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ABSTRACTS

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**Chlorfenapyr: A pyrrole insecticide for the control of pyrethroid or DDT resistant Anopheles gambiae (Diptera: Culicidae) mosquitoes.**

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Owing to the development and spread of pyrethroid resistance in Anopheles gambiae in Africa there is an urgent need to develop alternative insecticides to supplement the pyrethroids. Chlorfenapyr is a pyrrole insecticide first commercialized for the control of agricultural pests and termites. Performance against An. gambiae bearing kdr (pyrethroid and DDT resistance) or Ace-1(R) insensitive acetylcholinesterase (organophosphate and carbamate resistance) mechanisms was studied using a variety of adult bioassay tests including a simulated-experimental hut system (tunnel tests) that allows uninhibited mosquito behaviour/insecticide interactions. Strains resistant to pyrethroids and organophosphates showed no cross resistance to chlorfenapyr. In cone bioassays on treated netting the mortality of adult mosquitoes showed an unexpected curvilinear response, with highest mortality occurring at intermediate dosages. Adults expressed irritability to chlorfenapyr at higher dosages, which might explain the dosage-mortality trend. Toxic activity of chlorfenapyr was slow compared to conventional neurotoxic insecticides and additional mortality occurred between 24h and 72h. In tunnel tests, the dosage-mortality trend showed a more typical sigmoid response and most mortality occurred during the first 24h. Mosquito penetration through the holed, treated netting showed only limited inhibition and blood-feeding was not inhibited. Mortality rates in the kdr strain exposed to chlorfenapyr treated netting in tunnel tests were much higher than with permethrin treated netting over the same 100-500mg/m(2) dosage range. Chlorfenapyr has potential for malaria control in treated-net or residual spraying applications in areas where mosquitoes are pyrethroid resistant. For treated-net applications chlorfenapyr might be combined with pyrethroid as a mixture to provide personal protection as well as to give control of resistant mosquitoes.


**Chondroitin Sulfate Proteoglycan but Not Hyaluronic Acid Is the Receptor for the Adherence of Plasmodium falciparum-Infected Erythrocytes in Human Placenta, and Infected Red Blood Cell Adherence Up-Regulates the Receptor Expression.**

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A low-sulfated chondroitin sulfate proteoglycan (CSPG) has been shown to be the receptor for the adherence of Plasmodium falciparum-infected red blood cells (IRBCs) in human placenta. Recently, hyaluronic acid (HA) has been suggested as an additional receptor even though IRBC binding to HA and the presence of HA at locations where IRBCs adhere in the placenta have not been established. In this study, we investigated whether HA is also a receptor for IRBC binding. IRBCs from infected placentas as well as those from different laboratory strains could
bind to CSPG but not to HA. In a cell depletion assay, IRBCs from infected placentas could bind quantitatively to CSPG. Although CSPG is present both in the intervillous space and on the syncytiotrophoblast surface, HA is absent in these locations. These data conclusively demonstrate that CSPG, but not HA, is a receptor for IRBC adherence in the placenta. Our data also show, for the first time, that the IRBC-binding CSPG in the placenta is of fetal origin and that, in P. falciparum-infected placentas, the CSPG level is significantly increased, which could exacerbate IRBC adherence and placental pathogenesis. These results have important implications for the development of anti-IRBC adhesion-based vaccine for pregnancy-associated malaria.


High spatial resolution mapping of malaria transmission risk in the Gambia, west Africa, using LANDSAT TM satellite imagery.

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Understanding local variability in malaria transmission risk is critically important when designing intervention or vaccine trials. Using a combination of field data, satellite image analysis, and GIS modeling, we developed a high-resolution map of malaria entomological inoculation rates (EIR) in The Gambia, West Africa. The analyses are based on the variation in exposure to malaria parasites experienced in 48 villages in 1996 and 21 villages in 1997. The entomological inoculation rate (EIR) varied from 0 to 166 infective bites per person per rainy season. Detailed field surveys identified the major Anopheles gambiae s.l. breeding habitats. These habitats were mapped by classification of a LANDSAT TM satellite image with an overall accuracy of 85%. Village EIRs decreased as a power function based on the breeding areas size and proximity. We use this relationship and the breeding habitats to map the variation in EIR over the entire 2500-km(2) study area.


Effect of rice cultivation patterns on malaria vector abundance in rice-growing villages in Mali.


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Irrigation for rice cultivation increases the production of Anopheles gambiae, the main vector of malaria in Mali. Mosquito abundance is highly variable across villages and seasons. We examined whether rice cultivation patterns mapped using remotely sensed imagery can account for some of this variance. We collected entomologic data and mapped land use around 18 villages in the two cropping seasons during two years. Land use classification accuracy ranged between 70% and 86%. The area of young rice explained 86% of the inter-village variability in An. gambiae abundance in August before the peak in malaria transmission. Estimating rice in a 900-meter buffer area around the villages resulted in the best correlation with mosquito abundance, larger buffer areas were optimum in the October and dry season models. The quantification of the relationship
between An. gambiae abundance and rice cultivation could have management applications that merit further study.


**Plasmodium vivax malaria in the Republic of Korea during 2004-2005: changing patterns of infection.**

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Vivax malaria re-emerged in the Republic of Korea (ROK) in 1993. The annual incidence of this disease, which had increased rapidly through 2000 with geographic expansion, started to decrease in 2001, reaching 664 cases in 2004; however, the trends changed in 2005 when 1,304 cases were reported. Among 2,168 cases of vivax malaria reported from 2004 through 2005, 389 cases (17.9%) were ROK military personnel, 565 cases (26.1%) were veterans who had been discharged from the military within 2 years of report of infection, and 1,214 cases (56.0%) were civilians. Local transmission might have taken place during this period in the southern side of the Demilitarized Zone. Regional increase of vivax malaria in North Korea, increased local transmissions in ROK, and active transmission by vector mosquitoes during the transmission season might be important factors responsible for the re-increase of vivax malaria in ROK during 2005.


**Short report: rare Plasmodium falciparum merozoite surface protein 1 19-kda (msp-1(19)) haplotypes identified in mali using high-throughput genotyping methods.**

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Genetic diversity in malaria vaccine antigens may compromise malaria vaccine efficacy, so it is important to understand this diversity and the processes that generate it. By applying new high-throughput genotyping methods to a large sample of infections from Mali (N = 1369), seven new 19-kDa merozoite surface protein 1 (MSP-1(19)) haplotypes were identified. Herein we report the sequences of these new haplotypes and discuss their possible origins. Although they are present in < 1% of the samples examined, the existence of these rare haplotypes reveals a greater degree of diversity at this locus than previously reported and highlights the potential for Plasmodium to evolve under selective pressure from the immune system and from such interventions as vaccines and drugs.


**The importance of the period of malarial infection during pregnancy on birth weight in tropical Africa.**

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Malaria in pregnant women is related to low birth weight (LBW), a factor contributing to infant mortality. Which period of infection during pregnancy leads to the most harmful consequences is unclear. We analyzed data collected in Burkina Faso for 1190 pregnant women. Birth weight was analyzed through multivariate linear and logistic regressions. Infection after 6 months of pregnancy was related to a decrease in mean birth weight (-105 g, P = 0.02) and a higher risk of low birth weight (AOR = 1.8, P = 0.02). A trend was found between infection before 4 months of pregnancy and a decrease in birth weight (-68 g, P = 0.08). This suggests that the end of pregnancy is the most important period in terms of public health, but infection at the beginning of pregnancy may also have consequences. Malaria prevention policies should be started early in pregnancy, especially by implementing the systematic use of insecticide-treated nets.


Determinants of bed net use in the Gambia: implications for malaria control.

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Malaria is still one of the biggest health threats in the developing world, with an estimated 300 million episodes per year and one million deaths, most of which are in sub-Saharan Africa. Although the efficacy and cost-effectiveness of treated bed nets has been widely reported, little is known about the range, strength, or interaction between different factors that influence their demand at the household level. This study modeled the determinants of bed net ownership as well as the factors that influence the number of bed nets purchased. Data was collected from 1,700 randomly selected households in the Farafenni region of The Gambia. Interviews were also held with 129 community spokespersons to explore the extent to which community level factors such as the quality of roads and access to market centers also influence demand for bed nets. The results of each model of demand and their policy implications are discussed.

9: Anal Chem. 2007 May 4

Bioluminescence DNA Hybridization Assay for Plasmodium falciparum Based on the Photoprotein Aequorin.

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A bioluminescence DNA hybridization assay for the detection of Plasmodium falciparum, the most deadly species of malaria, using the photoprotein aequorin as a bioluminescent label has been developed. The current gold standard for the detection of malaria is light microscopy, which can detect down to approximately 50 parasites/μL of blood, but has low-throughput, high costs, and requires high skill, which limit the applicability of the method, especially in the developing regions where malaria detection is mostly needed. The utilization of aequorin as a bioluminescence label offers the advantages of high signal-to-noise ratio and reliable detection down to attomole levels, allowing for the development of highly sensitive and miniaturized high-throughput bioluminescence assays. Herein, we developed a DNA hybridization assay for the detection of P. falciparum based on the competition between the target DNA and the signal...
generating DNA streptavidin-aequorin for hybridization with the probe DNA. This bioluminescence hybridization assay demonstrated a detection limit of 3 pg/µL and was employed for the detection of target DNA in standard and spiked human serum samples. The DNA hybridization assay was developed in a microplate format without the need for sample PCR amplification, showing the potential suitability of this method in the parallel analysis of samples by low-trained personnel, such as that typically encountered in developing regions.


Detection of in-vivo chloroquine resistance in Plasmodium falciparum from Rameswaram Island, a pilgrim centre in southern India.


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Resistance to chloroquine (CQ) in Plasmodium falciparum is one of the main causes of the wide-spread resurgence of malaria in India and a challenge to the effective control of the disease. In the pilgrim centre of Rameswaram Island, malaria has persisted despite the various control measures undertaken over the years. When CQ resistance in Rameswaram was investigated in vivo, recrudescent parasitaemias were observed in 25 (58%) of the 43 study subjects who were given CQ and completed follow-up, all occurring between days 10 and 28 (late treatment failures). The results of the msp(1), msp(2) and glurp genotyping of paired samples of P. falciparum, collected on day 0 and the day of recrudescence from 23 of the apparent treatment failures, indicated that 21 (91%) of the 23 were probably true treatment failures. All of the paired samples harboured parasites with the K76T mutation in their pfcrt genes, and subsequent sequencing of nine day-0 samples revealed the SVMNT haplotype in all nine. This is the first report of in-vivo drug resistance in P. falciparum from Rameswaram Island. Such resistance, which is probably the result of the indiscriminate use of CQ and/or the import of malaria from mainland India, warrants a change in the drug regimen used locally for the first-line treatment of uncomplicated, P. falciparum malaria, to make treatment more effective and slow the development and spread of more foci of CQ resistance.


Ability of mothers to diagnose fever and anaemia in their young children, in a malaria-endemic region of West Africa.

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The rapid and correct diagnosis of fever and anaemia at the household level is a prerequisite for the successful management and control of life-threatening disease among young children, particularly in malaria-endemic areas of Africa. The ability of mothers to diagnose fever and anaemia in their young children has recently been explored, as part of a large, birth-cohort study in rural, north-western Burkina Faso. During a cross-sectional survey in six villages, 345 children aged <3 years and their mothers were investigated. Each mother was asked if she considered her child to be febrile and/or anaemic before that
child's temperature and haematocrit were measured, with an electronic thermometer and portable centrifuge, respectively. The recorded prevalences of fever (>=37.5 degrees C) and anaemia (haematocrit, <25%) in the children were 12.2% and 21.4%, respectively. The mothers' diagnoses had a sensitivity of 76.2% [95% confidence interval (CI)=60.6%-88.0%] for fever and 4.1% (CI=0.8%-11.4%) for anaemia, with corresponding specificities of 87.1% (CI=82.8%-90.7%) and 95.9% (CI=92.9%-98.0%). Mothers in rural Africa appear to be fairly accurate in detecting fever in their children but less accurate in detecting anaemia. While malaria control needs to employ a mix of preventive and curative measures, anaemia control will benefit from community-based malaria-control measures as well as broader approaches addressing the nutritional status of young children.


**Effect of artesunate plus sulfadoxine-pyrimethamine on haematological recovery and anaemia, in Kenyan children with uncomplicated, Plasmodium falciparum malaria.**


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Malaria-associated anaemia is a major public-health problem. Although the treatment of uncomplicated, Plasmodium falciparum malaria aims to clear the parasites, relieve the symptoms and permit haematological recovery, data on the impact of antimalarial treatment on haematological recovery are few. Haematological recovery and the prevalence of anaemia were therefore evaluated in 600 Kenyan children with uncomplicated malaria who were randomly assigned to one of three treatment groups. The children were given sulfadoxine-pyrimethamine (SP) on day 0, SP plus artesunate on day 0 (AS1), or SP on day 0 and artesunate on each of days 0-2 (AS3). Haemoglobin (Hb) concentrations were measured on days 0, 7, 14, 21 and 28, with haematological recovery defined as a day-28 Hb concentration of at least 11 g/dl. Only 96 (18%) of the 543 children who were anaemic (i.e. with <11.0 g Hb/dl) at enrolment achieved haematological recovery. The prevalence of anaemia fell from 91% on day 0 to 74% (252/340) by day 28 (P=0.065). Compared with SP alone, neither artesunate regimen resulted in higher Hb concentrations on day 28 (with means of 10.2, 9.9 and 10.2 g/dl for AS3, AS1 and SP, respectively; P=0.254), a higher frequency of haematological recovery (19%, 14% and 20% for AS3, AS1 and SP, respectively; P=0.301) or a greater reduction in the prevalence of anaemia (prevalences in the AS3, AS1 and SP arms falling from 90%, 89% and 93%, respectively, on day 0, to corresponding values of 71%, 82% and 69% on day 28; P=0.40). In fact, between days 0 and 7, the children in the AS3 arm showed a larger drop in mean Hb than the children in the other two treatment arms. In general, haematological recovery was most likely in older children who had mild anaemia at presentation and were parasitologically cured. Overall, the frequencies of haematological recovery were modest and not influenced by the artesunate treatments. Other factors contributing to anaemia need to be explored more fully.


**Comparative analysis of malaria parasite density using actual and assumed white blood cell counts.**

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AIM: To compare malaria parasite densities, calculated using the white cell counts (WBC) of individual children with a standard WBC count of 8.0 x 10^9/L. METHODS: In a cross-sectional study, the prevalence of malaria WBCs and malaria parasite densities were estimated in 240 healthy Nigerian children aged 1-8 years. RESULTS: Of 240 children, 75 (31.25%) were infected with malaria, 69 (28.75%) with P. falciparum and 9 (3.75%) with other species. The mean (SD) WBC count was 5.1 (2.0) x 10^9/L. There was an age-related significant difference in the mean WBC counts (t=2.000, p<0.05), with values higher in the under-5s [5.6 (2.0) x 10^9/L] than in the > or =5-years group [5.0 (1.8) x 10^9/L]. No significant difference was observed with regard to gender and malaria infection. The mean (SD) parasite densities of P. falciparum obtained using the assumed value of 8.0 x 10^9/L [1936 (1119.5)] was significantly higher than the parasite densities estimated using the individual WBC counts [1140 (862.8) for P. falciparum] (p<0.0001). CONCLUSION: Parasite density estimation using the assumed count of 8.0 x 10^9/L might result in over-estimation of the parasite burden. The WBCs of individual patients should always be estimated when parasite density is required.


**Comparative study of the effectiveness and pharmacokinetics of two rectal artesunate/oral mefloquine combination regimens for the treatment of uncomplicated childhood falciparum malaria.**


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BACKGROUND: Rectal artesunate has been shown to be an effective treatment for falciparum malaria and is useful in patients who cannot take medicine orally or when parenteral medication is inconvenient. A combination with mefloquine can decrease the duration of treatment, increase compliance and delay development of resistance. There are no clear data on whether a higher dosage of rectal artesunate results in a better clinical response. AIM: To assess two rectal artesunate/oral mefloquine regimens for treating uncomplicated multi-drug-resistant childhood falciparum malaria. METHODS: Seventy children aged 1-14 years with uncomplicated falciparum malaria were randomly assigned to receive either 10 (range 8-12) or 20 (range 16-24) mg/kg/day rectal artesunate for 3 days followed by 25 mg/kg oral mefloquine. The study endpoints were fever clearance time, parasite clearance time and proportion of patients with recrudescence. Serum levels of artesunate and dihydro-artemisinin were measured after the first dose of rectal artesunate in 16 subjects. RESULTS: Both regimens were safe and effective. The cure rate was 100% in the 53 patients who completed 28-day follow-up. All of the study endpoints were comparable between both treatment groups. CONCLUSION: A regimen of rectal artesunate 10 mg/kg/day for 3 days followed by mefloquine 25 mg/kg is optimal for the treatment of uncomplicated falciparum malaria. There was no definite benefit from increasing the dosage of rectal artesunate from 10 to 20 mg/kg/day.
A mass spectrometry based systems approach for the identification of inhibitors of Plasmodium falciparum fatty acid synthase.

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The emergence of strains of Plasmodium falciparum resistant to the commonly used antimalarials warrants the development of new antimalarial agents. The discovery of type II Fatty Acid Synthase (FAS) in Plasmodium, distinct from its human host (type I FAS) opened up new avenues for the development of novel antimalarials. The process of fatty acid synthesis takes place by iterative elongation of butyryl-acyl carrier protein (ACP) by two carbon units, by successive action of four enzymes constituting the elongation module of FAS till the desired acyl length is obtained. Study of fatty acid synthesis machinery of the parasite inside the red blood cell culture has always been a challenging task. Here, we report the in vitro reconstitution of the elongation module of the fatty acid synthase of malaria parasite involving all the four enzymes, FabB/F (beta-ketoacyl-ACP synthase), FabG (beta-ketoacyl-ACP reductase), FabZ (beta-ketoacyl-ACP dehydratase) and FabI (enoyl-ACP reductase) and its analysis by MALDI-TOF. That this in vitro systems approach completely mimics the in vivo machinery is confirmed by the distribution of acyl products. Using known inhibitors of the enzymes of elongation module, cerulenin, Triclosan, NAS-21/91 and (-)-catechin gallate, we demonstrate that accumulation of intermediates resulting from the inhibition of any of the enzymes can be unambiguously followed by MALDI-TOF. Thus this work not only offers a powerful tool for easier and faster throughput screening of inhibitors but also allows for the study of biochemical properties of the FAS pathway of the malaria parasite.

Identifying and characterising the Plasmodium falciparum RhopH3 Plasmodium vivax homologue.


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Four Plasmodium species cause malaria in humans, Plasmodium falciparum being the most widely studied to date. All Plasmodium species have paired club-shaped organelles towards their apical extreme named rhoptries that contain many lipids and proteins which are released during target cell invasion. P. falciparum RhopH3 is a rhoptry protein triggering important immune responses in patients from endemic regions. It has also been shown that anti-RhopH3 antibodies inhibit in vitro invasion of erythrocytes. Recent immunisation studies in mice with the Plasmodium yoelii and Plasmodium berghei RhopH3 P. falciparum homologue proteins found that they are able to induce protection in murine models. This study described identifying and characterising RhopH3 protein in Plasmodium vivax; it is encoded by a seven exon gene and expressed during the parasite’s asexual stage. PvRhopH3 has similar processing to its homologue in P. falciparum and presents a cellular immunolocalisation pattern characteristic of rhoptry.
proteins.

17: Bioorg Med Chem. 2007 May 18

**Computational modeling tools for the design of potent antimalarial bisbenzamidines: Overcoming the antimalarial potential of pentamidine.**

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Malaria is nowadays a worldwide and serious problem with a significant social, economic, and human cost, mainly in developing countries. In addition, the emergence and spread of resistance to existing antimalarial therapies deteriorate the global malaria situation, and lead thus to an urgent need toward the design and discovery of new antimalarial drugs. In this work, a QSAR predictive model based on GETAWAY descriptors was developed which is able to explain with, only three variables, more than 77% of the variance in antimalarial potency and displays a good internal predictive ability (of 73.3% and 72.9% from leave-one-out cross-validation and bootstrapping analyses, respectively). The performance of the proposed model was judged against other five methodologies providing evidence of the superiority of GETAWAY descriptors in predicting the antimalarial potency of the bisbenzamidine family. Moreover, a desirability analysis based on the final QSAR model showed that to be a useful way of selecting the predictive variable level necessary to obtain potent bisbenzamidines. From the proposed model it is also possible to infer that elevated high atomic masses/polarizabilities/van der Waals volumes could play a negative/positive/positive role in the molecular interactions responsible for the desired drug conformation, which is required for the optimal binding to the macromolecular target. The results obtained point out that our final QSAR model is statistically significant and robust as well as possessing a high predictive effectiveness. Thus, the model provides a feasible and practical tool for looking for new and potent antimalarial bisbenzamidines.


**Transcriptional analysis of an immune-responsive serine protease from Indian malarial vector, Anopheles culicifacies.**


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BACKGROUND: The main vector for transmission of malaria in India is the Anopheles culicifacies mosquito species, a naturally selected subgroup of which is completely refractory (R) to transmission of the malaria parasite, Plasmodium vivax; RESULTS: Here, we report the molecular characterization of a serine protease (acsp30)-encoding gene from A. culicifacies, which was expressed in high abundance in the refractory strain compared to the susceptible (S) strain. The transcriptional upregulation of acsp30 upon Plasmodium challenge in the refractory strain coincided with ookinete invasion of mosquito midgut. Gene
organization and primary sequence of acsp30 were identical in the R and S strains suggesting a divergent regulatory status of acsp30 in these strains. To examine this further, the upstream regulatory sequences of acsp30 were isolated, cloned and evaluated for the presence of promoter activity. The 702 bp upstream region of acsp30 from the two strains revealed sequence divergence. The promoter activity measured by luciferase-based reporter assay was shown to be 1.5-fold higher in the R strain than in the S. Gel shift experiments demonstrated a differential recruitment of nuclear proteins to upstream sequences of acsp30 as well as a difference in the composition of nuclear proteins in the two strains, both of which might contribute to the relative abundance of acsp30 in the R strain; CONCLUSION: The specific upregulation of acsp30 in the R strain only in response to Plasmodium infection is suggestive of its role in contributing the refractory phenotype to the A. culicifacies mosquito population.


Retrospective analysis of artemisinin pharmacokinetics: application of a semiphysiological autoinduction model.

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What is already known about this subject? * A previous semiphysiological model has been published, describing the time course of the autoinduction of artemisinin. * The model, which was based on saliva sampling, has been successfully applied in another set of saliva data. What this study adds? * In this report, we applied the same model to plasma data from six clinical studies involving healthy volunteers and malaria patients. * The model performed well and we suggest that it could be used as a general model in cases of time-dependent pharmacokinetics and drug-drug interactions. Aims To describe the time-course of the autoinduction of artemisinin by applying a semiphysiological pharmacokinetic model. Methods Plasma concentration-time data from six clinical studies involving oral administration of artemisinin to healthy subjects and malaria patients were included in the analysis. NONMEM was used to apply a semiphysiological model incorporating metabolizing enzymes and a pharmacokinetic model including a separate hepatic compartment. Results The model described the data well. The hepatic extraction ratio increased from 0.74 at pre-induced conditions to 0.98 after autoinduction of metabolism. Conclusions Our model successfully described the time-course of autoinduction of metabolism of artemisinin in subjects receiving oral artemisinin.

20: Cad Saude Publica. 2007 May;23(5):1099-1112.

Spatial analysis for stratification of priority malaria control areas, Mato Grosso State, Brazil.

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The goal of this study was to stratify priority areas for malaria control in the State of Mato Grosso, Brazil, based on spatial analysis. The variables used were: Annual Parasite Index (API), Plasmodium falciparum/Plasmodium vivax ratio, population variation, number of families settled, and percent of deforested
area. The Moran's I and Local Moran Test were applied, visualized with the Box Map and Moran Map, for 1986–1991, 1992-1997, and 1998-2003. Box Map identified areas with high, low, and intermediate priority for control, and Moran Map identified municipalities with significant autocorrelation. In the high priority area, located in the North of Mato Grosso, malaria incidencc decreased drastically despite the increase in the number of municipalities from the first to the last period. Other municipalities were added to the lower priority area, from the Southeast, Southwest, and Central-South of the State. The intermediate priority area was located along the border with neighboring States and municipalities classified as high and low priority. Spatial analysis showed the importance of the neighboring phenomenon between municipalities in defining priority areas, thus highlighting the technique's advantages for use in malaria control and surveillance.


Structure-activity-based design of a synthetic malaria Peptide eliciting sporozoite inhibitory antibodies in a virosomal formulation.


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The circumsporozoite protein (CSP) of Plasmodium falciparum is a leading candidate antigen for inclusion in a malaria subunit vaccine. We describe here the design of a conformationally constrained synthetic peptide, designated UK-39, which has structural and antigenic similarity to the NPNA-repeat region of native CSP. NMR studies on the antigen support the presence of helical turn-like structures within consecutive NPNA motifs in aqueous solution. Intramuscular delivery of UK-39 to mice and rabbits on the surface of reconstituted influenza virosomes elicited high titers of sporozoite crossreactive antibodies. Influenza virus proteins were crucially important for the immunostimulatory activity of the virosome-based antigen delivery system, as a liposomal formulation of UK-39 was not immunogenic. IgG antibodies elicited by UK-39 inhibited invasion of hepatocytes by P. falciparum sporozoites, but not by antigenically distinct P. yoelii sporozoites. Our approach to optimized virosome-formulated synthetic peptide vaccines should be generally applicable for other infectious and noninfectious diseases.


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Quinoline hexose analogs are expected to be useful as novel agents for treatment of chloroquine-resistant malaria. Here, we report preparation of 4-hydroxy quinoline-beta-glucosides from anilines in 4 steps.

23: Clin Chem. 2007 May 17
Profiling the Antibody Immune Response against Blood Stage Malaria Vaccine Candidates.

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BACKGROUND: The complexity and diversity of the antibody immune response to the antigen repertoire of a pathogen has long been appreciated. Although it has been recognized that the detection of antibodies against multiple antigens dramatically improves the clinical sensitivity and specificity of diagnostic assays, the prognostic value of serum reactivity profiles against multiple microbial antigens in protection has not been investigated. METHODS: Using malaria as a model we investigated whether antigen reactivity profiles in serum of children with different levels of clinical immunity to Plasmodium falciparum malaria correlated with protection. We developed a microarray immunoassay of 18 recombinant antigens derived from 4 leading blood-stage vaccine candidates for P. falciparum [merozoite surface protein 1 (MSP1), MSP2, MSP3, and apical membrane antigen (AMA)-1]. Associations between observed reactivity profiles and clinical status were sought using k-means clustering and phylogenetic networks. RESULTS: The antibody immune response was unexpectedly complex, with different combinations of antigens recognized in different children. Serum reactivity to individual antigens did not correlate with immune status. By contrast, combined recognition of AMA-1 and allelic variants of MSP2 was significantly associated with protection against clinical malaria. This finding was confirmed independently by k-means clustering and phylogenetic networking. CONCLUSIONS: The analysis of reactivity profiles provides a wealth of novel information about the immune response against microbial organisms that would pass unnoticed in analysis of reactivity to antigens individually. Extension of this approach to a large fraction of the proteome may expedite the identification of correlates of protection and vaccine development against microbial diseases.

Quantifying the economic burden of malaria in Nigeria using the willingness to pay approach.

Jimoh A, Sofola O, Petu AO, Okorosobo TK.

ABSTRACT: BACKGROUND: Malaria illness imposes great burden on the society as it has adverse effects on the physical, mental and social well being of the people as well as on the economic development of the nation. METHODS: The study uses the Willingness To Pay (WTP) approach to evaluate the burden of malaria in Nigeria. RESULTS: The results indicate that households would be prepared to pay an average of about Naira 1,112 (USD 9.3) per month for the treatment of malaria. This is about Naira 427 (USD 3.6) in excess of the average expenditure they currently make on malaria treatment per month. Similarly, households are willing to pay on the average a sum of Naira 7,324 (USD 61) per month for the control of malaria. Again, this is an excess of about Naira 2,715 (USD 22.6) over the cost they currently bear (protection, treatment and indirect costs), and it represents households' average valuation of their intangible costs of malaria illness. This amount represents about Naira 611.7 (USD 5.1) per head per month and Naira 7,340 (USD 61.2) per year. For a country with a population of about 120 million this translates to about Naira 880,801 million per annum representing about 12.0 per cent of Gross Domestic Product. Hence, the malaria
burden in Nigeria is enormous and has a devastating impact on economic growth. CONCLUSION: In the long term, it is important to recognize that health and poverty are closely linked. Reducing the burden of malaria in Nigeria will help to contribute to the economic well-being of communities; and poverty-reduction will be an essential input into improving health. National malaria control programme in Nigeria and their partners need to recognize these links, and identify mechanisms for ensuring that the poorest have access to essential health interventions.


New concepts in vaccine development in malaria.

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PURPOSE OF REVIEW: To focus on recent novel concepts in the development of malaria vaccines. RECENT FINDINGS: There is a renewed interest in whole attenuated sporozoite vaccines, either as irradiated or genetically modified sporozoites, because they consistently elicit solid protection against challenge infections. Enthusiasm about these vaccines is, however, tempered by technical, logistical, safety and even cultural hurdles that might need to be surmounted. Less than a score of Plasmodium falciparum proteins are currently in the development pipeline as malaria vaccines. There is an urgent need to ratchet up the process of candidate vaccine discovery, and reverse vaccinology and genome-wide surveys remain promising strategies. The development of malaria vaccines for placental malaria is an active area and chondroitin sulfate A-binding epitopes of the variant PfEMP1 have been identified. Live bacteria and viral vectors hold special promise for vaccine delivery. SUMMARY: Attenuated sporozoite vaccines have made a resurgence to center stage in malaria vaccine development. There is an urgent need to identify more subunit vaccine candidates that can enter into the development pipeline, identify surrogate markers of immunity and design vaccines which induce long-lasting immunity.

26: Ecotoxicol Environ Saf. 2007 May 25

Heavy metals in mosquito larval habitats in urban Kisumu and Malindi, Kenya, and their impact.

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Concentrations and distribution of cadmium, chromium, copper, iron, lead, manganese and zinc in mosquito larval habitats in urban Kisumu and Malindi, Kenya and their effect on the presence of Anopheles gambiae, Aedes aegypti, Culex quinquefasciatus and Anopheles funestus larvae were investigated. Manganese and iron were the most prevalent heavy metals in water of larval habitats in urban Kisumu and Malindi, respectively. Iron was the most prevalent heavy metal in bottom sediments in larval habitats in both cities. The highest concentrations of all heavy metals, except cadmium and iron, were recorded in the poorly planned-well drained stratum in the two cities. All heavy metals were more concentrated in human-made than in natural larval habitats. Copper was positively associated with the presence of Ae. aegypti, and lead was associated
with the presence of An. gambiae and Ae. aegypti in urban Kisumu. Absence of significant correlation between the other metals and mosquito species in both cities, despite relatively high concentrations, suggest that the local larval populations, including key malaria vectors have adapted to the detected levels of these metals.


Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin.

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The pyrethroid knockdown resistance gene (kdr) has become widespread in Anopheles gambiae in West Africa. A trial to test the continuing efficacy of insecticide-treated nets (ITN) and indoor residual spraying (IRS) was undertaken in experimental huts at 2 sites in Benin, the first where kdr is present at high frequency (Ladjji), the second-where An. gambiae is susceptible (Malanville). Holes were made in the nets to mimic worn nets. At Malanville, 96% of susceptible An. gambiae were inhibited from blood-feeding, whereas at Ladjji feeding was uninhibited by ITNs. The mortality rate of An. gambiae in ITN huts was 98% in Malanville but only 30% at Ladjji. The efficacy of IRS was equally compromised. Mosquitoes at Ladjji had higher oxidase and esterase activity than in a laboratory-susceptible strain, but this fact did not seem to contribute to resistance. Pyrethroid resistance in An. gambiae appears to threaten the future of ITN and IRS in Benin.


Role of TGF-beta and PGE(2 )in T cell responses during Plasmodium yoelii infection.

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During an acute blood-stage malaria infection, T cell responses to malaria and other bystander antigens are inhibited. Plasmodium infection induces strong cytokine responses that facilitate parasite clearance but may interfere with T cell functions, as some of the soluble immune mediators induced are also general inhibitors of T cell responses. Using a malaria mouse model, we have analyzed the cytokines produced by dendritic cells in response to P. yoelii infection that have potential T cell inhibitory activity. We found that during acute infection DC migrate to the spleen and secrete TGF-beta, prostaglandin E(2) (PGE(2)) and IL-10. We have analyzed the role of these general T cell inhibitors in a particular T cell response of evident importance in malaria infections: the CD8(+) T cells generated against the liver-stage of the disease. During blood-stage infection, inhibition of the activity of TGF-beta and PGE(2) restores the CD8(+) T cell responses generated by sporozoites, increasing protection against re-infection. Our findings suggest that the strong cytokine response induced by blood-stage P. yoelii infection affects host T cell responses, inhibiting protective CD8(+) T cells against the liver-stage of the disease.
ABSTRACT: BACKGROUND: The HIV epidemic has challenged our previous understanding of endemic Burkitts lymphoma. Despite the strong association of Burkitts lymphoma and HIV infection in the Developed world, and against previous postulations that the cancer is due to immunosupression among African children, the HIV epidemic in the Malaria belt has not been associated with a corresponding increase in incidence of childhood Burkitts lymphoma. Even outside the context of HIV infection, there is substantial evidence for a strong but skewed immune response towards a TH2 response in genesis of Burkitt lymphoma. Presentation of the Hypothesis: Rather than a global and/or profound immunosupression, the final common pathway in genesis of Burkitts lymphoma is the dysregulation of the immune response towards a TH2 response dominated by B-lymphocytes, and the concomitant suppression of the TH1 cell-mediated immune surveillance, driven by various viral/parasitic/bacterial infections. TESTING THE HYPOTHESIS: Case control studies comparing TH2 and TH1 immune responses in Burkitt lymphoma of different etiological types (sporadic, HIV-related, endemic and post-transplant) to demonstrate significant dominance of TH2 immune response in presence of poor CMI response as a common factor. Immunological profiling to evaluate differences between immune states that are associated (such as recurrent Malaria infection) and those that are not associated (such as severe protein-energy malnutrition) with Burkitt lymphoma. Prospective cohorts profiling chronology of immunological events leading to Burkitt lymphoma in children with EBV infection. Implications of the Hypothesis: The dysregulation of the immune response may be the missing link in our understanding of Burkitt lymphomagenesis. This will provide possibilities for determination of risk and for control of development of malignancy in individuals/populations exposed to the relevant infections.

Aggregation in malaria parasites places limits on mosquito infection rates.

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Gametocytes are responsible for the transmission of malaria parasites, Plasmodium spp., from man to mosquito. Although transmission success, as measured by the proportion of mosquitoes infected, generally increases with gametocyte density, the proportion of parasites that are gametocytes is always paradoxically only a few percent of the asexual blood parasites. To address this paradox, we analyse transmission data sets from an urban and an adjacent rural setting in Cameroon to elucidate whether there are discernable lower and upper limits to Plasmodium falciparum gametocyte density that are linked to transmission success. We find that there exists a lower gametocyte density at which mosquito infection rates considerably increase. In addition, we identify upper gametocyte densities at which mosquito infection rates level off. Greatest increases in infection rates occur at low gametocyte densities and coincide with
maximum oocyst aggregation within the infected mosquito population. This aggregated oocyst distribution remains despite increases in gametocyte density and ever-decreasing gains in mosquito infection rates. There is increasing suggestion that malaria parasites have evolved sex allocation strategies to ensure transmission in response to a changing, transmission-blocking environment. Here transmission-blocking immunity is proposed not only to ensure low density gametocyte transmission success but also to impose upper limits on transmission success.

31: Infect Genet Evol. 2007 Mar 31

Selection of pfmdr1 mutations after amodiaquine monotherapy and amodiaquine plus artemisinin combination therapy in East Africa.


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Despite the pharmacodynamic advantages with artemisinin-based combination therapy (ACT) and some potentially opposite molecular mechanisms of tolerance to amodiaquine (AQ)/desethylamodiaquine (DEAQ) and artesunate (ART), there is a risk for rapid decay in efficacy if the two drugs are unable to ensure mutual prevention against a selection and spread of drug-resistant parasites. We have studied if mutations in the pfcr7 and pfmdr1 genes selected in recurrent infections after AQ monotherapy are also selected after AQ plus ART combination therapy. Samples for molecular analysis were derived from three clinical trials on children <5 years old with uncomplicated Plasmodium falciparum malaria; one AQ monotherapy study conducted in Kenya 2003 and two AQ plus ART combination therapy studies conducted in Zanzibar 2002-2003 and 2005, respectively. The PCR-adjusted treatment failure rates in the three studies were 19%, 8% and 9%, respectively. After monotherapy there was a significant selection of pfcr7 76T in re-infections (OR not calculable; p=0.048) and of pfmdr1 86Y in recrudescent infections (OR 8.0; p=0.048). No such selection was found after combination therapy. A selection of pfmdr1 1246Y and the pfmdr1 haplotype (a.a 86, 184, 1246) YYY was found in recrudescent infections both after monotherapy (OR 7.6; p=0.009 and OR 3.1; p=0.029) and combination therapy in 2005 (OR 3.6; p=0.017 and OR 5.4; p<0.001). Hence, pfmdr1 1246Y with synergistic or compensatory addition of pfmdr1 86Y and 184Y appears to be involved in AQ/DEAQ resistance and treatment failure. Our results suggest that ART may protect against a selection of these SNPs initially, but maybe not after continuous drug pressure in a population. However, treatment failure rate and spread of pfmdr1 SNPs may remain at a low level because of the suggested opposite selection by ART and the pharmacodynamic advantages with ACT.

32: Infect Genet Evol. 2007 Mar 31

Chloroquine resistant P. falciparum prevalence is low and unchanged between 1990 and 2005 in Guinea-Bissau: An effect of high chloroquine dosage?

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Chloroquine resistant malaria was first reported in Guinea-Bissau in 1990 but chloroquine remains the most commonly used antimalarial in the country. Since
1990, we have conducted nearly annual standardized WHO in vitro micro-tests to assess chloroquine resistance. We have identified pfcrt 76T and other genetic polymorphisms in samples from 1992, 1993, 1995, 2004 and 2005. We have also monitored drug prescriptions for febrile illnesses. The mean proportion of in vitro tests indicating chloroquine resistance was 33% (range 14-54%) with the exception of an outlying value year 2000. The proportion of chloroquine resistant P. falciparum detected by in vitro testing did not increase over time. Pfcrt 76T was associated with chloroquine resistance but pfmdr1 86Y was not. The mean pfcrt 76T prevalence varied between 13% and 38%. The prevalence of SNPs at Pfcrt positions 76, 271, 326 and pfmdr1 position 86 did not change significantly between 1992 and 2005. In a health centre the median chloroquine dose prescribed for febrile illnesses between 1994 and 2003 was 63mg/kg. The genetic basis of chloroquine resistance appears to be the same in Guinea-Bissau as in other countries. Despite that, the prevalence of chloroquine resistant P. falciparum has not gradually increased between 1990 and 2005 in Guinea-Bissau. Chloroquine is commonly prescribed at more than double the normal dose in Guinea Bissau. It has previously been hypothesized that treatment with high doses of chloroquine may be effective. We discuss the possibility that the delayed spread of chloroquine resistant P. falciparum in Guinea-Bissau is the result of treatment with high and effective doses of chloroquine.

33: Infect Immun. 2007 May 25

**Induction of Nitric Oxide Synthase and Activation of Signaling Proteins in Anopheles Mosquitoes by Malaria Pigment Hemozoin.**

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Anopheles stephensi, a major vector for malaria parasite transmission, responds to Plasmodium infection by synthesis of inflammatory levels of nitric oxide (NO), which can limit parasite development in the midgut. We have previously shown that Plasmodium falciparum glycosylphosphatidylinositolos (PfGPIs) can induce A. stephensi NO synthase (AsNOS) expression in the midgut epithelium in vivo in a manner similar to the induction of cytokines and NO by PfGPIs in mammalian cells. In mosquito cells, signaling by PfGPIs and P. falciparum merozoites is mediated through Akt/protein kinase B (PKB), the mitogen-activated protein kinase kinase (MAPKK) DSOR1, and extracellular signal-regulated kinase (ERK). In mammalian cells, a second parasite factor, malaria pigment or hemozoin (Hz) signals NOS induction through ERK- and nuclear factor kappa B (NF-kappaB)-dependent pathways and has been demonstrated to be a novel proinflammatory ligand for Toll-like receptor 9 (TLR9). In this study, we demonstrate that Hz can also induce AsNOS gene expression in immortalized Anopheles stephensi and Anopheles gambiae cell lines in vitro and in A. stephensi midgut tissue in vivo. In mosquito cells, Hz signaling is mediated through transforming growth factor-beta (TGF-beta)-associated kinase 1 (TAK1), Akt/PKB, ERK and atypical protein kinase C zeta/lambda (aPKCzeta/lambda). Our results show that Hz is a prominent parasite-derived signal for Anopheles and that signaling pathways activated by PfGPIs and Hz have both unique and shared components. Taken together with our previous findings, our data indicate that parasite signaling of innate immunity is conserved in mosquito and mammalian cells.
Enhanced immunity to Plasmodium falciparum circumsporozoite protein using Salmonella Typhi expressing PfCSP and a PfCSP-encoding DNA vaccine in a heterologous prime-boost strategy.


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Two Salmonella enterica serovar Typhi (S. Typhi) strains that express and export a truncated version of Plasmodium falciparum circumsporozoite surface protein (tCSP) fused to S. Typhi Cytolysin A (ClyA) were constructed as a first step in the development of a pre-erythrocytic malaria vaccine. Synthetic codon-optimized genes (tcsp1 and tcsp2), containing immunodominant B- and T-cell epitopes present in native CSP, were fused in-frame to the carboxyl terminus of ClyA (clyA::tcsp) in genetically stabilized expression plasmids. Expression and export of ClyA-tCSP1 and ClyA-tCSP2 by S. Typhi vaccine strain CVD 908-htrA were demonstrated by immunoblotting of whole cell lysates and culture supernatants. The immunogenicity of these constructs was evaluated using a "heterologous prime-boost" approach, consisting of mucosal priming with S. Typhi expressing ClyA-tCSP1 and ClyA-tCSP2, followed by parenteral boosting with PfCSP DNA vaccines pVR2510 and pVR2571. Mice primed intranasally on days 0 and 28 with CVD 908-htrA(pSEC10tcsp2), and boosted intradermally on day 56 with PfCSP DNA vaccine pVR2571, induced high titers of serum NANP IgG (predominantly IgG2a); no serological responses to DNA vaccination were observed in the absence of S. Typhi-PfCSP priming. Mice primed with S. Typhi expressing tCSP2 and boosted with PfCSP DNA also developed high frequencies of IFN-gamma secreting cells, which surpassed those produced by PfCSP DNA in the absence of priming. A prime-boost regimen combining mucosal delivery of PfCSP exported from a Salmonella-based live vector vaccine, followed by parenteral PfCSP DNA boost, is a promising vaccine strategy in the development of a live vector-based malaria vaccine.

Inhibition of Dendritic Cell Maturation by Malaria is Dose-Dependent and Does Not Require Plasmodium falciparum Erythrocyte Membrane Protein 1 (PfEMP1).

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Plasmodium falciparum infected RBCs have been shown to modulate maturation of human monocyte-derived dendritic cells (DCs), interfering with their ability to activate T cells. Interaction between Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) and CD36 expressed by DCs is the proposed mechanism, but we show here that DC modulation does not require CD36 binding, PfEMP1 or contact between DCs and infected RBCs, and depends on infected RBC dose. Infected RBCs expressing a PfEMP1 variant that binds chondroitin sulfate A (CSA) but not CD36 were phagocytosed, inhibited LPS-induced phenotypic maturation and cytokine
secretion, and abrogated the ability of DCs to stimulate allogeneic T cell proliferation. CD36- and CSA-binding infected RBCs showed comparable inhibition. P. falciparum lines rendered deficient in PfEMP-1 expression by targeted gene knockout or knockdown also inhibited LPS-induced phenotypic maturation, and separation of DCs and infected RBCs in transwells showed inhibition was not contact-dependent. Inhibition was observed at infected RBC: DC ratios of 100:1 but not 10:1. High doses of infected RBCs were associated with apoptosis of DCs, which was not activation-induced. Lower doses of infected RBCs stimulated DC maturation sufficient to activate autologous T cell proliferation. In conclusion, modulation of DC maturation by P. falciparum is dose-dependent and does not require interaction between PfEMP1 and CD36. Inhibition and apoptosis of DCs by high dose infected RBCs may or may not be physiological. However, our observation that low dose infected RBCs initiate functional DC maturation warrants re-evaluation and further investigation of DC interactions with blood stage P. falciparum.

36: Insect Mol Biol. 2007 May 16

Genomic and evolutionary analyses of Tango transposons in Aedes aegypti, Anopheles gambiae and other mosquito species.

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Tango is a transposon of the Tc1 family and was originally discovered in the African malaria mosquito, Anopheles gambiae. Here we report a systematic analysis of the genome sequence of the yellow fever mosquito, Aedes aegypti, which uncovered three distinct Tango transposons. We name the only An. gambiae Tango transposon AgTango1 and the three Ae. aegypti Tango elements AeTango1-3. Like AgTango1, AeTango1 and AeTango2 elements both have members that retain characteristics of autonomous elements such as intact open reading frames and terminal inverted repeats (TIRs). AeTango3 is a degenerate transposon with no full-length members. All full-length Tango transposons contain subterminal direct repeats within their TIRs. AgTango1 and AeTango1-3 form a single clade among other Tc1 transposons. Within this clade, AgTango1 and AeTango1 are closely related and share approximately 80% identity at the amino acid level, which exceeds the level of similarity of the majority of host genes in the two species. A survey of Tango in other mosquito species was carried out using degenerate PCR. Tango was isolated and sequenced in all members of the An. gambiae species complex, Aedes albopictus and Ochlerotatus atropalpus. Oc. atropalpus contains a rich diversity of Tango elements, while Tango elements in Ae. albopictus and the An. gambiae species complex all belong to Tango1. No Tango was detected in Culex pipiens quinquefasciatus, Anopheles stephensi, Anopheles dirus, Anopheles farauti or Anopheles albimanus using degenerate PCR. Bioinformatic searches of the Cx. p. quinquefasciatus (~10 x coverage) and An. stephensi (0.33 x coverage) databases also failed to uncover any Tango elements. Although other evolutionary scenarios cannot be ruled out, there are indications that Tango underwent horizontal transfer among divergent mosquito species.

37: Int J Infect Dis. 2007 May 25

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OBJECTIVE: The main objective of the study was to assess the impact of a community-based delivery system of intermittent preventive treatment (IPT) for malaria in pregnancy with sulfadoxine-pyrimethamine (SP) on access, parasitemia, anemia and low birth weight as primary outcome measures. METHODS: A study was designed to test the community-based delivery system of IPT through traditional birth attendants (TBAs), drug-shop vendors (DSVs), community reproductive health workers (CRHWs) and adolescent peer mobilizers (APMs), and to compare these with IPT at health units in an area of high malaria transmission - Mukono District, Uganda. RESULTS: Two thousand seven hundred and eighty-five pregnant women participated in the study. The majority of the women (92.4%) at the community-based approaches received their first dose of IPT during their second trimester compared to 76.1% at health units (p<0.0001). At both health units and the community-based approaches, IPT increased mean hemoglobin by 6.7% (p<0.0001) for all parities and by 10.2% among primigravidae. IPT reduced the prevalence of severe anemia from 5.7% to 3.1% (p<0.04). The prevalence of parasitemia was reduced from 24.5% to 16.1% (p<0.001), and parasite density reduced significantly (p<0.02) after the first dose and remained stable with the second dose. Overall the proportion of low birth weight was 6.3% (8.3% at health units versus 6.0% at the community-based approaches, p<0.03) highlighting the importance of access and adherence to IPT. This intervention was acceptable to 89.6% of the women at the community-based approaches intending to use IPT in the future, while 48.1% of them had recommended it to other women. CONCLUSIONS: The community-based approaches increased access and adherence to IPT with an effect on anemia, severe anemia, parasitemia and low birth weight. However the reduced effect of IPT on parasitemia points to drug resistance with SP and this requires further evaluation; research into the identification of other more efficacious drugs for malaria prevention in pregnancy is also required.

38: Int J Parasitol. 2007 Apr 19

Plasmodium falciparum Pf34, a novel GPI-anchored rhoptry protein found in detergent-resistant microdomains.

Proellocks NI, Kovacevic S, Ferguson DJ, Kats LM, Morahan BJ, Black CG, Waller KL, Coppel RL.

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Apicomplexan parasites are characterised by the presence of specialised organelles, such as rhoptries, located at the apical end of invasive forms that play an important role in invasion of the host cell and formation of the parasitophorous vacuole. In this study, we have characterised a novel Plasmodium falciparum rhoptry protein, Pf34, encoded by a single exon gene located on chromosome 4 and expressed as a 34kDa protein in mature asexual stage parasites. Pf34 is expressed later in the life cycle than the previously described rhoptry protein, Rhoptry Associated Membrane Antigen (RAMA). Orthologues of Pf34 are present in other Plasmodium species and a potential orthologue has also been identified in Toxoplasma gondii. Indirect immunofluorescence assays show that Pf34 is located at the merozoite apex and localises to the rhoptry neck. Pf34, previously demonstrated to be glycosyl-phosphatidylinositol (GPI)-anchored [Gilson, P.R., Nebll, T., Vukcevic, D., Moritz, R.L., Sargeant, T., Speed, T.P.,
Schofield, L., Crabb, B.S. (2006) Identification and stoichiometry of GPI-anchored membrane proteins of the human malaria parasite Plasmodium falciparum. Mol. Cell. Proteomics 5, 1286-1299.], is associated with parasite-derived detergent-resistant microdomains (DRMs). Pf34 is carried into the newly invaded ring, consistent with a role for Pf34 in the formation of the parasitophorous vacuole. Pf34 is exposed to the human immune system during infection and is recognised by human immune sera collected from residents of malaria endemic areas of Vietnam and Papua New Guinea.

39: Int J Parasitol. 2007 Mar 30

**Minimum requirements for ookinete to oocyst transformation in Plasmodium.**

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During their passage through a mosquito vector, malaria parasites undergo several developmental transformations including that from a motile zygote, the ookinete, to a sessile oocyst that develops beneath the basal lamina of the midgut epithelium. This transformation process is poorly understood and the oocyst is the least studied of all the stages in the malaria life cycle. We have used an in vitro culture system to monitor morphological features associated with transformation of Plasmodium berghei ookinetes and the role of basal lamina components in this process. We also describe the minimal requirements for transformation and early oocyst development. A defined sequence of events begins with the break-up of the inner surface membrane, specifically along the convex side of the ookinete, where a protrusion occurs. A distinct form, the transforming ookinete or took, has been identified in vitro and also observed in vivo. Contrary to previous suggestions, we have shown that no basal lamina components are required to trigger ookinete to oocyst transformation in vitro. We have demonstrated that transformation does not occur spontaneously; it is initiated in the presence of bicarbonate added to PBS, but it is not mediated by changes in pH alone. Transformation is a two-step process that is not completed unless a range of nutrients are also present. A minimal medium is defined which supports transformation and oocyst growth from 7.8 to 11.4mum by day 5 with 84% viability. We conclude that ookinete transformation is mediated by bicarbonate and occurs in a similar manner to the differentiation of sporozoite to the hepatic stage.


**Synthesis of 5'-methylthio coformycins: specific inhibitors for malarial adenosine deaminase.**

Tyler PC, Taylor EA, Frohlich RF, Schramm VL.

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Transition state theory suggests that enzymatic rate acceleration (kcat/knon) is related to the stabilization of the transition state for a given reaction. Chemically stable analogues of a transition state complex are predicted to
convert catalytic energy into binding energy. Because transition state stabilization is a function of catalytic efficiency, differences in substrate specificity can be exploited in the design of tight-binding transition state analogue inhibitors. Coformycin and 2'-deoxycoformycin are natural product transition state analogue inhibitors of adenosine deaminases (ADAs). These compounds mimic the tetrahedral geometry of the ADA transition state and bind with picomolar dissociation constants to enzymes from bovine, human, and protozoan sources. The purine salvage pathway in malaria parasites is unique in that Plasmodium falciparum ADA (PfADA) catalyzes the deamination of both adenosine and 5'-methylthioadenosine. In contrast, neither human adenosine deaminase (HsADA) nor the bovine enzyme (BtADA) can deaminate 5'-methylthioadenosine. 5'-Methylthiocobalamin and 5'-methylthio-2'-deoxycoformycin were synthesized to be specific transition state mimics of the P. falciparum enzyme. These analogues inhibited PfADA with dissociation constants of 430 and 790 pM, respectively. Remarkably, they gave no detectable inhibition of the human and bovine enzymes. Adenosine deamination is involved in the essential pathway of purine salvage in P. falciparum, and prior studies have shown that inhibition of purine salvage results in parasite death. Inhibitors of HsADA are known to be toxic to humans, and the availability of parasite-specific ADA inhibitors may prevent this side-effect. The potent and P. falciparum-specific inhibitors described here have potential for development as antimalarials without inhibition of host ADA.

41: J Ethnobiol Ethnomedicine. 2007 May 1;3(1):18

Plants used traditionally to treat malaria in Brazil: the archives of Flora Medicinal.

Botsaris AS.

ABSTRACT: The archives of Flora Medicinal, an ancient pharmaceutical laboratory that supported ethnomedical research in Brazil for more than 30 years, were searched for plants claimed to have antimalarial activity. Forty plant species indicated to treat malaria were described by Dr. J. Monteiro da Silva (Flora Medicinal leader) and his co-workers. Eight species, Bathysa cuspidata, Cosmos sulphureus, Cecropia hololeuca, Erismum calcaratum, Gomphrena arborescens, Musa paradisiaca, Ocotea odorifera, and Pradosia lactescens, are quoted as antimalarial for the first time in ethnobotanical literature. Some species, including Mikania glomerata, Melampodium divaricatum, Galipea multiflora, Aspidosperma polyneuron, and Coutarea hexandra, are reported to have activity in malaria patients under clinical observation. In the information obtained there are also many details about the appropriate clinical use of each plant. For example, some plants are indicated to increase others potency, while others are traditionally employed for specific symptoms or conditions that often accompany malaria, such as weakness, renal failure or cerebral malaria. Many plants that have been considered to lack activity against malaria due to absence of in vitro activity against Plasmodium may have other mechanisms of action. Thus researchers should maybe carefully consider ethnomedical information before deciding the screening strategies to be used in the search for new antimalarial drugs.


Cutting Edge: conventional dendritic cells are the critical APC required for the induction of experimental cerebral malaria.

deWalick S, Amante FH, McSweeney KA, Randall LM, Stanley AC, Haque A, Kuns RD,
Cerebral malaria (CM) is a serious complication of Plasmodium falciparum infection, causing significant morbidity and mortality among young children and nonimmune adults in the developing world. Although previous work on experimental CM has identified T cells as key mediators of pathology, the APCs and subsets therein required to initiate immunopathology remain unknown. In this study, we show that conventional dendritic cells but not plasmacytoid dendritic cells are required for the induction of malaria parasite-specific CD4+ T cell responses and subsequent experimental CM. These data have important implications for the development of malaria vaccines and the therapeutic management of CM.


Discovery of a Rhodanine Class of Compounds as Inhibitors of Plasmodium falciparum Enoyl-Acyl Carrier Protein Reductase.


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Enoyl acyl carrier protein (ACP) reductase, one of the enzymes of the type II fatty acid biosynthesis pathway, has been established as a promising target for the development of new drugs for malaria. Here we present the discovery of a rhodanine (2-thioxothiazolidin-4-one) class of compounds as inhibitors of this enzyme using a combined approach of rational selection of compounds for screening, analogue search, docking studies, and lead optimization. The most potent inhibitor exhibits an IC50 of 35.6 nM against Plasmodium falciparum enoyl ACP reductase (PfENR) and inhibits growth of the parasite in red blood cell cultures at an IC50 value of 750 nM. Many more compounds of this class were found to inhibit PfENR at low nanomolar to low micromolar concentrations, expanding the scope for developing new antimalarial drugs. The structure-activity relationship of these rhodanine compounds is discussed.


Estimating the number of insecticide-treated nets required by African households to reach continent-wide malaria coverage targets.

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CONTEXT: African countries are scaling up malaria interventions, especially insecticide-treated nets (ITNs), for which ambitious coverage targets have been set. OBJECTIVE: To estimate how many ITNs are available in African households that are at risk of malaria and how many ITNs are needed to reach targets for use by children younger than 5 years and pregnant women. DATA SOURCES: Primary sources of data were the Multiple Indicator Cluster Surveys II, the Demographic and Health Surveys, or other nationally representative or large-scale household
surveys that measured household possession and use of nets or ITNs among children younger than 5 years. DATA EXTRACTION: Data from 42 household surveys between 1999 and 2006 on net and ITN coverage (either household possession or use) and average numbers of nets and ITNs per household were compared with populations and households at risk. Data are included for 43 sub-Saharan African countries. DATA SYNTHESIS: For the median survey year 2003, the population-weighted mean proportion of households possessing at least 1 ITN was 6.7% (range among countries, 0.1%-71.0%) and was 23.8% (range, 5.0%-91.2%) for any type of net. Based on an average of 0.13 ITNs per household, we estimated that 53.6 million nets, of which 16.7 million were ITNs, were available in households at risk of malaria. Between 130 million and 264 million ITNs are required in 2007 to reach the 80% coverage target for about 133 million children younger than 5 years and pregnant women living in 123 million households in risk areas; the exact number depends on usage patterns (best estimate, assuming 55% of owned ITNs are used by the target groups, 192 million ITNs). CONCLUSION: To achieve the targeted ITN usage rates, numbers of ITNs available to African households must be dramatically increased.


Burden, features, and outcome of neurological involvement in acute falciparum malaria in Kenyan children.


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CONTEXT: Plasmodium falciparum appears to have a particular propensity to involve the brain but the burden, risk factors, and full extent of neurological involvement have not been systematically described. OBJECTIVES: To determine the incidence and describe the clinical phenotypes and outcomes of neurological involvement in African children with acute falciparum malaria. DESIGN, SETTING, AND PATIENTS: A review of records of all children younger than 14 years admitted to a Kenyan district hospital with malaria from January 1992 through December 2004. Neurological involvement was defined as convulsive seizures, agitation, prostration, or impaired consciousness or coma. MAIN OUTCOME MEASURES: The incidence, pattern, and outcome of neurological involvement. RESULTS: Of 58,239 children admitted, 19,560 (33.6%) had malaria as the primary clinical diagnosis. Neurological involvement was observed in 9313 children (47.6%) and manifested as seizures (6563/17,517 [37.5%]), agitation (316/11,193 [2.8%]), prostration (3223/15,643 [20.6%]), and impaired consciousness or coma (2129/16,080 [13.2%]). In children younger than 5 years, the mean annual incidence of admissions with malaria was 2694 per 100,000 persons and the incidence of malaria with neurological involvement was 1156 per 100,000 persons. However, readmissions may have led to a 10% overestimate in incidence. Children with neurological involvement were older (median, 26 [interquartile range {IQR}, 15-41] vs 21 [IQR, 10-40] months; P<.001), had a shorter duration of illness (median, 2 [IQR, 1-3] vs 3 [IQR, 2-3] days; P<.001), and a higher geometric mean parasite density (42.0 [95% confidence interval {CI}, 40.0-44.1] vs 30.4 [95% CI, 29.0-31.8] x 10^3/microL; P<.001). Factors independently associated with neurological involvement included past history of seizures (adjusted odds ratio [AOR], 3.50; 95% CI, 2.78-4.42), fever lasting 2 days or less (AOR, 2.02; 95% CI, 1.64-2.49), delayed capillary refill time (AOR, 3.66; 95% CI, 2.40-5.56), metabolic acidosis (AOR, 1.55; 95% CI, 1.29-1.87), and hypoglycemia (AOR, 2.11; 95% CI, 1.31-3.37).
Mortality was higher in patients with neurological involvement (4.4% [95% CI, 4.2%-5.1%] vs 1.3% [95% CI, 1.1%-1.5%]; P<.001). At discharge, 159 (2.2%) of 7281 patients had neurological deficits. CONCLUSIONS: Neurological involvement is common in children in Kenya with acute falciparum malaria, and is associated with metabolic derangements, impaired perfusion, parasitemia, and increased mortality and neurological sequelae. This study suggests that falciparum malaria exposes many African children to brain insults.


Improved diagnostic testing and malaria treatment practices in Zambia.


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CONTEXT: Improving the accuracy of malaria diagnosis with rapid antigen-detection diagnostic tests (RDTs) has been proposed as an approach for reducing overtreatment of malaria in the current era of widespread implementation of artemisinin-based combination therapy in sub-Saharan Africa. OBJECTIVE: To assess the association between use of microscopy and RDT and the prescription of antimalarials. DESIGN, SETTING, AND PARTICIPANTS: Cross-sectional, cluster sample survey, carried out between March and May 2006, of all outpatients treated during 1 working day at government and mission health facilities in 4 sentinel districts in Zambia. MAIN OUTCOME MEASURE: Proportions of patients undergoing malaria diagnostic procedures and receiving antimalarial treatment. RESULTS: Seventeen percent of the 104 health facilities surveyed had functional microscopy, 63% had RDTs available, and 73% had 1 or more diagnostics available. Of patients with fever (suspected malaria), 27.8% (95% confidence interval [CI], 13.1%-42.5%) treated in health facilities with malaria diagnostics were tested and 44.6% had positive test results. Of patients with negative blood smear results, 58.4% (95% CI, 36.7%-80.2%) were prescribed an antimalaria drug, as were 35.5% (95% CI, 16.0%-55.0%) of those with a negative RDT result. Of patients with fever who did not have diagnostic tests done, 65.9% were also prescribed antimalarials. In facilities with artemether-lumefantrine in stock, this antimalarial was prescribed to a large proportion of febrile patients with a positive diagnostic test result (blood smear, 75.0% [95% CI, 51.7%-98.3%]; RDT, 70.4% [95% CI, 39.3%-100.0%]), but also to some of those with a negative diagnostic test result (blood smear, 30.4% [95% CI, 8.0%-52. 9%]; RDT, 26.7% [95% CI, 5.7%-47.7%]). CONCLUSIONS: Despite efforts to expand the provision of malaria diagnostics in Zambia, they continue to be underused and patients with negative test results frequently receive antimalarials. Provision of new tools to reduce inappropriate use of new expensive antimalarial treatments must be accompanied by a major change in clinical treatment of patients presenting with fever but lacking evidence of malaria infection.


Hemoglobin variants and disease manifestations in severe falciparum malaria.

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CONTEXT: The geographical distributions of hemoglobin S (HbS), hemoglobin C
(HbC), and alpha-thalassemia (-alpha) strongly suggest balancing selection with malaria. However, whereas several studies indicate that the HbS carrier state protects against all major forms of clinical malaria, malaria protection on clinical grounds has been more difficult to confirm for HbC and -alpha, and questions remain as to whether it applies to all forms of the disease.

OBJECTIVE: To assess the association between major clinical forms of severe falciparum malaria and HbS, HbC, and -alpha. DESIGN, SETTING, AND PARTICIPANTS: Case-control study of 2591 children with severe falciparum malaria enrolled at a tertiary referral center in Ghana, West Africa, and 2048 age-, sex-, and ethnicity-matched control participants recruited by community surveys. MAIN OUTCOME MEASURES: Frequencies of HbS, HbC, and -alpha in patients and controls, including stratifications of patients for signs of disease. RESULTS: Patients presented with partly overlapping signs of disease, including severe anemia (64%), cerebral malaria (22%), respiratory distress (30%), hyperparasitemia (32%), prostration (52%), acidosis (59%), and hyperlactatemia (56%). Carrier states of HbS, HbC, and -alpha were found in 1.4%, 9.4%, and 25.2% of the patients, respectively, and 14.8%, 8.7%, and 27.3% of controls. The HbS carrier state was negatively associated with all forms of the disease studied (overall odds ratio [OR], 0.08; 95% confidence interval [CI], 0.06-0.12). The HbC carrier state showed a negative association selectively with cerebral malaria (OR, 0.64; 95% CI, 0.45-0.91), and the -alpha carrier state showed a negative association selectively with severe anemia (OR, 0.82; 95% CI, 0.69-0.96). CONCLUSION: Whereas the HbS carrier state was found to be negatively associated with all major forms of severe falciparum malaria, the negative associations of the carrier states of HbC and -alpha appeared to be limited to cerebral malaria and severe anemia, respectively.


Combination therapy for uncomplicated falciparum malaria in Ugandan children: a randomized trial.


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CONTEXT: Combination therapy is now widely advocated as first-line treatment for uncomplicated malaria in Africa. However, it is not clear which treatment regimens are optimal or how to best assess comparative efficacies in highly endemic areas. OBJECTIVE: To compare the efficacy and safety of 3 leading combination therapies for the treatment of uncomplicated malaria. DESIGN, SETTING, AND PARTICIPANTS: Single-blind randomized clinical trial, conducted between November 2004 and June 2006, of treatment for all episodes of uncomplicated malaria in children in an urban community in Kampala, Uganda. A total of 601 healthy children (aged 1-10 years) were randomly selected and were followed up for 13 to 19 months, receiving all medical care at the study clinic. INTERVENTIONS: Study participants were randomized to receive 1 of 3 combination therapies (amodiaquine plus sulfadoxine-pyrimethamine, amodiaquine plus artesunate, or artemether-lumefantrine) when diagnosed with their first episode of uncomplicated malaria. The same assigned treatment was given for all subsequent episodes. MAIN OUTCOME MEASURE: 28-Day risk of parasitological failure (unadjusted and adjusted by genotyping to distinguish recrudescence from new infection) for each episode of uncomplicated malaria treated with study drugs. RESULTS: Of enrolled children, 329 of 601 were diagnosed with at least 1 episode of uncomplicated malaria, and 687 episodes of Plasmodium falciparum
malaria were treated with study drugs. The 28-day risk of treatment failure (unadjusted by genotyping) for individual episodes of malaria were 26.1% (95% CI, 21.1%-32.1%) for amodiaquine plus sulfadoxine-pyrimethamine, 17.4% (95% CI, 13.1%-23.1%) for amodiaquine plus artesunate, and 6.7% (95% CI, 3.9%-11.2%) for artemether-lumefantrine (P<.05 for all pairwise comparisons). When only recrudescent treatment failures were considered, the risks of failure were 14.1% (95% CI, 10.3%-19.2%), 4.6% (95% CI, 2.5%-8.3%), and 1.0% (95% CI, 0.3%-4.0%) for the same order of study drugs, respectively (P< or = .008 for all pairwise comparisons, except amodiaquine plus artesunate vs artemether-lumefantrine, P = .05). There were no deaths or cases of severe malaria. Significant reductions in anemia (9.3% [95% CI, 7.0%-12.0%] at enrollment vs 0.6% [95% CI, 0.1%-2.2%] during the last 2 months of follow-up; P<.001) and asymptomatic parasitemia (18.6% [95% CI, 15.5%-22.1%] at enrollment vs 2.3% [95% CI, 1.5%-3.5%] during the last 2 months of follow-up; P<.001) were observed according to routine testing. CONCLUSIONS: Artemether-lumefantrine was the most efficacious treatment for uncomplicated malaria in the study population. With all study regimens, the provision of prompt and reasonably effective facility-based treatment was associated with good outcomes in long-term health measures. TRIAL REGISTRATION: isrctn.org Identifier: ISRCTN37517549.


Sulfadoxine-pyrimethamine, chlorproguanil-dapsone, or chloroquine for the treatment of Plasmodium vivax malaria in Afghanistan and Pakistan: a randomized controlled trial.

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CONTEXT: In areas where Plasmodium falciparum and Plasmodium vivax coexist and treatments for the 2 species differ, misdiagnosis can lead to poor outcomes in either disease. A unified therapy effective against both species would reduce reliance on species-specific diagnosis, which in many areas is difficult to maintain. The antifolates are an important and affordable antimalarial class to which it is often assumed P vivax malaria is intrinsically resistant. OBJECTIVE: To test the relative efficacy and safety of 2 antifolate drugs against P vivax malaria and compare each with chloroquine. DESIGN, SETTING, AND PATIENTS: An open-label randomized controlled trial comparing chloroquine, sulfadoxine-pyrimethamine, and chlorproguanil-dapsone for the treatment of P vivax malaria was conducted in eastern Afghanistan and northwestern Pakistan, areas in which P vivax malaria predominates. A total of 20,410 patients older than 3 years were screened; 767 patients (315 in Pakistan and 452 in Afghanistan) with confirmed P vivax malaria were enrolled and followed up daily for 4 days, then weekly for 28 days, between March 2004 and June 2006. MAIN OUTCOME MEASURES: Complete clearance of parasites with no recrudescence by day 14. Secondary outcomes included being parasite-free by day 28, clinical failure, and anemia. RESULTS: By day 14, only 1 patient in the sulfadoxine-pyrimethamine group had parasites. By day 28, failure rates were found in 2 of 153 patients (1.3%) in the chloroquine group, 5 of 290 patients (1.7%) in the sulfadoxine-pyrimethamine group, and 27 of 272 patients (9.9%) in the chlorproguanil-dapsone group. Chlorproguanil-dapsone was less effective than sulfadoxine-pyrimethamine (adjusted odds ratio [OR], 6.4; 95% confidence interval [CI], 2.4-17.0; P<.001) and chloroquine (adjusted OR, 8.4; 95% CI, 2.0-36.5; P = .004). Chloroquine and sulfadoxine-pyrimethamine were equivalent in efficacy at day 28 (adjusted OR, 1.3; 95% CI, 0.3-7.0; P = .73). Chloroquine
cleared gametocytes and asexual parasites more rapidly than sulfadoxine-pyrimethamine or chlorproguanil-dapsone did. All drugs were well tolerated. CONCLUSIONS: Although chloroquine remains the drug of choice, antifolates are effective against P vivax malaria in South Asia. These drugs may be appropriate for unified treatment where species-specific diagnosis is unavailable, most likely in combination with other drugs. TRIAL REGISTRATION: clinicaltrials.gov Identifier: NCT00158561.


Malaria prevention measures in coalition troops in Afghanistan.

Croft AM, Darbyshire AH, Jackson CJ, van Thiel PP.

(no abstract available)

51: Malar J. 2007 May 26;6(1):71

Assessing the risk of self-diagnosis malaria in urban informal settlements of Nairobi using self-reported morbidity survey.

Ye Y, Kimani-Murage E, Kebaso J, Mugisha F.

ABSTRACT: BACKGROUND: Because of the belief that Nairobi is a low risk zone for malaria, little empirical data exists on malaria risk in the area. The aim of this study was to explore the risk of perceived malaria and some associated factors in Nairobi informal settlements using self-reported morbidity survey. METHODS: The survey was conducted from May to August 2004 on 7,288 individuals in two informal settlements of Nairobi. Participants were asked to report illnesses they experienced in the past 15 days. Logistic regression was used to estimate the odds of perceived-malaria. The model included variables such as site of residence, age, ethnicity and number of reported symptoms. RESULTS: Participants reported 165 illnesses among which malaria was the leading cause (28.1%). The risk of perceived-malaria was significantly higher in Viwandani compared to Korogocho (OR 1.61, 95%CI: 1.10-2.26). Participants in age group 25-39 years had significantly higher odds of perceived-malaria compared to those under-five years (OR 2.07, 95%CI: 1.43-2.98). The Kikuyu had reduced odds of perceived-malaria compared to other ethnic groups. Individuals with five and more symptoms had high odds compared to those with no symptoms (OR 23.69, 95%CI: 12.98-43.23). CONCLUSION: Malaria was the leading cause of illness as perceived by the residents in the two informal settlements. This was rational as the number of reported symptoms is highly associated with the risk of reporting the illness. These results highlight the need for a more comprehensive assessment of malaria epidemiology in Nairobi to be able to offer evidence-based guidance to policy on malaria in Kenya and particularly in Nairobi.

52: Malar J. 2007 May 25;6(1):70

Pharmacokinetics and pharmacodynamics of fosmidomycin monotherapy and combination therapy with clindamycin in the treatment of multidrug resistant falciparum malaria.

Na-Bangchang K, Ruengweerayut R, Karbwang J, Chauemung A, Hutchinson D.

ABSTRACT: BACKGROUND: The study investigated the pharmacokinetics of fosmidomycin when given alone and in combination with clindamycin in patients with acute uncomplicated falciparum malaria. METHODS: A total of 15 and 18
patients with acute uncomplicated Plasmodium falciparum malaria who fulfilled the enrollment criteria were recruited from out-patient department of Mae Sot Hospital, Tak Province, Thailand. Patients were treated with monotherapy with fosmidomycin at the dose of 1,200 mg every 8 hours for 7 days (n=15) or combination therapy with fosmidomycin (900 mg every 12 hours for 7 days) and clindamycin (600 mg every 12 hours for 7 days) (n=18). Blood samples were taken for pharmacokinetic investigations of clindamycin and/or fosmidomycin and 24-hour urine samples were collected during dosing period. Efficacy assessments included clinical and parasitological evaluation. Safety and tolerability were assessed based on clinical and laboratory assessments. RESULTS: Both mono- and combination therapy regimens of fosmidomycin were well tolerated with no serious adverse events. Combination therapy with fosmidomycin and clindamycin was proven highly effective with 100% cure rate, whereas cure rate of monotherapy was 22% (28-day follow up). Pharmacokinetics of fosmidomycin following mono- and combination therapy were similar except Vz/F and CL/F, which were significantly smaller in the combination regimen. Plasma concentration-time profiles of both fosmidomycin and clindamycin were best fit with a one-compartment open model with first-order absorption and elimination and with absorption lag time. Steady-state plasma concentrations of fosmidomycin and clindamycin were attained at about the second or third dose. There was no evidence of dose accumulation during multiple dosing. Urinary recovery of fosmidomycin was 18.7 and 20% following mono- and combination therapy, respectively. CONCLUSION: Pharmacokinetic dose optimization of fosmidomycin-clindamycin combination therapy with the course of treatment of not longer than three days is required to obtain a regimen which is safe and produced 100% cure for multidrug-resistant P. falciparum.

53: Malar J. 2007 May 22;6(1):68

Acceptability and efficacy of intra-rectal quinine alkaloids as a pre-transfer treatment of severe malaria in peripheral health care facilities in Mopti, Mali.

Thera MA, Keita F, Sissoko MS, Traore OB, Coulibaly D, Sacko M, Lameyre V, Ducret JP, Doumbo OK.

ABSTRACT: BACKGROUND: The acceptability and efficacy of a new kit with a new formulation of quinine alkaloids designed for the intra-rectal administration in the treatment of non-per os malaria was assessed in the peripheral health care system of Mopti, Mali. METHODS: A single-arm trial was conducted from August 2003 to January 2004. An initial dose of diluted quinine alkaloids (20mg/kg Quinimax(R)) was administered by the intra-rectal route to children with presumptive non per-os malaria at six peripheral heath care centres. The children were then referred to two referral hospitals where standard inpatient care including intravenous route were routinely provided. A malaria thick smear was done at inclusion and a second dose of intra-rectal quinine alkaloids. Primary outcome was acceptability of the intra-rectal route by children and their parents as well as the ease to handle the kit by health care workers. RESULTS: The study included 134 children with a median age of 33 months and 53.7% were male. Most of the children (67%) and 92% of parents or guardians readily accepted the intra-rectal route; 84% of health care workers found the kit easy to use. At the peripheral health care centres, 32% of children had a coma score [less than or equal to]3 and this was reduced to 10% at the referral hospital, following one dose of intra-rectal quinine alkaloids (IRQA). The mean
time to availability of oral route treatment was 1.8+/−1.1 days. Overall, 73% of cases were confirmed severe malaria and for those the case fatality rate was 7.2%. CONCLUSIONS: IRQA was well accepted by children, their parents/guardians and by the health workers at peripheral health facilities in Mopti, Mali. There was also a quick recovery from deep coma and a reduced case fatality rate in severe malaria.

54: Malar J. 2007 May 22;6(1):65

Randomized clinical trial of artemisinin versus non-artemisinin combination therapy for uncomplicated falciparum malaria in Madagascar.

Menard D, Andrianina NN, Randriamarosoa Z, Randriamantena A, Rasoarilalao N, Jahevitra M, Ratsimbasa A, Tuseo L, Raveloson A.

ABSTRACT: BACKGROUND: Data concerning antimalarial combination treatment for uncomplicated malaria in Madagascar are largely lacking. Randomized clinical trial was designed to assess therapeutic efficacies of chloroquine (CQ), amodiaquine (AQ), sulphadoxine-pyrimethamine (SP), amodiaquine plus sulphadoxine-pyrimethamine combination (AQ+SP) and artemisinin plus amodiaquine combination (AQ+AS). METHODS: 287 children between 6 months and 15 years of age, with uncomplicated falciparum malaria, were enrolled in the study. Primary endpoints were the day-14 and day-28 risks of parasitological failure, either unadjusted or adjusted by genotyping. RESULTS: All treatment regimens, except for CQ treatment, gave clinical cure rates above 97% by day-14 and 92% by day-28 (PCR-corrected). AQ+SP was as effective as AQ+AS. The risk of new infection within the month after therapy was generally higher for AQ+AS than AQ+SP. CONCLUSIONS: These findings show that the inexpensive and widely available combination AQ+SP may be valuable in for the treatment of uncomplicated malaria in Madagascar and could have an important role in this country, where much of the drugs administered go to patients who do not have malaria.

55: Malar J. 2007 May 22;6(1):63

Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae.

Okumu FO, Knols BG, Fillinger U.

ABSTRACT: BACKGROUND: Larviciding is a key strategy used in many vector control programmes around the world. Costs could be reduced if larvicides could be manufactured locally. The potential of natural products as larvicides against the main African malaria vector, Anopheles gambiae s.s was evaluated. METHODS: To assess the larvicidal efficacy of a neem (Azadirachta indica) oil formulation (azadirachtin content of 0.03% w/v) on An. gambiae s.s., larvae were exposed as third and fourth instars to a normal diet supplemented with the neem oil formulations in different concentrations. A control group of larvae was exposed to a corn oil formulation in similar concentrations. RESULTS: Neem oil had an LC50 value of 11 ppm after 8 days, which was nearly five times more toxic than the corn oil formulation. Adult emergence was inhibited by 50% at a concentration of 6 ppm. Significant reductions on growth indices and pupation, besides prolonged larval periods, were observed at neem oil concentrations above 8 ppm. The corn oil formulation, in contrast, produced no growth disruption within the tested range of concentrations. CONCLUSION: Neem oil has good larvicidal properties for An. gambiae s.s. and suppresses successful adult emergence at very low concentrations. Considering the wide distribution and availability of this tree and its products along the East African coast, this
may prove a readily available and cheap alternative to conventional larvicides.

**56: Malar J. 2007 May 21;6(1):60**

Genetic structure of *Plasmodium falciparum* field isolates in eastern and north-eastern India.


ABSTRACT: BACKGROUND: Molecular techniques have facilitated the studies on genetic diversity of *Plasmodium* species particularly from field isolates collected directly from patients. Msp-1 and msp-2 are highly polymorphic markers and the large allelic polymorphism has been reported in the block 2 of the msp-1 gene and the central repetitive domain (block3) of the msp-2 gene. Families differing in nucleotide sequences and in number of repetitive sequences (length variation) were used for genotyping purposes. As limited reports are available on the genetic diversity existing among *Plasmodium falciparum* population of India, this report evaluates the extent of genetic diversity in the field isolates of *P. falciparum* in eastern and north-eastern region of India. METHODS: A study was designed to assess the diversity of msp-1 and msp-2 among the field isolates from India using allele specific nested PCR assays and sequence analysis. Field isolates were collected from five sites distributed in three states namely, Assam, West Bengal and Orissa. RESULTS: *P. falciparum* isolates of the study sites are highly diverse in respect of length as well as sequence motifs with prevalence of all the reported allelic families of msp-1 and msp-2. Prevalence of identical allelic composition as well as high level of sequence identity of alleles suggests a considerable amount of gene flow between the states. A comparatively higher proportion of multiclonal isolates as well as multiplicity of infection (MOI) was observed among isolates of district Karbi Anglong (Assam) and district Sundergarh (Orissa), highly malarious districts. In all the five sites, R033 family of msp-1 was observed to be monomorphic with an allele size of 150/160bp. Observed above 80-90% sequence identity of Indian isolates with data of other regions suggest that Indian *P. falciparum* population is a mixture of different strains. CONCLUSION: The present study shows that field isolates of eastern and north-eastern regions of India are highly diverse in respect of msp-1 (block 2) and msp-2 (central repeat region, block 3). As expected Indian isolates present a picture of diversity closer to south-east Asia, Papua New Guinea and Latin American countries, regions with low to meso-endemicity of malaria in comparison to African regions of hyper- to holo-endemicity.

**57: Malar J. 2007 May 16;6(1):58**

Paracheck-Pf(R) accuracy and recently treated *Plasmodium falciparum* infections: Is there a risk of over-diagnosis?

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ABSTRACT: BACKGROUND: An assessment of the accuracy of Paracheck Pf(R), a malaria rapid diagnostic test (RDT) detecting histidine rich protein 2, was undertaken amongst children aged 6-59 months in eastern Democratic Republic of Congo. METHODS: This RDT assessment occurred in conjunction with an ACT efficacy trial. Febrile children were simultaneously screened with both RDT and high quality microscopy and those meeting inclusion criteria were followed for 35 days. RESULTS: 358 febrile children were screened with 180 children recruited for five weeks follow-up. On screening, the RDT accurately diagnosed all 235
true malaria cases, indicating 100% RDT sensitivity. Of the 123 negative slides, the RDT gave 59 false-positive results, indicating 52.0% (64/123) RDT specificity. During follow-up after treatment with an artemisinin-based combination therapy, 98.2% (110/112), 94.6% (106/112), 92.0% (103/112) and 73.5% (50/68) of effectively treated children were still false-positive by RDT at days 14, 21, 28 and 35, respectively. CONCLUSIONS: Results show that though the use of Paracheck-Pf(R) is as sensitive as microscopy in detecting true malaria cases, a low specificity did present a high frequency of false-positive RDT results. What's more, a duration of RDT false-positivity was found that significantly surpassed the 'fortnight' after effective treatment reported by its manufacturer. Though further research is needed in assessing RDT accuracy, study results showing the presence of frequent false positivity should be taken into consideration to avoid clinicians inappropriately focusing on malaria, not identifying the true cause of illness, and providing unnecessary treatment.


Use of over-the-counter malaria medicines in children and adults in three districts in Kenya: implications for private medicine retailer interventions.

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BACKGROUND: Global malaria control strategies highlight the need to increase early uptake of effective antimalarials for childhood fevers in endemic settings, based on a presumptive diagnosis of malaria in this age group. Many control programmes identify private medicine sellers as important targets to promote effective early treatment, based on reported widespread inadequate childhood fever treatment practices involving the retail sector. Data on adult use of over-the-counter (OTC) medicines is limited. This study aimed to assess childhood and adult patterns of OTC medicine use to inform national medicine retailer programmes in Kenya and other similar settings. METHODS: Large-scale cluster randomized surveys of treatment seeking practices and malaria parasite prevalence were conducted for recent fevers in children under five years and recent acute illnesses in adults in three districts in Kenya with differing malaria endemicity. RESULTS: A total of 12, 445 households were visited and data collected on recent illnesses in 11, 505 children and 19, 914 adults. OTC medicines were the most popular first response to fever in children with fever (47.0%; 95% CI 45.5, 48.5) and adults with acute illnesses (56.8%; 95% CI 55.2, 58.3). 36.9% (95% CI 34.7, 39.2) adults and 22.7% (95% CI 20.9, 24.6) children using OTC medicines purchased antimalarials, with similar proportions in low and high endemicity districts. 1.9% (95% CI 0.8, 4.2) adults and 12.1% (95% CI 16.3,34.2) children used multidose antimalarials appropriately. Although the majority of children and adults sought no further treatment, self-referral to a health facility within 72 hours of illness onset was the commonest pattern amongst those seeking further help. CONCLUSION: In these surveys, OTC medicines were popular first treatments for fever in children or acute illnesses in adults. The proportions using OTC antimalarials were similar in areas of high and low malaria endemicity. In all districts, adults were more likely to self-treat with OTC antimalarial medicines than febrile children were to receive them, and less likely to use them in recommended ways. Government health centres were the most common second resort for treatment and were often used within 72 hours. In view of these practices, more research is needed to assess the impact on the popularity of private medicine sellers of strengthened public sector policies on access to malaria treatment and insecticide-treated bed nets.
Improved targeting of OTC antimalarials to high risk groups, better communication strategies regarding adult as well as children's dosages, and facilitating more rapid referral to trained health workers where needed are important challenges to private medicine seller programmes.

59: Malar J. 2007 May 8;6(1):56

Quantification of the efficiency of treatment of Anopheles gambiae breeding sites with petroleum products by local communities in areas of insecticide resistance in the Republic of Benin.

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ABSTRACT: BACKGROUND: The emergence of Anopheles populations capable of withstanding lethal doses of insecticides has weakened the efficacy of most insecticide based strategies of vector control and, has highlighted the need for developing new insecticidal molecules or, improving the efficacy of existing insecticides or abandoning those to which resistance has emerged. The use of petroleum products (PP) against mosquito larvae had an immense success during early programmes of malaria control, but these compounds were abandoned and replaced in the 1950s by synthetic insecticides probably because of the high performances given by these new products. In the current context of vector resistance, it is important to elucidate the empirical use of PP by quantifying their efficiencies on resistant strains of Anopheles. METHODS: Larvae of Anopheles Ladji a local resistant strain were exposed to increasing concentrations of various PP (kerosene, petrol and engine oils) for 24 hours and the lethal activities recorded. The highest concentration (HiC) having no lethal activity (also referred as the NOEL or no effect level) and the lowest concentration (LoC100) yielding 100% mortality were rated for each PP on the Ladji strain. Prior to laboratory analysis, KAP studies were conducted in three traditional communities were insecticide resistance is clearly established to confirm the use of PP against mosquitoes. RESULTS: Laboratory analysis of petrol, kerosene and engine oils, clearly established their lethal activities on resistant strains of Anopheles larvae. Contrary to existing references, this research revealed that exposed larvae of Anopheles were mostly killed by direct contact toxicity and not by suffocation as indicated in some earlier reports. CONCLUSION: This research could serve as scientific basis to backup the empirical utilisation of PP on mosquito larvae and to envisage possibilities of using PP in some traditional settings where Anopheles have developed resistance to currently used insecticides.

60: Malar J. 2007 May 4;6:55.

Antimalarial drug prescribing practice in private and public health facilities in South-east Nigeria: a descriptive study.


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BACKGROUND: Nigeria's national standard has recently moved to artemisinin combination treatments for malaria. As clinicians in the private sector are responsible for attending a large proportion of the population ill with malaria, this study compared prescribing in the private and public sector in one State in Nigeria prior to promoting ACTs. OBJECTIVE: To assess prescribing for
uncomplicated malaria in government and private health facilities in Cross River State. **METHOD:** Audit of 665 patient records at six private and seven government health facilities in 2003. **RESULTS:** Clinicians in the private sector were less likely to record history or physical examination than those in public facilities, but otherwise practice and prescribing were similar. Overall, 45% of patients had a diagnostic blood slide; 77% were prescribed monotherapy, either chloroquine (30.2%), sulphadoxine-pyrimethamine (22.7%) or artemisinin derivatives alone (15.8%). Some 20.8% were prescribed combination therapy; the commonest was chloroquine with sulphadoxine-pyrimethamine. A few patients (3.5%) were prescribed sulphadoxine-pyrimethamine-mefloquine in the private sector, and only 3.0% patients were prescribed artemisinin combination treatments. **CONCLUSION:** Malaria treatments were varied, but there were not large differences between the public and private sector. Very few are following current WHO guidelines. Monotherapy with artemisinin derivatives is relatively common.

61: Malar J. 2007 May 3;6:54.

**Efficacy of antimalarial treatment in Guinea: in vivo study of two artemisinin combination therapies in Dabola and molecular markers of resistance to sulphadoxine-pyrimethamine in N’Zerekore.**

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**BACKGROUND:** In the last five years, countries have been faced with changing their malaria treatment policy to an artemisinin-based combination therapy (ACT), many with no national data on which to base their decision. This is particularly true for a number of West African countries, including Guinea, where these studies were performed. Two studies were conducted in 2004/2005 in programmes supported by Médecins Sans Frontières, when chloroquine was still national policy, but artesunate (AS)/sulphadoxine-pyrimethamine (SP) had been used in refugee camps for two years. **METHODS:** In Dabola (central Guinea), 220 children aged 6-59 months with falciparum malaria were randomized to receive either AS/amodiaquine (AQ) or AS/SP. In vivo efficacy was assessed following the 2003 World Health Organization guidelines. In a refugee camp in Laine (south of Guinea), where an in vivo study was not feasible due to the unstable context, a molecular genotyping study in 160 patients assessed the prevalence of mutations in the dihydrofolate reductase (dhfr) (codons 108, 51, 59) and dihydropteroate synthase (dhps) (codons 436, 437, 540) genes of Plasmodium falciparum, which have been associated with resistance to pyrimethamine and sulphadoxine, respectively. **RESULTS:** In Dabola, after 28 days of follow-up, Polymerase Chain Reaction (PCR)-adjusted failure rates were 1.0% (95%CI 0-5.3) for AS/AQ and 1.0% (95%CI 0-5.5) for AS/SP. In the refugee camp in Laine, the molecular genotyping study found three dhfr mutations in 85.6% (95% CI 79.2-90.7) patients and quintuple dhfr/dhps mutations in 9.6% (95% CI 5.2-15.9). **CONCLUSION:** Both AS/AQ and AS/SP are highly efficacious in Dabola, whereas there is molecular evidence of established SP resistance in Laine. This supports the choice of the national programme of Guinea to adopt AS/AQ as first line antimalarial treatment. The results highlight the difficulties faced by control programmes, which have gone through the upheaval of implementing ACTs, but cannot predict how long their therapeutic life will be, especially in countries which have chosen drugs also available as monotherapies.
Measures of clinical malaria in field trials of interventions against Plasmodium falciparum.

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BACKGROUND: Standard methods for defining clinical malaria in intervention trials in endemic areas do not guarantee that efficacy estimates will be unbiased, and do not indicate whether the intervention has its effect by modifying the force of infection, the parasite density, or the risk of pathology at given parasite density. METHODS: Three different sets, each of 500 Phase IIb or III malaria vaccine trials were simulated corresponding to each of a pre-erythrocytic, blood stage, and anti-disease vaccine, each in a population with 80% prevalence of patent malaria infection. Simulations considered only the primary effects of vaccination in a homogeneous trial population. The relationships between morbidity and parasite density and the performance of different case definitions for clinical malaria were analysed using conventional likelihood ratio tests to compare incidence of episodes defined using parasite density cut-offs. Bayesian latent class models were used to compare the overall frequencies of clinical malaria episodes in analyses that did not use diagnostic cut-offs. RESULTS: The different simulated interventions led to different relationships between clinical symptoms and parasite densities. Consequently, the operating characteristics of parasitaemia cut-offs in general differ between vaccine and placebo arms of the simulated trials, leading to different patterns of bias in efficacy estimates depending on the type of intervention effect. Efficacy was underestimated when low parasitaemia cut-offs were used but the efficacy of an asexual blood stage vaccine was overestimated when a high parasitaemia cut-off was used. The power of a trial may be maximal using case definitions that are associated with substantial bias in efficacy. CONCLUSION: Secondary analyses of the data of malaria intervention trials should consider the relationship between clinical symptoms and parasite density, and attempt to estimate overall numbers of clinical episodes and the degree of bias of the primary efficacy measure. Such analyses would help to clarify whether the effect of an intervention corresponds to that anticipated on the basis of the parasite stage that is targeted, and would highlight whether the primary measure of efficacy results from unexpected behaviour in the parasitological and clinical data used to estimate it.

Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea.

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BACKGROUND: A comprehensive malaria control intervention was initiated in February 2004 on Bioko Island, Equatorial Guinea. This manuscript reports on the continuous entomological monitoring of the indoor residual spray (IRS) programme during the first two years of its implementation. METHODS: Mosquitoes were captured daily using window traps at 16 sentinel sites and analysed for species
identification, sporozoite rates and knockdown resistance (kdr) using polymerase chain reaction (PCR) to assess the efficacy of the vector control initiative from December 2003 to December 2005. RESULTS: A total of 2,807 and 10,293 Anopheles funestus and Anopheles gambiae s.l. respectively were captured throughout the study period. Both M and S molecular forms of An. gambiae s.s. and Anopheles melas were identified. Prior to the first round of IRS, sporozoite rates were 6.0, 8.3 and 4.0 for An. gambiae s.s., An. melas and An. funestus respectively showing An. melas to be an important vector in areas in which it occurred. After the third spray round, no infective mosquitoes were identified. After the first spray round using a pyrethroid spray the number of An. gambiae s.s. were not reduced due to the presence of the kdr gene but An funestus and An. melas populations declined from 23.5 to 3.1 and 5.3 to 0.8 per trap per 100 nights respectively. After the introduction of a carbamate insecticide in the second round, An. gambiae s.s. reduced from 25.5 to 1.9 per trap per 100 nights and An. funestus and An. melas remained at very low levels. Kdr was found only in the M-form of An. gambiae s.s. with the highest frequency at Punta Europa (85%). CONCLUSION: All three vectors that were responsible for malaria transmission before the start of the intervention were successfully controlled once an effective insecticide was used. Continuous entomological surveillance including resistance monitoring is of critical importance in any IRS based malaria vector control programme. This paper demonstrates that sufficient resources for such monitoring should be included in any proposal in order to avoid programme failures.

64: Malar J. 2007 Apr 30;6:50.

Larval habitats of Anopheles gambiae s.s. (Diptera: Culicidae) influences vector competence to Plasmodium falciparum parasites.

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BACKGROUND: The origin of highly competent malaria vectors has been linked to productive larval habitats in the field, but there isn't solid quantitative or qualitative data to support it. To test this, the effect of larval habitat soil substrates on larval development time, pupation rates and vector competence of Anopheles gambiae to Plasmodium falciparum were examined. METHODS: Soils were collected from active larval habitats with sandy and clay substrates from field sites and their total organic matter estimated. An. gambiae larvae were reared on these soil substrates and the larval development time and pupation rates monitored. The emerging adult mosquitoes were then artificially fed blood with infectious P. falciparum gametocytes from human volunteers and their midguts examined for oocyst infection after seven days. The wing sizes of the mosquitoes were also measured. The effect of autoclaving the soil substrates was also evaluated. RESULTS: The total organic matter was significantly different between clay and sandy soils after autoclaving (P = 0.022). A generalized liner model (GLM) analysis identified habitat type (clay soil, sandy soil, or lake water) and autoclaving (that reduces presence of microbes) as significant factors affecting larval development time and oocyst infection intensities in adults. Autoclaving the soils resulted in the production of significantly smaller sized mosquitoes (P = 0.008). Autoclaving clay soils resulted in a significant reduction in Plasmodium falciparum oocyst intensities (P = 0.041) in clay soils (un autoclaved clay soils (4.28 +/- 0.18 oocysts/midgut; autoclaved clay soils = 1.17 +/- 0.55 oocysts/midgut) although no difference (P = 0.480) in infection rates was observed between clay soils (10.4%), sandy soils (5.3%) or lake water.
CONCLUSION: This study suggests an important nutritional role for organic matter and microbial fauna on mosquito fitness and vector competence. It shows that the quality of natural aquatic habitats of mosquito larvae may influence malaria parasite transmission potential by An. gambiae. This information can be important in targeting larval habitats for malaria control.


[Atypical etiology of malaria: local perceptions and practices for treatment and prevention in the department of Gaoua, Burkina Faso] [Article in French]
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Malaria is still a public health problem in many sub-Saharan countries. This study was undertaken to understand and analyze the relationship between local perceptions of malaria and practices for prevention and management in the department of Gaoua in Burkina Faso. The goal was to improve the effectiveness of prevention and management of malaria in the target population, i.e., children under the age of five. Individual interviews and focus groups using a semi-structured guide were carried out with mothers, traditional healthcare providers and elderly persons in four villages of the department of Gaoua. Findings showed that practices used for treatment and prevention were directly related to perceptions about malaria. Due to poverty, inadequate health service delivery and ignorance, people do not always seek medical attention and express doubts about the efficacy of modern care. Endogenous practices for malaria prevention are directly related to causes described by the population. Modern preventive techniques are not used by the population. For instance nets are misused to protect corpses from flies or for shelter during funerals.

66: Mol Biochem Parasitol. 2007 Apr 20

Pharmacogenomic analyses of targeting the AT-rich malaria parasite genome with AT-specific alkylating drugs.
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Human malaria parasites, including the most lethal Plasmodium falciparum, are increasingly resistant to existing antimalarial drugs. One remarkable opportunity to selectively target P. falciparum stems from the unique AT-richness of its genome (80% A/T, relative to 60% in human DNA). To rationally explore this opportunity, we used drugs (adozelesin and bizelesin) which distinctly target AT-rich minisatellites and an in silico approach for genome-wide analysis previously experimentally validated in human cells [Woynarowski JM, Trevino AV, Rodriguez KA, Hardies SC, Benham CJ. AT-rich islands in genomic DNA as a novel target for AT-specific DNA-reactive antitumor drugs. J Biol Chem 2001;276:40555-66]. Both drugs demonstrate a potent, rapid and irreversible inhibition of the cultured P. falciparum (50% inhibition at 110 and 10+/-2.3pM, respectively). This antiparasital activity reflects most likely drug binding to specific super-AT-rich regions. Relative to the human genome, the P. falciparum genome shows 3.9- and 7-fold higher frequency of binding sites for adozelesin and bizelesin, respectively. The distribution of these sites is non-random with the most prominent clusters found in large unique minisatellites...
[median size 3.5kbp of nearly pure A/T, with multiple converging repeats but no shared consensus other than (A/T)(n)]. Each of the fourteen P. falciparum chromosomes contains only one such "super-AT island" located within approximately 3-7.5kbp of gene-free and nucleosome-free loci. Important functions of super-AT islands are suggested by their exceptional predicted potential to serve as matrix attachment regions (MARs) and a precise co-localization with the putative centromeres. CONCLUSION: Super-AT islands, identified as unique domains in the P. falciparum genome with presumably crucial functions, offer therapeutically exploitable opportunity for new antimalarial strategies.

67: Mol Biochem Parasitol. 2007 Apr 7

Nuclear gyrB encodes a functional subunit of the Plasmodium falciparum gyrase that is involved in apicoplast DNA replication.

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The DNA replication machinery of the Plasmodium falciparum apicoplast is a validated drug target. Nuclear-encoded gyrase subunits are predicted to play a critical role in maintaining DNA topology during the D-loop/bi-directional ori replication process of the parasite. We show the presence of P. falciparum gyrase subunits in parasite lysates by using antibodies generated against recombinant gyrases A and B. The ATPase activity of PfGyrB was inhibited by novobiocin that also caused parasite death in culture. Reduction of apicoplast/nuclear DNA ratio in the presence of novobiocin indicated that the drug targets apicoplast DNA replication. Molecular modeling of gyrases A and B subunits revealed extensive fold conservation with the Escherichia coli counterparts as well as the presence of a long disordered loop adjacent to the ATPase domain of PfGyrB. Our results have implications for development of PfGyrB as a drug target against malaria.

68: Parasitol Res. 2007 May 29

Prenatal immune responses to Plasmodium falciparum erythrocyte membrane protein 1 DBL-alpha domain in Gabon.


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In areas where malaria transmission is stable, infants are often born to mothers who had Plasmodium falciparum infections during pregnancy. A significant number become exposed to infected erythrocytes or soluble parasite products with subsequent fetal immune priming or tolerance in utero. We performed ELISA to assay IgG and IgM seropositivity rates against three PfEMP1 DBL-alpha domains from 42 maternal-cord paired samples obtained at delivery from a hyperendemic area in Gabon. IgG was present in up to 80% of the cord serum samples, while IgM was found in only 20% of the same samples. These levels were not dependent on the parity of the mother or the peripheral and placental infectious status. The presence of IgM against DBL-alpha domain in cord serum samples suggests that this component is able to cross the placental barrier and mount a fetal immune response.
The impact of IgG antibodies to recombinant Plasmodium falciparum 732var CIDR-1alpha domain in mothers and their newborn babies.

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Different domains of a novel full-length var gene (termed 732var) isolated from a placenta of a malaria-infected woman were expressed in Escherichia coli as recombinant proteins and analysed biochemically and immunologically. Two of these, the Duffy binding-like (DBL)-3gamma domain and the cysteine-rich interdomain region (CIDR)-1alpha were able to bind chondroitin sulfate A and CD36, respectively. The DBL-3gamma domain was investigated in a previous study and confirmed here to exhibit anti-disease characteristics related to pregnancy-associated malaria. Mothers with high anti-DBL-3gamma antibody levels were protected from placental infection. The novel finding in this study is that babies born to mothers carrying anti-CIDR-1alpha antibodies had a delayed time to the first infection.

Medicinal plants used by the people of Northeast India for curing malaria.

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The present study showed that the people of the Northeastern region of India use at least 65 plants belonging to 38 families to treat malaria. Different plant parts such as the leaf, root, bark and fruit and in some cases the whole plant were used for making the herbal preparations. All crude preparations were made using water as the medium. The preparations were orally administered either as a plant crude extract, juice and decoction or leaf infusion. Of the 65 plants, 21 were found to be used in the form of a decoction. The hard parts of the herbs such as the root and bark were taken in the form of a decoction. In some cases the ingredients of the herbal preparation also included honey or sugar. The present investigation also indicated that most of the preparations made for curing malaria were derived from single plant sources.

Artemether-Lumefantrine versus Dihydroartemisinin-Piperaquine for Treatment of Malaria: A Randomized Trial.


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OBJECTIVES: To compare the efficacy and safety of artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) for treating uncomplicated falciparum
malaria in Uganda. DESIGN: Randomized single-blinded clinical trial. SETTING: Apac, Uganda, an area of very high malaria transmission intensity. PARTICIPANTS: Children aged 6 mo to 10 y with uncomplicated falciparum malaria. INTERVENTION: Treatment of malaria with AL or DP, each following standard 3-d dosing regimens. OUTCOME MEASURES: Risks of recurrent parasitemia at 28 and 42 d, unadjusted and adjusted by genotyping to distinguish recrudescences and new infections.

RESULTS: Of 421 enrolled participants, 417 (99%) completed follow-up. The unadjusted risk of recurrent falciparum parasitemia was significantly lower for participants treated with DP than for those treated with AL after 28 d (11% versus 29%; risk difference [RD] 18%, 95% confidence interval [CI] 11%-26%) and 42 d (43% versus 53%; RD 9.6%, 95% CI 0%-19%) of follow-up. Similarly, the risk of recurrent parasitemia due to possible recrudescence (adjusted by genotyping) was significantly lower for participants treated with DP than for those treated with AL after 28 d (1.9% versus 8.9%; RD 7.0%, 95% CI 2.5%-12%) and 42 d (6.9% versus 16%; RD 9.5%, 95% CI 2.8%-16%). Patients treated with DP had a lower risk of recurrent parasitemia due to non-falciparum species, development of gametocytemia, and higher mean increase in hemoglobin compared to patients treated with AL. Both drugs were well tolerated; serious adverse events were uncommon and unrelated to study drugs. CONCLUSION: DP was superior to AL for reducing the risk of recurrent parasitemia and gametocytemia, and provided improved hemoglobin recovery. DP thus appears to be a good alternative to AL as first-line treatment of uncomplicated malaria in Uganda. To maximize the benefit of artemisinin-based combination therapy in Africa, treatment should be integrated with aggressive strategies to reduce malaria transmission intensity.


The importance of human FcgammaRI in mediating protection to malaria.


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The success of passive immunization suggests that antibody-based therapies will be effective at controlling malaria. We describe the development of fully human antibodies specific for Plasmodium falciparum by antibody repertoire cloning from phage display libraries generated from immune Gambian adults. Although these novel reagents bind with strong affinity to malaria parasites, it remains unclear if in vitro assays are predictive of functional immunity in humans, due to the lack of suitable animal models permissive for P. falciparum. A potentially useful solution described herein allows the antimalarial efficacy of human antibodies to be determined using rodent malaria parasites transgenic for P. falciparum antigens in mice also transgenic for human Fc-receptors. These human IgG1s cured animals of an otherwise lethal malaria infection, and protection was crucially dependent on human FcgammaRI. This important finding documents the capacity of FcgammaRI to mediate potent antimalaria immunity and supports the development of FcgammaRI-directed therapy for human malaria.


Bacteria of the genus Asaia stably associate with Anopheles stephensi, an Asian malarial mosquito vector.

Favia G, Ricci I, Damiani C, Raddadi N, Crotti E, Marzorati M, Rizzi A, Urso R,
Here, we show that an alpha-proteobacterium of the genus Asaia is stably associated with larvae and adults of Anopheles stephensi, an important mosquito vector of Plasmodium vivax, a main malaria agent in Asia. Asaia bacteria dominate mosquito-associated microbiota, as shown by 16S rRNA gene abundance, quantitative PCR, transmission electron microscopy and in situ-hybridization of 16S rRNA genes. In adult mosquitoes, Asaia sp. is present in high population density in the female gut and in the male reproductive tract. Asaia sp. from An. stephensi has been cultured in cell-free media and then transformed with foreign DNA. A green fluorescent protein-tagged Asaia sp. strain effectively lodged in the female gut and salivary glands, sites that are crucial for Plasmodium sp. development and transmission. The larval gut and the male reproductive system were also colonized by the transformed Asaia sp. strain. As an efficient inducible colonizer of mosquitoes that transmit Plasmodium sp., Asaia sp. may be a candidate for malaria control.

74: Proc Natl Acad Sci U S A. 2007 May 21

Effect of plasmodial RESA protein on deformability of human red blood cells harboring Plasmodium falciparum.


During intraerythrocytic development, Plasmodium falciparum exports proteins that interact with the host cell plasma membrane and subplasma membrane-associated spectrin network. Parasite-exported proteins modify mechanical properties of host RBCs, resulting in altered cell circulation. In this work, optical tweezers experiments of cell mechanical properties at normal physiological and febrile temperatures are coupled, for the first time, with targeted gene disruption techniques to measure the effect of a single parasite-exported protein on host RBC deformability. We investigate Pf155/Ring-infected erythrocyte surface antigen (RESA), a parasite protein transported to the host spectrin network, on deformability of ring-stage parasite-harboring human RBCs. Using a set of parental, gene-disrupted, and revertant isogenic clones, we found that RESA plays a major role in reducing deformability of host cells at the early ring stage of parasite development, but not at more advanced stage. We also show that the effect of RESA on deformability is more pronounced at febrile temperature, which ring-stage parasite-harboring RBCs can be exposed to during a malaria attack, than at normal body temperature.


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We investigated patterns of genetic diversity of Plasmodium falciparum associated with its two main African vectors: Anopheles gambiae and Anopheles funestus. We dissected 10,296 wild-caught mosquitoes from three tropical sites, two in Cameroon (Simbock and Tibati, separated by 320 km) and one in Kenya (Rota, >2,000 km from the other two sites). We assayed seven microsatellite loci in 746 oocysts from 183 infected mosquito guts. Genetic polymorphism was very high in parasites isolated from both vector species. The expected heterozygosity (H(E)) was 0.79 in both species; the observed heterozygosities (H(O)) were 0.32 in A. funestus and 0.42 in A. gambiae, indicating considerable inbreeding within both vector species. Mean selfing (s) between genetically identical gametes was s = 0.33. Differences in the rate of inbreeding were statistically insignificant among sites and between the two vector species. As expected, because of the high rate of inbreeding, linkage disequilibrium was very high; it was significant for all 21 loci pairs in A. gambiae and for 15 of 21 pairs in A. funestus, although only two pairwise comparisons were between loci on the same chromosome. Overall, the genetic population structure of P. falciparum, as evaluated by F statistics, was predominantly clonal rather than panmictic, a population structure that facilitates the spread of antimalarial drug and vaccine resistance and thus may impair the effectiveness of malaria control efforts.

High prevalence of malaria infection in Amazonas state, Venezuela.

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This study was carried out to determine the incidence of malaria in an endemic region of Amazonas State, Venezuela. For this, 200 random samples were collected from symptomatic and asymptomatic individuals from San Fernando de Atabapo and Santa Barbara. Epidemiological factors were related to malaria infection, which was diagnosed by microscopy observation and amplification of the 18S rDNA sequence by PCR. Malaria prevalence in these populations was 28.5%, whilst P. vivax and P. falciparum prevalences were 12 and 17%, respectively. No infection by P. malariae was found. A mixed infection was found on an asymptomatic individual. Prevalence patterns differed between age groups depending on the Plasmodium species. We found that 34.8% of the P. vivax and 15.2% of the P. falciparum infections were asymptomatic. The use of nets was helpful to prevent P. vivax infection, but did not protect against P. falciparum infection. The results suggest the presence of more than one mosquito vector in the area, displaying a differential pattern of infection for each Plasmodium species. There appear to be risk factors associated with malaria infections in some individuals. The population based approach and PCR diagnosis improved the accuracy of the statistical analysis in the study.
[Evaluation of the Optimal-IT and ICT P.f./P.v. rapid dipstick tests for diagnosing malaria within primary healthcare in the municipality of Manaus, Amazonas] [Article in Portuguese]

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Malaria is routinely diagnosed using the thick blood smear test. However, this technique requires the training of microscopists and may be time-consuming. A concordance study was conducted on two dipstick tests (Optimal-IT and ICT P.f./P.v.) and the thick blood smear test, within primary healthcare in Manaus.

Congenital malaria: The least known consequence of malaria in pregnancy.

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Congenital malaria is the least known manifestation of malaria and a very neglected area of research. Most of the existing information is limited to case reports in children born to non-immune women. With the use of molecular techniques, congenital infection is being increasingly detected among infants born to semi-immune women in endemic countries. However, many gaps in knowledge remain. The mechanisms and timing of infection are unclear. Furthermore, there is a lack of information on the impact of congenital malaria infection on the subsequent risk of malaria and general morbidity in the infant. There is also a lack of consensus on the clinical guidelines for its management. More research is needed in order to establish adequate preventive and management recommendations to avoid this consequence of malaria in pregnancy.

Significant association between TNF-alpha (TNF) promoter allele (-1031C, -863C, and -857C) and cerebral malaria in Thailand.


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We examined a possible association of three single nucleotide polymorphisms (SNPs) of the tumor necrosis factor alpha (TNF) promoter -1031T>C (rs1799964), -863C>A (rs1800630), and -857C>T (rs1799724) with severe malaria in 466 adult patients having Plasmodium falciparum malaria in northwest Thailand. Four TNF promoter alleles comprising these three SNPs were detected in the studied population. The frequency of the TNF U04 allele designated -1031C, -863C, and -857C was found to be significantly greater in patients with cerebral malaria than in patients with mild malaria (12.6%; cerebral malaria vs 5.6%; mild malaria; odds ratio =2.5; P=0.002). The association of U04 with susceptibility
High residual chloroquine blood levels in African children with severe malaria seeking healthcare.

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Despite widespread resistance, chloroquine remains widely used in West Africa, particularly in home treatment. We examined chloroquine blood levels on admission to a referral hospital with respect to the manifestation of severe malaria in 290 Ghanaian children. Of the patients, 78% exhibited chloroquine concentrations (subtherapeutic, 35%; therapeutic, 37%; supratherapeutic, 6%) and 11% died. Most parasites (78%) carried the pfcrt-T76 chloroquine resistance mutation. High drug concentrations correlated with reduced parasitaemia but also with selection of resistant parasites, lower respiratory and heart rates, increased plasma lactate levels and impaired consciousness. Geometric mean chloroquine concentrations tended to be higher in children who died than in survivors (1.135 vs. 0.778nmol/l; P=0.09). Supratherapeutic drug levels (>5000nmol/l) were associated with fatal outcome (odds ratio 8.6; 95% CI 1.4-51.7). Residual chloroquine concentrations were found to be abundant in children with severe malaria and to be associated with alterations in the clinical manifestation of the disease and its case fatality. This may result from toxic effects of the drug and/or reflect preceding overtreatment in children with acute life-threatening disease. In areas of intense chloroquine resistance and frequent pre-treatment, additional administration of chloroquine at hospital admission is not only ineffective but may even further endanger patients.

Extension of indoor residual spraying for malaria control into high transmission settings in Africa.

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Contrary to previous consensus, a recent WHO statement recommends a more dominant role for indoor residual spraying (IRS) in malaria control in high transmission settings of sub-Saharan Africa and re-emphasises the role of DDT. We review the issues related to this change in recommendation. In high transmission settings, IRS must be implemented indefinitely and at high quality to achieve control. As current infrastructure limitations and unpredictable funding make this unlikely, each country must carefully consider the role of IRS. There remains a need to support ongoing insecticide-treated net scale-up. Insecticide choice is hampered by the lack of economic costing data.
Examining the evidence of under-five mortality reduction in a community-based programme in Gaza, Mozambique.

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Effective implementation of programmes with the community Integrated Management of Childhood Illness model has demonstrated improvements in care-seeking behaviours and utilisation of health services. The child survival programme implemented in Chokwe district of Gaza province, Mozambique, achieved high coverage for bed net use (80%), oral rehydration therapy for children with diarrhoea (94%) and prompt care-seeking from trained providers for children with danger signs. The project also instituted a community-based vital registration and health information system for routine surveillance of births, deaths and childhood illnesses using an extensive network of 2300 volunteers. Evidence from this system indicated a 66% reduction in infant mortality and a 62% reduction in under-five mortality. To check the reliability of the findings, an independent mortality assessment was carried out using a pregnancy history questionnaire with a sample population of 998 women using standard methodologies applied in the Demographic and Health Surveys. The mortality survey showed reductions of 49% and 42% in infant and under-five mortality, respectively. The leading causes of death identified by verbal autopsies were malaria (30%), neonatal causes (17%) and pneumonia (21.3%). These findings suggest that effective community-based partnerships that support the delivery of health services can contribute to mortality reductions.