Part A – Articles with links to the full-text
(29 studies)


The protein-phosphatome of the human malaria parasite Plasmodium falciparum.

Full-text - http://www.biomedcentral.com/1471-2164/9/412

Wilkes JM, Doerig C.

ABSTRACT: BACKGROUND: Malaria, caused by the parasitic protist Plasmodium falciparum, represents a major public health problem in the developing world. The P. falciparum genome has been sequenced, which provides new opportunities for the identification of novel drug targets. We report an exhaustive analysis of the P. falciparum genomic database (PlasmoDB) aimed at identifying and classifying all protein phosphatases (PP) in this organism. RESULTS: Using a variety of bioinformatics tools, we identified 26 malarial putative PP sequences within the four major established PP families, plus 7 sequences that we predict to dephosphorylate "non-protein" substrates. We constructed phylogenetic trees to position these sequences relative to PPs from other organisms representing all major eukaryotic phyla except Cercozoans (for which no full genome sequence is available). Predominant observations were: (i) P. falciparum possessed the smallest phosphatome of any of the organisms investigated in this study; (ii) no malarial PP clustered with the tyrosine-specific subfamily of the PTP group (iii) a cluster of 7 closely related members of the PPM/PP2C family is present, and (iv) some P. falciparum protein phosphatases are present in clades lacking any human homologue. CONCLUSION: The considerable phylogenetic distance between Apicomplexa and other Eukaryotes is reflected by profound divergences between the phosphatome of malaria parasites and those of representative organisms from all major eukaryotic phyla, which might be exploited in the context of efforts for the discovery of novel targets for antimalarial chemotherapy.


Bed net ownership, use, and perceptions among women seeking antenatal care in Kinshasa, Democratic Republic of the Congo (DRC): opportunities for improved maternal and child health.

Full-text - http://www.biomedcentral.com/1471-2164/9/412


ABSTRACT: BACKGROUND: To describe malaria knowledge, attitudes toward malaria and bed net use, levels of ownership and use of bed nets, and factors associated with ownership and use among pregnant women attending their first antenatal care (ANC) visit in Kinshasa, DRC. METHODS: Women attending their first ANC visit at one maternity in Kinshasa were recruited to take part in a study where they were given free insecticide treated bed nets(ITNs) and then followed up at delivery and
6 months post delivery to assess ITN use. This study describes the baseline levels of bed net ownership and use, attitudes towards net use and factors associated with net use. RESULTS: Among 351 women interviewed at baseline, 115 (33%) already owned a bed net and 86 (25%) reported to have slept under the net the previous night. Cost was reported as the reason for not owning a net by 48% of the 236 women who did not own one. In multivariate analyses, women who had secondary school or higher education were 3.4 times more likely to own a net (95% CI 1.6, 7.3) and 2.8 times more likely to have used a net (95% CI 1.3, 6.0) compared to women with less education. CONCLUSION: Distribution of ITNs in antenatal clinics in this setting is needed and feasible. The potential for ITN use by this target population is high.

Malaria prevalence and mosquito net coverage in Oromia and SNNPR regions of Ethiopia.

Full-text - http://www.biomedcentral.com/1471-2458/8/321


The Carter Center, Addis Ababa, Ethiopia. estifanos_b@yahoo.com

BACKGROUND: Malaria transmission in Ethiopia is unstable and seasonal, with the majority of the country's population living in malaria-prone areas. Results from DHS 2005 indicate that the coverage of key malaria interventions was low. The government of Ethiopia has set the national goal of full population coverage with a mean of 2 long-lasting insecticidal nets (LLINs) per household through distribution of about 20 million LLIN by the end of 2007. The aim of this study was to generate baseline information on malaria parasite prevalence and coverage of key malaria control interventions in Oromia and SNNPR and to relate the prevalence survey findings to routine surveillance data just before further mass distribution of LLINs. METHODS: A 64 cluster malaria survey was conducted in January 2007 using a multi-stage cluster random sampling design. Using Malaria Indicator Survey Household Questionnaire modified for the local conditions as well as peripheral blood microscopy and rapid diagnostic tests, the survey assessed net ownership and use and malaria parasite prevalence in Oromia and SNNPR regions of Ethiopia. Routine surveillance data on malaria for the survey time period was obtained for comparison with prevalence survey results. RESULTS: Overall, 47.5% (95% confidence interval (CI) 33.5-61.9%) of households had at least one net, and 35.1% (95% CI 23.1-49.4%) had at least one LLIN. There was no difference in net ownership or net utilization between the regions. Malaria parasite prevalence was 2.4% (95% CI 1.6-3.5%) overall, but differed markedly between the two regions: Oromia, 0.9% (95% CI 0.5-1.6); SNNPR, 5.4% (95% CI 3.4-8.5), p < 0.001. This difference between the two regions was also reflected in the routine surveillance data. CONCLUSION: Household net ownership exhibited nearly ten-fold increase compared to the results of Demographic and Health Survey 2005 when fewer than 5% of households in these two regions owned any nets. The results of the survey as well as the routine surveillance data demonstrated that malaria continues to be a significant public health challenge in these regions—and more prevalent in SNNPR than in Oromia.

Obstacles to prompt and effective malaria treatment lead to low community-coverage in two rural districts of Tanzania.

Full-text - http://www.biomedcentral.com/1471-2458/8/317

ABSTRACT: BACKGROUND: Malaria is still a leading child killer in sub-Saharan Africa. Yet, access to prompt and effective malaria treatment, a mainstay of any malaria control strategy, is sub-optimal in many settings. Little is known about obstacles to treatment and community-effectiveness of case-management strategies. This research quantified treatment seeking behaviour and access to treatment in a highly endemic rural Tanzanian community. The aim was to provide a better understanding of obstacles to treatment access in order to develop practical and cost-effective interventions. METHODS: We conducted community-based treatment-seeking surveys including 226 recent fever episodes in 2004 and 2005. The local Demographic Surveillance System provided additional household information. A census of drug retailers and health facilities provided data on availability and location of treatment sources. RESULTS: After intensive health education, the biomedical concept of malaria has largely been adopted by the community. 87.5% (78.2-93.8) of the fever cases in children and 80.7% (68.1-90.0) in adults were treated with one of the recommended antimalarials (at the time SP, amodiaquine or quinine). However, only 22.5% (13.9-33.2) of the children and 10.5% (4.0-21.5) of the adults received prompt and appropriate antimalarial treatment. Health facility attendance increased the odds of receiving an antimalarial (OR=7.7) but did not have an influence on correct dosage. The exemption system for under-fives in public health facilities was not functioning and drug expenditures for children were as high in health facilities as with private retailers. CONCLUSIONS: A clear preference for modern medicine was reflected in the frequent use of antimalarials. Yet, quality of case-management was far from satisfactory as was the functioning of the exemption mechanism for the main risk group. Private drug retailers played a central role by complementing existing formal health services in delivering antimalarial treatment. Health system factors like these need to be tackled urgently in order to translate the high efficacy of newly introduced artemisinin-based combination therapy (ACT) into equitable community-effectiveness and health-impact.

Use of a Drosophila Model to Identify Genes Regulating Plasmodium Growth in the Mosquito.

Full-text - http://www.genetics.org/cgi/rapidpdf/genetics.108.089748v1

Brandt SM, Jaramillo-Gutierrez G, Kumar S, Barillas-Mury C, Schneider DS.

Institute of Environmental Science and Research Limited.

We performed a forward genetic screen, using Drosophila as a surrogate mosquito, to identify host factors required for the growth of the avian malaria parasite, Plasmodium gallinaceum. We identified 18 presumed loss-of-function mutants that reduced the growth of the parasite in flies. Presumptive mutation sites were identified in 14 of the mutants on the basis of the insertion site of a transposable element. None of the identified genes have been previously implicated in innate immune responses or interactions with Plasmodium. The functions of 5 Anopheles gambiae homologs were tested by using RNAi to knock down gene function followed by measuring the growth of the rodent parasite, Plasmodium berghei. Loss-of-function of four of these genes in the mosquito affected Plasmodium growth, suggesting that Drosophila can be used effectively as a surrogate mosquito to identify relevant host factors in the mosquito.
Adaptation costs for climate change-related cases of diarrhoeal disease, malnutrition, and malaria in 2030.

Full-text - http://www.globalizationandhealth.com/content/4/1/9

Ebi KL.

ESS, LLC, Alexandria, VA 22304, USA. krisebi@essllc.org.

ABSTRACT: BACKGROUND: Climate change has begun to negatively affect human health, with larger burdens projected in the future as weather patterns continue to change. The climate change-related health consequences of diarrhoeal diseases, malnutrition, and malaria are projected to pose the largest risks to future populations. Limited work has been done to estimate the costs of adapting to these additional health burdens. METHODS: The costs of treating diarrhoeal diseases, malnutrition (stunting and wasting only), and malaria in 2030 were estimated under three climate scenarios using (1) the current numbers of cases; (2) the projected relative risks of these diseases in 2030; and (3) current treatment costs. The analysis assumed that the number of annual cases and costs of treatment would remain constant. There was limited consideration of socioeconomic development.

RESULTS: Under a scenario assuming emissions reductions resulting in stabilization at 750 ppm CO2 equivalent in 2210, the costs of treating diarrhoeal diseases, malnutrition, and malaria in 2030 were estimated to be $4 to 12 billion. This is almost as much as current total annual overseas development assistance for health.

CONCLUSION: The investment needs in the health sector to address climate-sensitive health outcomes are large. Additional human and financial resources will be needed to prevent and control the projected increased burden of health outcomes due to climate change.

A study of the distribution and abundance of the adult malaria vector in western Kenya highlands.

Full-text - http://www.ij-healthgeographics.com/content/7/1/50

Li L, Bian L, Yan G.

ABSTRACT: BACKGROUND: A sharp rise in the malaria mortality rate has been observed recently in western Kenya. Malaria is transmitted by mosquito vectors. Malaria control strategies can be more successful if the distribution and abundance of mosquito vectors is predicted. However, how mosquito vectors are distributed in space remain poor understood, and this question is rarely studied using spatial methods. This study aims to provide a better understanding of the distribution and abundance of mosquito vectors. To achieve this objective, spatial and non-spatial methods were employed. The data on the distribution of adult mosquitoes, and mosquito breeding habitats in a study area in western Kenya, and environmental variables were analyzed. RESULTS: The models developed using spatial methods outperformed the models developed using non-spatial methods. Houses close to locations where mosquito breeding habitats were repeatedly observed had more abundant adult female mosquitoes. Distance to high-order streams was identified as an effective predictor for the distribution of adult mosquitoes. CONCLUSIONS: The spatial method is more effective in modeling the distribution of adult mosquitoes than the non-spatial method. The results of this study can be used to facilitate decision-making related to mosquito surveillance and malaria prevention.
Evolution of virulence in malaria.

Full-text - http://jbiol.com/content/7/6/22

Penman B, Gupta S.
Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, UK. sunetra.gupta@zoo.ox.ac.uk.

ABSTRACT: The pathogenesis of severe malarial disease is not yet fully understood. It is clear that host immunopathology plays a central role, and a recent paper in BMC Evolutionary Biology suggests that the ability of the parasite to stimulate interleukin-10 production is a major factor and speculates on its impact on the coevolution of host and parasite.

Evidence of increasing Leu-Phe knockdown resistance mutation in Anopheles gambiae from Niger following a nationwide long-lasting insecticide-treated nets implementation.

Full-text - http://www.malariajournal.com/content/7/1/189

Czeher C, Labbo R, Arzika I, Duchemin JB.

ABSTRACT: BACKGROUND: At the end of 2005, a nationwide long-lasting insecticide-treated net (LLIN) distribution targeting the most vulnerable populations was implemented throughout Niger. A large number of studies in Africa have reported the existence of anopheline populations resistant to various insecticides, partly due to knockdown resistance (kdr) mutations, but few operational wide-scale control programmes werecoupled with the monitoring of such mutations. The distribution of the kdr-west (kdr-w) Leu-Phe mutation was studied in Anopheles gambiae s.l. populations from Niger and temporal variations were monitored following the nationwide LLIN implementation. METHODS: Mosquitoes were collected from 14 localities during the wet seasons of 2005, 2006 and 2007 with additional sampling in the capital city, Niamey. After morphological identification of Anopheles gambiae s.l. specimens, DNA extracts were used for the determination of species and molecular forms of the Anopheles gambiae complex and for the detection of the kdr-w mutation. RESULTS: Around 1,500 specimens collected in the three consecutive years were analysed. All Anopheles arabiensis specimens analysed were homozygous susceptible, whereas the few Anopheles gambiae S forms exhibited a high overall kdr-w frequency. The M form samples exhibited a lower overall kdr-w frequency before the LLIN distribution, that increased significantly in the two wet season collections following the LLIN distribution. Higher kdr frequencies were repeatedly noticed within host-seeking females compared to resting ones in indoor collections. In addition, preliminary results in M form urban populations from Niamey showed far higher kdr frequencies than in all of the rural sites studied. DISCUSSION: This study describes the first case of kdr mutation in Anopheles gambiae populations from Niger. It is suspected that the LLIN have caused the important temporal increase of kdr-w mutation observed during this study. While the kdr mutation is still found at a low level, this rapid increase could potentially lead to high kdr frequencies within a few years. CONCLUSIONS: These results are of prime importance in the effort to document multiple effects of operational control programmes on mosquito vectors, and to conceive sustainable control strategies for future malaria control programmes.
Dynamics of multiple insecticide resistance in the malaria vector Anopheles gambiae in a rice growing area in south-western Burkina Faso.


ABSTRACT: BACKGROUND: Insecticide resistance of the main malaria vector, Anopheles gambiae, has been reported in south-western Burkina Faso, West Africa. Cross-resistance to DDT and pyrethroids was conferred by alterations at site of action in the sodium channel, the Leu-Phe kdr mutation; resistance to organophosphates and carbamates resulted from a single point mutation in the oxyanion hole of the acetylcholinesterase enzyme designed as ace-1R. METHODS: An entomological survey was carried out during the rainy season of 2005 at Vallee du Kou, a rice growing area in south-western Burkina Faso. At the Vallee du Kou, both insecticide resistance mechanisms have been previously described in the M and S molecular forms of An. gambiae. This survey aimed i) to update the temporal dynamics and the circumsporozoite infection rate of the two molecular forms M and S of An. gambiae; ii) to update the frequency of the Leu-Phe kdr mutation within these forms; and, finally iii) to investigate the occurrence of the ace-1R mutation. Mosquitoes collected by indoor residual collection and by human landing catches were counted and morphologically identified. Species and molecular forms of An. gambiae, ace-1R and Leu-Phe kdr mutations were determined using PCR techniques. The presence of the circumsporozoite protein of Plasmodium falciparum was determined using ELISA. RESULTS: Anopheles gambiae populations were dominated by the M form. However the S form occurred in relative important proportion towards the end of the rainy season with a maximum peak in October at 51%. Sporozoite rates were similar in both forms. The frequency of the Leu-Phe kdr mutation in the S form reached a fixation level while it is still spreading in the M form. Furthermore, the ace-1R mutation prevailed predominately in the S form and has just started spreading in the M form. The two mutations occurred concomitantly both in M and S populations. CONCLUSION: These results showed that the Vallee du Kou, a rice growing area formerly occupied mainly by M susceptible populations, is progressively colonized by S resistant populations living in sympatry with the former. As a result, the distribution pattern of insecticide resistance mutations shows the occurrence of both resistance mechanisms concomitantly in the same populations. The impact of multiple resistance mechanisms in M and S populations of An. gambiae on vector control measures against malaria transmission, such as insecticide-treated nets (ITNs) and indoor residual spraying (IRS), in this area is discussed.
isotypes and their association with different malaria clinical expressions.

METHODS: Different isotypes against P. falciparum blood stages, IgG, IgG1, IgG2, IgG3, IgG4, IgM, IgE and IgA, were determined by ELISA. The results were based on the analysis of different clinical expressions of malaria (complicated, uncomplicated and asymptomatic) and factors related to prior malaria exposure such as age and the number of previous clinical malaria attacks. The occurrence of the H131 polymorphism of the FcgammaIotaIotaA receptor was also investigated in part of the studied population. RESULTS: The highest levels of IgG, IgG1, IgG2 and IgG3 antibodies were observed in infected individuals, while uninfected individuals showed the highest levels of IgG4 antibodies. Individuals reporting more than five previous clinical malaria attacks presented a predominance of IgG1, IgG2 and IgG3 antibodies, while IgM, IgA and IgE antibodies predominated among individuals reporting five or less previous clinical malaria bouts. Among individuals with uncomplicated and asymptomatic malaria, there was a predominance of high-avidity IgG, IgG1, IgG2 antibodies and low-avidity IgG3 antibodies, while IgG4, IgE and IgM antibodies were predominant among individuals with complicated malaria. The H131 polymorphism was found in 44.4% of the individuals, and the highest IgG2 levels were observed among asymptomatic individuals with this allele, suggesting the protective role of IgG2 in this population. CONCLUSIONS: Together, the results suggest a differential regulation in the anti-P. falciparum antibody pattern in different clinical expressions of malaria and showed that even in unstable transmission areas, protective immunity against malaria can be observed, when the appropriated antibodies are produced.


MalVac: Database of malarial vaccine candidates.

Full-text - [http://www.malariajournal.com/content/7/1/184](http://www.malariajournal.com/content/7/1/184)

Chaudhuri R, Ahmed S, Ansari FA, Singh HV, Ramachandran S.

ABSTRACT: BACKGROUND: The sequencing of genomes of the Plasmodium species causing malaria, offers immense opportunities to aid in the development of new therapeutics and vaccine candidates through Bioinformatics tools and resources. METHODS: The starting point of MalVac database is the collection of known vaccine candidates and a set of predicted vaccine candidates identified from the whole proteome sequences of Plasmodium species provided by PlasmoDb 5.4 release (31st October 2007). These predicted vaccine candidates are the adhesins and adhesin-like proteins from Plasmodium species, Plasmodium falciparum, Plasmodium vivax and Plasmodium yoelii. Subsequently, these protein sequences were analysed through 20 publicly available algorithms to obtain Orthologs, Paralogs, BetaWraps, TargetP, TMHMM, SignalP, CDDSearch, BLAST with Human Ref. Proteins, T-cell epitopes, B-cell epitopes, Discotopes, and allergen predictions. All of this information was collected and organized with the ORFids of the protein sequences as primary keys. This information is relevant from the view point of Reverse Vaccinology in facilitating decision making on the most probable choice for vaccine strategy. RESULTS: Detailed information on the patterning of the epitopes and other motifs of importance from the viewpoint of reverse vaccinology has been obtained on the most probable protein candidates for vaccine investigation from three major malarial species. Analysis data are available on 161 adhesin proteins from P. falciparum, 137 adhesin proteins from P. vivax and 34 adhesin proteins from P. yoelii. The results are displayed in convenient tabular format and a facility to export the entire data has been provided. The MalVac database is a "community resource". Users are encouraged to export data and further contribute by value addition. Value added data may be sent back to the community through either MalVac or PlasmoDB. CONCLUSION: A web server MalVac for facilitation of the identification of probable vaccine candidates has been developed and can be freely accessed.
Transformation of the rodent malaria parasite Plasmodium chabaudi and generation of a stable fluorescent line PcGFPCON.

Full-text - http://www.malariajournal.com/content/7/1/183

Reece SE, Thompson J.

ABSTRACT: BACKGROUND: The rodent malaria parasite Plasmodium chabaudi has proven of great value in the analysis of fundamental aspects of host-parasite-vector interactions implicated in disease pathology and parasite evolutionary ecology. However, the lack of gene modification technologies for this model has precluded more direct functional studies. METHODS: The development of in vitro culture methods to yield P. chabaudi schizonts for transfection and conditions for genetic modification of this rodent malaria model are reported. RESULTS: Independent P. chabaudi gene-integrant lines that constitutively express high levels of green fluorescent protein throughout their life cycle have been generated. CONCLUSIONS: Genetic modification of P. chabaudi is now possible. The production of genetically distinct reference lines offers substantial advances to our understanding of malaria parasite biology, especially interactions with the immune system during chronic infection.

Anopheles gambiae complex along The Gambia river, with particular reference to the molecular forms of An. gambiae s.s.

Full-text - http://www.malariajournal.com/content/7/1/182

Caputo B, Nwakanma D, Jawara M, Adiamoh M, Dia I, Konate L, Petrarca V, Conway DJ, Della Torre A.

ABSTRACT: BACKGROUND: The geographic and temporal distribution of M and S molecular forms of the major Afrotropical malaria vector species Anopheles gambiae s.s. at the western extreme of their range of distribution has never been investigated in detail. Materials and methods Collections of indoor-resting An. gambiae s.l. females were carried out along a ca. 400 km west to east transect following the River Gambia from the western coastal region of The Gambia to south-eastern Senegal during 2005 end of rainy season/early dry season and the 2006 rainy season. Specimens were identified to species and molecular forms by PCR-RFLP and the origin of blood-meal of fed females was determined by ELISA test. RESULTS: Over 4,000 An. gambiae s.l. adult females were collected and identified, 1041 and 3038 in 2005 and 2006, respectively. M-form was mainly found in sympatry with Anopheles melas and S-form in the western part of the transect, and with Anopheles arabiensis in the central part. S-form was found to prevail in rural Sudano-Guinean savannah areas of Eastern Senegal, in sympatry with An. arabiensis. Anopheles melas and An. arabiensis relative frequencies were generally lower in the rainy season samples, when An. gambiae s.s. was prevailing. No large seasonal fluctuations were observed for M and S-forms. In areas where both M and S were recorded, the frequency of hybrids between them ranged from to 0.6% to 7%. DISCUSSION: The observed pattern of taxa distribution supports the hypothesis of a better adaptation of M-form to areas characterized by water-retaining alluvial deposits along the Gambia River, characterized by marshy vegetation, mangrove woods and rice cultivations. In contrast, the S-form seems to be better adapted to free-draining soil, covered with open woodland savannah or farmland, rich in temporary larval breeding sites characterizing mainly the eastern part of the transect, where the environmental impact of the Gambia River is much less profound and agricultural activities are mainly rain-dependent. Very interestingly, the observed frequency of hybridization between the molecular forms along the whole transect was much higher than has been reported so far for other areas.
CONCLUSIONS: The results support a bionomic divergence between the M and S-forms, and suggest that the western extreme of An. gambiae s.s. geographical distribution may represent an area of higher-than-expected hybridization between the two molecular forms.


Malaria case-management under artemether-lumefantrine treatment policy in Uganda.

Full-text - [http://www.malariajournal.com/content/7/1/181](http://www.malariajournal.com/content/7/1/181)


Malaria Public Health and Epidemiology Group, KEMRI/Wellcome Trust Research Programme, Nairobi, Kenya. dzurovac@nairobi.kemri-wellcome.org

BACKGROUND: Case-management with artemether-lumefantrine (AL) is one of the key strategies to control malaria in many African countries. Yet, the reports on translation of AL implementation activities into clinical practice are scarce. Here the quality of AL case-management is reported from Uganda; approximately one year after AL replaced combination of chloroquine and sulphadoxine-pyrimethamine (CQ+SP) as recommended first line treatment for uncomplicated malaria. METHODS: A cross-sectional survey, using a range of quality of care assessment tools, was undertaken at all government and private-not-for-profit facilities in four Ugandan districts. Main outcome measures were AL prescribing, dispensing and counseling practices in comparison with national guidelines, and factors influencing health workers decision to 1) treat for malaria, and 2) prescribe AL. RESULTS: 195 facilities, 232 health workers and 1,763 outpatient consultations were evaluated. Of 1,200 patients who needed treatment with AL according to guidelines, AL was prescribed for 60%, CQ+SP for 14%, quinine for 4%, CQ for 3%, other antimalarials for 3%, and 16% of patients had no antimalarial drug prescribed. AL was prescribed in the correct dose for 95% of patients. Only three out of seven AL counseling and dispensing tasks were performed for more than 50% of patients. Patients were more likely to be treated for malaria if they presented with main complaint of fever (OR = 5.22; 95% CI: 3.61-7.54) and if they were seen by supervised health workers (OR = 1.63; 95% CI: 1.06-2.50); however less likely if they were treated by more qualified health workers (OR = 0.61; 95% CI: 0.40-0.93) and presented with skin problem (OR = 0.29; 95% CI: 0.15-0.55). AL was more likely prescribed if the appropriate weight-specific AL pack was in stock (OR = 6.15; 95% CI: 3.43-11.05) and when CQ was absent (OR = 2.16; 95% CI: 1.09-4.28). Routine AL implementation activities were not associated with better performance. CONCLUSION: Although the use of AL was predominant over non-recommended therapies, the quality of AL case-management at the point of care is not yet optimal. There is an urgent need for innovative quality improvement interventions, which should be rigorously tested. Adequate availability of ACTs at the point of care will, however, ultimately determine the success of any performance interventions and ACT policy transitions.

16: Malar J. 2008 Sep 18;7(1):180

A community effectiveness trial of strategies promoting intermittent preventive treatment with sulphadoxine-pyrimethamine in pregnant women in rural Burkina Faso.

Full-text - [http://www.malariajournal.com/content/7/1/180](http://www.malariajournal.com/content/7/1/180)

Gies S, Coulibaly SO, Ouattara FT, Ky C, Brabin BJ, D'Alessandro U.

ABSTRACT: BACKGROUND: Intermittent preventive treatment with sulphadoxine-pyrimethamine for pregnant women (IPTp-SP) is currently being scaled up in many countries in sub-Saharan Africa. Despite high antenatal clinic (ANC) attendance, coverage with the required two doses of SP remains low. The study investigated
whether a targeted community-based promotion campaign to increase ANC attendance and SP uptake could effectively improve pregnancy outcomes in the community.

METHODS: Between 2004 and 2006 twelve health centres in Boromo Health District, Burkina Faso were involved in this study. Four were strategically assigned to community promotion in addition to IPTp-SP (Intervention A) and eight were randomly allocated to either IPTp-SP (Intervention B) or weekly chloroquine (Control). Primi- and secundigravidae were enrolled at village level and thick films and packed cell volume (PCV) taken at 32 weeks gestation and at delivery. Placental smears were prepared and newborns weighed. Primary outcomes were peripheral parasitaemia during pregnancy and at delivery, placental malaria, maternal anaemia, mean and low birth weight. Secondary outcomes were the proportion of women with [greater than or equal to] 3 ANC visits and [greater than or equal to] 2 doses of SP. Intervention groups were compared using logistic and linear regression with linearized variance estimations to correct for the cluster-randomized design. RESULTS: SP uptake ([greater than or equal to]2 doses) was higher with (Intervention A: 70%) than without promotion (Intervention B: 49%) (OR 2.45 95%CI 1.25-4.82 p=0.014). Peripheral (33.3%) and placental (30.3%) parasite rates were significantly higher in the control arm compared to Intervention B (peripheral: 20.1% OR 0.50 95%CI 0.37-0.69 p=0.001; placental: 20.5% OR 0.59 95%CI 0.44-0.78 p=0.002) but did not differ between Intervention A (17.4%; 18.1%) and Intervention B (20.1; 20.5%) (peripheral: OR 0.84 95%CI 0.60-1.18 p=0.280; placental: OR 0.86 95%CI 0.58-1.29 p=0.430). Mean PCV and birth weight and prevalence of anaemia and low birth weight did not differ between study arms. CONCLUSIONS: The promotional campaign resulted in a major increase in IPTp-coverage, with two thirds of women at delivery having received [greater than or equal to] 2 doses of SP. Despite lower prevalence of malaria infection this did not translate into a significant difference in maternal anaemia or birth weight. This data provides evidence that, as with immunization programmes, extremely high coverage is essential for effectiveness. This critical threshold of coverage needs to be defined, possibly on a regional basis.


Ototoxicity of artemether/lumefantrine in the treatment of falciparum malaria: a randomized trial.

Full-text - http://www.malariajournal.com/content/7/1/179

Gurkov R, Eshetu T, Barreto Miranda I, Behrens-Riha N, Mamo Y, Girma T, Krause E, Schmidt M, Hempel JM, Loscher T.

ABSTRACT: BACKGROUND: Due to increasing drug resistance, artemisinin-based combination chemotherapy (ACT) has become the first-line treatment of falciparum malaria in many endemic countries. However, irreversible ototoxicity associated with artemether/lumefantrine (AL) has been reported recently and suggested to be a serious limitation in the use of ACT. The aim of the study was to compare ototoxicity, tolerability, and efficacy of ACT with that of quinine and atovaquone/proguanil in the treatment of uncomplicated falciparum malaria. METHODS: Ninety-seven patients in south-west Ethiopia with slide-confirmed malaria were randomly assigned to receive either artemether/lumefantrine or quinine or atovaquone/proguanil and followed-up for 90 days. Comprehensive audiovestibular testing by pure tone audiometry (PTA), transitory evoked (TE) and distortion product (DP) otoacoustic emissions (OAE) and brain stem evoked response audiometry (BERA) was done before enrolment and after seven, 28 and 90 days. RESULTS: PTA and DP-OAE levels revealed transient significant cochlear hearing loss in patients treated with quinine but not in those treated with artemether/lumefantrine or atovaquone/proguanil. TE-OAE could be elicited in all examinations, except for three patients in the Q group on day 7, who suffered a transient hearing loss greater than 30 dB. There was no evidence of drug-induced brain stem lesions by BERA measurements. CONCLUSIONS: There was no detrimental effect of a standard oral regimen of artemether/lumefantrine on peripheral hearing or brainstem auditory
pathways in patients with uncomplicated falciparum malaria. In contrast, transient hearing loss is common after quinine therapy and due to temporary outer hair cell dysfunction.


Antibody response dynamics to the Plasmodium falciparum conserved vaccine candidate antigen, merozoite surface protein-1 C-terminal 19kD, in Peruvians exposed to hypoendemic malaria transmission.

Full-text - [http://www.malariajournal.com/content/7/1/173](http://www.malariajournal.com/content/7/1/173)

Torres KJ, Clark EH, Hernandez JN, Soto-Cornejo KE, Gamboa D, Branch OH.

ABSTRACT: BACKGROUND: In high-transmission areas, developing immunity to symptomatic Plasmodium falciparum infections requires 2-10 years of uninterrupted exposure. Delayed malaria-immunity has been attributed to difficult-to-develop and then short-lived antibody responses. METHODS: In a study area, with <0.5 P. falciparum infections/person/year, antibody responses to the merozoite surface protein-1 C-terminal 19kD (MSP1-19kD) antigen were evaluated and associations with P. falciparum infections in children and adults. In months surrounding and during the malaria seasons of 2003-2004, 1,772 participants received >6 active visits in one study-year. Community-wide surveys were conducted at the beginning and end of each malaria season, and weekly active visits were completed for randomly-selected individuals each month. There were 79 P. falciparum infections with serum samples collected during and approximately one month before and after infection. Anti-MSP1-19kD IgG levels were measured by ELISA. Results The infection prevalence during February-July was similar in children (0.02-0.12 infections/person/month) and adults (0.03-0.14 infections/person/month) and was negligible in the four-month dry season. In children and adults, the seroprevalence was maintained in the beginning (children=28.9%, adults=61.8%) versus ending malaria-season community survey (children=26.7%, adults=64.6%). Despite the four-month non-transmission season, the IgG levels in Plasmodium-negative adults were similar to P. falciparum-positive adults. Although children frequently responded upon infection, the transition from a negative/low level before infection to a high level during/after infection was slower in children. Adults and children IgG-positive before infection had reduced symptoms and parasite density. CONCLUSIONS: Individuals in low transmission areas can rapidly develop and maintain alphaMSP1-19kD IgG responses for >4 months, unlike responses reported in high transmission study areas. A greater immune capacity might contribute to the frequent asymptomatic P. falciparum infections in this Peruvian population.


Efficacy, safety and tolerability of artesunate-mefloquine in the treatment of uncomplicated Plasmodium falciparum malaria in four geographic zones of Nigeria.

Full-text - [http://www.malariajournal.com/content/7/1/172](http://www.malariajournal.com/content/7/1/172)

Agomo PU, Meremikwu MM, Watila IM, Omalu IJ, Odey FA, Oguche S, Ezeiru VI, Aina OO.

Malaria Unit, Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research, PMB 2013, Yaba-Lagos, Nigeria. puagomo@hotmail.com

BACKGROUND: The combination of artesunate and mefloquine has been reported to be effective against multi-drug resistant Plasmodium falciparum malaria, which has been reported in Nigeria. The objective of this multi-centre study was to evaluate the efficacy, safety and tolerability of the co-packaged formulation of artesunate and mefloquine in the treatment of uncomplicated malaria in two weight groups: those between 15 - 29 kg and > or = 30 kg respectively. METHODS: The trial was...
conducted in rural communities in the north-east, north-central, south-west and south-eastern parts of Nigeria. The WHO protocol for testing antimalarial drugs was followed. Outpatients having amongst other criteria, parasite density of > or =1,000 microl were enrolled. The co-packaged drugs were administered for 3 days at a dosage of artesunate, 4 mg/kg body wt/day and mefloquine, 25 mg/kg/body wt total) on days 0, 1 and 2. Patients were followed up for 28 days with the assessment of the parasitological parameters on days 1, 2, 3, 7, and 28. RESULTS: Four hundred and forty-six (446) patients were enrolled and 431 completed the study. Cure rates in both treatment groups was >90% at day 28. The mean parasite clearance times in treatment groups I and II were 40.1 and 42.4 hours respectively. The combination of artesunate and mefloquine showed good gametocidal activity, (gametocyte clearance time of 42.0 & 45.6 hours in treatment groups I and II respectively). There were no serious adverse events. Other adverse events observed were headache, dizziness, vomiting and abdominal discomfort. There was no significant derangement in the haematological and biochemical parameters. CONCLUSION: This co-packaged formulation of artesunate + mefloquine (Artequin) is highly efficacious, safe and well-tolerated. It is recommended for the treatment of uncomplicated P. falciparum malaria in Nigeria.

20: Malar J. 2008 Sep 8;7:171.

Dynamics of positional warfare malaria: Finland and Korea compared.

Full-text - http://www.malariajournal.com/content/7/1/171

Huldén L, Huldén L.

Department of Forest Ecology, Faculty of Agriculture and Forestry, University of Helsinki, Finland. lena.hulden@helsinki.fi

BACKGROUND: A sudden outbreak of vivax malaria among Finnish troops in SE-Finland and along the front line in Hanko peninsula in the southwest occurred in 1941 during World War II. The common explanation has been an invasion of infective Anopheles mosquitoes from the Russian troops crossing the front line between Finland and Soviet Union. A revised explanation is presented based on recent studies of Finnish malaria. METHODS: The exact start of the epidemic and the phenology of malaria cases among the Finnish soldiers were reanalyzed. The results were compared with the declining malaria in Finland. A comparison with a corresponding situation starting in the 1990's in Korea was performed. RESULTS AND DISCUSSION: The malaria cases occurred in July in 1941 when it was by far too early for infective mosquitoes to be present. The first Anopheles mosquitoes hatched at about the same time as the first malaria cases were observed among the Finnish soldiers. It takes about 3-6 weeks for the completion of the sporogony in Finland. The new explanation is that soldiers in war conditions were suddenly exposed to uninfected mosquitoes and those who still were carriers of hypnozoites developed relapses triggered by these mosquitoes. It is estimated that about 0.5% of the Finnish population still were carriers of hypnozoites in the 1940's. A corresponding outbreak of vivax malaria in Korea in the 1990's is similarly interpreted as relapses from activated hypnozoites among Korean soldiers. The significance of the mosquito induced relapses is emphasized by two benefits for the Plasmodium. There is a synchronous increase of gametocytes when new mosquitoes emerge. It also enables meiotic recombination between different strains of the Plasmodium. CONCLUSION: The malaria peak during the positional warfare in the 1940's was a short outbreak during the last phase of declining indigenous malaria in Finland. The activation of hypnozoites among a large number of soldiers and subsequent medication contributed to diminishing the reservoir of malaria and speeded up the eradication of the Finnish malaria. A corresponding evolution of Korean malaria is anticipated with relaxed tensions and decreasing troop concentrations along the border between South and North Korea.
Var2CSA DBL6-epsilon domain expressed in HEK293 induces limited cross-reactive and blocking antibodies to CSA binding parasites.

Full-text - http://www.malariajournal.com/content/7/1/170


Institut Pasteur, Unité de Biologie des Interactions Hôte-Parasite, CNRS URA2581, Batiment Nicolle, 25 rue du Docteur Roux, F-75724 Paris Cedex 15, France.

BACKGROUND: Pregnancy-associated malaria (PAM) is a serious consequence of Plasmodium falciparum-infected erythrocytes sequestration in the placenta through the adhesion to the placental receptor chondroitin sulfate A (CSA). Although women become resistant to PAM as they acquire transcending inhibitory immunity against CSA-binding parasites, hundreds of thousands of lives could be saved if a prophylactic vaccine targeting the surface proteins of placental parasites could be designed. Recent works point to the variant protein var2CSA as the key target for the development of a pregnancy-associated malaria vaccine. However, designing such a prophylactic vaccine has been hindered by the difficulty in identifying regions of var2CSA that could elicit broadly neutralizing and adhesion-blocking antibodies. METHODS: Var2CSA is a very large protein with an estimated molecular weight of 350 kDa, and can be divided into six cysteine rich Duffy binding-like domains (DBL). The human embryonic kidney 293 cell line (HEK293) was used to produce secreted soluble recombinant forms of var2CSA DBL domains. The Escherichia coli expression system was also assessed for the domains not expressed or expressed in low amount in the HEK293 system. To investigate whether var2CSA binding DBL domains can induce biologically active antibodies recognizing the native var2CSA and blocking the interaction, mice were immunized with the refolded DBL3-X or the HEK293 secreted DBL6-epsilon domains. RESULTS: Using the HEK293 expression system, DBL1-X, DBL4-epsilon and DBL6-epsilon were produced at relatively high levels in the culture supernatant, while DBL3-X and DBL5-epsilon were produced at much lower levels. DBL2-X and DBL3-X domains were obtained after refolding of the inclusion bodies produced in E. coli. Importantly, mice antisera raised against the recombinant DBL6-epsilon domain, specifically reacted against the surface of CSA-binding parasites and revealed adhesion blocking activity. CONCLUSION: This is the first report showing inhibitory binding antibodies obtained through a var2CSA recombinant DBL domain immunization protocol. These results support the current strategies using var2CSA as immunogen in the aim of blocking placental sequestration of malaria parasites. This work is a step towards the development of a var2CSA based vaccine that will prevent pregnancy-associated malaria and improve pregnancy outcomes.

Plasmodium falciparum gametocyte sex ratios in children with acute, symptomatic, uncomplicated infections treated with amodiaquine.

Full-text - http://www.malariajournal.com/content/7/1/169

Sowunmi A, Balogun ST, Gbotosho GO, Happi CT.

Department of Pharmacology & Therapeutics and Institute for Medical Research and Training, University of Ibadan, Ibadan, Nigeria. akinsowunmi@hotmail.com

BACKGROUND: Amodiaquine is frequently used as a partner drug in combination therapy or in some setting as monotherapy, but little is known about its effects on gametocyte production and sex ratio and its potential influence on transmission in Africa. The effects of amodiaquine on sexual stage parasites and gametocyte sex ratio, and the factors associated with a male-biased sex ratio were evaluated in
612 children with uncomplicated Plasmodium falciparum malaria who were treated with amodiaquine during the period 2000 - 2006 in an endemic area. METHODS: Clinical, parasitological and laboratory parameters were evaluated before treatment and during follow-up for 28-42 days, and according to standard methods. Gametocyte sex ratio was defined as the proportion of peripheral gametocytes that are male. RESULTS: Clinical recovery from illness occurred in all children. Gametocytaemia was detected in 66 patients (11%) before treatment and in another 56 patients (9%) after treatment. Gametocyte densities were significantly higher by days 3-7 following treatment compared with pre-treatment (P < 0.0001). Overall, mean gametocyte sex ratio increased significantly during follow-up and over the study periods from 2000-2006 (P < 0.001 in both cases), but was female-biased at enrolment throughout the study periods. Absence of fever, a haematocrit < 25%, asexual parasitaemia > 20,000/microL, gametocytaemia < 18/microL, and enrolment in 2006 were associated with a male-biased sex ratio pre-treatment. Anaemia and high parasitaemia were independent predictors of gametocyte maleness 7 days post treatment. CONCLUSION: Amodiaquine may significantly increase gametocyte carriage, density and sex ratio, and may potentially influence transmission. It is possible that anaemia could have contributed to the increased sex ratio. These findings may have implications for malaria control efforts in Africa.


Distribution systems of insecticide-treated bed nets for malaria control in rural Burkina Faso: cluster-randomized controlled trial.


Department of Tropical Hygiene and Public Health, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany. olaf.mueller@urz.uni-heidelberg.de

BACKGROUND: Insecticide-impregnated bed nets (ITNs) have been shown to be a highly effective tool against malaria in the endemic regions of sub-Saharan Africa (SSA). There are however different opinions about the role of ITN social marketing and ITN free distribution in the roll-out of ITN programmes. The objective of this study was to evaluate the effects of free ITN distribution through antenatal care services in addition to an ITN social marketing programme in an area typical for rural SSA. METHODS: A cluster-randomised controlled ITN trial took place in the whole Kossi Province in north-western Burkina Faso, an area highly endemic for malaria. Twelve clusters were assigned to long-term ITN (Serena brand) social marketing plus free ITN (Serena brand) distribution to all pregnant women attending governmental antenatal care services (group A), and 13 clusters to ITN social marketing only (group B). The intervention took place during the rainy season of 2006 and thereafter. The trial was evaluated through a representative household survey at baseline and after one year. Serena ITN household ownership was the primary outcome measure. FINDINGS: A total of 1052 households were visited at baseline in February 2006 and 1050 at follow-up in February 2007. Overall Serena ITN household ownership increased from 16% to 28% over the study period, with a significantly higher increase in group A (13% to 35%) than in group B (18% to 23%) (p<0.001). INTERPRETATION: The free distribution of ITNs to pregnant women through governmental antenatal care services in addition to ITN social marketing substantially improved ITN household ownership in rural Burkina Faso. TRIAL REGISTRATION: Controlled-Trials.com ISRCTN07985309.
What should vaccine developers ask? Simulation of the effectiveness of malaria vaccines.

Penny MA, Maire N, Studer A, Schapira A, Smith TA.

Swiss Tropical Institute, Basel, Switzerland.

BACKGROUND: A number of different malaria vaccine candidates are currently in pre-clinical or clinical development. Even though they vary greatly in their characteristics, it is unlikely that any of them will provide long-lasting sterilizing immunity against the malaria parasite. There is great uncertainty about what the minimal vaccine profile should be before registration is worthwhile; how to allocate resources between different candidates with different profiles; which candidates to consider combining; and what deployment strategies to consider. METHODS AND FINDINGS: We use previously published stochastic simulation models, calibrated against extensive epidemiological data, to make quantitative predictions of the population effects of malaria vaccines on malaria transmission, morbidity and mortality. The models are fitted and simulations obtained via volunteer computing. We consider a range of endemic malaria settings with deployment of vaccines via the Expanded program on immunization (EPI), with and without additional booster doses, and also via 5-yearly mass campaigns for a range of coverages. The simulation scenarios account for the dynamic effects of natural and vaccine induced immunity, for treatment of clinical episodes, and for births, ageing and deaths in the cohort. Simulated pre-erythrocytic vaccines have greatest benefits in low endemic settings (<EIR of 10.5) where between 12% and 14% of all deaths are averted when initial efficacy is 50%. In some high transmission scenarios (>EIR of 84) PEV may lead to increased incidence of severe disease in the long term, if efficacy is moderate to low (<70%). Blood stage vaccines (BSV) are most useful in high transmission settings, and are comparable to PEV for low transmission settings. Combinations of PEV and BSV generally perform little better than the best of the contributing components. A minimum half-life of protection of 2-3 years appears to be a precondition for substantial epidemiological effects. Herd immunity effects can be achieved with even moderately effective (>20%) malaria vaccines (either PEV or BSV) when deployed through mass campaigns targeting all age-groups as well as EPI, and especially if combined with highly efficacious transmission-blocking components. CONCLUSIONS: We present for the first time a stochastic simulation approach to compare likely effects on morbidity, mortality and transmission of a range of malaria vaccines and vaccine combinations in realistic epidemiological and health systems settings. The results raise several issues for vaccine clinical development, in particular appropriateness of vaccine types for different transmission settings; the need to assess transmission to the vector and duration of protection; and the importance of deployment additional to the EPI, which again may make the issue of number of doses required more critical. To test the validity and robustness of our conclusions there is a need for further modeling (and, of course, field research) using alternative formulations for both natural and vaccine induced immunity. Evaluation of alternative deployment strategies outside EPI needs to consider the operational implications of different approaches to mass vaccination.
Cationic liposomes formulated with synthetic mycobacterial cordfactor (CAF01): a versatile adjuvant for vaccines with different immunological requirements.


Adjuvant Research, Department of Infectious Disease Immunology, Statens Serum Institut, Copenhagen, Denmark. eag@ssi.dk

BACKGROUND: It is now emerging that for vaccines against a range of diseases including influenza, malaria and HIV, the induction of a humoral response is insufficient and a substantial complementary cell-mediated immune response is necessary for adequate protection. Furthermore, for some diseases such as tuberculosis, a cellular response seems to be the sole effector mechanism required for protection. The development of new adjuvants capable of inducing highly complex immune responses with strong antigen-specific T-cell responses in addition to antibodies is therefore urgently needed.

METHODS AND FINDINGS: Herein, we describe a cationic adjuvant formulation (CAF01) consisting of DDA as a delivery vehicle and synthetic mycobacterial cordfactor as immunomodulator. CAF01 primes strong and complex immune responses and using ovalbumin as a model vaccine antigen in mice, antigen specific cell-mediated- and humoral responses were obtained at a level clearly above a range of currently used adjuvants (Aluminium, monophosphoryl lipid A, CFA/IFA, Montanide). This response occurs through Toll-like receptor 2, 3, 4 and 7-independent pathways whereas the response is partly reduced in MyD88-deficient mice. In three animal models of diseases with markedly different immunological requirement; Mycobacterium tuberculosis (cell-mediated), Chlamydia trachomatis (cell-mediated/humoral) and malaria (humoral) immunization with CAF01-based vaccines elicited significant protective immunity against challenge.

CONCLUSION: CAF01 is potentially a suitable adjuvant for a wide range of diseases including targets requiring both CMI and humoral immune responses for protection.

Hyperbaric oxygen prevents early death caused by experimental cerebral malaria.

Full-text: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003126


Department of Microbiology & Immunology, State University of Campinas, Campinas, São Paulo, Brazil.

BACKGROUND: Cerebral malaria (CM) is a syndrome characterized by neurological signs, seizures and coma. Despite the fact that CM presents similarities with cerebral stroke, few studies have focused on new supportive therapies for the disease. Hyperbaric oxygen (HBO) therapy has been successfully used in patients with numerous brain disorders such as stroke, migraine and atherosclerosis.

METHODOLOGY/PRINCIPAL FINDINGS: C57BL/6 mice infected with Plasmodium berghei ANKA (PbA) were exposed to daily doses of HBO (100% O2, 3.0 ATA, 1-2 h per day) in conditions well-tolerated by humans and animals, before or after parasite establishment. Cumulative survival analyses demonstrated that HBO therapy protected 50% of PbA-infected mice and delayed CM-specific neurological signs when administrated after patent parasitemia. Pressurized oxygen therapy reduced peripheral parasitemia, expression of TNF-alpha, IFN-gamma and IL-10 mRNA levels and percentage of gammadelta and alphabeta CD4(+) and CD8(+) T lymphocytes.
sequestered in mice brains, thus resulting in a reduction of blood-brain barrier (BBB) dysfunction and hypothermia. CONCLUSIONS/SIGNIFICANCE: The data presented here is the first indication that HBO treatment could be used as supportive therapy, perhaps in association with neuroprotective drugs, to prevent CM clinical outcomes, including death.


Computational analysis of constraints on noncoding regions, coding regions and gene expression in relation to Plasmodium phenotypic diversity.

Full-text: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0003122

Essien K, Hannenhalli S, Stoeckert CJ Jr.

Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America.

BACKGROUND: Malaria-causing Plasmodium species exhibit marked differences including host choice and preference for invading particular cell types. The genetic bases of phenotypic differences between parasites can be understood, in part, by investigating constraints on gene expression and genic sequences, both coding and regulatory. METHODOLOGY/PRINCIPAL FINDINGS: We investigated the evolutionary constraints on sequence and expression of parasitic genes by applying comparative genomics approaches to 6 Plasmodium genomes and 2 genome-wide expression studies. We found that the coding regions of Plasmodium transcription factor and sexual development genes are relatively less constrained, as are those of genes encoding CCCH zinc fingers and invasion proteins, which all play important roles in these parasites. Transcription factors and genes with stage-restricted expression have conserved upstream regions and so do several gene classes critical to the parasite's lifestyle, namely, ion transport, invasion, chromatin assembly and CCCH zinc fingers. Additionally, a cross-species comparison of expression patterns revealed that Plasmodium-specific genes exhibit significant expression divergence. CONCLUSIONS/SIGNIFICANCE: Overall, constraints on Plasmodium's protein coding regions confirm observations from other eukaryotes in that transcription factors are under relatively lower constraint. Proteins relevant to the parasite's unique lifestyle also have lower constraint on their coding regions. Greater conservation between Plasmodium species in terms of promoter motifs suggests tight regulatory control of lifestyle genes. However, an interspecies divergence in expression patterns of these genes suggests that either expression is controlled via genomic or epigenomic features not encoded in the proximal promoter sequence, or alternatively, the combinatorial interactions between motifs confer species-specific expression patterns.


The cysteine-rich interdomain region from the highly variable plasmodium falciparum erythrocyte membrane protein-1 exhibits a conserved structure.

Full-text: http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000147

Klein MM, Gittis AG, Su HP, Makobongo MO, Moore JM, Singh S, Miller LH, Garboczi DN.

Structural Biology Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, United States of America.
Plasmodium falciparum malaria parasites, living in red blood cells, express proteins of the erythrocyte membrane protein-1 (PfEMP1) family on the red blood cell surface. The binding of PfEMP1 molecules to human cell surface receptors mediates the adherence of infected red blood cells to human tissues. The sequences of the 60 PfEMP1 genes in each parasite genome vary greatly from parasite to parasite, yet the variant PfEMP1 proteins maintain receptor binding. Almost all parasites isolated directly from patients bind the human CD36 receptor. Of the several kinds of highly polymorphic cysteine-rich interdomain region (CIDR) domains classified by sequence, only the CIDR1alpha domains bind CD36. Here we describe the CD36-binding portion of a CIDR1alpha domain, MC179, as a bundle of three alpha-helices that are connected by a loop and three additional helices. The MC179 structure, containing seven conserved cysteines and 10 conserved hydrophobic residues, predicts similar structures for the hundreds of CIDR sequences from the many genome sequences now known. Comparison of MC179 with the CIDR domains in the genome of the P. falciparum 3D7 strain provides insights into CIDR domain structure. The CIDR1alpha three-helix bundle exhibits less than 20% sequence identity with the three-helix bundles of Duffy-binding like (DBL) domains, but the two kinds of bundles are almost identical. Despite the enormous diversity of PfEMP1 sequences, the CIDR1alpha and DBL protein structures, taken together, predict that a PfEMP1 molecule is a polymer of three-helix bundles elaborated by a variety of connecting helices and loops. From the structures also comes the insight that DBL1alpha domains are approximately 100 residues larger and that CIDR1alpha domains are approximately 100 residues smaller than sequence alignments predict. This new understanding of PfEMP1 structure will allow the use of better-defined PfEMP1 domains for functional studies, for the design of candidate vaccines, and for understanding the molecular basis of cytoadherence.


Refractive index maps and membrane dynamics of human red blood cells parasitized by Plasmodium falciparum.

Full-text - http://hwmaint.pnas.org/cgi/content/full/105/37/13730


G. R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Parasitization by malaria-inducing Plasmodium falciparum leads to structural, biochemical, and mechanical modifications to the host red blood cells (RBCs). To study these modifications, we investigate two intrinsic indicators: the refractive index and membrane fluctuations in P. falciparum-invaded human RBCs (Pf-RBCs). We report experimental connections between these intrinsic indicators and pathological states. By employing two noninvasive optical techniques, tomographic phase microscopy and diffraction phase microscopy, we extract three-dimensional maps of refractive index and nanoscale cell membrane fluctuations in isolated RBCs. Our systematic experiments cover all intraerythrocytic stages of parasite development under physiological and febrile temperatures. These findings offer potential, and sufficiently general, avenues for identifying, through cell membrane dynamics, pathological states that cause or accompany human diseases.

Part B – Citations and abstracts (Alphabetical by journal title)


Genetic diversity in the C-terminal 42kDa region of merozoite surface protein-1 of Plasmodium vivax (PvMSP-1(42)) among Indian isolates.

Thakur A, Alam MT, Sharma YD.
Department of Biotechnology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India.

Plasmodium vivax merozoite surface protein 1 (PvMSP-1) is a leading malaria vaccine candidate. This protein is processed to give rise to various sized fragments during merozoite maturation. Here, we describe the analysis of genetic diversity in the 42kDa C-terminal part of this protein among 33 Indian P. vivax isolates. A total of 27 haplotypes with 72 mutations and 0.0212+/-0.0005S.D. over all pi nucleotide diversity were observed among the isolates. Twenty-six of 27 haplotypes reported here were new as they have not been reported so far from any other country. The difference between non-synonymous (dN) and synonymous (dS) mutations was found to be positive (0.0081+/-0.0051) for the entire 42kDa region. Further analysis revealed that 33kDa (MSP-1(33)) fragment of the MSP-1(42) was highly polymorphic with pi nucleotide diversity 0.0290+/-.0.0007S.D. The dN-dS for this region of MSP-1 was also positive (0.0114+/-0.0071S.E.). On the other hand, there was no non-synonymous mutation in the 19kDa (MSP-1(19)) fragment of the MSP-1(42) and thus it was highly conserved. In conclusion, MSP-1(33) fragment was highly polymorphic and appeared to be under diversifying selection whereas there was no selection at MSP-1(19) region among the isolates. Present study will be helpful for the development of PvMSP-1 based vaccine against P. vivax malaria.


Yunnan Institute of Parasitic Diseases/Malaria Research Center of Yunnan/Institute of Vector-Pathogen Biology for Dali University, Pu'er City, Yunnan 665000, China.

The emergence and spread of drug resistant malaria parasites are an important factor contributing to the global resurgence of malaria, demonstrating the essence of drug resistance surveillance in endemic areas. In the malarious border regions of Yunnan Province, China, we have selected three study sites to monitor in vitro and in vivo resistance of Plasmodium falciparum parasites to chloroquine (CQ) from 1981 to 2006. In vitro studies using the microtest clearly showed high degree of CQ resistance in the early 1980s, when CQ was replaced by artesunate monotherapy for falciparum malaria. In subsequent in vitro surveys performed in the early 1990s and 2003-2004, we found reductions in both the concentrations inhibiting 50% parasite growth (IC(50)s) and the percentage of resistant parasites at all study sites, although the degrees of the reduction varied among sites. Even though amodiaquine has never been used in this area, there were consistently high levels of resistance to this drug, confirming crossresistance between CQ and amodiaquine. In vivo clinical studies were consistent with the results of the in vitro assays. The overall rate of resistant clinical cases decreased from 97% in 1981-1983 to 40% in 2005-2006. Collectively, whereas a general trend of reduction in CQ resistance was observed in Yunnan, variations among sites existed in this relatively small area, probably as the result of both geographical heterogeneity of malaria epidemiology in Yunnan and different levels of CQ resistance in neighboring countries.


Analysis of TPI gene promoter variation in three sub-Saharan Africa population samples.

CIAS/Departamento de Antropologia, Universidade de Coimbra, Portugal.

Population samples from Angola, Mozambique, and S. Tomé e Príncipe were screened for the TPI gene promoter variants -5A-->G, -8G-->A and -24T-->G. Three haplotypes were identified in the three populations: the haplotype -5A-8G-24T (average frequency 65.3%) and two less common haplotypes -5G-8G-24T (average frequency 24.7%) and -5G-8A-24T (average frequency 10.0%). A population sample from Central Portugal showed the haplotype -5A-8G-24T in 139 chromosomes and one subject heterozygous for haplotype -5G-8A-24G. The exact test of sample differentiation among three groups of malaria-infected individuals classified according to the severity of the disease showed no significant differences. We confirmed TPI gene diversity in sub-Saharan Africa, but we could not detect any association between TPI promoter variation and a malarial protective effect. Larger scale epidemiological studies are thus required to clarify this putative mechanism of natural host defense against this worldwide public health problem. Am. J. Hum. Biol., 2008. (c) 2008 Wiley-Liss, Inc.


Parachuting Cats and Crushed Eggs: The Controversy Over the Use of DDT to Control Malaria.

Oshaughnessy PT.

The University of Iowa.

The use of DDT to control malaria has been a contentious practice for decades. This controversy centers on concerns over the ecological harm caused by DDT relative to the gains in public health from its use to prevent malaria. Given the World Health Organization's recent policy decisions concerning the use of DDT to control malaria, it is worth reviewing the historical context of DDT use. Ecological concerns focused on evidence that DDT ingestion by predatory birds resulted in eggs with shells so thin they were crushed by adult birds. In addition, DDT spraying to control malaria allegedly resulted in cats being poisoned in some areas, which led to increased rodent populations and, in turn, the parachuting of cats into the highlands of the island of Borneo to kill the rodents, a story that influenced the decision to ban DDT spraying. I focus on this story with the intention of grounding the current debate on lessons from the past.


Duffy Antigen/Receptor for Chemokines Gene is Associated with Asthma and IgE in Three Populations.


Division of Allergy and Clinical Immunology, Department of Medicine, The Johns Hopkins University (JHU), Baltimore, Maryland, United States; Institute for Immunological Research, University of Cartagena, Cartagena, Colombia.

RATIONALE: Asthma prevalence and severity are high among underserved minorities, including those of African descent. The Duffy antigen/receptor for chemokines is the receptor for Plasmodium vivax on erythrocytes and functions as a chemokine clearing receptor. Unlike European populations, decreased expression of the receptor on erythrocytes is common among populations of African descent, and results from a functional T-46C polymorphism (rs2814778) in the promoter. This
variant provides an evolutionary advantage in malaria-endemic regions, because Duffy antigen/receptor for chemokines-negative erythrocytes are more resistant to infection by Plasmodium vivax. OBJECTIVES: To determine the role of the rs2814778 polymorphism in asthma and atopy as measured by total serum IgE levels among four populations of African descent (African Caribbean, African American, Brazilian, and Colombian) and a European American population. METHODS: Family-based association tests were performed in each of the five populations to test for association between the rs2814778 polymorphism and asthma or total IgE concentrations. MEASUREMENTS AND MAIN RESULTS: Asthma was significantly associated with the rs2814778 polymorphism in the African Caribbean, Colombian and Brazilian families (P<0.05). High total IgE levels were associated with this variant in African Caribbean and Colombian families (P<0.05). The variant allele was not polymorphic among European Americans. CONCLUSIONS: Susceptibility to asthma and atopy among certain populations of African descent is influenced by a functional polymorphism in Duffy Antigen/Receptor for Chemokines gene. This genetic variant, which confers resistance to malarial parasitic infection, may also partially explain ethnic differences in morbidity of asthma.


Acute respiratory distress syndrome in a case of Plasmodium ovale malaria.

Rojo-Marcos G, Cuadros-González J, Mesa-Latorre JM, Culebras-López AM, de Pablo-Sánchez R.

Department of Internal Medicine, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain. grojo.hupa@salud.madrid.org

Acute respiratory distress syndrome is a well-known complication in Plasmodium falciparum infection. It is less frequently described in Plasmodium vivax, and only one case is reported in Plasmodium ovale. Here we present the second description of this pulmonary complication in a P. ovale acute infection.


Challenges in routine implementation and quality control of rapid diagnostic tests for malaria-Rufiji District, Tanzania.

McMorrow ML, Masanja MI, Abdulla SM, Kahigwa E, Kachur SP.

Malaria Branch, Division of Parasitic Diseases, National Center for Zoonotic Vector-borne and Enteric Diseases, US Centers for Disease Control and Prevention, USA. MMcmorrow@cdc.gov

Rapid diagnostic tests (RDTs) represent an alternative to microscopy for malaria diagnosis and have shown high sensitivity and specificity in a variety of study settings. Current World Health Organization (WHO) guidelines for quality control of RDTs provide detailed instructions on pre-field testing, but offer little guidance for quality assurance once RDTs are deployed in health facilities. From September 2006 to April 2007, we introduced a histidine-rich protein II (HRP2)-based RDT (Paracheck) for suspected malaria cases five years of age and older in nine health facilities in Rufiji District, Tanzania, to assess sensitivity and specificity of RDTs in routine use at rural health facilities. Thick blood smears were collected for all patients tested with RDTs and stained and read by laboratory personnel in each facility. Thick smears were subsequently reviewed by a reference microscopist to determine RDT sensitivity and specificity. In all nine health facilities, there were significant problems with the quality of staining and microscopy. Sensitivity and specificity of RDTs were difficult to assess given the poor quality of routine blood smear staining. Mean operational sensitivity of RDTs based on reference microscopy was 64.8%, but varied greatly by health facility, range 18.8-85.9%. Sensitivity of RDTs
increased with increasing parasite density. Specificity remained high at 87.8% despite relatively poor slide quality. Institution of quality control of RDTs based on poor quality blood smear staining may impede reliable measurement of sensitivity and specificity and undermine confidence in the new diagnostic. There is an urgent need for the development of alternative quality control procedures for rapid diagnostic tests that can be performed at the facility level.


Effects of different antimalarial drugs on gametocyte carriage in P. vivax malaria.


Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

The gametocytocidal and asexual stage activities of eight antimalarial and eight antibiotic-containing regimens were evaluated in 349 adult patients with P. vivax malaria. Gametocytemia was found in 63% of patients (22% before and 41% after treatment). The median (range) gametocyte clearance time was 24 hours (range, 2-504 hours) and correlated with asexual parasite clearance time (r = 0.52, P < 0.001). Gametocytemia in vivax malaria was more common in patients with admission parasitemia > 10,000/microL and after treatment with drugs which have weak antimalarial activity, and was also associated with an increased rate of vivax reappearance (29.4% versus 14.1%, P = 0.002). Sexual stage activities corresponded with asexual stage activity for all tested regimens. Treatment with potent antimalarial drugs reduces the transmission potential of P. vivax.


Does cotrimoxazole prophylaxis for the prevention of HIV-associated opportunistic infections select for resistant pathogens in Kenyan adults?


Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

mhamel@ke.cdc.gov

We assessed the effect of daily cotrimoxazole, essential for HIV care, on development of antifolate-resistant Plasmodium falciparum, naso-pharyngeal Streptococcus pneumoniae (pneumococcus), and commensal Escherichia coli.

HIV-positive subjects with CD4 cell count < 350 cells/muL (lower-CD4; N = 692) received cotrimoxazole; HIV-positive with CD4 cell count > or = 350 cells/muL (higher-CD4; N = 336) and HIV-negative subjects (N = 132) received multivitamins. Specimens were collected at baseline, 2 weeks, monthly, and at sick visits during 6 months of follow-up to compare changes in resistance, with higher-CD4 as referent. P. falciparum parasitemia incidence density was 16 and 156/100 person-years in lower-CD4 and higher-CD4, respectively (adjusted rate ratio [ARR] = 0.11; 95% confidence interval [CI] = 0.06-0.15; P < 0.001) and 97/100 person-years in HIV-negative subjects (ARR = 0.62; 95% CI = 0.44-0.86; P = 005).

Incidence density of triple and quintuple dihydrofolate-reductase/dihydropteroate-synthetase mutations was 90% reduced in lower-CD4 compared with referent. Overall, cotrimoxazole non-susceptibility was high among isolated pneumococcus (92%) and E. coli (76%) and increased significantly in lower-CD4 subjects by Week 2 (P < 0.005). Daily cotrimoxazole prevented malaria and reduced incidence of antifolate-resistant P. falciparum but contributed to increased pneumococcus and commensal Escherichia coli resistance.

A randomized, comparative study of supervised and unsupervised artesunate-amodiaquine, for the treatment of uncomplicated malaria in Ghana.


Navrongo Health Research Centre, P.O. Box 114, Navrongo, Ghana.

Although the use of artesunate-amodiaquine treatment is growing in Africa, data on its effectiveness are limited. In only the second published comparison of supervised and unsupervised treatments with this combination, Ghanaian children with uncomplicated malaria have recently been investigated in an open-label, randomized, comparative study. Children aged 6-120 months attending the Navrongo War Memorial hospital between November 2005 and December 2006 were enrolled if they had uncomplicated Plasmodium falciparum malaria and at least one of their parents/guardians gave their informed consent. Overall, 638 patients were screened, 357 were found to have P. falciparum infection, and 308 of these satisfied the other selection criteria and were enrolled. The subjects were divided randomly into two treatment arms. All the children were scheduled to receive 10 mg amodiaquine/kg and 4 mg artesunate/kg daily for 3 days but only 154 (the 'supervised') were given all their treatments in hospital, with each dose directly observed. Although the other 154 children (the 'unsupervised') were given their first dose in hospital, under supervision, they were then sent home with the tablets they required to complete treatment. Study participation lasted for 28 days, with follow-up on days 3, 7, 14, 21 and 28. During follow-up, axillary temperatures, any emergent signs and symptoms, and concomitant drug consumption were recorded and haemoglobin concentrations and malarial parasitaemias and gametocytaemias were measured. All but seven of the 308 subjects completed the study. At enrolment the subjects had a mean age of 45.0 months, a mean weight of 14.8 kg, a mean axillary temperature of 37.9 degrees C and a geometric mean parasitaemia of 11,367 asexual stages/mul. About 55% of the children investigated were girls. There were no significant baseline differences between the two treatment arms. Although there was also no difference in the clearance of fever and parasitaemia between the two arms by day 14, a supervised child was significantly more likely to show an adequate clinical and parasitological response, by day 21 (91.3% v. 84.1%; P= 0.05) or day 28 (80.0% v. 64.9%; P<0.01), than an unsupervised child. The reported adverse effects following treatment and the trend in haemoglobin recovery were, however, similar in the two arms. Although artesunate-amodiaquine appeared very effective in the treatment of uncomplicated P. falciparum malaria in children, whether supervised or not, it appears that supervised treatment provided stronger prevention against re-infection and recrudescence. At least in the present study, treatment at home, without medical supervision, probably led to relatively poor compliance.


Therapeutic responses of Plasmodium vivax and P. falciparum to chloroquine, in an area of western India where P. vivax predominates.

Srivastava HC, Yadav RS, Joshi H, Valecha N, Mallick PK, Prajapati SK, Dash AP.

National Institute of Malaria Research (ICMR), Field Station, Civil Hospital, Nadiad – 387001, Gujarat, India. hcsrv_52@rediffmail.com

In 2003-2005, following an increase in the local incidence of human malaria, the therapeutic efficacy of chloroquine (CQ) in the treatment of Plasmodium vivax and P. falciparum malaria was evaluated in the Anand district of Gujarat state, in western India. After oral administration of CQ, clinical and parasitological responses were measured over a follow-up period of 28 days, following the
standard protocol of the World Health Organization. Most of the recurrent infections were checked, by genotyping, to see whether they were the result of treatment failure or re-infection during the follow-up. At the primary health centre (PHC) in Deva, all 57 P. vivax cases included in the study responded to CQ within 3 days. At the Pansora PHC, however, only 59 [90.8%, with a 95% confidence interval (CI) of 83.7%-97.8%] of the 65 P. vivax cases appeared to respond completely, recurrent infections being observed in the other six cases (9.2%; CI=2.2%-16.3%). Of the four recurrent infections checked by genotyping, however, only two appeared to be the result of true treatment failure. Twenty-seven (81.8%; CI=67.2%-94.4%) of the 33 P. falciparum cases who were enrolled in the study, all from Pansora PHC also showed apparent treatment failure, with one early failure, 17 late clinical failures and nine late parasitological failures. All 23 P. falciparum cases that showed apparent treatment failure and were investigated by genotyping appeared to be true cases of failure, none showing any evidence of re-infection during follow-up. The mean parasite-clearance times for those infected with P. falciparum, both those considered CQ-sensitive and the treatment failures, exceeded 2 days. These results indicate the presence of CQ-resistant P. vivax and P. falciparum in Anand district. The high frequency of CQ failure against P. falciparum observed in this study led to a change in the drug policy at the Pansora PHC, with artemisinin-based combination therapy now being used for the first-line treatment of P. falciparum malaria. Chloroquine remains the recommended first-line treatment for P. vivax infections in the area but the treatment failure seen in at least two P. vivax cases indicates a need for further monitoring of the therapeutic efficacy of CQ against such infections, in central Gujarat and elsewhere.


Antigenic Variation in Plasmodium falciparum.

Scherf A, Lopez-Rubio JJ, Riviere L.

Biology of Host-Parasite Interactions Unit, CNRS URA2581, Institut Pasteur 75724 Paris, France; email: ascherf@pasteur.fr, jjlopez@pasteur.fr, lriviere@pasteur.fr.

The persistence of the human malaria parasite Plasmodium falciparum during blood stage proliferation in its host depends on the successive expression of variant molecules at the surface of infected erythrocytes. This variation is mediated by the differential control of a family of surface molecules termed PfEMP1 encoded by approximately 60 var genes. Each individual parasite expresses a single var gene at a time, maintaining all other members of the family in a transcriptionally silent state. PfEMP1/var enables parasitized erythrocytes to adhere within the microvasculature, resulting in severe disease. This review highlights key regulatory mechanisms thought to be critical for monoallelic expression of var genes. Antigenic variation is orchestrated by epigenetic factors including monoallelic var transcription at separate spatial domains at the nuclear periphery, differential histone marks on otherwise identical var genes, and var silencing mediated by telomeric heterochromatin. In addition, controversies surrounding var genetic elements in antigenic variation are discussed.


Placental malaria increases malaria risk in the first 30 months of life.


Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon, South Africa.
BACKGROUND: Plasmodium falciparum infection during pregnancy is associated with stillbirth, fetal growth restriction, and low birth weight. An additional consequence may be increased risk of malaria in early life, although the epidemiological evidence of this consequence is limited. METHODS: A cohort of 527 children were observed actively every month for 30 months after delivery. Offspring of mothers with microscopically detectable placental P. falciparum infection at the time of delivery were defined as exposed. The outcome measure was malaria (parasitemia and fever). Analyses were performed using Cox proportional hazard models and were stratified by gravidity. RESULTS: Overall, offspring of mothers with placental P. falciparum infection had a significantly higher risk of clinical malaria during the first 30 months of life (adjusted hazard ratio, 2.1; 95% confidence interval [CI], 1.2-3.7). The adjusted hazard ratio for offspring of multigravidae was 2.6 (95% CI, 1.3-5.3), and that for primigravidae was 1.5 (95% CI, 0.6-3.8). The offspring of placenta-infected primigravidae had no episodes of malaria during the first year of life. CONCLUSIONS: Our findings show that active placental P. falciparum infection detected at delivery is associated with an approximately 2-fold greater risk of malaria during early life, compared with noninfection. The fact that persons born to infected multigravidae rather than primigravidae appear to be at greater risk emphasizes the importance of preventing malaria in mothers of all gravidities.


Serological evaluation of MPL17 and MPL21 leptospiral recombinant antigens by enzyme linked immunosorbent assays.

Oliveira TR, Longhi MT, de Morais ZM, Romero EC, Blanco RM, Kirchgatter K, Vasconcellos SA, Nascimento AL.

Centro de Biotecnologia, Instituto Butantan, Avenida Vital Brazil, 1500, 05503-900, São Paulo, SP, Brazil; Laboratório de Zoonoses Bacterianas do VPS, Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, Brazil; Divisão de Biologia Médica, Instituto Adolfo Lutz, São Paulo, Brazil; Núcleo de Estudos em Malária, Superintendência de Controle de Endemias (SUCEN)/Instituto de Medicina Tropical de São Paulo, Universidade de São Paulo, São Paulo, Brazil; Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, USP, São Paulo, Brazil.

Leptospirosis is a zoonosis of multisystem involvement caused by pathogenic strains of the genus Leptospira. In the last few years, intense studies aimed the development of a vaccine have provided important knowledge on the nature of the host immunological mechanisms. The purpose of this study was to analyze the immune response of individuals during infection against two recombinant proteins (MPL17/LIC10765 and MPL21/LIC13131) of Leptospira interrogans serovar Copenhageni. The recombinant proteins expressed in Escherichia coli as 6xHis-tag fusion proteins were purified from the soluble bacterial fraction by affinity chromatography using Ni(2+)-charged resin. The recombinant proteins were used to evaluate their ability to bind to IgG (and subclass) or IgM antibodies of serum samples from early and convalescent phases of patients diagnosed with leptospirosis (n= 52) by ELISA. The prevalence of total IgG antibodies against MPL17 and MPL21 were 38.5% and 21.2%, respectively. Statistically significant higher titers were achieved with MPL17 when compared to the reference microscopic agglutination test (MAT). The specificity of the assay was estimated to be 95.5% for MPL17 and 80.6% for MPL21 when examined with serum samples from individuals with unrelated febrile diseases and control healthy donors. The proteins are conserved among Leptospira strains that cause human and animal diseases. MPL17/LIC10765 and MPL21/LIC13131 are most likely new surface proteins of leptospires as revealed in liquid-phase immunofluorescence assays with living organisms. Our results demonstrated that these recombinant proteins are highly immunogenic and together might be useful to diagnose the disease.

**IMMUNE RESPONSES TO AN IMPROVED MULTI-EPI TOPE, MULTI-STAGE MALARIA VACCINE CANDIDATE ANTIGEN FALVAC-1A IN DIFFERENT GENETIC BACKGROUNDS OF MICE.**


Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, 30341, USA; Atlanta Research and Education Foundation, Decatur, GA, 30033, USA; Division of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD, 20708.

FALVAC-1A is a second-generation multi-target, multi-epitope synthetic candidate vaccine against Plasmodium falciparum, incorporating elements designed to yield a stable and immunogenic molecule. Characteristics of the immunogenicity of FALVAC-1A were evaluated in congenic (H-2(b), H-2(k), H-2(d)) and outbred strains of mice. The influences of four adjuvants (aluminum phosphate, QS-21, Montanide ISA-720 and copolymer CRL-1005) on different aspects of the immune responses were also assessed. FALVAC-1A generated strong antibody responses in all mouse strains. The highest mean ELISA antibody concentrations against FALVAC-1A were observed in the outbred ICR mice, followed by B10.BR, B10.D2 and C57BL/6, though this order varied between adjuvants with no statistical differences between mouse strains. In all mouse strains, the highest anti-FALVAC-1A ELISA antibody titers were induced by FALVAC-1A in copolymer and ISA-720 formulations, followed by QS-21 and AlPO4. These antibodies were of all 4 subclasses, though IgG1 predominated except with QS-21 adjuvant which induced predominantly IgG2c responses. Both sporozoites and blood stages of P. falciparum were recognized by anti-FALVAC-1A sera in the immunofluorescence assay. In addition to antibody, cellular immune responses exemplified by splenic IFN-gamma and IL-4 ELISPOTs were detected. In summary, FALVAC-1A was found to be highly immunogenic and elicited functionally relevant antibodies that can recognize sporozoites and blood stage parasites in diverse genetic backgrounds.


**Health facility and health worker readiness to deliver new national treatment policy for malaria in Kenya.**

Njogu J, Akhwale W, Hamer DH, Zurovac D.

Malaria Public Health and Epidemiology Group, KEMRI/Wellcome Trust Research Programme, P.O. Box 43640-00100, Nairobi, Kenya.

OBJECTIVE: To evaluate health facility and health worker readiness to deliver new artemether-lumefantrine (AL) treatment policy for uncomplicated malaria in Kenya. DESIGN: Cross-sectional survey. SETTING: Health facilities in four sentinel districts in Kenya. PARTICIPANTS: All government facilities in study districts (n = 211) and all health workers performing outpatient consultations (n = 654). MAIN OUTCOME MEASURES: Availability of antimalarial drugs on the survey day, stock-outs in past six months, presence of AL wall charts, health worker's exposure to in-service training on AL and access to new national malaria guidelines. RESULTS: The availability of any tablets of AL, sulfadoxine-pyrimethamine and amodiaquine was nearly universal on the survey day. However, only 61% of facilities stocked all four weight-specific packs of AL. In the past six months, 67% of facilities had stock-out of at least one AL tablet pack and 15% were out of stock for all four packs at the same time. Duration of stock-out was substantial for all AL packs (median range: 27-39% of time). During the same period, the stock-outs of sulfadoxine-pyrimethamine and amodiaquine were rare. Only 19% of facilities had all AL wall charts displayed, AL in-service training was provided to 47% of health workers and 59% had access to the new...
guidelines. CONCLUSION: Health facility and health worker readiness to implement AL policy is not yet optimal. Continuous supply of all four AL pack sizes and removal of not recommended antimalarials is needed. Further coordinated efforts through the routine programmatic activities are necessary to improve delivery of AL at the point of care.


Molecular Surveillance for Multidrug-Resistant Plasmodium falciparum, Cambodia.


University of North Carolina School of Public Health, Chapel Hill, North Carolina, USA (N.K. Shah, A.P. Alker, S.R. Meshnick); National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia (R. Sem, S. Muth, S. Duong); Pasteur Institute of Cambodia, Phnom Penh, (R. Sem, F. Ariey); and US Naval Medical Research Unit No. 2, Jakarta, Indonesia (A.I. Susanti, J.D. Maguire, C. Wongsrichanalai).

We conducted surveillance for multidrug-resistant Plasmodium falciparum in Cambodia during 2004-2006 by assessing molecular changes in pfmdr1. The high prevalence of isolates with multiple pfmdr1 copies found in western Cambodia near the Thai border, where artemisinate-mefloquine therapy failures occur, contrasts with isolates from eastern Cambodia, where this combination therapy remains highly effective.


Deforestation and Vectorial Capacity of Anopheles gambiae Giles Mosquitoes in Malaria Transmission, Kenya.

Afrane YA, Little TJ, Lawson BW, Githeko AK, Yan G.

Kenya Medical Research Institute, Kisumu, Kenya (Y.A. Afrane, A.K. Githeko); University of Edinburgh, Edinburgh, Scotland, UK (T.J. Little); Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (Y.A. Afrane, B.W. Lawson); and University of California, Irvine, California, USA (G. Yan).

We investigated the effects of deforestation on microclimates and sporogonic development of Plasmodium falciparum parasites in Anopheles gambiae mosquitoes in an area of the western Kenyan highland prone to malaria epidemics. An. gambiae mosquitoes were fed with P. falciparum-infected blood through membrane feeders. Fed mosquitoes were placed in houses in forested and deforested areas in a highland area (1,500 m above sea level) and monitored for parasite development. Deforested sites had higher temperatures and relative humidities, and the overall infection rate of mosquitoes was increased compared with that in forested sites. Sporozoites appeared on average 1.1 days earlier in deforested areas. Vectorial capacity was estimated to be 77.7% higher in the deforested site than in the forested site. We showed that deforestation changes microclimates, leading to more rapid sporogonic development of P. falciparum and to a marked increase of malaria risk in the western Kenyan highland.


Biological control of mosquito populations through frogs: Opportunities & constrains.

Raghavendra K, Sharma P, Dash AP.

National Institute of Malaria Research (ICMR), Delhi, India.
The use of frogs and tadpoles for disease vector control is still largely unexplored. Frogs are an important part of the ecosystem with a role for insect and pest control including mosquitoes. Available information suggests the existence of many direct and indirect factors affecting the growth and survival of both prey and predators. Other controphic species that have influence on this relationship also show considerable effect. Still, the associations of different prey and predator relationships in the environment to assess the feasibility of use of a species as biocontrol agent for vector control and management. However, frogs cannot be used as an independent intervention for disease vector control and more research is needed to use them effectively for mosquito control.


Experimental Malaria Infection Triggers Early Expansion of Natural Killer Cells.

Kim CC, Parikh S, Sun JC, Myrick A, Lanier LL, Rosenthal PJ, Derisi JL.

Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco CA; Department of Medicine, University of California San Francisco, San Francisco CA; Department of Microbiology and Immunology and the Cancer Research Institute, University of California San Francisco, San Francisco CA; Biology Scholars Program, University of California San Francisco, San Francisco CA; Cancer Research Institute, University of California San Francisco, San Francisco CA; Howard Hughes Medical Institute.

In order to gain a better understanding of gene expression during early malaria infection, we conducted microarray analysis of early blood responses in mice infected with erythrocytic stage Plasmodium chabaudi. Immediately following infection, we observed coordinated and sequential waves of immune responses, with interferon-associated gene transcripts dominating by 16 hours post-infection, followed by strong increases in natural killer (NK) cell-associated and MHC class I-related transcripts by 32 hours post-infection. We showed by flow cytometry that the observed elevation in NK cell-associated transcripts was the result of a dramatic increase in the proportion of NK cells in the blood during infection. Subsequent microarray analysis of NK cells isolated from the peripheral blood of infected mice revealed a cell proliferation expression signature consistent with the observation that NK cells replicate in response to infection. Early proliferation of NK cells was directly observed in studies with adoptively transferred cells in infected mice. These data indicate that the early response to P. chabaudi infection of the blood is marked by a primary wave of interferon with a subsequent response by NK cells.


An in vivo/in vitro model of Plasmodium falciparum rosetting and autoagglutination mediated by varO, a group A var gene encoding a frequent serotype.


Institut Pasteur, Unité d'Immunologie Moléculaire des Parasites, URA CNRS 2581, F-75015 Paris, France; Institut Pasteur de la Guyane, BP 6010, 97306 Cayenne Cedex, French Guiana; Institut Pasteur, Unité d'Immunologie Structurale, CNRS URA 2185, F-75015 Paris, France; Institut Pasteur, Plate-forme de Production de Protéines recombinantes et d'Anticorps, F-75015 Paris, France; Institut Pasteur de Dakar, Unité d'Epidémiologie des Maladies Infectieuses, BP 220, Dakar Sénégal.

In the Saimiri sciureus monkey, erythrocytes infected with the varO antigenic
variant of the Plasmodium falciparum Palo Alto 89F5 clone bind uninfected red blood cells ("rosetting"), form autoagglutinates and display a high multiplication rate, three phenotypic characteristics associated with severe malaria in human patients. We report here that varO parasites express a var gene presenting the characteristics of group A var genes, and show that the varO-DBL1alpha domain is implicated in the rosetting of both Saimiri sciureus and human erythrocytes. The soluble varO-NTS-DBL1alpha recombinant domain, produced in the baculovirus/insect cell system, induced high titers of antibodies that reacted with varO-infected red blood cells and disrupted varO rosettes. VarO parasites were culture-adapted in vitro using human erythrocytes. They formed rosettes and autoagglutinates, displayed the same surface serotype and expressed the same varO gene as the monkey-propagated parasites. To develop an in vitro model with highly homogeneous varO parasites, rosette purification was combined with positive selection by panning on a varO-NTS-DBL1alpha-specific mouse monoclonal antibody. These single variant, clonal parasites were used to analyse seroprevalence to varO at the village level in a holoendemic setting (Dielmo, Senegal). We found 93.6% (95% CI: 89.7-96.4) seroprevalence of varO surface-reacting antibodies and 86.7% (95%CI: 82.8-91.6) seroprevalence to the recombinant NTS-DBL1alpha domain, with virtually all permanent residents having seroconverted by the age of five years. These data imply that varO represents a relevant in vivo/in vitro model of rosetting and autoagglutination for the rationale development of vaccine candidates and therapeutic strategies aimed at preventing malaria pathology.


Low prevalence of antibodies to pre-erythrocytic but not blood-stage Plasmodium falciparum antigens in an area of unstable as compared to stable malaria transmission.


University of Minnesota, Minneapolis, MN, USA; Case Western Reserve University, Cleveland, OH, USA; Kenya Medical Research Institute, Kisumu, Kenya; National Institutes of Health, Bethesda, MD, USA; Walter Reed Army Institute for Research, Silver Spring, MD, USA.

In areas where transmission of Plasmodium falciparum is high and stable, age-related acquisition of high-level IgG antibodies to pre-erythrocytic circumsporozoite protein (CSP) and liver-stage antigen-1 (LSA-1) has been associated with protection from clinical malaria. In contrast, age-related protection from malaria develops slowly or not at all in residents of epidemic-prone areas of unstable low malaria transmission. We hypothesized that this suboptimal clinical and parasitologic immunity may in part be due to reduced antibodies to CSP or LSA-1 and/or vaccine candidate blood-stage antigens. Frequencies and levels of IgG antibodies to CSP, LSA-1, thrombospondin-related adhesive protein (TRAP), apical membrane antigen-1 (AMA-1), erythrocyte binding antigen-175 (EBA-175), and merozoite surface protein-1 (MSP-1) were compared in 243 Kenyans living in a highland area of unstable transmission and 210 residents of a nearby lowland area of stable transmission. Antibody levels to CSP, LSA-1, TRAP and AMA-1 in the oldest age group (>40 years) in the unstable transmission area were lower than or similar to those of children 2-6 years old in the stable transmission area. Only 3.3% of individuals in the unstable transmission area had high-level IgG (arbitrary units >2) to both CSP and LSA-1, as compared to 43.3% of individuals in the stable transmission area. In contrast, antibody levels and frequencies to MSP-1 and EBA-175 were similar in adults in areas of stable and unstable malaria transmission. Suboptimal immunity to malaria in areas of unstable malaria transmission may relate in part to infrequent high-level antibodies to pre-erythrocytic antigens and AMA-1.
Polymorphic Variability in the Interleukin (IL)-1beta Promoter Conditions Susceptibility to Severe Malarial Anemia and Functional Changes in IL-1beta Production.

Ouma C, Davenport GC, Awandare GA, Keller CC, Were T, Otieno MF, Vulule JM, Martinson J, Ong’echa JM, Ferrell RE, Perkins DJ.

Interleukin (IL)-1beta is a cytokine released as part of the innate immune response to Plasmodium falciparum. Because the role played by IL-1beta polymorphic variability in conditioning the immunopathogenesis of severe malarial anemia (SMA) remains undefined, relationships between IL-1beta promoter variants (-31C/T and -511A/G), SMA (hemoglobin [Hb] level <6.0 g/dL), and circulating IL-1beta levels were investigated in children with parasitemia ([Formula: see text]) from western Kenya. The IL-1beta promoter haplotype -31C/-511A (CA) was associated with increased risk of SMA (Hb level <6.0 g/dL; odds ratio [OR], 1.98 [95% confidence interval (CI), 1.55-2.27]; [Formula: see text]) and reduced circulating IL-1beta levels ([Formula: see text]). The TA (-31T/-511A) haplotype was nonsignificantly associated with protection against SMA (OR, 0.52 [95% CI, 0.18-1.16]; [Formula: see text]) and elevated IL-1beta production ([Formula: see text]). Compared with the non-SMA group, children with SMA had significantly lower IL-1beta levels and nonsignificant elevations in both IL-1 receptor antagonist (IL-1Ra) and the ratio of IL-1Ra to IL-1beta. The results presented demonstrate that variation in IL-1beta promoter conditions susceptibility to SMA and functional changes in circulating IL-1beta levels.

Amplification of pvmdr1 Associated with Multidrug-Resistant Plasmodium vivax.


Background. Multidrug-resistant strains of Plasmodium vivax are emerging in Southeast Asia. Methods. In vitro drug susceptibility and pvmdr1 genotype were determined in P. vivax field isolates from Indonesia and Thailand. Results. Increased pvmdr1 copy number was present in 21% of isolates from
Thailand (15/71) and none from Indonesia (0/114; [Formula: see text]). Compared with Indonesian isolates, the median IC(50) of Thai isolates was lower for chloroquine (36 vs. 114 nmol/L; [Formula: see text]) but higher for amodiaquine (34 vs. 13.7 nmol/L; [Formula: see text]), artesunate (8.33 vs. 1.58 nmol/L; [Formula: see text]), and mefloquine (111 vs. 9.87 nmol/L; [Formula: see text]). In 11 cryopreserved Thai isolates, those with increased pvmdr1 copy number had a higher IC(50) for mefloquine (78.6 vs. 38 nmol/L for single-copy isolates; [Formula: see text]). Compared with isolates with the wild-type allele, the Y976F mutation of pvmdr1 was associated with reduced susceptibility to chloroquine (154 nmol/L [range, 4.6-3505] vs. 34 nmol/L [range, 6.7-149]; [Formula: see text]) but greater susceptibility to artesunate (1.8 vs. 9.5 nmol/L; [Formula: see text]) and mefloquine (14 vs. 121 nmol/L; [Formula: see text]). Conclusions. Amplification of pvmdr1 and single-nucleotide polymorphisms are correlated with susceptibility of P. vivax to multiple antimalarial drugs. Chloroquine and mefloquine appear to exert competitive evolutionary pressure on pvmdr1, similar to that observed with pfmdr1 in Plasmodium falciparum.


Complexity of Plasmodium falciparum Clinical Samples from Uganda during Short-Term Culture.


Departments of Medicine and Microbiology, Makerere University, Kampala, Uganda; Department of Medicine, University of California, San Francisco.

We cultured Plasmodium falciparum parasites from 98 Ugandan children with malaria and determined the complexity of infection (COI) on the basis of msp-2 polymorphisms daily for 9 days. The mean COI decreased during culture from 1.73 to 1.56. New strains appeared after day 0 in 20 cultures. Strains disappeared after day 0 in 56% of 45 cultures that were initially mixed; persisting strains more commonly had wild-type dhfr (C59) and dhps (K540) sequences and mutant pfmdr1 (86Y) sequences. Thus, initial genotypes offer an imperfect representation of clinical COI. Loss of strains in culture may be due to diminished fitness of some drug-resistant strains.


Operational impact of DDT reintroduction for malaria control on Anopheles arabiensis in Mozambique.

Coleman M, Casimiro S, Hemingway J, Sharp B.

Malaria Research Programme, Medical Research Council, Ridge Rd., Durban, South Africa. mcoleman@liverpool.ac.uk

ABSTRACT With the increase in indoor residual spraying in many internationally and nationally funded malaria control programs, and affirmation by World Health Organization (WHO) that DDT is appropriate for use in the absence of longer lasting insecticide formulations in some malaria endemic settings, DDT has been reintroduced as a major malaria control intervention in Africa. Indoor residual spraying with DDT was reintroduced into Mozambique for malaria control in 2005, and it is increasingly becoming the main insecticide used for malaria vector control in Mozambique. The selection of DDT in Mozambique is evidence-based, taking account of the susceptibility of Anopheles arabiensis (Patton) and Anopheles gambiae (Giles) s.s. to all the available insecticide choices, as well as relative costs of the insecticide and the logistical costs of spraying. Before this time in Mozambique, DDT was replaced by h-cyhalothrin in 1993. Resistance occurred quickly to this insecticide, and in 2000 the pyrethroid was phased out and the carbamate bendiocarb was introduced. Low-level resistance was detected by

Environmental Health at USAID – Malaria Bulletin, October 2008
biochemical assay to bendiocarb in 1999 in both Anopheles funestus (Giles) and An. arabiensis, although this was not evident in WHO bioassays of the same population. In the 2000-2006 surveys the levels of bendiocarb resistance had been selected to a higher level in An. arabiensis, with resistance detectable by both biochemical and WHO bioassay. The insecticide resistance monitoring program includes assessment of field populations by standard WHO insecticide susceptibility assays and biochemical assays. Monitoring was established in 1999, and it was maintained as part of an operational monitoring and evaluation program thereafter.


Potential distribution of two species in the medically important Anopheles minimus complex (Diptera: Culicidae).

Foley DH, Rueda LM, Peterson AT, Wilkerson RC.

Department of Entomology, Walter Reed Army Institute of Research, 503 Robert Grant Ave., Silver Spring, MD 20910, USA. foleydes@si.edu

Anopheles minimus Theobald (=An. minimus A) and possibly Anopheles harrisoni Harbach & Manguin (=An. minimus C) are important malaria vector species in the Minimus Complex in Southeast Asia. The distributions of these species are poorly known, although detailed information could benefit malaria vector incrimination and control. We used published collection records of these species and environmental geospatial data to construct consensus ecological niche models (ENM) of each species' potential geographic distribution. The status of the Indian taxon An. fluviatilis S as a species distinct from An. harrisoni has been debated in the literature, so we tested for differentiation in ecological niche characteristics. The predicted potential distribution of An. minimus is more southerly than that of An. harrisoni: Southeast Asia is predicted to be more suitable for An. minimus, and China and India are predicted more suitable for An. harrisoni, so An. harrisoni seems to dominate under cooler conditions. The distribution of An. minimus is more continuous than that of An. harrisoni: disjunction in the potential distribution of the latter is suggested between India and Southeast Asia Anopheles fluviatilis S occurrences are predicted within the An. harrisoni ecological potential, so we do not document ecological differentiation that might reject conspecificity. Overall, model predictions offer a synthetic view of the distribution of this species complex across the landscapes of southern and eastern Asia.


Species composition and distribution of adult Anopheles (Diptera: Culicidae) in Panama.

Loaiza JR, Bermingham E, Scott ME, Rovira JR, Conn JE.

Department of Natural Resource Sciences, 21,111 Lakeshore Road, ste. Anne de Bellevue, Quebec, Canada. Jose.loaiza@mail.mcgill.ca

Anopheles (Diptera: Culicidae) species composition and distribution were studied using human landing catch data over a 35-yr period in Panama. Mosquitoes were collected from 77 sites during 228 field trips carried out by members of the National Malaria Eradication Service. Fourteen Anopheles species were identified. The highest average human biting rates were recorded from Anopheles (Nyssorhynchus) albimanus (Wiedemann) (9.8 bites/person/night) and Anopheles (Anopheles) punctimacula (Dyar and Knab) (6.2 bites/person/night). These two species were also the most common, present in 99.1 and 74.9%, respectively, of the sites. Anopheles (Nyssorhynchus) aquasalis (Curry) was encountered mostly in the indigenous Kuna Yala Comarca along the eastern Atlantic coast, where malaria
case history and average human biting rate (9.3 bites/person/night) suggest a local role in malaria transmission. An. albimanus, An. punctimacula, and Anopheles (Anopheles) vestitipennis (Dyar and Knab) were more abundant during the rainy season (May-December), whereas An. aquasalis was more abundant in the dry season (January-April). Other vector species collected in this study were Anopheles (Kerteszia) neivai (Howard, Dyar, and Knab) and Anopheles (Anopheles) pseudopunctipennis s.l. (Theobald). High diversity of Anopheles species and six confirmed malaria vectors in endemic areas of Panama emphasize the need for more detailed studies to better understand malaria transmission dynamics.


**Four New Species of Plasmodium from New Guinea Lizards: Integrating Morphology and Molecules.**

Perkins SL, Austin C.

New Guinea is one of the most biodiverse regions of the world, particularly in terms of the herpetofauna present, yet surprisingly little is known about the parasites that infect these organisms. A survey of diverse scinid and agamid lizard hosts from this country showed a diversity of malaria parasites infecting these hosts. We combined morphological and morphometric observations of the parasites (primarily gametocytes) along with DNA sequence data from the mitochondrial cytochrome b and cytochrome oxidase I genes, and here describe 4 new species of Plasmodium, i.e. Plasmodium minuoviride n. sp., Plasmodium koreafense n. sp., Plasmodium megalotrypa n. sp., and Plasmodium gemini n. sp. A fifth species, Plasmodium lacertiliae Thompson and Hart 1946, is redescribed based on new observations of hosts and localities and adding molecular data. This combined morphological and molecular approach is advised for all future descriptions of new malaria parasite species, particularly in light of situations where every life history stage is not available.


**Malaria prevention practices among mothers delivering in an urban hospital in southwest Nigeria.**

Yusuf OB, Dada-Adegbola HO, Ajayi IO, Falade CO.

Department of Epidemiology, Medical Statistics & Environmental Health, University of Ibadan, Ibadan, Nigeria.

BACKGROUND & OBJECTIVES: The pregnant woman is more prone to malaria than her non-pregnant counterpart with grave consequences for both mother and baby. This study aims at determining the malaria prevention practices among pregnant women in an area hyper-endemic for malaria. METHODS: For the study 983 parturient mothers were enrolled in Ibadan, southwest Nigeria. Information was collected on sociodemographic characteristics, use of malaria chemoprophylaxis, use of anti-vector measures, and malaria parasitaemia. RESULTS: Most mothers [956/972 (98.4%)] reported the use of anti-vector measures for malaria prevention. These include, window screens (78.9%), insecticides spray (69.9%), mosquito coils (25.3%), untreated bednets (2.5%), and insecticide-treated nets (1.1%). Most mothers used anti-vector measures either singly or in combination. About 86% (840/972) of the mothers used drugs for chemoprophylaxis. Thirteen (1.3%) mothers used chemoprophylaxis alone (CP), 135 (13.9%) used anti-vector measures alone (AV) while 820 (84.4%) used chemoprophylaxis plus anti-vector (CPAV). Weekly dose of pyrimethamine [214 (25%)] and intermittent preventive treatment with sulphadoxine-pyrimethamine [598 (71.2%)] were the widely used chemoprophylactic drugs. The prevalence of patent parasitaemia at delivery was 7.7% (1/13), 12.1% (99/820) and 16.3% (22/135) among CP, CPAV and AV groups respectively. Geometric mean parasite densities among the respective groups were 7840/microl, 1228/microl
and 8936/microl. CONCLUSION: Window screens and insecticide sprays were widely used for malaria prevention while the use of ITN was very low among enrolled mothers. There is a need to pay concerted efforts to improve ITN usage rate in Nigeria.


Development of a behaviour change communication strategy for a vaccination-linked malaria control tool in southern Tanzania.


ABSTRACT: BACKGROUND: Intermittent preventive treatment of malaria in infants (IPTi) using sulphadoxine-pyrimethamine and linked to the expanded programme on immunization (EPI) is a promising strategy for malaria control in young children. As evidence grows on the efficacy of IPTi as public health strategy, information is needed so that this novel control tool can be put into practice promptly, once a policy recommendation is made to implement it. This paper describes the development of a behaviour change communication strategy to support implementation of IPTi by the routine health services in southern Tanzania, in the context of a five-year research programme evaluating the community effectiveness of IPTi. METHODS: Mixed methods including a rapid qualitative assessment and quantitative health facility survey were used to investigate communities' and providers' knowledge and practices relating to malaria, EPI, sulphadoxine-pyrimethamine and existing health posters. Results were applied to develop an appropriate behaviour change communication strategy for IPTi involving personal communication between mothers and health staff, supported by a brand name and two posters. RESULTS: Malaria in young children was considered to be a nuisance because it causes sleepless nights. Vaccination services were well accepted and their use was considered the mother's responsibility. Babies were generally taken for vaccination despite complaints about fevers and swellings after the injections. Sulphadoxine-pyrimethamine was widely used for malaria treatment and intermittent preventive treatment of malaria in pregnancy, despite widespread rumours of adverse reactions based on hearsay and newspaper reports. Almost all health providers said that they or their spouse were ready to take SP in pregnancy (96%, 223/242). A brand name, key messages and images were developed and pre-tested as behaviour change communication materials. The posters contained public health messages, which explained the intervention itself, how and when children receive it and safety issues. Implementation of IPTi started in January 2005 and evaluation is ongoing. CONCLUSIONS: Behaviour Change Communication (BCC) strategies for health interventions must be both culturally appropriate and technically sound. A mixed methods approach can facilitate an interactive process among relevant actors to develop a BCC strategy.


Effectiveness of artemisinin-based combination therapy used in the context of home management of malaria: A report from three study sites in sub-Saharan Africa.


ABSTRACT: BACKGROUND: The use of artemisinin-based combination therapy (ACT) at the community level has been advocated as a means to increase access to effective antimalarial medicines by high risk groups living in underserved areas, mainly in sub-Saharan Africa. This strategy has been shown to be feasible and acceptable to the community. However, the parasitological effectiveness of ACT when dispensed by community medicine distributors (CMDs) within the context of home management of malaria (HMM) and used unsupervised by caregivers at home has not been

Environmental Health at USAID – Malaria Bulletin, October 2008
evaluated. METHODS: In a sub-set of villages participating in a large-scale study on feasibility and acceptability of ACT use in areas of high malaria transmission in Ghana, Nigeria and Uganda, thick blood smears and blood spotted filter paper were prepared from finger prick blood samples collected from febrile children between six and 59 months of age reporting to trained CMDs for microscopy and PCR analysis. Presumptive antimalarial treatment with ACT (artesunate-amodiaquine in Ghana, artemether-lumefantrine in Nigeria and Uganda) was then initiated. Repeat finger prick blood samples were obtained 28 days later for children who were parasitaemic at baseline. For children who were parasitaemic at follow-up, PCR analyses were undertaken to distinguish recrudescence from re-infection. The extent to which ACTs had been correctly administered was assessed through separate household interviews with caregivers having had a child with fever in the previous two weeks. RESULTS: Over a period of 12 months, a total of 1,740 children presenting with fever were enrolled across the study sites. Patent parasitaemia at baseline was present in 1,189 children (68.3%) and varied from 60.1% in Uganda to 71.1% in Ghana. A total of 606 children (51% of infected children) reported for a repeat test 28 days after treatment. The crude parasitological failure rate varied from 3.7% in Uganda (C.I. 1.2%-6.2%) to 41.8% in Nigeria (C.I. 35%-49%). The PCR adjusted parasitological cure rate was greater than 90% in all sites, varying from 90.9% in Nigeria (C.I. 86%-95%) to 97.2% in Uganda (C.I. 95%-99%). Reported adherence to correct treatment in terms of dose and duration varied from 81% in Uganda (C.I. 67%-95%) to 97% in Ghana (C.I. 95%-99%) with an average of 94% (C.I. 91%-97%). CONCLUSION: While follow-up rates were low, this study provides encouraging data on parasitological outcomes of children treated with ACT in the context of HMM and adds to the evidence base for HMM as a public health strategy as well as for scaling-up implementation of HMM with ACTs.


The Ross-Macdonald model in a patchy environment.

Auger P, Kouokam E, Sallet G, Tchuente M, Tsanou B.

IRD, UR GEODES, 32 Avenue Henri Varagnat, 3143 Bondy Cedex, France.

We generalize to n patches the Ross-Macdonald model which describes the dynamics of malaria. We incorporate in our model the fact that some patches can be vector free. We assume that the hosts can migrate between patches, but not the vectors. The susceptible and infectious individuals have the same dispersal rate. We compute the basic reproduction ratio R(0). We prove that if R(0)<1, then the disease-free equilibrium is globally asymptotically stable. When R(0)>1, we prove that there exists a unique endemic equilibrium, which is globally asymptotically stable on the biological domain minus the disease-free equilibrium.


Synthesis and antimalarial activity of novel side chain modified antimalarial agents derived from 4-aminoquinoline.

Solomon VR, Haq W, Smilkstein M, Srivastava K, Rajakumar S, Puri SK, Katti SB.

Division of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226 001, India.

Malaria is one of the foremost public health problems in developing countries affecting nearly 40% of the global population. Apart from this, the past two decade's emergence of drug resistance has severely limited the choice of available antimalarial drugs. Furthermore, the general trend emerging from the SAR-studies is that chloroquine resistance does not involve any change to the target of this class of drugs but involves compound specific efflux mechanism.
Based on this premise a number of groups have developed short chain analogues of 4-aminoquinoline, which are active against CQ-resistant strains of P. falciparum in vitro studies. However, these derivatives undergo biotransformation (de-alkylation) significantly affecting lipid solubility of the drug. In view of this background information, we thought that it would be interesting to study the effect of additional lipophilicity and cationic charge at the lateral side chain of 4-aminoquinoline. This prompted us to explore the cationic amino acid conjugates namely, lysine and ornithine of 4-aminoquinoline with a view to achieve improved antimalarial activity and to the best of our knowledge such amino acid conjugates have not been hitherto reported in the literature in the case of 4-aminoquinolines. In the present study, a new series of side-chain modified 4-aminoquinolines have been synthesized and found active against both susceptible and multidrug resistant strains of P. falciparum in vitro and P. yoelli in vivo. The seminal finding of the present study is that a new series of compounds having significantly more activity against CQ resistant parasites has been identified.


Dihydroethanoanthracene derivatives reverse in vitro quinoline resistance in Plasmodium falciparum malaria.


Unité de Recherche en Biologie et Epidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Boulevard Charles Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France.

The capacity of ten molecules for reversing resistance in Plasmodium falciparum in vitro to quinoline antimalarial drugs, such as chloroquine (CQ), quinine (QN), mefloquine (MQ) and monodesethylamodiaquine (MDAQ), was assessed against 27 Plasmodium falciparum isolates. Four of these compounds were 9,10-dihydroethanoanthracene derivatives (DEAs). These DEAs reversed 75 to 92% of the CQ resistant strains. These synthetic compounds were more effective in combination with CQ than verapamil, ketotifen, chlorpromazine, reserpine or nicardipine, which reversed less than 50% of the CQ resistant strains. DEAs significantly reversed 67 to 100% of the MDAQ resistant parasites. These compounds were more effective in combination with MDAQ than ketotifen (60% of reversal), chlorpromazine (45%), verapamil (33%), reserpine (30%) or nicardipine (9%). The reversal activity of MQ resistance was less pronounced, regardless of the molecule tested, and was homogeneous with a rate ranging from 42% for ketotifen to 58% for reserpine, nicardipine, verapamil and cyproheptadine. The four DEAs significantly reversed 50 to 55% of the parasites resistant to MQ. Fifty-six to 78 % of the QN resistant parasites were reversed by the synthetic DEAs. There were few differences in the rate of reversal activity on QN resistant strains between the ten compounds, with rates ranging between 56 to 78% for the ten chemosensitizers. The use of DEAs in combination with quinoline seems to be thus a promising strategy for limiting the development of drug resistant strains and for treating patients in drug resistant areas.


[Anopheles mosquitoes (Diptera, Culicidae) of the Tien Shan: morphological, cytogenetic, and molecular genetic analysis] [Article in Russian]

An. artemievi, An. claviger, An. hyrcanus, An. messeae, and An. superpictus were detected in the Western Tien Shan. An. artemievi was first recorded in Kazakhstan. An. artemievi, An. claviger, and An. superpictus were noted in the Inferior Tien Shan. An. messeae was first observed in the Issyk Kul hollow. An. artemievi, An. claviger, and An. superpictus were habitants of the foothills of the South-Western Tien Shan. An. artemievi, An. hyrcanus, An. superpictus, and An. pulcherrimus were in the plain. An. pulcherrimus and An. superpicts mosquitoes are regarded as important vectors in the new malaria foci of the Fergana regions. The role of An. artemievi in the transmission of malaria is to be specified.


[Malaria mosquitoes of the Anopheles maculipennis (Diptera, Culicidae) complex in Georgia] [Article in Russian]

[No authors listed]

Malaria mosquito larvae and imagoes underwent morphological, cytogenetic, and molecular genetic analyses in West and East Georgia. In the areas under study, the authors identified three related types of malaria mosquitoes of the maculipennis complex: An. maculipennis Meigen, 1818; An. melanoon Hacket, 1934; An. sacharovi Favre, 1903. The authors revealed the types An. maculipennis and An. melanoon in the Kolchida cavity (West Georgia) and An. maculipennis and An. sacharovi in the Iveria cavity (East Georgia). The morphology of eggs was defined in the study types of mosquitoes. An. melanoon ovipositions similar in the exochorion pattern with An. messeae eggs were found in West Georgia.


Inflammatory pathways in malaria infection: TLRs share the stage with other components of innate immunity.

Erdman LK, Finney CA, Liles WC, Kain KC.

McLaughlin-Rotman Centre for Global Health, McLaughlin Centre for Molecular Medicine, University of Toronto, Toronto, Ontario, Canada.

Severe forms of malaria infection claim over 1 million lives annually. One aspect of severe malaria pathogenesis is an excessive or dysregulated inflammatory response to infection. With the characterization of Toll-like receptors (TLRs), which initiate inflammation upon detection of microbial products, involvement of TLRs in the host response to malaria has undergone intense investigation. While TLRs appear to mediate inflammation in malaria infection and may contribute to development of severe malaria, it is unlikely that they operate in isolation from other components of innate immunity. Here, we highlight recent findings implicating other innate immune mechanisms in the host inflammatory response to malaria, propose how they may integrate and synergize with TLR pathways, and discuss opportunities and challenges associated with anti-inflammatory adjunctive therapy for the treatment of severe malaria.


Diagnosis of malaria: challenges for clinicians in endemic and non-endemic regions.

Bronzan RN, McMorrow ML, Kachur SP.

United States Public Health Service, Malaria Branch, Division of Parasitic Diseases, National Center for Zoonotic Vector-Borne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.
Malaria is a leading cause of morbidity and mortality worldwide. Prompt diagnosis and treatment are critical factors in reducing morbidity and mortality, as delayed treatment of malaria increases the risk of death. Microscopy has long been the standard of malaria diagnosis, but newer diagnostic tests now offer advantages in certain settings. Malaria diagnosis is complicated by the fact that acquired immunity to malaria can result in asymptomatic infections. In a symptomatic (febrile) patient, no existing malaria diagnostic test can distinguish malarial illness from parasitemia with concomitant fever of another cause. In this review we discuss the available malaria diagnostic tests, appropriate applications for each, and the challenges of malaria diagnosis in both endemic and non-endemic settings.


Reverse genetics screen identifies six proteins important for malaria development in the mosquito.

Ecker A, Bushell ES, Tewari R, Sinden RE.

Division of Cell and Molecular Biology, Imperial College London, London SW7 2AZ, UK.

Transmission from the vertebrate host to the mosquito vector represents a major population bottleneck in the malaria life cycle that can successfully be targeted by intervention strategies. However, to-date only about 25 parasite proteins expressed during this critical phase have been functionally analysed by gene disruption. We describe the first systematic, larger scale generation and phenotypic analysis of Plasmodium berghei knockout (KO) lines, characterizing 20 genes encoding putatively secreted proteins expressed by the ookinete, the parasite stage responsible for invasion of the mosquito midgut. Of 12 KO lines that were generated, six showed significant reductions in parasite numbers during development in the mosquito, resulting in a block in transmission of five KOs. While expression data, time-point of essential function and mutant phenotype correlate well in three KOs defective in midgut invasion, in three KOs that fail at sporulation, maternal inheritance of the mutant phenotype suggests that essential function occurs during ookinete formation and thus precedes morphological abnormalities by several days.


Knowledge, use and promotion of insecticide treated nets by health workers in a suburban town in south western Nigeria.

Iyaniwura CA, Ariba A, Runshewe-Abiodun T.

Department of Community Health and Primary Care, Olabisi Onabanjo University Teaching Hospital, Sagamu. christywura@yahoo.com

BACKGROUND: Morbidity and mortality associated with malaria can be significantly reduced by widespread use of insecticide treated nets. Health workers can increase acceptability of ITN by promoting its use and serving as role model.

OBJECTIVE: To assess the knowledge, use and promotion of insecticide treated bed-net by health workers.

METHOD: This descriptive, cross sectional study was carried out among health care workers in Sagamu (Ogun State) between November 2004 and January 2005. Data was collected from 263 health workers using a pretested, structured questionnaire.

RESULT: Two hundred and forty six (93.5%) were aware of insecticide treated bednets (ITN) but many did not have adequate knowledge about it, only 52 (20.9%) knew that ITN should be retreated every 6 months. Sixty (22.8%) were currently using ITN. In the homes where they were currently using ITN, children were the main users (59%). The major reasons given
for not using an ITN were that it had not occurred to them (23.2%), 13.3% were satisfied with the method they were using and 12.1% felt it was not convenient to use. Less than one-third (32.3%) indicated that ITN was available in their health facility. Fifty-seven percent (56.7%) had recommended it for patients before. The main reasons given by those who had not recommended it before were: lack of knowledge about it (52.5%), while 20% indicated that they were not familiar with it. CONCLUSION: Awareness about ITN is high among the health workers but the knowledge about it is inadequate. The major challenges to use and promotion of ITN by health workers are lack of conviction about the unique benefits of ITN, inadequate knowledge and poor access to the nets.


Antioxidant status of bilirubin and uric acid in patients diagnosed with Plasmodium falciparum malaria in Douala.

Bertrand KE, Mathieu N, Inocent G, Honore FK.

Department of Molecular Biology and Biotechnology, International Centre of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya.

Oxidative stress and changes in antioxidant status have been implicated in the pathogenesis of malaria. To assess the antioxidant level of bilirubin and uric acid associated with falciparum malaria infection, 60 untreated patients (30 men and 30 women) in Douala, Cameroon were screened for the study. Sixty five healthy individuals (29 men and 36 women) were used as controls. Total and conjugated bilirubin were calculated using Jendrassik-Grof method while uric acid was determined using Barham-Trinder method. It was observed that total and conjugated bilirubins were significantly (p < 0.001) higher in malaria patients (10.722 +/- 4.043 and 3.627 +/- 1.571 mg L(-1), respectively) when compared to control (6.830 +/- 2.436 and 1.777 +/- 0.729 mg L(-1)) and these bilirubin levels increased significantly with parasite count (p < 0.050). There was also significant increased (p = 0.021) of uric acid in malaria patients (56.262 +/- 13.963 mg L(-1)) compared to controls (49.838 +/- 15.419 mg L(-1)). No significant differences based on sex were observed on uric acid, parasite count, total and conjugated bilirubins in malaria patients. Positive correlations were obtained between parasite count and total bilirubin (r = 0.320, p < 0.050), conjugated bilirubin (r = 0.477, p < 0.001), uric acid (r = 0.060, p > 0.050) and between total and conjugated bilirubin (r = 0.729, p < 0.001). From this study, it has been hypothesized that the augmentation of plasma level of bilirubin and uric acid could provide more protection against oxidative stress induced by malaria.


Incidence of human malaria infection in northern hilly region of Balochistan, adjoining with NWFP, Pakistan: district Zhob.

Yasinzai MI, Kakarsulemankhel JK. Department of Zoology, University of Balochistan, Quetta, Pakistan.

This study was conducted to investigate the incidence of malarial infections in human population in 37 localities of district Zhob, Balochistan, Pakistan. Malarial parasites were identified in the blood slides of suspected patients of the disease from July, 2004 to June, 2006 and encompassed 7748 subjects. Out of 7748 suspected cases of malaria, 3240 (41.8%) were found to be positive for malarial parasite in blood smear slides. Out of positive cases, 1681 (51.8%) were identified as Plasmodium vivax infection and 1559 (48.1%) cases with P. falciparum. However, seasonal variation was also noted with the highest (85.4%: 141/165) infection of P. vivax in March and lowest (18.6%: 59/316) in October while infection of P. falciparum was highest (81.3%: 257/316) in October and lowest (14.5%: 24/165) in March. Infection with P. vivax in male was 75.7%
In March and in female 26.3% (58/220) in May whereas infection of P. falciparum in male was 61.5% (245/398) in July and in female was 20.5% (65/316) in October. These results are compared with those of other studies done in Pakistan. Cases of P. malariae and P. ovale were not found in the present study. In conclusion it can be pointed out that the high incidence rate of P. vivax (51.8%:1681/3240) in Zhob district poses a significant health hazard because it may also lead to cerebral malaria as it was suggested by previous workers.

Towards a vaccine against pregnancy-associated malaria.

Tuikue Ndam N, Deloron P.

Institut de Recherche pour le Développement, UR010, Laboratoire de Parasitologie, Université Paris Descartes, IFR 71, 4, avenue de l’Observatoire, 75006 Paris, France. Nicaise.Ndam@ird.fr

The consequences of pregnancy-associated malaria on pregnant women (anaemia), their babies (birth weight reduction), and infants (increased morbidity and mortality) are well documented. Field observations during the last decade have underlined the key role of the interactions between P. falciparum variable surface antigens expressed on infected erythrocytes and a novel receptor: chondroitin sulfate A (CSA) for the placental sequestration of infected erythrocytes. Identification of a distinct P. falciparum erythrocyte membrane protein 1 (PfEMP1) variant, VAR2CSA, as the dominant variant surface antigen and as a clinically important target for protective immune response to pregnancy-associated malaria has raised hope for developing a new preventive strategy based on inducing these immune responses by vaccination. However, despite particular structure and interclonal conservation of VAR2CSA among other PfEMP1, significant challenges still exist concerning the development of a VAR2CSA-based vaccine with profound efficacy.

PCR-based methods to the diagnosis of imported malaria.

Berry A, Benoit-Vical F, Fabre R, Cassaing S, Magnaval JF.

Service de Parasitologie-Mycologie, Hôpital Rangueil, Centre Hospitalier Universitaire de Toulouse, TSA 50032, 31059 Toulouse 9, France. berry.a@chu-toulouse.fr

Rapid and precise diagnosis of malaria is needed to take care febrile patient returning from endemic areas. Since the first description of the diagnosis of Plasmodium infection by polymerase-chain-reaction (PCR), the role of this kind of molecular method in the laboratory diagnosis of imported malaria is still a topical question. PCR-based assays were found to be more sensitive and more specific than all conventional methods. The highest contribution of the molecular diagnosis is that a PCR negative result would ascertain the lack of any malaria infection, thus quickly orienting the investigations toward other aetiology. This technique should be now considered as the gold standard for the diagnosis of imported malaria.

Protective immunity against malaria liver stage after vaccination with live parasites.

Rénia L.
Despite nearly 100 years of research and control efforts, malaria remains one of the most important infectious diseases. An efficient vaccine would be a powerful tool to reduce mortality and morbidity. Experimentally, induction of sterile immunity in humans after vaccination with attenuated sporozoites has been obtained. This observation has spurred the search for subunit vaccines that aim to reproduce this protection. As yet none of the current candidate subunit vaccines achieved complete protection reproducibly. This failure coupled to the recent advent of genetically modified Plasmodium parasites has led to a renewed interest in the use of live parasites for vaccination against malaria pre-erythrocytic stages. In this article, we review and discuss the recent developments in this field.


Discovery of new targets for antimalarial chemotherapy.

Grellier P, Depoix D, Schrével J, Florent I.

National Museum of Natural History, USM504-EA3335, Functional biology of protozoa, Department RDDM, CP 52, 61, rue Buffon, F-75231 Paris Cedex 05, France.
grellier@mnhn.fr

The understanding of the biology and the biochemistry of malaria parasites has considerably increased over the past two decades with the discovery of many potential targets for new antimalarial drugs. The decrypted genomes of several Plasmodium species and the new post-genomic tools further enriched our "reservoir" of targets and increased our ability to validate potential drug targets or to study the entire parasite metabolism. This review discusses targets involved in calcium metabolism, protein prenylation and apicoplast functions that have emerged by different approaches.


Protective immunity induced by daily bites from irradiated mosquitoes infected with Plasmodium yoelii.

Wong KA, Zhou A, Rodriguez A.

Department of Medical Parasitology, New York University School of Medicine, New York, NY 10010, USA.

Individuals in malaria endemic regions do not develop fully protective immune responses against Plasmodium liver stage infections. In high transmission areas, individuals can be exposed to more than two infective mosquito bites daily. Their exposure to Plasmodium sporozoites, therefore, is in the form of small and frequent doses. This is very different from individuals studied in controlled immunization trials where the delivery of large numbers of radiation-attenuated sporozoites in a limited number of doses can induce sterile protective immunity. Using irradiated mosquitoes infected with Plasmodium yoelii 17XNL, we tested whether daily bites from a few mosquitoes can induce a protective immune response in mice. This immunization strategy successfully induced a protective response, preventing the development of liver stages when mice were challenged with nonirradiated sporozoites. These results provide further support for the development of liver stage vaccines. They are also a call for further study into why fully protective responses against the liver stage are not seen in individuals from endemic regions.

Treatment and control of mycoplasma contamination in Plasmodium falciparum culture.

Singh S, Puri SK, Srivastava K.

Division of Parasitology, Central Drug Research Institute, Lucknow, India.

A comparative efficacy of four antibiotics, plasmocin (macrolid), Biomyc-1, -2, (tetracycline), and Biomyc-3, and Mycoplasma Removing Agent (quinolone derivatives) was determined for elimination of mycoplasma from Plasmodium falciparum culture. Presence of mycoplasma was detected using enzyme-PCR-based mycoplasma detection kit and survival of malaria parasite was determined in Giemsa's stained smear made from treated and untreated cultures. It was observed that a combination of Biomyc-1 and -2 killed malaria parasites within 24 h, whereas plasmocin and Biomyc-3 caused slow death of malaria parasite stretched over a period of 6 days. The only compound which did not kill malaria parasite and eradicated mycoplasma from P. falciparum culture was observed to be MRA.

50: Parasitol Res. 2008 Sep 16.

Mediation of oviposition responses in the malaria mosquito Anopheles stephensi Liston by certain fatty acid esters.


Defence Research & Development Establishment, Jhansi Road, Gwalior, 474 002, India.

The chemical factors involved in oviposition site selection by mosquitoes have become the focus of interest in recent years, and considerable attention is paid to the chemical cues influencing mosquito oviposition. Studies on synthetic oviposition attractants/repellents of long-chain fatty acid esters against Anopheles stephensi are limited. Screening and identification of chemicals which potentially attract/repel the gravid females to/or from oviposition site could be exploited for eco-friendly mosquito management strategies. The ester compounds demonstrated their ability to repel and attract the gravid A. stephensi females in the treated substrates. Significant level of concentration-dependent negative oviposition response of mosquitoes to octadecyl propanoate, heptadecyl butanoate, hexadecyl pentanoate, and tetradeccyl heptanoate were observed. In contrast, decyl undecanoate, nonyl dodecanoate, pentyl hexadecanoate, and propyl octadecanoate elicited concentration-dependent positive oviposition responses from the gravid mosquitoes. Forcing a female to retain her eggs due to unavailability of a suitable oviposition site and attracting them to lay the eggs in a baited ovitraps shall ensure effective control of mosquito breeding and population buildup because the oviposition bioassay target the most susceptible stage of an insect life cycle. Treating relatively smaller natural breeding sites with an effective repellent and placing ovitraps containing an attractant in combination with insect-growth regulator (IGR)/insecticide would be a promising method of mosquito management.


Genetic characterization, distribution and prevalence of avian pox and avian malaria in the Berthelot's pipit (Anthus berthelotii) in Macaronesia.

Illera JC, Emerson BC, Richardson DS.

Island Ecology and Evolution Research Group, IPNA, CSIC, C/ Astrofisico Francisco Sánchez, 3, E-38206, La Laguna, Tenerife, Canary Islands, Spain,
Exotic pathogens have been implicated in the decline and extinction of various native-island-bird species. Despite the fact that there is increasing concern about the introduction of diseases in island ecosystems, little is known about parasites in the islands of Macaronesia. We focus on Berthelot's pipit (Anthus berthelotii), an endemic and widespread Macaronesian bird species, using a combination of field studies and molecular techniques to determine: (1) the range and prevalence of avian pox and malaria in Berthelot's pipits throughout the species' distribution, (2) the genetic characterization of both parasites in order to ascertain the level of host specificity. We sampled 447 pipits across the 12 islands inhabited by this species. Overall, 8% of all individuals showed evidence of pox lesions and 16% were infected with avian malaria, respectively. We observed marked differences in the prevalence of parasites among islands both within and between archipelagos. Avian pox prevalence varied between 0-54% within and between archipelagos and avian malaria prevalence varied between 0-64% within and between archipelagos. The diversity of pathogens detected was low: only two genetic lineages of avian malaria and one lineage of avian pox were found to infect the pipit throughout its range. Interestingly, both avian malaria parasites found were Plasmodium spp. that had not been previously reported in the Macaronesian avifauna (but that had been observed in the lesser kestrel Falco naumanni), while the avian pox was a host specific lineage that had previously been reported on two of the Canary Islands.


**Intracellular calcium levels in the Plasmodium berghei ookinete.**

Sidén-Kiamos I, Louis C.

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, N. Plastiras 110, Vassilika Vouton, 700 13 Heraklion, Crete, Greece.

SUMMARY: Ookinetes are the motile and invasive stages of Plasmodium parasites in the mosquito host. Here we explore the role of intracellular Ca2+ in ookinete survival and motility as well as in the formation of oocysts in vitro in the rodent malaria parasite Plasmodium berghei. Treatment with the Ca2+ ionophore A23187 induced death of the parasite, an effect that could be prevented if the ookinetes were co-incubated with insect cells before incubation with the ionophore. Treatment with the intracellular calcium chelator BAPTA/AM resulted in increased formation of oocysts in vitro. Calcium imaging in the ookinete using fluorescent calcium indicators revealed that the purified ookinetes have an intracellular calcium concentration in the range of 100 nm. Intracellular calcium levels decreased substantially when the ookinetes were incubated with insect cells and their motility was concomitantly increased. Our results suggest a pleiotropic role for intracellular calcium in the ookinete.


**Inferring malaria parasite population structure from serological networks.**

Buckee CO, Bull PC, Gupta S.

Department of Zoology, University of Oxford, Tinbergen Building, South Parks Road, Oxford OX1 3PS, UK Wellcome Collaborative Research Program, KEMRI, Kilifi 80108, Kenya Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501, USA.

The malaria parasite Plasmodium falciparum is characterized by high levels of genetic diversity at antigenic loci involved in virulence and immune evasion. Knowledge of the population structure and dynamics of these genes is important...
for designing control programmes and understanding the acquisition of immunity to malaria; however, high rates of homologous and non-homologous recombination as well as complex patterns of expression within hosts have hindered attempts to elucidate these structures experimentally. Here, we analyse serological data from Kenya using a novel network technique to deconstruct the relationships between patients' immune responses to different parasite isolates. We show that particular population structures and expression patterns produce distinctive signatures within serological networks of parasite recognition, which can be used to discriminate between competing hypotheses regarding the organization of these genes. Our analysis suggests that different levels of immune selection occur within different groups of the same multigene family leading to mixed population structures.


An age-structured model to evaluate the potential of novel malaria-control interventions: a case study of fungal biopesticide sprays.

Hancock PA, Thomas MB, Godfray HC.

NERC Centre for Population Biology, Imperial College London, Silwood Park Campus, Ascot, Berks SL5 7PY, UK.

It has recently been proposed that mosquito vectors of human diseases, particularly malaria, may be controlled by spraying with fungal biopesticides that increase the rate of adult mortality. Though fungal pathogens do not cause instantaneous mortality, they can kill mosquitoes before they are old enough to transmit disease. A model is developed (i) to explore the potential for fungal entomopathogens to reduce significantly infectious mosquito populations, (ii) to assess the relative value of the many different fungal strains that might be used, and (iii) to help guide the tactical design of vector-control programmes. The model follows the dynamics of different classes of adult mosquitoes with the risk of mortality due to the fungus being assumed to be a function of time since infection (modelled using the Weibull distribution). It is shown that substantial reductions in mosquito numbers are feasible for realistic assumptions about mosquito, fungus and malaria biology and moderate to low daily fungal infection probability. The choice of optimal fungal strain and spraying regime is shown to depend on local mosquito and malaria biology. Fungal pathogens may also influence the ability of mosquitoes to transmit malaria and such effects are shown to further reduce vectorial capacity.


Blood-stage Plasmodium infection induces CD8+ T lymphocytes to parasite-expressed antigens, largely regulated by CD8alpha+ dendritic cells.


Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia.

Although CD8(+)-positive lymphocytes do not contribute to protection against the blood stage of Plasmodium infection, there is mounting evidence that they are principal mediators of murine experimental cerebral malaria (ECM). At present, there is no direct evidence that the CD8(+)-positive T cells mediating ECM are parasite-specific or, for that matter, whether parasite-specific CD8(+)-positive T cells are generated in response to blood-stage infection. To resolve this and to define the cellular requirements for such priming, we generated transgenic P. berghei parasites expressing model T cell epitopes. This approach was necessary as MHC class I-restricted antigens to blood-stage infection have not been defined. Here, we
show that blood-stage infection leads to parasite-specific CD8(+) and CD4(+) T cell responses. Furthermore, we show that P. berghei-expressed antigens are cross-presented by the CD8α(+) subset of dendritic cells (DC), and that this induces pathogen-specific cytotoxic T lymphocytes (CTL) capable of lysing cells presenting antigens expressed by blood-stage parasites. Finally, using three different experimental approaches, we provide evidence that CTL specific for parasite-expressed antigens contribute to ECM.


**Natural selection of FLT1 alleles and their association with malaria resistance in utero.**

Muehlenbachs A, Fried M, Lachowitzer J, Mutabingwa TK, Duffy PE.

Mother-Offspring Malaria Studies Project, Seattle Biomedical Research Institute, Seattle, WA 98109, USA.

Placental malaria (PM) caused by Plasmodium falciparum contributes significantly to infant mortality in sub-Saharan Africa and is associated with pregnancy loss. We hypothesized that fetal genes that modify PM would be associated with fetal fitness. During PM, placental trophoblasts produce soluble fms-like tyrosine kinase 1 (sFlt1), also known as soluble VEGF receptor 1, an angiogenesis inhibitor associated with preeclampsia. Here we present a study examining the genotype of the fms-related tyrosine kinase 1 (FLT1) 3' UTR in Tanzanian mother-infant pairs. First-time mothers suffer the most PM, and newborn FLT1 genotype distribution differed by birth order, with newborns of first-time mothers outside of Hardy-Weinberg equilibrium (HWE) during peak PM season. Among first-time but not other mothers, maternal FLT1 genotype was associated with a history of prior pregnancy loss. During PM, newborn FLT1 genotype was associated with low birth weight and placental inflammatory gene expression. FLT1 genotype was also associated with Flt1 levels among study subjects and in vitro. Thus, FLT1 variants confer fetal fitness in utero and are associated with the maternal immune response during PM. This indicates that FLT1 is under natural selection in a malaria endemic area and that human exposure to malaria can influence the evolutionary genetics of the maternal-fetal relationship.


**Memory CD8 T cell responses exceeding a large but definable threshold provide long-term immunity to malaria.**


Department of Microbiology, University of Iowa, Iowa City, IA 52242, USA.

Infection of mice with sporozoites of Plasmodium berghei or Plasmodium yoelii has been used extensively to evaluate liver-stage protection by candidate preerythrocytic malaria vaccines. Unfortunately, repeated success of such vaccines in mice has not translated readily to effective malaria vaccines in humans. Thus, mice may be used better as models to dissect basic parameters required for immunity to Plasmodium-infection than as preclinical vaccine models. In turn, this basic information may aid in the rational design of malaria vaccines. Here, we describe a model of circumsporozoite-specific memory CD8 T cell generation that protects mice against multiple P. berghei sporozoite challenges for at least 19 months. Using this model we defined a threshold frequency of memory CD8 T cells in the blood that predicts long-term sterilizing immunity against liver-stage infection. Importantly, the number of Plasmodium-specific memory CD8 T cells required for immunity greatly exceeds the...
number required for resistance to other pathogens. In addition, this model allowed us to identify readily individual immunized mice that exceed or fall below the protective threshold before infection, information that should greatly facilitate studies to dissect basic mechanisms of protective CD8 T cell memory against liver-stage Plasmodium infection. Furthermore, the extremely large threshold in memory CD8 T cell frequencies required for long-term protection in mice may have important implications for development of effective malaria vaccines.


Performance of the OptiMAL dipstick in the diagnosis of malaria infection in pregnancy.

Tagbor H, Bruce J, Browne E, Greenwood B, Chandramohan D.

Department of Community Health, School of Medical Sciences, Kwame Nkrumah University of Science and Technology Kumasi, Ghana.

The accuracy of OptiMAL(R) dipsticks was compared with that of microscopy in the diagnosis of malaria infection in pregnancy. During the course of a clinical trial of antimalarial drugs in pregnancy, we screened 4500 pregnant women of all parities who accessed antenatal clinic services at St. Theresa's Hospital's in Nkoranza, Ghana, between March 2003 and December 2004 with OptiMAL(R) dipsticks and confirmed the diagnosis of malaria with microscopy. We determined the sensitivity, specificity, positive and negative predictive values, and the area under receiver operating characteristic (ROC) curve for the OptiMAL(R) antigen test compared to microscopy for the diagnosis of malaria infection in pregnancy. OptiMAL(R) dipsticks had a sensitivity of 96.6%, specificity of 85.4%, a positive predictive value of 92.7%, a negative predictive value of 92.6%, and an area under the ROC curve of 0.91 (95% CI of 0.90-0.92). The diagnostic accuracy of the OptiMAL(R) dipstick is high and the test may have practical use in the diagnosis of malaria infection in pregnancy in malaria endemic countries.


Intermittent preventive treatment for malaria and anaemia control in Tanzanian infants; the development and implementation of a public health strategy.


Ifakara Health Institute (IHI), P.O. Box 78373, Dar es Salaam, Tanzania.

Minimizing the time between efficacy studies and public health action is important to maximize health gains. We report the rationale, development and implementation of a district-based strategy for the implementation of intermittent preventive treatment in infants (IPTi) for malaria and anaemia control in Tanzania. From the outset, a research team worked with staff from all levels of the health system to develop a public-health strategy that could continue to function once the research team withdrew. The IPTi strategy was then implemented by routine health services to ensure that IPTi behaviour-change communication materials were available in health facilities, that health workers were trained to administer and to document doses of IPTi, that the necessary drugs were available in facilities and that systems were in place for stock management and supervision. The strategy was integrated into existing systems as far as possible and well accepted by health staff. Time-and-motion studies documented that IPTi implementation took a median of 12.4min (range 1.6-28.9) per nurse per vaccination clinic. The collaborative approach between researchers and health staff effectively translated research findings into a strategy fit for public health implementation.
Plasmodium vivax circumsporozoite variants and Duffy blood group genotypes in the Brazilian Amazon region.


University of São Paulo State Júlio Mesquita Filho, Rua Cristóvão Colombo 2265, 15054-000 São José do Rio Preto, São Paulo State, Brazil; Faculty of Medicine of São José do Rio Preto, Avenida Brigadeiro Faria Lima 5416, Vila São Pedro, São José do Rio Preto, São Paulo State, Brazil.

The circumsporozoite protein (CSP) of the Plasmodium vivax infective sporozoite is considered to be a major target for the development of recombinant malaria vaccines. The Duffy blood group molecule acts as the red blood cell receptor for P. vivax. We review the frequency of P. vivax CSP variants and report their association with the Duffy blood group genotypes from Brazilian Amazon patients carrying P. vivax malaria. Peripheral blood samples were collected from 155 P. vivax-infected individuals from five Brazilian malaria-endemic areas. The P. vivax CSP variants and the Duffy blood group genotypes were assessed using PCR/RFLP. In single infections, the VK210 variant was the commonest followed by the P. vivax-like variant. The typing of P. vivax indicated that the frequency of variants among the study areas was significantly different from one to another. This is the first detection of the VK247 and P. vivax-like variant in single infections in endemic areas of Brazil. Association of the CSP P. vivax variants with the heterozygous Duffy blood group system genotype was significant for VK210 single infection. These observations provide additional data on the Plasmodium-host interactions concerning the Duffy blood group and P. vivax capability of causing human malaria.

Dynamics of Plasmodium falciparum alleles in children with normal haemoglobin and with sickle cell trait in western Uganda.

Kiwanuka GN, Joshi H, Isharaza WK, Eschrich K.

Department of Biochemistry, Faculty of Medicine, Mbarara University of Science and Technology, P.O. Box 1410 Mbarara, Uganda.

We describe the diversity of Plasmodium falciparum populations in western Uganda and assess the role that asymptomatic malaria carriers with sickle cell trait (HbAS) may be playing on the Plasmodium population structure. We genotyped P. falciparum in 291 samples using merozoite surface protein (MSP) 1 and 2 loci. Extensive genetic diversity was detected among symptomatic children in Mbarara (20 MSP1 alleles; 31 MSP2 alleles) and Kagando, Kasese (19 MSP1 alleles; 30 MSP2 alleles). Multiplicity of infection (MOI) was significantly higher in Kagando, Kasese than in Mbarara, with 2.7 and 2.1 genotypes/PCR positive sample with MSP2 marker, respectively. Similar strains were circulating in the two sites; however, a few strains specific to individual sites were observed. Prevalence of HbAS was 36% (12/33) among asymptomatic children in Kisinga sub-county, Kasese. In asymptomatic children, MOI was age-dependent and higher in HbAS carriers than HbAA, suggesting that HbAS carriers harbour a wider range of P. falciparum genotypes. Sickle cell trait may influence rapid acquisition of premunition by creating a reservoir of variant parasite strains in the host. The high level of genetic diversity demonstrated here shows that even in areas with low or seasonal transmission, high levels of parasite polymorphism can occur.
Increasing incidence of malaria in Kurseong, Darjeeling District, West Bengal, India, 2000-2004.

Sharma PK, Ramakrishnan R, Hutin YJ, Gupte MD.

Field Epidemiology Training Programme (FETP), National Institute of Epidemiology (NIE), Indian Council of Medical Research (ICMR), Chennai, Tamil Nadu, India.

In Kurseong, Darjeeling District, India, malaria caused concern but insufficient information was available. We analysed surveillance data to estimate the burden of malaria and to examine trends. Confirmed malaria reports were reviewed and climatic records were collected. The annual parasite incidence (API; number of cases/population) and the annual blood examination rate (ABER; number of slides examined/population) were calculated to assess case detection activities, and the slide positivity rate (SPR; number of slides positive/total number examined) was calculated to assess transmission trends. The API increased from 2 to 7.8 per 1000 population between 2000 and 2004 (no deaths), with a high incidence among all age groups. Two foothill areas with forests and slow-moving streams accounted for 88% of the 697 cases in 2004. The average 2000-2004 ABER was 4.8%, below the 10% examination target of the National Anti-Malaria Programme. The proportion of Plasmodium falciparum increased from 62% in 2000 to 77% in 2004. More than 50% of P. falciparum in the area were chloroquine resistant. The SPR increased from 8.1% in 2000 to 11.9% in 2004 and peaked during monsoons. Annual rainfall increased from 2000 to 2003. Malaria transmission increased, with an increasing proportion of P. falciparum in a context of resistance to chloroquine. We recommend increasing case detection and using artemisinin-based combination therapy to treat P. falciparum malaria.

Asymptomatic Plasmodium parasitaemia in pregnant Nigerian women: almost a decade after Roll Back Malaria.

Nwagha UI, Ugwu VO, Nwagha TU, Anyaehie BU.

Department of Physiology/Obstetrics and Gynaecology, University of Nigeria, Enugu Campus, Enugu, Nigeria.

Malaria during pregnancy is a major cause of fetal and maternal morbidity and mortality. In malaria-endemic areas, the condition may remain asymptomatic but is still associated with complications. The objective of this study was to determine the prevalence of asymptomatic malaria parasitaemia and its relationship with various sociodemographic characteristics. The study was performed at three hospitals in Enugu, the centre of southeast Nigeria, during the rainy season between March 2006 and October 2007. Pregnant women attending the antenatal clinic at the index pregnancy were randomly selected and counseled, and peripheral blood samples were collected for malaria parasite and packed cell volume estimation. Age, parity, gestational age at booking, degree of anaemia and parasite density were recorded. Of 125 pregnant women tested, 73 had microscopic Plasmodium parasitaemia, giving a prevalence of 58.4%. Asymptomatic malaria parasitaemia was more common in primigravidae, in the second trimester and in the younger age group. Anaemia in pregnancy was prevalent (55.2%) and there was no significant difference in the density of parasitaemia in those with mild, moderate and severe anaemia. The prevalence of Plasmodium parasitaemia in pregnant Nigerian women is still very high nearly a decade after Roll Back Malaria. It is therefore pertinent to reappraise Roll Back Malaria strategies or to design a more effective programme for the prevention and treatment of malaria in pregnancy.
Switching *Plasmodium falciparum* genes on and off for erythrocyte invasion.

Cortés A. ICRES and Institute for Research in Biomedicine, Barcelona Science Park, Barcelona 08028, Catalonia, Spain.

Culture-adapted lines of the malaria parasite *Plasmodium falciparum* use alternative pathways for the invasion of erythrocytes. The expression of parasite ligands that are involved in the different pathways varies among parasite lines. Recently, several studies have attempted to characterize the use of different invasion pathways and the expression of specific invasion ligands in field isolates, opening the way to understand how invasion occurs in natural infections. In this review, these findings are discussed in the context of the most recent data on invasion by culture-adapted parasites to describe the current understanding of how wild parasites invade, how the variant expression of invasion ligands relates to switching between alternative invasion pathways and why so many different pathways are needed.

Malaria: some considerations regarding parasite productivity.

Rosenberg R.

Division of Vector Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, 80521, USA.

The complicated life cycle of *Plasmodium* is characterized by proliferative stages in each of its hosts - mosquito and vertebrate - that are interrupted by restrictive steps as it moves from one to the other. Productivity at each stage affects not only pathology but also the probability for successful transmission. This Opinion article briefly assesses what is known about productivity at each step and attempts, with limited success, to put each in the context of an entire cycle, sporozoite to sporozoite.

*Plasmodium falciparum*: a paradigm for alternative folate biosynthesis in diverse microorganisms?

Hyde JE, Dittrich S, Wang P, Sims PF, de Crécy-Lagard V, Hanson AD.

Manchester Interdisciplinary Biocentre, Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK.

Folates have a key role in metabolism, and the folate-dependent generation of DNA precursors in the form of deoxythymidine 5'-phosphate is particularly important for the replication of malaria parasites. Although *Plasmodium falciparum* can synthesize folate derivatives de novo, a long-standing mystery has been the apparent absence of a key enzyme, dihydroneopterin aldolase, in the classical folate biosynthetic pathway of this organism. The discovery that a different enzyme, pyruvoyltetrahydropterin synthase, can produce the necessary substrate for the subsequent step in folate synthesis raises the question of whether this solution is unique to *P. falciparum*. Bioinformatic analyses suggest otherwise and indicate that an alternative route to folate could be widespread among diverse microorganisms and could be a target for novel drugs.

Gametocytes: insights gained during a decade of molecular monitoring.
In vertebrate hosts, malaria parasites produce specialized male and female sexual stages (gametocytes). Soon after being taken up by a mosquito, gametocytes rapidly produce gametes and, once mated, they infect their vector and can be transmitted to new hosts. Despite being the parasite stages that were first identified (over a century ago), gametocytes have remained elusive, and basic questions remain concerning their biology. However, the postgenomic era has substantiated information on the specialized molecular machinery of gametocytogenesis and expedited the development of molecular tools to detect and quantify gametocytes. The application of such highly sensitive and specific tools has opened up novel approaches and provided new insights into gametocyte biology. Here, we review the discoveries made during the past decade, highlight unanswered questions and suggest new directions.

**68: Trends Parasitol. 2008 Sep 7.**

**alpha(+)–thalassaemia and malarial anaemia.**

Danquah I, Mockenhaupt FP.

Institute of Tropical Medicine and International Health, Charité – University Medicine Berlin, Spandauer Damm 130, 14050 Berlin, Germany.

The mechanisms by which alpha(+)–thalassaemia protects against severe malaria, and severe malarial anaemia in particular, are poorly understood. A recent report proposes that the increased count of microcytic and hypochromic erythrocytes in alpha(+)–thalassaemia reduces the haemoglobin decline during acute malaria and, thus, reduces the risk of anaemia. This mechanism might add to further alpha(+)–thalassaemic attributes that are involved in the attenuation of anaemia caused by both acute and chronic Plasmodium infections.

**69: Trends Parasitol. 2008 Sep 2.**

**Mutually exclusive var gene expression in the malaria parasite: multiple layers of regulation.**

Chookajorn T, Ponsuwanna P, Cui L.

Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

As a major factor in Plasmodium falciparum malaria pathogenesis, the var gene family has been the focus of extensive research, which has contributed to our current understanding of Plasmodium antigenic variation. In recent years, sophisticated molecular tools have enabled the generation of interesting data regarding the regulation of mutually exclusive var expression. Although their results are still inconclusive, these studies have demonstrated the existence of multiple layers of control over gene activation, silencing, memory and 'counting'. This review attempts to summarize recent findings and dissect the different layers of var regulation.

**70: Trop Med Int Health. 2008 Sep 16.**

**Analysis of circulating populations of Plasmodium falciparum in mild and severe**
malaria in two different epidemiological patterns in Madagascar.


Laboratoire de Parasitologie Mycologie, AP-HP Hôpital Avicenne, Paris, Cedex, France.

Objective To investigate whether the severity of Plasmodium falciparum attack in endemic areas was associated with the multiplicity of infection (MOI) and/or with a particular genotype(s). Method In two areas of different malaria transmission pattern in Madagascar (Sainte-Marie - mesoendemic and Tsiroanomandidy – hypoendemic) the number and the proportions of msp-2 genotypes within isolates were determined for each patient using a capillary electrophoresis genotyping method. DNA sequencing was performed to identify the msp-2 allelic family of dominant clones. Results Eighty six uncomplicated and 33 severe cases were included in Sainte-Marie and 48 uncomplicated and 69 severe cases were included in Tsiroanomandidy. We found no association between the MOI and severity of malaria as the same mean number of msp-2 genotypes was found in isolates from uncomplicated and from severe malaria cases (3.72 and 3.73, respectively, P>0.05). The study of the association of dominant clones with clinical status showed no particular genotype or allelic family associated with malaria severity. Conclusions Severity of malaria was not associated with higher MOI in our study. Severity did not appear restricted to some particular genotypes either. On the contrary, severe malaria appeared to be caused by very common genotypes in the studied areas. More comprehensive explorations including immunity and genetic factors of the host are needed to acquire new information about this complex condition.


Effect of iron or multiple micronutrient supplements on the prevalence of anaemia among anaemic young children of a malaria-endemic area: a randomized double-blind trial.

Ouédraogo HZ, Dramaix-Wilmet M, Zeba AN, Hennart P, Donnen P.

Biomedical and Public Health Department, Institute of Research in Health Sciences, Ouagadougou, Burkina Faso.

Objective To assess the effect of supplementation with iron or multiple micronutrients (MM) on the prevalence of anaemia in a malaria-endemic area. Methods A community-based randomized double-blind trial was conducted in rural Burkina Faso, including children aged 6-23 months with haemoglobin (Hb) concentrations of 70-109 g/l who were randomized into an iron group (Fe, n = 96), an iron and zinc group (IZ, n = 100) or an MM group (MM, n = 100), 5 days/week for 6 months. All children were provided with insecticide-treated bednets; those who had a Plasmodium falciparum (PF) positive-smear at baseline and/or at each monthly checking received antimalarial therapy. Results The mean (SD) endpoint Hb concentration was higher in the MM group [113.2 (13.6) g/l] than in the IZ group [106.3 (15.6) g/l] and the Fe group [107.1 (12.9) g/l] (P = 0.001). Children in the MM group were more likely to recover from anaemia than those in the Fe group [prevalence rate ratios, PRR (95% confidence interval, CI) = 1.62 (1.22-2.15), P < 0.001]. The IZ group did not differ from the Fe group [PRR (95% CI) = 0.94 (0.65-1.35), P = 0.72]. None of the interactions on the effect of supplementation of baseline age (0.13), or baseline height-for-age z-score (P = 0.33), or incident PF parasitemia (P = 0.99), was significant. Conclusion In this malaria-endemic area, in combination with malaria management, the MM supplement was more efficacious than the Fe supplement and the IZ supplement for reducing anaemia. Further investigation into limiting factors and amounts of
micronutrients that would be more efficacious for reducing anaemia is recommended.

72: Vaccine. 2008 Sep 17.

**Mixed allele malaria vaccines: Host protection and within-host selection.**

Barclay VC, Chan BH, Anders RF, Read AP.

School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, UK; Centre for Infectious Disease Dynamics, Departments of Biology and Entomology, The Pennsylvania State University, University Park 16802, USA.

Malaria parasites are frequently polymorphic at the antigenic targets of many candidate vaccines, presumably as a consequence of selection pressure from protective immune responses. Conventional wisdom is therefore that vaccines directed against a single variant could select for non-target variants, rendering the vaccine useless. Many people have argued that a solution is to develop vaccines containing the products of more than one variant of the target. However, we are unaware of any evidence that multi-allele vaccines better protect hosts against parasites or morbidity. Moreover, selection of antigen-variants is not the only evolution that could occur in response to vaccination. Increased virulence could also be favored if more aggressive strains are less well controlled by vaccine-induced immunity. Virulence and antigenic identity have been confounded in all studies so far, and so we do not know formally from any animal or human studies whether vaccine failure has been due to evasion of protective responses by variants at target epitopes, or whether vaccines are just less good at protecting against more aggressive strains. Using the rodent malaria model Plasmodium chabaudi and recombinant apical membrane antigen-1 (AMA-1), we tested whether a bi-allelic vaccine afforded greater protection from parasite infection and morbidity than did vaccination with the component alleles alone. We also tested the effect of mono- and bi-allelic vaccination on within-host selection of mixed P. chabaudi infections, and whether parasite virulence mediates pathogen titres in immunized hosts. We found that vaccination with the bi-allelic AMA-1 formulation did not afford the host greater protection from parasite infection or morbidity than did mono-allelic AMA-1 immunization. Mono-allelic immunization increased the frequency of heterologous clones in mixed clone infections. There was no evidence that any type of immunization regime favored virulence. A single AMA-1 variant is a component of candidate malaria vaccines current in human trials; our results suggest that adding extra AMA-1 alleles to these vaccines would not confer clinical benefits, but that that mono-allelic vaccines could alter AMA-1 allele frequencies in natural populations.

73: Vaccine. 2008 Sep 17.

**New malaria vaccine candidates based on the Plasmodium vivax Merozoite Surface Protein-1 and the TLR-5 agonist Salmonella Typhimurium FliC flagellin.**

Bargieri DY, Rosa DS, Braga CJ, Carvalho BO, Costa FT, Espíndola NM, Vaz AJ, Soares IS, Ferreira LC, Rodrigues MM.

Centro Interdisciplinar de Terapia Gênica (CINTERGEN), Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo 04044-010, SP, Brazil; Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, Escola Paulista de Medicina, Rua Mirassol, 207, São Paulo 04044-010, SP, Brazil.

The present study evaluated the immunogenicity of new malaria vaccine formulations based on the 19kDa C-terminal fragment of Plasmodium vivax Merozoite Surface Protein-1 (MSP1(19)) and the Salmonella enterica serovar Typhimurium.
flagellin (FliC), a Toll-like receptor 5 (TLR5) agonist. FliC was used as an adjuvant either admixed or genetically linked to the P. vivax MSP1(19) and administered to C57BL/6 mice via parenteral (s.c.) or mucosal (i.n.) routes. The recombinant fusion protein preserved MSP1(19) epitopes recognized by sera collected from P. vivax infected humans and TLR5 agonist activity. Mice parenterally immunized with recombinant P. vivax MSP1(19) in the presence of FliC, either admixed or genetically linked, elicited strong and long-lasting MSP1(19)-specific systemic antibody responses with a prevailing IgG1 subclass response. Incorporation of another TLR agonist, CpG ODN 1826, resulted in a more balanced response, as evaluated by the IgG1/IgG2c ratio, and higher cell-mediated immune response measured by interferon-gamma secretion. Finally, we show that MSP1(19)-specific antibodies recognized the native protein expressed on the surface of P. vivax parasites harvested from infected humans. The present report proposes a new class of malaria vaccine formulation based on the use of malarial antigens and the innate immunity agonist FliC. It contains intrinsic adjuvant properties and enhanced ability to induce specific humoral and cellular immune responses when administered alone or in combination with other adjuvants.

74: Vaccine. 2008 Sep 16.

**Complete protection against P. berghei malaria upon heterologous prime/boost immunization against circumsporozoite protein employing Salmonella type III secretion system and Bordetella adenylate cyclase toxoid.**


Department of Immunology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Sterile immunity against malaria can be achieved by the induction of IFNgamma-producing CD8(+) T cells that target infected hepatocytes presenting epitopes of the circumsporozoite protein (CSP). In the present study we evaluate the protective efficacy of a heterologous prime/boost immunization protocol based on the delivery of the CD8(+) epitope of Plasmodium berghei CSP into the MHC class I presentation pathway, by either a type III secretion system of live recombinant Salmonella and/or by direct translocation of a recombinant Bordetella adenylate cyclase toxoid fusion (ACT-CSP) into the cytosol of professional antigen-presenting cells (APCs). A single intraperitoneal application of the recombinant ACT-CSP toxoid, as well as a single oral immunization with the Salmonella vaccine, induced a specific CD8(+) T cell response, which however conferred only a partial protection on mice against a subsequent sporozoite challenge. In contrast, a heterologous prime/boost vaccination with the live Salmonella followed by ACT-CSP led to a significant enhancement of the CSP-specific T cell response and induced complete protection in all vaccinated mice.

75: Vaccine. 2008 Sep 16.

**Production, quality control, stability and pharmacotoxicity of cGMP-produced Plasmodium falciparum AMA1 FVO strain ectodomain expressed in Pichia pastoris.**


Department of Parasitology, Biomedical Primate Research Center, Lange Kleiweg 139, 2288 GJ Rijswijk, The Netherlands.

Plasmodium falciparum apical membrane antigen 1 (PfAMA1) is a leading asexual blood stage vaccine candidate for malaria. In preparation for clinical trials, PfAMA1 ectodomain (amino acid 25-545, FVO strain) was produced in Pichia pastoris.
by 35L scale fed batch fermentation under current Good Manufacturing Practice (cGMP). Fermentation was followed by a three-step chromatographic purification procedure resulting in a yield of 5.8g of purified protein. As judged by size exclusion chromatography, the cGMP-product comprised >95% PfAMA1 monomer, the remainder being predominantly PfAMA1 dimer. In SDS-PAGE two main bands of 68 and 70kDa and some minor bands were evident. Under reducing conditions a site of limited proteolytic cleavage within a disulphide bonded region became evident; less than 15% of the protein had this internal cleavage. By mass-spectrometric analysis, all bands analyzed in overloaded SDS-PAGE gels comprised PfAMA1 derived products. The protein was quantitatively bound by immobilized 4G2, a monoclonal antibody reactive with a reduction sensitive conformational determinant. The lyophilized product was stable for over 1 year. Immunopotency did not diminish, and storage did not lead to alterations in the behaviour of the protein upon formulation with adjuvants selected for Phase I clinical evaluation. These formulations also showed no pharmacotoxicity in rabbits. The final product conformed to preset criteria and was judged suitable for use in human clinical trials.


Virulence evolution in response to vaccination: the case of malaria.

Mackinnon MJ, Gandon S, Read AF.

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK. mmackinnon@kilifi.kemri-wellcome.org

One theory of why some pathogens are virulent (i.e., they damage their host) is that they need to extract resources from their host in order to compete for transmission to new hosts, and this resource extraction can damage the host. Here we describe our studies in malaria that test and support this idea. We go on to show that host immunity can exacerbate selection for virulence and therefore that vaccines that reduce pathogen replication may select for more virulent pathogens, eroding the benefits of vaccination and putting the unvaccinated at greater risk. We suggest that in disease contexts where wild-type parasites can be transmitted through vaccinated hosts, evolutionary outcomes need to be considered.