in Kabalo, the failure rate on day 28 after PCR-genotyping adjustment was 0 [1-sided 95% CI: 5.8] for AS+SP and AS+AQ [1-sided 95% CI: 6.2]. It was 19.6% [95% CI: 11.4-32.7] for SP monotherapy. CONCLUSION: The finding of
varying efficacy of the same combinations at two sites in one country highlights one difficulty of implementing a uniform national treatment policy in a large country. The poor efficacy of AS+AQ in Boende should alert the national programme to foci of resistance and emphasizes the need for systems for the prospective monitoring of treatment efficacy at sentinel sites in the country.


Community response to intermittent preventive treatment of malaria in infants (IPTi) delivered through the expanded programme of immunization in five African settings.


ABSTRACT: BACKGROUND: IPTi delivered through EPI has been shown to reduce the incidence of clinical malaria by 20-59%. However, new health interventions can only be effective if they are also socially and culturally acceptable. It is also crucial to ensure that attitudes to IPTi do not negatively influence attitudes to and uptake of immunization, or that people do not misunderstand IPTi as immunization against malaria and neglect other preventive measures or delay treatment seeking. METHODS: These issues were studied in five African countries in the context of clinical trials and implementation studies of IPTi. Mixed methods were used, including structured questionnaires (1,296), semi-structured interviews (168), in-depth interviews (748) and focus group discussions (95) with mothers, fathers, health workers, community members, opinion leaders, and traditional healers. Participant observation was also carried out in the clinics. RESULTS: IPTi was widely acceptable because it resonated with existing traditional preventive practices and a general concern about infant health and good motherhood. It also fit neatly within already widely accepted routine vaccination. Acceptance and adherence were further facilitated by the hierarchical relationship between health staff and mothers and by the fact that clinic attendance had a social function for women beyond acquiring health care. Type of drug and regimen were important, with newer drugs being seen as more effective, but potentially also more dangerous. Single dose infant formulations delivered in the clinic seem to be the most likely to be both acceptable and adhered to. There was little evidence that IPTi per se had a negative impact on attitudes to EPI or that it had any affect on EPI adherence. There was also little evidence of IPTi having a negative impact on health seeking for infants with febrile illness or existing preventive practices. CONCLUSIONS: IPTi is generally acceptable across a wide range of settings in Africa and involving different drugs and regimens, though there is a strong preference for a single dose infant formulation. IPTi does not appear to have any negative effect on attitudes to EPI, and it is not interpreted as immunization against malaria.


Longitudinal survey of malaria morbidity over 10 years in Saharevo (Madagascar): further lessons for strengthening malaria control.

BACKGROUND: Madagascar has been known for having bio-geo-ecological diversity which is reflected by a complex malaria epidemiology ranging from hyperendemic to malaria-free areas. Malaria-related attacks and infection are frequently recorded both in children and adults living in areas of low malaria transmission. To integrate this variability in the national malaria control policy, extensive epidemiological studies are required to up-date previous records and adjust strategies. METHODS: A longitudinal malaria survey was conducted from July 1996 to June 2005 among an average cohort of 214 villagers in Saharevo, located at 900 m above the sea. Saharevo is a typical eastern foothill site at the junction between a costal wet tropical area (equatorial malaria pattern) and a drier high-altitude area (low malaria transmission). RESULTS: Passive and active malaria detection revealed that malaria transmission in Saharevo follows an abrupt seasonal variation. Interestingly, malaria was confirmed in 45% (1,271/2,794) of malaria-presumed fevers seen at the health centre. All four Plasmodia that infect humans were also found: Plasmodium falciparum; Plasmodium vivax, Plasmodium malariae and Plasmodium ovale. Half of the malaria-presumed fevers could be confirmed over the season with the highest malaria transmission level, although less than a quarter in lower transmission time, highlighting the importance of diagnosis prior to treatment intake. P. falciparum malaria has been predominant (98%). The high prevalence of P. falciparum malaria affects more particularly under 10 years old children in both symptomatic and asymptomatic contexts. Children between two and four years of age experienced an average of 2.6 malaria attacks with P. falciparum per annum. Moreover, estimated incidence of P. falciparum malaria tends to show that half of the attacks (15 attacks) risk to occur during the first 10 years of life for a 60-year-old adult who would have experienced 32 malaria attacks. CONCLUSION: The incidence of malaria decreased slightly with age but remained important among children and adults in Saharevo. These results support that a premunition against malaria is slowly acquired until adolescence. However, this claims for a weak premunition among villagers in Saharevo and by extension in the whole eastern foothill area of Madagascar. While the Malagasy government turns towards malaria elimination plans nowadays, choices and expectations to up-date and adapt malaria control strategies in the foothill areas are discussed in this paper.

Malar J. 2009 Aug 5;8(1):188.

Epileptogenic potential of mefloquine chemoprophylaxis: a pathogenic hypothesis.

Nevin RL.

ABSTRACT: BACKGROUND: Mefloquine has historically been considered safe and well-tolerated for long-term malaria chemoprophylaxis, but prescribing it requires careful attention in order to rule out contraindications to its use. Contraindications include a history of certain neurological conditions that might increase the risk of seizure and other adverse events. The precise pathophysiological mechanism by which mefloquine might predispose those with such a history to seizure remains unclear. PRESENTATION OF THE HYPOTHESIS: Studies have demonstrated that mefloquine at doses consistent with chemoprophylaxis accumulates at high levels in brain tissue, which results in altered neuronal calcium homeostasis, altered gap-junction functioning, and contributes to neuronal cell death. This paper reviews the scientific evidence associating mefloquine with alterations in neuronal function, and it suggests the novel hypothesis that among those with the prevalent EPM1 mutation, inherited and
mefloquine-induced impairments in neuronal physiologic safeguards might increase risk of GABAergic seizure during mefloquine chemoprophylaxis. Testing and implications of the hypothesis Consistent with case reports of tonic-clonic seizures occurring during mefloquine chemoprophylaxis among those with family histories of epilepsy, it is proposed here that a new contraindication to mefloquine use be recognized for people with EPM1 mutation and for those with a personal history of myoclonus or ataxia, or a family history of degenerative neurologic disorder consistent with EPM1. Recommendations and directions for future research are presented.


Status of insecticide susceptibility in Anopheles gambiae s.l. from malaria surveillance sites in The Gambia.

Betson M, Jawara M, Awolola TS.

ABSTRACT: BACKGROUND: Vector control is an effective way of reducing malaria transmission. The main vector control methods include the use of insecticide-treated bed nets and indoor residual spraying (IRS). Both interventions rely on the continuing susceptibility of Anopheles to a limited number of insecticides. However, insecticide resistance, in particular pyrethroid-DDT cross-resistance, is a challenge facing malaria vector control in Africa because pyrethroids represent the only class of insecticides approved for treating bed nets and DDT is commonly used for IRS. Here baseline data are presented on the insecticide susceptibility levels of malaria vectors prior to The Gambian indoor residual spraying intervention programme. METHODS: Anopheles larvae were collected from six malaria surveillance sites (Brikama, Essau, Farafenni, Mansakonko, Kuntaur and Basse) established by the National Malaria Control Programme and the UK Medical Research Council Laboratories in The Gambia. The mosquitoes were reared to adulthood and identified using morphological keys and a species-specific polymerase chain reaction assay. Two- to three-day old adult female mosquitoes were tested for susceptibility to permethrin, deltamethrin and DDT using standard WHO protocols, insecticide susceptibility test kits and treated papers. RESULTS: All Anopheles mosquitoes tested belonged to the Anopheles gambiae complex. Anopheles arabiensis was predominant (54.1%), followed by An. gambiae s.s. (26.1%) and Anopheles melas (19.8%). Anopheles gambiae s.s. and An. arabiensis were found at all six sites. Anopheles melas was recorded only at Brikama. Mosquitoes from two of the six sites (Brikama and Basse) were fully susceptible to all three insecticides tested. However, DDT resistance was found in An. gambiae from Essau where the 24 hours post-exposure mortality was <80% but 88% for permethrin and 92% for deltamethrin. CONCLUSIONS: This current survey of insecticide resistance in Anopheles provides baseline information for monitoring resistance in The Gambia and highlights the need for routine resistance surveillance as an integral part of the proposed nation wide IRS intervention using DDT.

Malar J. 2009 Aug 5;8(1):186. [Epub ahead of print]

Defining the relationship between Plasmodium falciparum parasite rate and clinical disease: statistical models for disease burden estimation.

Patil AP, Okiro EA, Gething PW, Guerra CA, Sharma SK, Snow RW, Hay SI.

ABSTRACT: BACKGROUND: Clinical malaria has proven an elusive burden to enumerate. Many cases go undetected by routine disease recording systems. Epidemiologists
have, therefore, frequently defaulted to actively measuring malaria in population cohorts through time. Measuring the clinical incidence of malaria longitudinally is labour-intensive and impossible to undertake universally. There is a need, therefore, to define a relationship between clinical incidence and the easier and more commonly measured index of infection prevalence: the "parasite rate". This relationship can help provide an informed basis to define malaria burdens in areas where health statistics are inadequate. METHODS: Formal literature searches were conducted for Plasmodium falciparum malaria incidence surveys undertaken prospectively through active case detection at least every 14 days. The data were abstracted, standardized and geo-referenced. Incidence surveys were time-space matched with modelled estimates of infection prevalence derived from a larger database of parasite prevalence surveys and modelling procedures developed for a global malaria endemicity map. Several potential relationships between clinical incidence and infection prevalence were then specified in a non-parametric Gaussian process model with minimal, biologically informed, prior constraints. Bayesian inference was then used to choose between the candidate models. RESULTS: The suggested relationships with credible intervals are shown for the Africa and a combined America and Central and South East Asia regions. In both regions clinical incidence increased slowly and smoothly as a function of infection prevalence. In Africa, when infection prevalence exceeded 40%, clinical incidence reached a plateau of 500 cases per thousand of the population per annum. In the combined America and Central and South East Asia regions, this plateau was reached at 250 cases per thousand of the population per annum. A temporal volatility model was also incorporated to facilitate a closer description of the variance in the observed data. CONCLUSION: It was possible to model a relationship between clinical incidence and P. falciparum infection prevalence but the best-fit models were very noisy reflecting the large variance within the observed opportunistic data sample. This continuous quantification allows for estimates of the clinical burden of P. falciparum of known confidence from wherever an estimate of P. falciparum prevalence is available.

Malar J. 2009 Aug 5;8:185.

**Spatial malaria epidemiology in Bangladeshi highlands.**


International Center for Diarrhoeal Disease Research Bangladesh, Dhaka, Bangladesh. ubydu1@icddrb.org

BACKGROUND: Malaria is a major public health burden in the south-eastern part of Bangladesh, particularly in the Chittagong Hill Tracts region. In 2007, BRAC and ICDDR,B carried out a malaria prevalence survey in the endemic regions including the Khagrachari District. METHODS: This study was done to detect clusters of malaria and identify the geographic risk factors. Thirty mauzas (the lowest administrative unit/bigger than village in Bangladesh that has polygon boundary) from the area were selected for the survey using probability proportional to size (PPS) sampling. Twenty-five households within each mauza were then randomly selected for screening, with a GPS point being recorded at each household. Rapid diagnostic tests were used to diagnose malaria. RESULTS: The average malaria prevalence in the District was 15.47% (n = 750). SaTScan detected five geographic clusters of malaria, one of which was highly significant (p = 0.001). Malaria cases were significantly associated with proximity to water bodies and forests. CONCLUSION: The data presented in this paper are the first step to understanding malaria in southeastern Bangladesh from a micro-geographic perspective. The study results suggest that there are 'malaria hot-spots' in the study area. The
government of Bangladesh and non-governmental organizations involved in malaria control should consider these results when planning malaria control measures. In particular, malaria maps should be updated on a regular basis as new data become available.

Malar J. 2009 Aug 4;8:184.

Sequence variation of PfEMP1-DBLalpha in association with rosette formation in Plasmodium falciparum isolates causing severe and uncomplicated malaria.

Horata N, Kalambaheti T, Craig A, Khusmith S.

Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. h_natharinee@hotmail.com

BACKGROUND: Rosetting and cytoadherence of Plasmodium falciparum-infected red blood cells have been associated with severity of malaria. ICAM-1 and CD36 are the main host cell receptors, while PfEMP1-DBLalpha is a major parasite ligand, which can contribute to rosette formation. This study is aimed at demonstrating whether the highly polymorphic PfEMP1-DBLalpha sequences occurring among Thai isolates causing severe and uncomplicated malaria are associated with their ability to form rosettes and reflected the clinical outcome of the patients.

METHODS: Two hundred and ninety five PfEMP1-DBLalpha sequences from Thai clinical isolates causing severe and uncomplicated malaria were evaluated by sequencing and direct comparison using the specific text string analysis functions in Microsoft Excel and Perl. The relationships between the PfEMP1-DBLalpha sequences were also analysed by network analysis. The binding abilities of parasitized red blood cells (PRBCs) to CD36, wild type ICAM-1, ICAM-1Kilifi and ICAM-1S22/A under static condition were included.

RESULTS: Two hundred and eighty one non-identical amino acid sequences were identified (< 95% sequence identity). When the distributions of semi-conserved features (PoLV1-4 and sequence group) within the rosetting domain PfEMP1-DBLalpha were observed, close similarity was found between isolates from the two disease groups. The sequence group 1 representing uncomplicated malaria was significantly different from the sequence group 3 representing the majority of severe malaria (p = 0.027). By using a simple non-phylogenetic approach to visualize the sharing of polymorphic blocks (position specific polymorphic block, PSPB) and cys/PoLV among DBLalpha sequences, the sequence group 1 was split from the other five sequence groups. The isolates belonging to sequence group 5 gave the highest mean rosetting rate (21.31%). However, within sequence group 2 and group 6, the isolates causing severe malaria had significantly higher rosetting rate than those causing uncomplicated malaria (p = 0.014, p = 0.007, respectively).

CONCLUSION: This is the first report of PfEMP1-DBLalpha analysis in clinical Thai isolates using semi-conserved features (cys/PoLV and PSPBs). The cys/PoLV group 5 gave the highest rosetting rate. PfEMP1-DBLalpha domains in Thai isolates are highly diverse, however, clinical isolates from severe and uncomplicated malaria shared common sequences.

Malar J. 2009 Aug 3;8:183.

MSP-1p42-specific antibodies affect growth and development of intra-erythrocytic parasites of Plasmodium falciparum.

Bergmann-Leitner ES, Duncan EH, Angov E.

US Military Malaria Vaccine Program, Walter Reed Army Institute of Research,
BACKGROUND: Antibodies are the main effector molecules in the defense against blood stages of the malaria parasite Plasmodium falciparum. Understanding the mechanisms by which vaccine-induced anti-blood stage antibodies work in protecting against malaria is essential for vaccine design and testing. METHODS: The effects of MSP-1p42-specific antibodies on the development of blood stage parasites were studied using microscopy, flow cytometry and the pLDH assay. To determine allele-specific effects, if present, allele-specific antibodies and the various parasite clones representative of these alleles of MSP-1 were employed. RESULTS: The mode of action of anti-MSP-1p42 antibodies differs among the parasite clones tested: anti-MSP-1p42 sera act mainly through invasion-inhibitory mechanisms against FVO parasites, by either preventing schizonts from rupturing or agglutinating merozoites upon their release. The same antibodies do not prevent the rupture of 3D7 schizonts; instead they agglutinate merozoites and arrest the development of young parasites at the early trophozoite stage, thus acting through both invasion- and growth inhibitory mechanisms. The second key finding is that antibodies have access to the intra-erythrocytic parasite, as evidenced by the labeling of developing merozoites with fluorochrome-conjugated anti-MSP-1p42 antibodies. Access to the parasite through this route likely allows antibodies to exert their inhibitory activities on the maturing schizonts leading to their inability to rupture and be released as infectious merozoites. CONCLUSION: The identification of various modes of action by which anti-MSP-1 antibodies function against the parasite during erythrocytic development emphasizes the importance of functional assays for evaluating malaria vaccines and may also open new avenues for immunotherapy and vaccine development.

Publication Types:
  Research Support, U.S. Gov't, Non-P.H.S.

PMID: 19650894 [PubMed - in process]

60: Malar J. 2009 Aug 1;8:182.

Implementation of a novel PCR based method for detecting malaria parasites from naturally infected mosquitoes in Papua New Guinea.

Hasan AU, Suguri S, Sattabongkot J, Fujimoto C, Amakawa M, Harada M, Ohmae H.

Department of International Medical Zoology, Faculty of Medicine, Kagawa University, 1750-1, Ikenobe, Miki, Kita, Kagawa, 761-0793 Japan.
ahasan@med.kagawa-u.ac.jp

BACKGROUND: Detection of Plasmodium species in mosquitoes is important for designing vector control studies. However, most of the PCR-based detection methods show some potential limitations. The objective of this study was to introduce an effective PCR-based method for detecting Plasmodium vivax and Plasmodium falciparum from the field-caught mosquitoes of Papua New Guinea. METHODS: A method has been developed to concurrently detect mitochondrial cytochrome b (Cyt b) of four human Plasmodium species using PCR (Cytb-PCR). To particularly discriminate P. falciparum from P. vivax, Plasmodium ovale and Plasmodium malariae, a polymerase chain reaction-repeated fragment length polymorphism (PCR-RFLP) has further been developed to use with this method. However, due to limited samples number of P. ovale and P. malariae; this study was mainly confined to P. vivax and P. falciparum. The efficiency of Cytb-PCR was evaluated by comparing it with two 'gold standards' enzyme linked immunosorbent
assay specific for circumsporozoite protein (CS-ELISA) using artificially infected mosquitoes; and nested PCR specific for small subunit ribosomal RNA (SSUrRNA) using field caught mosquitoes collected from three areas (Kaboibus, Wingei, and Jawia) of the East Sepic Province of Papua New Guinea. RESULTS: A total of 90 mosquitoes were artificially infected with three strains of Plasmodium: P. vivax-210 (n = 30), P. vivax-247 (n = 30) and P. falciparum (n = 30). These infected mosquitoes along with another 32 unfed mosquitoes were first checked for the presence of Plasmodium infection by CS-ELISA, and later the same samples were compared with the Cytb-PCR. CS-ELISA for P. vivax-210, P. vivax-247 and P. falciparum detected positive infection in 30, 19 and 18 mosquitoes respectively; whereas Cytb-PCR detected 27, 16 and 16 infections, respectively. The comparison revealed a close agreement between the two assays (kappa = 0.862, 0.842 and 0.894, respectively for Pv-210, Pv-247 and P. falciparum groups). It was found that the eight CS-ELISA-positive mosquitoes detected negative by Cytb-PCR were false-positive results. The lowest detection limit of this Cytb-PCR was 10 sporozoites. A highly concordance result was also found between nested PCR and Cytb-PCR using 107 field caught mosquitoes, and both tests concordantly detected P. falciparum in an Anopheles punctulatus mosquito collected from Kaboibus. Both tests thus suggested an overall sporozoite rate of 0.9% (1/107) in the study areas. Subsequently, PCR-RFLP efficiently discriminated P. falciparum from P. vivax for all of the Cytb-PCR positive samples. CONCLUSION: A single step PCR based method has been introduced here that is highly sensitive, efficient and reliable for identifying P. vivax and P. falciparum from mosquitoes. The reliability of the technique was confirmed by its ability to detect Plasmodium as efficiently as those of CS-ELISA and nested PCR. Application of the assay offers the opportunity to detect vector species of Papua New Guinea and may contribute for designing further vector control programmes.

Malar J. 2009 Jul 31;8:180.

Space-time variation of malaria incidence in Yunnan province, China.

Clements AC, Barnett AG, Cheng ZW, Snow RW, Zhou HN.

University of Queensland, School of Population Health, Queensland, Australia. a.clements@uq.edu.au

BACKGROUND: Understanding spatio-temporal variation in malaria incidence provides a basis for effective disease control planning and monitoring. METHODS: Monthly surveillance data between 1991 and 2006 for Plasmodium vivax and Plasmodium falciparum malaria across 128 counties were assembled for Yunnan, a province of China with one of the highest burdens of malaria. County-level Bayesian Poisson regression models of incidence were constructed, with effects for rainfall, maximum temperature and temporal trend. The model also allowed for spatial variation in county-level incidence and temporal trend, and dependence between incidence in June-September and the preceding January-February. RESULTS: Models revealed strong associations between malaria incidence and both rainfall and maximum temperature. There was a significant association between incidence in June-September and the preceding January-February. Raw standardised morbidity ratios showed a high incidence in some counties bordering Myanmar, Laos and Vietnam, and counties in the Red River valley. Clusters of counties in south-western and northern Yunnan were identified that had high incidence not explained by climate. The overall trend in incidence decreased, but there was significant variation between counties. CONCLUSION: Dependence between incidence in summer and the preceding January-February suggests a role of intrinsic host-pathogen dynamics. Incidence during the summer peak might be predictable
based on incidence in January-February, facilitating malaria control planning, scaled months in advance to the magnitude of the summer malaria burden. Heterogeneities in county-level temporal trends suggest that reductions in the burden of malaria have been unevenly distributed throughout the province.


**Plasmodium falciparum enolase: stage-specific expression and sub-cellular localization.**

Pal Bhowmick I, Kumar N, Sharma S, Coppens I, Jarori GK.

ABSTRACT: BACKGROUND: In an earlier study, it was observed that the vaccination with Plasmodium falciparum enolase can confer partial protection against malaria in mice. Evidence has also build up to indicate that enolases may perform several non-glycolytic functions in pathogens. Investigating the stage-specific expression and sub-cellular localization of a protein may provide insights into its moonlighting functions. METHODS: Sub-cellular localization of P. falciparum enolase was examined using immunofluorescence assay, immuno-gold electron microscopy and western blotting. RESULTS: Enolase protein was detected at every stage in parasite life cycle examined. In asexual stages, enolase was predominantly (greater then or equal to 85-90%) present in soluble fraction, while in sexual stages it was mostly associated with particulate fraction. Apart from cytosol, enolase was found to be associated with nucleus, food vacuole, cytoskeleton and plasma membrane. CONCLUSIONS: Diverse localization of enolase suggests that apart from catalyzing the conversion of 2-phosphoglyceric acid into phosphoenolpyruvate in glycolysis, enolase may be involved in a host of other biological functions. For instance, enolase localized on the merozoite surface may be involved in red blood cell invasion; vacuolar enolase may be involved in food vacuole formation and/or development; nuclear enolase may play a role in transcription.


**Discovery: an interactive resource for the rational selection and comparison of putative drug target proteins in malaria.**

Joubert F, Harrison CM, Koegelenberg RJ, Odendaal CJ, de Beer TA.

Bioinformatics and Computational Biology Unit, Department of Biochemistry, University of Pretoria, Pretoria, South Africa. fourie.joubert@up.ac.za

BACKGROUND: Up to half a billion human clinical cases of malaria are reported each year, resulting in about 2.7 million deaths, most of which occur in sub-Saharan Africa. Due to the over-and misuse of anti-malarials, widespread resistance to all the known drugs is increasing at an alarming rate. Rational methods to select new drug target proteins and lead compounds are urgently needed. The Discovery system provides data mining functionality on extensive annotations of five malaria species together with the human and mosquito hosts, enabling the selection of new targets based on multiple protein and ligand properties. METHODS: A web-based system was developed where researchers are able to mine information on malaria proteins and predicted ligands, as well as perform comparisons to the human and mosquito host characteristics. Protein features used include: domains, motifs, EC numbers, GO terms, orthologs, protein-protein interactions, protein-ligand interactions and host-pathogen interactions among others. Searching by chemical structure is also available. RESULTS: An in silico
system for the selection of putative drug targets and lead compounds is presented, together with an example study on the bifunctional DHFR-TS from Plasmodium falciparum. CONCLUSION: The Discovery system allows for the identification of putative drug targets and lead compounds in Plasmodium species based on the filtering of protein and chemical properties.

Malar J. 2009 Jul 30;8:177.

Chloroquine-resistant Plasmodium vivax malaria in Serbo town, Jimma zone, south-west Ethiopia.

Ketema T, Bacha K, Birhanu T, Petros B.

Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia. tsiqeketema@gmail.com

BACKGROUND: Ethiopia has the highest proportion of vivax malaria, approximately 40% of all malaria infections, in contrast to African countries. Chloroquine (CQ) is the drug of choice for the treatment of Plasmodium vivax infection in the country, although CQ resistant P. vivax (CRPv) has started to challenge the efficacy of the drug. The present study was conducted to assess the current status of CRPv at Serbo, Jimma zone, south-west Ethiopia. METHODS: A 28-day in vivo therapeutic efficacy test was conducted from October 2007 to January 2008. Recurrence of parasitaemia and the clinical condition of patients were assessed on each visit during the follow-up. The levels of haemoglobin (Hb) in the study participants were determined. The patients' blood drug levels were measured using HPLC. Data was analysed using SPSS for windows version 10.0. HPLC data was computed using Chem Station for LC 3D systems software. RESULTS: Of the total 84 patients included in the study, 78 completed their 28-day follow-up, six of whom being excluded for different reasons. In three children (aged 7, 12 and 13 years), parasitaemia reappeared within the 28-days follow-up in spite of adequate absorption of the drug and absence of malaria symptom. In addition, on the day of recurrence of parasitaemia the levels of chloroquine-desethylchloroquine (CQ-DCQ) were above the minimum effective concentration (>or=100 etag/ml) in all the three cases, showing that treatment failure could not be attributed to low level of drug in the patients blood. CONCLUSION: Reappearance of the parasite within the 28 days of follow-up is due to parasite resistance to CQ. The 3.6% (95% CI = -0.038 - 0.0758) prevalence of CRPv malaria in the study area signals the need for launching monitory activities for CQ resistant P. vivax. Moreover, as former report from the same country, Debrezeit, also showed the occurrence of CRPv, survey on CRPv malaria should be made in P. vivax endemic areas in order to estimate the level of burden across the country.

Malar J. 2009 Jul 29;8:176.

Treatment of malaria from monotherapy to artemisinin-based combination therapy by health professionals in urban health facilities in Yaoundé, central province, Cameroon.

Sayang C, Gausseres M, Vernazza-Licht N, Malvy D, Bley D, Millet P.

Department of Tropical Medicine, Centre René Labusquière, University of Bordeaux 2, Bordeaux, France. sayangcollins@yahoo.fr

BACKGROUND: After adoption of artesunate-amodiaquine (AS/AQ) as first-line therapy for the treatment of uncomplicated malaria by the malaria control
programme, this study was designed to assess the availability of anti-malarial drugs, treatment practices and acceptability of the new protocol by health professionals, in the urban health facilities and drugstores of Yaoundé city, Cameroon. METHODS: Between April and August 2005, retrospective and current information was collected by consulting registers and interviewing health practitioners in urban health facilities using a structured questionnaire. RESULTS: In 2005, twenty-seven trade-named drugs have been identified in drugstores; quinine tablets (300 mg) were the most affordable anti-malarial drugs. Chloroquine was restricted to food market places and no generic artemisinin derivative was available in public health centres. In public health facilities, 13.6% of health professionals were informed about the new guidelines; 73.5% supported the use of AS-AQ as first-line therapy. However, 38.6% apprehended its use due to adverse events attributed to amodiaquine. Malaria treatment was mainly based on the diagnosis of fever. Quinine (300 mg tablets) was the most commonly prescribed first-line anti-malarial drug in adults (44.5%) and pregnant women (52.5%). Artequin was the most cited artemisinin-based combination therapy (ACT) (9.9%). Medical sales representatives were the main sources of information on anti-malarials. CONCLUSION: The use of AS/AQ was not implemented in 2005 in Yaoundé, despite the wide range of anti-malarials and trade-named artemisinin derivatives available. Nevertheless, medical practitioners will support the use of this combination, when it is available in a paediatric formulation, at an affordable price. Training, information and participation of health professionals in decision-making is one of the key elements to improve adherence to new protocol guidelines. This baseline information will be useful to monitor progress in ACT implementation in Cameroon.

Malar J. 2009 Jul 29;8:175.

Decreased motivation in the use of insecticide-treated nets in a malaria endemic area in Burkina Faso.

Toé LP, Skovmand O, Dabiré KR, Diabaté A, Diallo Y, Guiguemdé TR, Doannio JM, Akogbeto M, Baldet T, Gruénais ME.

Institut de Recherche en Science de Santé/Centre Muraz, BP 390, Bobo-Dioulasso, Burkina Faso. lea_toe@yahoo.com

BACKGROUND: The use of insecticide-treated nets (ITN) is an important tool in the Roll Back Malaria (RBM) strategy. For ITNs to be effective they need to be used correctly. Previous studies have shown that many factors, such as wealth, access to health care, education, ethnicity and gender, determine the ownership and use of ITNs. Some studies showed that free distribution and public awareness campaigns increased the rate of use. However, there have been no evaluations of the short- and long-term impact of such motivation campaigns. A study carried out in a malaria endemic area in south-western Burkina Faso indicated that this increased use declined after several months. The reasons were a combination of the community representation of malaria, the perception of the effectiveness and usefulness of ITNs and also the manner in which households are organized by day and by night. METHODS: PermaNet 2.0 and Olyset were distributed in 455 compounds at the beginning of the rainy season. The community was educated on the effectiveness of nets in reducing malaria and on how to use them. To assess motivation, qualitative tools were used: one hundred people were interviewed, two hundred houses were observed directly and two houses were monitored monthly throughout one year. RESULTS: The motivation for the use of bednets decreased after less than a year. Inhabitants' conception of malaria and the inconvenience of using bednets in small houses were the major reasons. Acceptance that ITNs
were useful in reducing malaria was moderated by the fact that mosquitoes were considered to be only one of several factors which caused malaria. The appropriate and routine use of ITNs was adversely affected by the functional organization of the houses, which changed as between day and night. Bednets were not used when the perceived benefits of reduction in mosquito nuisance and of malaria were considered not to be worth the inconvenience of daily use.

CONCLUSION: In order to bridge the gap between possession and use of bednets, concerted efforts are required to change behaviour by providing accurate information, most particularly by convincing people that mosquitoes are the only source of malaria, whilst recognising that there are other diseases with similar symptoms, caused in other ways. The medical message must underline the seriousness of malaria and the presence of the malaria vector in the dry season as well as the wet, in order to encourage the use of bednets whenever transmission can occur. Communities would benefit from impregnated bednets and other vector control measures being better adapted to their homes, thus reducing the inconvenience of their use.

*Malar J. 2009 Jul 29;8:174.*

**Treatment of malaria from monotherapy to artemisinin-based combination therapy by health professionals in rural health facilities in southern Cameroon.**

Sayang C, Gausseres M, Vernazza-Licht N, Malvy D, Bley D, Millet P.

Department of Tropical Medicine, Centre René Labusquière, University of Bordeaux 2, Bordeaux, France. sayangcollins@yahoo.fr

BACKGROUND: One year after the adoption of artesunate-amodiaquine (AS/AQ) as first-line therapy for the treatment of uncomplicated malaria, this study was designed to assess the treatment practices regarding anti-malarial drugs at health facilities in four rural areas in southern Cameroon. METHODS: Between April and August 2005, information was collected by interviewing fifty-two health professionals from twelve rural health facilities, using a structured questionnaire. RESULTS: In 2005, only three anti-malarial drugs were used in rural health facilities, including: amodiaquine, quinine and sulphadoxine-pyrimethamine. Only 2.0% of the health professionals prescribed the recommended AS/AQ combination. After reading the treatment guidelines, 75.0% were in favour of the treatment protocol with the following limitations: lack of paediatric formulations, high cost and large number of tablets per day. Up to 21.0% of professionals did not prescribe AS/AQ because of the level of adverse events attributed to the use of amodiaquine as monotherapy. CONCLUSION: The present study indicates that AS/AQ was not available in the public health facilities at the time of the study, and health practitioners were not informed about the new treatment guidelines. Results of qualitative analysis suggest that prescribers should be involved as soon as possible in projects related to the optimization of treatment guidelines and comply with new drugs. Adapted formulations should be made available at the international level and implemented locally before new drugs and treatments are proposed through a national control programme. This baseline information will be useful to monitor progresses in the implementation of artemisinin-based combination therapy in Cameroon.

*Malar J. 2009 Jul 29;8:173.*

**Knowledge on the transmission, prevention and treatment of malaria among two endemic populations of Bangladesh and their health-seeking behaviour.**
Ahmed SM, Haque R, Haque U, Hossain A.
BRAC Research and Evaluation Division, BRAC Centre, 75 Mohakhali, Dhaka-1212 Bangladesh. ahmed.sm@brac.net

BACKGROUND: Data on sociological and behavioural aspects of malaria, which is essential for an evidence-based design of prevention and control programmes, is lacking in Bangladesh. This paper attempts to fill this knowledge gap by using data from a population-based prevalence survey conducted during July to November 2007, in 13 endemic districts of Bangladesh. METHODS: A two-stage cluster sampling technique was used to select study respondents randomly from 30 mauzas in each district for the socio-behavioural inquiry (n = 9,750). A pre-tested, semi-structured questionnaire was used to collect data in face-to-face interview by trained interviewers, after obtaining informed consent. RESULTS: The overall malaria prevalence rate in the 13 endemic districts was found to be 3.1% by the Rapid Diagnostic Test 'FalciVax' (P. falciparum 2.73%, P. vivax 0.16% and mixed infection 0.19%), with highest concentration in the three hill districts (11%). Findings revealed superficial knowledge on malaria transmission, prevention and treatment by the respondents. Poverty and level of schooling were found as important determinants of malaria knowledge and practices. Allopathic treatment was uniformly advocated, but the 'know-do' gap became especially evident when in practice majority of the ill persons either did not seek any treatment (31%) or practiced self-treatment (12%). Of those who sought treatment, the majority went to the village doctors and drugstore salespeople (around 40%). Also, there was a delay beyond twenty-four hours in beginning treatment of malaria-like fever in more than half of the instances. In the survey, gender divide in knowledge and health-seeking behaviour was observed disfavouring women. There was also a geographical divide between the high endemic south-eastern area and the low-endemic north-eastern area, the former being disadvantaged with respect to different aspects of malaria studied. CONCLUSION: The respondents in this study lacked comprehensive knowledge on different aspects of malaria, which was influenced by level of poverty and education. A gender and geographical divide in knowledge was observed disfavouring women and south-eastern area respectively. They preferred allopathic treatment for malaria, although a substantial proportion did not seek any treatment or sought self-treatment for malaria-like fever. Delay in seeking care was common. The implications of these findings for programme development are discussed.


A stratified random survey of the proportion of poor quality oral artesunate sold at medicine outlets in the Lao PDR - implications for therapeutic failure and drug resistance.


ABSTRACT: BACKGROUND: Counterfeit oral artesunate has been a major public health problem in mainland SE Asia, impeding malaria control. A countrywide stratified random survey was performed to determine the availability and quality of oral artesunate in pharmacies and outlets (shops selling medicines) in the Lao PDR (Laos). METHODS: In 2003, 'mystery' shoppers were asked to buy artesunate tablets from 180 outlets in 12 of the 18 Lao provinces. Outlets were selected using stratified random sampling by investigators not involved in sampling. Samples were analysed for packaging characteristics, by the Fast Red Dye test,
high-performance liquid chromatography (HPLC), mass spectrometry (MS), X-ray diffractometry and pollen analysis. RESULTS: Of 180 outlets sampled, 25 (13.9%) sold oral artesunate. Outlets selling artesunate were more commonly found in the more malarious southern Laos. Of the 25 outlets, 22 (88%; 95%CI 68-97%) sold counterfeit artesunate, as defined by packaging and chemistry. No artesunate was detected in the counterfeits by any of the chemical analysis techniques and analysis of the packaging demonstrated seven different counterfeit types. There was complete agreement between the Fast Red dye test, HPLC and MS analysis. A wide variety of wrong active ingredients were found by MS. Of great concern, 4/27 (14.8%) fakes contained detectable amounts of artemisinin (0.26-115.7mg/tablet). CONCLUSION: This random survey confirms results from previous convenience survey that counterfeit artesunate is a severe public health problem. The presence of artemisinin in counterfeits may encourage malaria resistance to artemisinin derivatives. With increasing accessibility of artemisinin-derivative combination therapy (ACT) in Laos, the removal of artesunate monotherapy from pharmacies may be an effective intervention.

Malar J. 2009 Jul 28;8:171.

Major variations in malaria exposure of travellers in rural areas: an entomological cohort study in western Côte d'Ivoire.


Unité d'Entomologie Médicale-Unité mixte de Recherche 6236, Institut de Médecine Tropicale du Service de Santé des Armées (IMTSSA), Marseille, France. eve.pradines@yahoo.fr

BACKGROUND: Malaria remains a major threat, to both travellers and military personnel deployed to endemic areas. The recommendations for travellers given by the World Health Organization is based on the incidence of malaria in an area and do not take the degree of exposure into account. The aim of this article is to evaluate the exposure of travellers by entomologic methods, which are the commonly used measures of the intensity of malaria transmission. METHODS: From February 2004 to June 2004, five groups of 30 military personnel were stationed in up to 10 sites in western Côte d'Ivoire, from one week to several months. Adult mosquitoes were collected by human landing catches at each site during the five months and the level of exposure to malaria transmission of each group was estimated. RESULTS: The level of transmission varied from one site to another one from less than one to approximately more than 100 infective bites per month. In the majority of sites, at least two anopheline species were involved in transmission. The cumulative EIR over the study period varied according to the groups from 29 infected bites per person/per mission to 324. CONCLUSION: The level of malaria transmission and malaria risk varies widely (varying by a factor of eleven) between groups of travellers travelling in the same region and at the same time. Physicians involved in travel medicine or supporting expatriated populations or refugees should consider this heterogeneity and emphasize the importance of combining appropriate measures, such as chemoprophylaxis and protective measures against mosquitoes.


Artemisinin resistance in Plasmodium falciparum malaria.

Dondorp AM, Nosten F, Yi P, Das D, Phyio AP, Tarning J, Lwin KM, Ariey F,
BACKGROUND: Artemisinin-based combination therapies are the recommended first-line treatments of falciparum malaria in all countries with endemic disease. There are recent concerns that the efficacy of such therapies has declined on the Thai-Cambodian border, historically a site of emerging antimalarial-drug resistance. METHODS: In two open-label, randomized trials, we compared the efficacies of two treatments for uncomplicated falciparum malaria in Pailin, western Cambodia, and Wang Pha, northwestern Thailand: oral artesunate given at a dose of 2 mg per kilogram of body weight per day, for 7 days, and artesunate given at a dose of 4 mg per kilogram per day, for 3 days, followed by mefloquine at two doses totaling 25 mg per kilogram. We assessed in vitro and in vivo Plasmodium falciparum susceptibility, artesunate pharmacokinetics, and molecular markers of resistance. RESULTS: We studied 40 patients in each of the two locations. The overall median parasite clearance times were 84 hours (interquartile range, 60 to 96) in Pailin and 48 hours (interquartile range, 36 to 66) in Wang Pha (P<0.001). Recrudescence confirmed by means of polymerase-chain-reaction assay occurred in 6 of 20 patients (30%) receiving artesunate monotherapy and 1 of 20 (5%) receiving artesunate-mefloquine therapy in Pailin, as compared with 2 of 20 (10%) and 1 of 20 (5%), respectively, in Wang Pha (P=0.31). These markedly different parasitologic responses were not explained by differences in age, artesunate or dihydroartemisinin pharmacokinetics, results of isotopic in vitro sensitivity tests, or putative molecular correlates of P. falciparum drug resistance (mutations or amplifications of the gene encoding a multidrug resistance protein [PfMDR1] or mutations in the gene encoding sarco-endoplasmic reticulum calcium ATPase6 [PfSERCA]). Adverse events were mild and did not differ significantly between the two treatment groups. CONCLUSIONS: P. falciparum has reduced in vivo susceptibility to artesunate in western Cambodia as compared with northwestern Thailand. Resistance is characterized by slow parasite clearance in vivo without corresponding reductions on conventional in vitro susceptibility testing. Containment measures are urgently needed. (ClinicalTrials.gov number, NCT00493363, and Current Controlled Trials number, ISRCTN64835265.) 2009 Massachusetts Medical Society


Comment in:


Center for Global Health and Diseases, Case Western Reserve University, Cleveland, Ohio, USA.

BACKGROUND: Malaria in pregnancy can expose the fetus to malaria-infected erythrocytes or their soluble products, thereby stimulating T and B cell immune responses to malaria blood stage antigens. We hypothesized that fetal immune
priming, or malaria exposure in the absence of priming (putative tolerance), affects the child’s susceptibility to subsequent malaria infections. METHODS AND FINDINGS: We conducted a prospective birth cohort study of 586 newborns residing in a malaria-holoendemic area of Kenya who were examined biannually to age 3 years for malaria infection, and whose malaria-specific cellular and humoral immune responses were assessed. Newborns were classified as (i) sensitized (and thus exposed), as demonstrated by IFNgamma, IL-2, IL-13, and/or IL-5 production by cord blood mononuclear cells (CBMCs) to malaria blood stage antigens, indicative of in utero priming (n = 246), (ii) exposed not sensitized (mother Plasmodium falciparum [Pf]+ and no CBMC production of IFNgamma, IL-2, IL-13, and/or IL-5, n = 120), or (iii) not exposed (mother Pf-, no CBMC reactivity, n = 220). Exposed not sensitized children had evidence for prenatal immune experience demonstrated by increased IL-10 production and partial reversal of malaria antigen-specific hyporesponsiveness with IL-2+IL-15, indicative of immune tolerance. Relative risk data showed that the putatively tolerant children had a 1.61 (95% confidence interval [CI] 1.10-2.43; p = 0.024) and 1.34 (95% CI 0.95-1.87; p = 0.097) greater risk for malaria infection based on light microscopy (LM) or PCR diagnosis, respectively, compared to the not-exposed group, and a 1.41 (95% CI 0.97-2.07, p = 0.074) and 1.39 (95% CI 0.99-2.07, p = 0.053) greater risk of infection based on LM or PCR diagnosis, respectively, compared to the sensitized group. Putatively tolerant children had an average of 0.5 g/dl lower hemoglobin levels (p = 0.01) compared to the other two groups. Exposed not sensitized children also had 2- to 3-fold lower frequency of malaria antigen-driven IFNgamma and/or IL-2 production (p<0.001) and higher IL-10 release (p<0.001) at 6-month follow-ups, when compared to sensitized and not-exposed children. Malaria blood stage-specific IgG antibody levels were similar among the three groups. CONCLUSIONS: These results show that a subset of children exposed to malaria in utero acquire a tolerant phenotype to blood-stage antigens that persists into childhood and is associated with an increased susceptibility to malaria infection and anemia. This finding could have important implications for malaria vaccination of children residing in endemic areas.


Preerythrocytic, live-attenuated Plasmodium falciparum vaccine candidates by design.


Seattle Biomedical Research Institute, Seattle, WA 98109, USA.

Falciparum malaria is initiated when Anopheles mosquitoes transmit the Plasmodium sporozoite stage during a blood meal. Irradiated sporozoites confer sterile protection against subsequent malaria infection in animal models and humans. This level of protection is unmatched by current recombinant malaria vaccines. However, the live-attenuated vaccine approach faces formidable obstacles, including development of accurate, reproducible attenuation techniques. We tested whether Plasmodium falciparum could be attenuated at the early liver stage by genetic engineering. The P. falciparum genetically attenuated parasites (GAPs) harbor individual deletions or simultaneous deletions of the sporozoite-expressed genes P52 and P36. Gene deletions were done by double-cross-over recombination to avoid genetic reversion of the knockout parasites. The gene deletions did not affect parasite replication throughout the erythrocytic cycle, gametocyte production, mosquito infections, and sporozoite production rates. However, the
deletions caused parasite developmental arrest during hepatocyte infection. The double-gene deletion line exhibited a more severe intrahepatocytic growth defect compared with the single-gene deletion lines, and it did not persist. This defect was assessed in an in vitro liver-stage growth assay and in a chimeric mouse model harboring human hepatocytes. The strong phenotype of the double knockout GAP justifies its human testing as a whole-organism vaccine candidate using the established sporozoite challenge model. GAPs might provide a safe and reproducible platform to develop an efficacious whole-cell malaria vaccine that prevents infection at the preerythrocytic stage.


Understanding the link between malaria risk and climate.

Paaijmans KP, Read AF, Thomas MB.

Center for Infectious Disease Dynamics;

The incubation period for malaria parasites within the mosquito is exquisitely temperature-sensitive, so that temperature is a major determinant of malaria risk. Epidemiological models are increasingly used to guide allocation of disease control resources and to assess the likely impact of climate change on global malaria burdens. Temperature-based malaria transmission is generally incorporated into these models using mean monthly temperatures, yet temperatures fluctuate throughout the diurnal cycle. Here we use a thermodynamic malaria development model to demonstrate that temperature fluctuation can substantially alter the incubation period of the parasite, and hence malaria transmission rates. We find that, in general, temperature fluctuation reduces the impact of increases in mean temperature. Diurnal temperature fluctuation around means >21 degrees C slows parasite development compared with constant temperatures, whereas fluctuation around <21 degrees C speeds development. Consequently, models which ignore diurnal variation overestimate malaria risk in warmer environments and underestimate risk in cooler environments. To illustrate the implications further, we explore the influence of diurnal temperature fluctuation on malaria transmission at a site in the Kenyan Highlands. Based on local meteorological data, we find that the annual epidemics of malaria at this site cannot be explained without invoking the influence of diurnal temperature fluctuation. Moreover, while temperature fluctuation reduces the relative influence of a subtle warming trend apparent over the last 20 years, it nonetheless makes the effects biologically more significant. Such effects of short-term temperature fluctuations have not previously been considered but are central to understanding current malaria transmission and the consequences of climate change.


The origin of malignant malaria.


Laboratory of Medical Zoology, Division of Entomology (PSIS), University of Massachusetts, Amherst, MA 01003;

Plasmodium falciparum, the causative agent of malignant malaria, is among the most severe human infectious diseases. The closest known relative of P. falciparum is a chimpanzee parasite, Plasmodium reichenowi, of which one single
isolate was previously known. The co-speciation hypothesis suggests that both parasites evolved separately from a common ancestor over the last 5-7 million years, in parallel with the divergence of their hosts, the hominin and chimpanzee lineages. Genetic analysis of eight new isolates of P. reichenowi, from wild and wild-born captive chimpanzees in Cameroon and Côte d'Ivoire, shows that P. reichenowi is a geographically widespread and genetically diverse chimpanzee parasite. The genetic lineage comprising the totality of global P. falciparum is fully included within the much broader genetic diversity of P. reichenowi. This is inconsistent with the co-speciation hypothesis. Phylogenetic analysis indicates that all extant P. falciparum populations originated from P. reichenowi, likely by a single host transfer, which may have occurred as early as 2-3 million years ago, or as recently as 10,000 years ago. The evolutionary history of this relationship may be explained by two critical genetic mutations. First, inactivation of the CMAH gene in the human lineage rendered human ancestors unable to generate the sialic acid Neu5Gc from its precursor Neu5Ac, and likely made humans resistant to P. reichenowi. More recently, mutations in the dominant invasion receptor EBA 175 in the P. falciparum lineage provided the parasite with preference for the overabundant Neu5Ac precursor, accounting for its extreme human pathogenicity.


Comment in:

Malaria and HIV co-infection in pregnancy in sub-Saharan Africa: impact of treatment using antimalarial and antiretroviral agents.

Uneke CJ, Ogbonna A.

Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria. unekecj@yahoo.com

Malaria and HIV infection represent severe public health problems in sub-Saharan Africa, and pregnant women are at increased risk because the two diseases intersect in pregnancy, causing adverse perinatal outcome. As access to antiretroviral drugs is increasing in the sub-region, and new combinations of antimalarial drugs are being implemented while more are being evaluated, there is potential for interactions between these therapies. In this report, the impact of treatment using antimalarial and antiretroviral agents in pregnant women with malaria and HIV co-infection was reviewed, using scientific publications identified through a Medline Entrez-Pubmed search with reference to sub-Saharan Africa. The safety and operational feasibility of use of antimalarial and antiretroviral agents to treat co-infected pregnant women were evaluated. Although use of these therapies was shown to improve the health of pregnant women with co-infection, low adherence, poor-quality drugs, resource scarcity, lack of infrastructure and inadequate treatment in sub-Saharan Africa continue to hamper treatment outcome. The absence of studies on interaction between antimalarials and antiretrovirals, as well as mounting evidence of treatment failure due to drug resistance and adverse drug reactions, in most parts of sub-Saharan Africa, make the establishment of new guidelines for the prevention of malaria and HIV infection during pregnancy imperative.

Vaccine. 2009 Aug 20;27(38):5187-94.

Plasmodium berghei HAP2 induces strong malaria transmission-blocking immunity in
Fertilization in Plasmodium is a complex process that occurs in the gut of the female Anopheles mosquito upon uptake of a bloodmeal. It requires the emergence of the gametocyte from the RBC and release of eight flagellate male gametes from each male cell, and subsequent fertilization of a similarly emerged immotile extracellular female macrogamete. Previous studies have demonstrated that antibodies against male gamete surface proteins ingested from the blood of an infected and immunized host inhibit parasite transmission. Gene disruption studies in Plasmodium berghei and complimentary studies on the green alga Chlamydomonas have shown that a conserved male gamete sterility gene, HAP2, is essential for fusion of male and female gametes. Genetic disruption of the HAP2 locus revealed that parasite fertilization is prevented, yet hap2 KO male gametes still retained the ability to form tight pre-fusion membrane attachments with females. We demonstrate that heterologous expression of the P. berghei HAP2 protein in Escherichia coli, and subsequent immunization of rabbits, has produced anti-sera that react specifically with recombinant HAP2, and with the native protein on the male gamete. Additionally, anti-HAP2 sera reduces in vitro formation of ookinetes by up to 81%, and, using standard membrane feeding assays, reduces oocyst burden within the mosquito host by up to 81.1%, and prevalence of in vivo infection by up to 34%. Inhibition is dose dependent. These results indicate that HAP2 should be considered as a potential target for any future anti-malarial transmission-blocking vaccine.


Plasmodium falciparum apical membrane antigen 1 vaccine elicits multifunctional CD4 cytokine-producing and memory T cells.

Huaman MC, Mullen GE, Long CA, Mahanty S.

Laboratory of Malaria and Vector Research and Malaria Vaccine Development Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852, USA. huamanc@niaid.nih.gov

The Plasmodium falciparum apical membrane antigen 1 (AMA1) is a leading vaccine candidate and was tested for safety and immunogenicity in human Phase I Clinical Trials. PBMC from vaccine recipients were analyzed by flow cytometric methods to determine the nature of T-cell responses and AMA1-reactive memory T cells. Both CD4 and CD8 T cells produced a number of cytokines following AMA1 re-stimulation, with IL-5-producing cells at the highest frequency, consistent with a Th2 bias. The relative frequency of multifunctional cells synthesizing Th1 cytokines IFN-gamma, IL-2 and TNF-alpha changed after each vaccination. Interestingly, median fluorescence intensity measurements revealed that cells producing more than one cytokine contributed greater quantities of each cytokine than cell populations that produced each of the cytokines alone. AMA1 vaccination also elicited the development of memory cell populations, and both central and effector memory T cells were identified concurrently after the AMA1 vaccination. The detailed profile of multifunctional T-cell responses to AMA1 presented here will advance our ability to assess the immunogenicity of human malarial vaccines.
Evaluation of a prime-boost vaccine schedule with distinct adenovirus vectors against malaria in rhesus monkeys.


Crucell Holland BV, PO Box 2048, 2301 CA Leiden, The Netherlands.

A vaccine that elicits both specific antibodies and IFN-gamma-producing T cells is required to protect against pre-erythrocytic malaria. Among the most promising approaches to induce such complex immunity are heterologous prime-boost vaccination regimens, in particular ones containing live viral vector. We have demonstrated previously that adenovectors serotype 35 (Ads35) encoding the circumsporozoite (CS) antigen or liver-stage antigen-1 (LSA-1) are highly effective in improving the T-cell responses induced by immunizations with protein-based vaccines in a heterologous prime-boost schedule. Here we evaluated the potential of a heterologous prime-boost vaccination that combines the Ad35.CS vector with the serologically distinct adenovector Ad5.CS, in rhesus macaques, after establishing the potency in mice. We show that the heterologous Ad35.CS/Ad5.CS prime-boost regimen elicits both antibody responses and robust IFN-gamma-producing CD8(+) T-cell responses against the CS antigen. Analysis of the quality of the antibody responses in rhesus macaques, using indirect immunofluorescence assay (IFA) with Plasmodium falciparum-coated slides, demonstrated that this heterologous prime-boost regimen elicits a high titer of antibodies that are able to bind to P. falciparum sporozoites. Level of the IFA response was superior to the response measured with sera of an adult human population living in endemic malaria region. In conclusion, the combination of Ad35.CS, a vaccine based on a rare serotype adenovirus, with Ad5.CS or possibly another adenovector of a distinct serotype, induces a complex immune response that is required for protection against malaria, and is thus a highly promising approach for pediatric vaccination.