# 2014 World Malaria Day

**Friday, April 25, 2014**

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<th>Time</th>
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<tr>
<td>8:00 a.m.</td>
<td>Registration</td>
<td>E. Monument St. entrance Feinstone Hall E2030</td>
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<td>9:00 a.m.</td>
<td><strong>Dean Michael Klag</strong>, Johns Hopkins Bloomberg School of Public Health Welcome Address</td>
<td>Sommer Hall W1214</td>
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<td><strong>GOVERNMENT ORGANIZATIONS</strong></td>
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<td>9:10 - 9:30 a.m.</td>
<td><strong>KEYNOTE:</strong> Rear Admiral Tim Ziemer, United States Navy (Retired) U.S. Global Malaria Coordinator, President’s Malaria Initiative &quot;Fighting Malaria with effective interventions and effective collaboration&quot;</td>
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<td>9:30 - 10:00 a.m.</td>
<td><strong>PANEL RESPONSE</strong> with Broemmelsiek, McCully, Thuma and Ziemer Moderator: William Moss</td>
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<td>10:00 - 10:30 a.m.</td>
<td><strong>BREAK &amp; POSTERS</strong></td>
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<td><strong>FAITH BASED ORGANIZATIONS</strong></td>
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<td>10:30 - 11:00 a.m.</td>
<td>Michele Broemmelsiek, Catholic Relief Services &quot;Investing In Partnerships to Defeat Malaria”</td>
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<td>11:00 - 11:30 a.m.</td>
<td>Timothy McCully, Lutheran World Relief &quot;Faith and malaria: Community Driven Programming”</td>
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<td>11:30 - 12:00 p.m.</td>
<td>Philip E. Thuma, Macha Research Trust, Zambia &quot;Decline in Malaria at Macha Mission Hospital in Southern Zambia – Who Gets the Credit?”</td>
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<td>12:00 - 1:00 p.m.</td>
<td><strong>LUNCH, POSTERS and TABLE DISPLAYS</strong></td>
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<td><strong>NATIONAL PRIORITIES, UNIVERSITY PROGRAMS &amp; NGOS</strong></td>
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<td>1:00 - 1:30 p.m.</td>
<td>S. Patrick Kachur, Centers for Disease Control and Prevention &quot;Public health science and values in the global malaria effort”</td>
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<td>1:30 - 2:00 p.m.</td>
<td>Christopher Helfrich, Nothing But Nets &quot;A Grassroots Movement to Save Lives”</td>
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<td>2:00 - 2:30 p.m.</td>
<td>Susan Krenn, Center for Communication Programs &quot;Communication- the Tie that Binds”</td>
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<td>2:30 - 3:00 p.m.</td>
<td>Koki Agarwal, Jhpiego &quot;Working to Defeat Malaria: Investing in Women and Children&quot;</td>
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<td>3:00 - 3:30 p.m.</td>
<td>William Moss, Johns Hopkins Malaria Research Institute &quot;The Southern Africa International Centers of Excellence for Malaria Research: Research to Policy&quot;</td>
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<td>3:30 - 3:40 p.m.</td>
<td>Closing remarks Peter Agre, Johns Hopkins Malaria Research Institute Bill Brieger, Jhpiego Matt Lynch, Center for Communication Programs</td>
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<td>3:40 - 5:00 p.m.</td>
<td><strong>POSTERS &amp; REFRESHMENTS</strong></td>
<td>Feinstone Hall E2030</td>
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Malaria and febrile illness care seeking in Bauchi State, Nigeria: context for improving case management at the primary level

William R. Brieger, MPH, CHES, DrPH ¹, Bright Orji, MPH ², Masduk Abdulkarim ³,
(1) International Health, Bloomberg School of Public Health, The John Hopkins University, 615 N Wolfe St, Baltimore, MD 21205 (and Jhpiego). (2) Jhpiego, Thames St, Baltimore, MD 21231. (3) Targeted States High Impact Project USAID Nigeria, Bauchi, Nigeria.

Seeking of appropriate and quality care for childhood illnesses is a major challenge in much of Africa including Bauchi State, Nigeria. In advance of an intervention to improve available care in the most common points of service (POS), government primary health care centers (PHCs) and patent medicine vendors (PMV), a survey was done of child caregivers in four districts concerning responses to febrile illness, suspected malaria, acute respiratory disease and diarrhea. The ethical review committee in the Bauchi State Ministry of Health approved of the study. A total of 3077 children below the age of five were identified in the households sampled. Their mothers, fathers or other caregivers consented and were interviewed. Among the children 74% had any illness, 57% had fever, 26% had cough, and 15% had diarrhea. Only 8.7% of 1186 febrile children had their blood tested. Care seeking from PMVs varied from 45% with fever, 40% with cough to 36% with diarrhea. Care from public sector POS varied from 26-33%. Treatment that might be considered ‘appropriate’ for each also varied with 30% receiving antimalarial drugs for suspected malaria, 20% getting oral rehydration solution for diarrhoea and 50% being given an antibiotic for a suspected acute respiratory illness. The results show that providing quality case management with appropriate commodities through PHCs and PMVs can improve the illness care of a majority of children in Bauchi State, and interventions are currently being planned to do this.
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*Plasmodium falciparum* infection and *Anopheles* multiple blood feeding rate during the wet and dry seasons in Nchelenge District, Zambia

Smita Das¹, Mbanga Muleba², Samantha Eng³, Maureen Kessler¹, and Douglas E. Norris¹

¹ Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, ² Tropical Disease Research Centre, Ndola, Zambia, ³ University of Maryland Baltimore Campus, Baltimore, MD

As part of the Southern Africa International Centers for Excellence in Malaria Research (ICEMR) project, mosquito collections were performed during August-September 2012 and March-May 2013 in Nchelenge District, Luapula Province, Zambia. Located along the environs of Lake Mweru and Kenani Stream, Nchelenge experiences hyperendemic transmission and has the highest malaria infection rate in children under the age of 5 years despite implementation of indoor residual spraying (IRS) and long-lasting insecticide-treated net (LLIN) distribution. Center for Disease Control light traps (CDC LTs) and pyrethroid spray catches (PSCs) were performed at three villages along Lake Mweru and two villages along Kenani Stream. The collections revealed that during the dry season, *Anopheles funestus sensu stricto* is the dominant vector near both the lake and stream. In contrast, during the wet season, *Anopheles gambiae* s.s. is the dominant vector in the lakeside villages, whereas *An. funestus* s.s. is the primary vector with secondary contribution from *An. gambiae* s.s. in the streamside villages. Both vector species are highly anthropophilic and *An. funestus* has a higher *Plasmodium falciparum* sporozoite rate than *An. gambiae*. In the wet and dry seasons, it was found that the *P. falciparum* infection rate of the vector populations was higher near the streamside villages than those of the lakeside villages. The multiple blood feeding rate for *An. funestus* is higher during the dry season, whereas the multiple blood feeding rate for *An. gambiae* is higher in the following wet season. As a result, the entomological inoculation rates (EIRs) for each vector during the dry and wet seasons are underestimated. The results also suggest that there are spatial differences in vector composition and their multiple blood feeding rates, which contribute to differences in human malaria risk during the dry and wet seasons. Overall, the vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and implications for malaria control in high-risk areas.
Malaria research within the Walter Reed Army Institute of Research Entomology Division

Silas A. Davidson¹, Lindsey S. Garver¹, Daniel E. Szumlas³
¹Walter Reed Army Institute of Research

Malaria is considered the most important infectious disease threat to the U.S military and therefore considerable efforts and funds are directed to studying this disease at WRAIR. The Entomology Division maintains a large mosquito insectary and two *Plasmodium falciparum* culture laboratories. These resources allow the Division to operate the world's most active Controlled Human Malaria Infection (CHMI) center for evaluation of new vaccines and drugs. The Division also houses the U.S. national mosquito collection as part of the Walter Reed Biosystematics Unit. A recent effort has been to barcode the *Anopheles* species of the world. WRAIR has access to overseas field sites in Thailand, Kenya, Egypt, Ghana, Cambodia and Peru. Insectaries are maintained at these sites and the most important local malaria vectors are reared. Many of these sites have, or are developing, protocols to infect mosquitoes with local strains of Plasmodium. Transmission studies are currently ongoing at these sites with a focus on *Anopheles*-*P. vivax* interactions. Also, the sites in Thailand and Kenya have recently built large semi-field enclosures that allow mosquitoes to be released and their behaviors studied and products evaluated in near natural environments.
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**Vectored antibody gene delivery protects mice against sporozoite challenge**

Cailin Deal¹, Alejandro B. Balazs², Diego Espinosa¹, Fidel Zavala¹, David Baltimore³ and Gary Ketner¹

¹Johns Hopkins Bloomberg School of Public Health, Baltimore, MD  ²Ragon Institute, Cambridge, MA  ³California Institute of Technology, Pasadena, CA

*Plasmodium* sporozoites can be neutralized *in vitro* by monoclonal antibodies (MAb) against the *circumsporozoite* protein (CSP). Passively transferred MAb against *P. falciparum* CSP can block liver invasion by sporozoites of a transgenic rodent parasite that expresses *P. falciparum* CSP (Pb-Pf), preventing infection in mice. Despite this, attempts at targeting CSP for a vaccine have fallen short of expectations, in part due to inability to induce durable high-titer antibodies. A single un-neutralized sporozoite can initiate infection, necessitating sustained high-titer neutralizing antibodies for lasting protection.

Recently, David Baltimore’s laboratory developed an adeno-associated virus type 8 (AAV8) platform that efficiently delivers pre-formed MAb genes *in vivo* and directs sustained, high-level MAb production. With the Baltimore lab, we have adopted that technology to express humanized MAbs against the central repeat region of the CSP protein of *P. falciparum* in mice. Mice developed high titer human IgG antibodies as early as 1 week post transduction and levels have remained constant for more than 20 weeks at 200 to 1000 µg of IgG/ml. In 70 percent of mice transduced with CSP MAb humanized 2A10 (h2A10), and challenged intravenously with $10^5$ Pb-Pf sporozoites, parasite liver burden was reduced to below the level of detection. h2A10-transduced mice challenged by infected mosquito bite displayed a statistically significant delay in time to patency, and in a separate experiment, 70 percent of mice were steriley protected. Examination of antibody levels in individual mice revealed that all mice with human IgG concentrations above 1mg/mL were completely protected. This suggests that exceeding this antibody threshold results in consistent sterile protection and establishes that vectored MAb gene delivery has the potential to be an effective form of malaria control.
The circumsporozoite protein (CSP), the most abundant protein on the surface of malaria sporozoites, is one of the best studied plasmodial antigens and is considered a major malaria vaccine candidate. Due to its immunodominant nature, CSP is capable of eliciting both T-cell and antibody responses. RTS,S, the most effective vaccine candidate to date, is based on the \textit{P. falciparum} CSP, hence underscoring CSP’s immunogenic properties. Despite RTS,S relative success, there is still a need for more effective vaccine candidates. Using a rodent chimeric \textit{P. berghei} strain, we independently assessed the protective efficacy of two different CSP-based vaccine formulations, soluble recombinant CSP preparations and a CSP-based Virus-Like Particle (VLP). We found significant differences between two different soluble recombinant CSP formulations; while CSP A was capable of inducing comparable antibody responses to CSP B, it failed to elicit significant CD4$^+$ T-cell responses. In addition, CSP B showed a stronger inhibitory effect on sporozoite infection. In separate experiments, we show that immunization of mice with a next generation CSP-based VLP results in robust antibody responses to CSP, which conferred sterile protection to mice challenged against chimeric sporozoites delivered by mosquito bites. Overall, our results show that chimeric parasites are instrumental tools for the pre-clinical evaluation of novel malaria vaccine candidates and underscore their usefulness for identifying effective vaccine formulations under stringent conditions.
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Vector Bionomics of Anopheles funestus Complex in Mutasa District, Zimbabwe

Tyler C Henning¹, Nzira Lukwa², Lovemore Gwanzura³, and Douglas E Norris¹
¹Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, ²National Institute of Health Research, Harare, Zimbabwe, ³University of Zimbabwe, Harare, Zimbabwe

Mutasa District, Zimbabwe is an area marked by seasonal malaria transmission with severe outbreaks occurring during the wet season. A research arm of the International Centers of Excellence in Malaria Research (ICEMR) in Southern Africa began in 2012 to assess malaria burden in the region. This area is characterized as a region of resurgent malaria, having had increasing rates in recent years despite being previously considered as under control. In order to assess the reasons for this transmission dynamic, the ICEMR project seeks to analyze malaria epidemiology, parasite genomics, and entomology, which is of particular importance for this study. Samples were collected during the wet seasons of 2012-2014 using CDC light traps and pyrethroid spray catches, and morphological identification indicates that the predominant malaria vector is Anopheles funestus senso lato. Molecular identification of samples from the 2012-2013 collections confirms these results, with samples being An. funestus senso stricto or Anopheles leesoni, both members of the An. funestus species complex. Blood meal analysis indicates that these mosquitoes feed predominantly on human populations. Sporozoite infection rates for 2012-2013 were approximately 5%. Of additional interest is the high level of pyrethroid resistant An. funestus as the dominant malaria vector in the region. During collections in December 2013, 80% of mosquitoes were collected in houses that had been recently treated (within 2 months) with pyrethroids used for IRS control methods. Of these samples, 93% were morphologically observed to be blood fed or gravid, suggesting that these mosquitoes had been resting on treated surfaces for many hours. Although the findings are preliminary in nature, they suggest that potentially insecticide-resistant An. funestus is the dominant malaria vector in the region and may be contributing to the resurgence of malaria in spite of control efforts. Further collections are scheduled and will help to further elucidate the vector component of this study. With this knowledge it may be possible to determine areas of highest malaria risk and provide information on how to most effectively deploy limited resources to achieve control.
Visualizing the function of two *Plasmodium* sporozoite surface proteins by *in vivo* imaging of the dermal inoculation site

Christine S. Hopp¹, Ahmed Salman², Shahid M. Khan², Chris J. Janse², Photini Sinnis¹
¹ Department of Molecular Microbiology & Immunology, Johns Hopkins School of Public Health;
² Leiden Malaria Research Group

Following injection by a female *Anopheles* mosquito into the skin of the mammalian host, *Plasmodium* sporozoites move within the dermis, penetrate the vascular endothelium to enter the blood vessel and travel to the liver. Two major surface molecules that play key roles in sporozoite infectivity and motility are the circumsporozoite protein (CSP) and the thrombospondin-related anonymous protein (TRAP). Recent work from the Sinnis lab has shown that both of these proteins have important roles in the exit of *P. berghei* sporozoites from the dermal inoculation site. Proteolytic processing of CSP leads to the exposure of a cell-adhesion domain and mutant PbCSΔN sporozoites, expressing a constitutively processed CSP, displayed normal infectivity when inoculated intravenously, however, infectivity was dramatically decreased when sporozoites were injected intradermally, suggesting that PbCSΔN parasites are impaired in their ability to exit the dermis. Similar to the PbCSΔN mutant, PbTRAP-VAL sporozoites carrying mutations in the rhomboid-cleavage site of TRAP have a much more dramatic reduction in their infectivity after intradermal inoculation compared to intravenous inoculation and sporozoites display slow, staccato motility *in vitro*. Using intravital microscopy of fluorescent PbCSΔN and PbTRAP-VAL sporozoites, we studied their motility at the dermal inoculation site. Visualizing endothelial cells by labeling PECAM1 (CD31) pointed to a potential role of CSP in the interaction of sporozoites with blood vessels. Using LysM-EGFP mice, we are visualizing the early influx of neutrophils to the site of infection and preliminary data suggests that sporozoite blood vessel invasion precedes the peak of neutrophil infiltration. By imaging neutrophils in conjunction with PbCSΔN and PbTRAP-VAL sporozoites we are trying to understand the mechanism sporozoites use to escape the early innate immune response.
A putative amino acid transporter in Plasmodium berghei has been cloned. Based on homology search, it shares the highest similarity with human solute carrier family (SLC38), which is also named sodium-coupled neutral amino acid transporters (SNAT) by some researchers. As specific transporters for certain amino acids, SNATs play many physiological roles including the transfer of glutamine from astrocyte to neuron in CNS and gluconeogenesis in liver. So, SLC38 members can be therapeutic targets in neoplasia. Here we have confirmed the expression of a putative SNAT family member (assigned as pb1430) in P. berghei RBC stages on mRNA level. This gene has been interrupted by targeted deletion from P. berghei genome. Delayed growth of parasites during RBC stage is observed. Physiological roles of pb1430 are being investigated. We’ve also cloned its homolog in human malaria parasites P. falciparum. Our data suggest pb1430 and its family member in P. falciparum can be new targets for malaria control.
Antimalarial chemotherapy: Artemisinin-derived dimer carbamates, carbonates, and thiocarbonates


Department of Chemistry, School of Arts and Sciences, The Johns Hopkins University, W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, The Johns Hopkins University, The Johns Hopkins Malaria Research Institute, Bloomberg School of Public Health, The Johns Hopkins University

Ongoing efforts have been made toward improving the oral bioavailability and minimizing the metabolic shortcomings of artemisinin and its first generation derivatives. In pursuit of an efficacious and robust antimalarial chemotherapy, twenty-nine novel artemisinin-derived 2-carbon-linked trioxane dimers were prepared and evaluated for antimalarial activity in P. berghei infected mice. All novel 2-carbon-linked dimer derivatives, combined with mefloquine and administered in a low single oral dose, emerged as potent antimalarial drug candidates. Additionally, several derivatives prolonged the survival times of malaria-infected mice to an average of 30 days, which was more effective compared to the popular antimalarial trioxane monomer drug artemether plus mefloquine.
Factors Associated with decreased *Plasmodium falciparum* infection risk in Malian children

Anna A. Minta, Tuan M. Tran, Aissata Ongoiba, Jill Coursen, Cecile Crosnier, Ababacar Diouf, Chingu-Yu Huang, Shanping Li, Safiatou Doumbo, Didier Doumtabe, Younoussou Kone, Aboudramane Bathily, Seydou Dia, Moussa Niangaly, Charles Dara, Jules Sangala, Louis H. Miller, Ogobara K. Doumbo, Kassoum Kayentao, Carole A. Long, Kazutoyo Miura, Gavin J. Wright, Boubacar Traore and Peter D. Crompton

1Johns Hopkins Hospital, Baltimore, Maryland, USA, 2Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA, 3Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali, 4Cell Surface Signalling Laboratory, Wellcome Trust Sanger Institute, Cambridge, United Kingdom, 5Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland, USA, 6Research Program in Quantitative Sciences Division of Oncology Biostatistics/Bioinformatics, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University, Baltimore, Maryland, USA

Nearly half of the world’s population is at risk for malaria, a disease that causes tremendous morbidity and mortality. Malaria disproportionately affects children under 5 years of age living in sub-Saharan Africa, and they are at risk for severe anemia, respiratory distress, coma, and death. The efforts to reduce malaria incidence have been hampered by many issues, including the development of resistance by the parasite to anti-malaria medications and resistance by the mosquito to insecticides. Malaria control efforts have also suffered significantly due to a lack of a highly efficacious vaccine. An understanding of malaria immunology is critical in the development of such a vaccine. Unfortunately, there are many gaps in knowledge in the field of malaria immunology. A longitudinal cohort study has been set up by our research team in the rural village of Kalifabougou, Mali to study immunologic and epidemiologic factors associated with *Plasmodium falciparum* (*Pf*) risk. Analysis of the 2011-2012 study cohort revealed that children under 4 years of age were less likely to be infected with *Pf* infection than older children and adults. Although the risk of disease varies by age, it is unexpected that the risk of infection would vary by age. In order to investigate this issue, we designed a study to analyze the association between *Pf* exposure, measured in three ways, and protection from *Pf* infection in these children. The antibody response to gSG6, an *Anopheles* specific salivary protein, was measured before and after the malaria season. Children who were protected from *Pf* infection were less likely to be bitten by the mosquito vector over the course of the malaria season, as measured by antibody response to the gSG6 protein (p=0.02). Exposure was also measured using a survey to assess bed net use, and analysis revealed no association between bed net use and protection from infection. The last measure of exposure will be at the “hotspot” level, which measures the association between geographic location at a level that is more refined than a region or town, and *Pf* infection. In addition to differential exposure, it is possible that some young children have the ability to block malaria transmission at the liver stage. This will be assessed by measuring antibody response to *Pf* antigens at various stages of the *Pf* life cycle using a protein microarray including 1000 malaria antigens.
In 2011, Benin’s National Malaria Control Program (NMCP) introduced a new policy whereby all suspected malaria cases need to undergo diagnostic testing prior to dispensing treatment. Considering that a large proportion of Benin’s population is Animist (Voodoo culture), taking blood from an individual is often seen as taking part of someone soul, and therefore with the introduction of rapid diagnostic testing (RDT) for malaria nationwide, it is crucial that an adapted communications strategy is developed.

To support this new national malaria policy, Catholic Relief Services (CRS) is working with Benin’s National Health Research Institute to provide the NMCP with recommendations for solutions to culturally based health behaviors related to childhood malaria prevention and management. The operations research component of the project aims to address key barriers to community RDT use and acceptance of results, as well as technical and social-cultural skills needed by community-based organizations (CBOs) to improve uptake and adherence to community rapid diagnostic testing. Results are being used to develop and test the effectiveness of behavior change strategies to increase care seeking, accept results and associated treatment outcomes. CRS will then work with the NMCP to use this evidence to improve and expand community RDT services nationwide through an adapted communications strategy.
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Historical insights into the neurotoxicity of the 8-aminoquinolines: Implications for the development of tafenoquine and for global malaria control efforts

Remington L. Nevin
Department of Mental Health, Johns Hopkins Bloomberg School of Public Health

The 8-aminoquinoline (8-AQ) class of antimalarials includes the currently licensed drug primaquine, the historical drugs pamaquine and plasmocid, the experimental World War II (WWII) era drug pentaquine, and the recently synthesized drug tafenoquine. The 8-AQs are the only class of antimalarial drug effective against relapsing forms of disease, and are therefore considered an essential component of global malaria control efforts. Although broader use of the 8-AQ class of antimalarials has traditionally been limited out of concern for their risk of hemolytic toxicity, historically the widespread deployment of this class of drug has also been limited by concerns of neurotoxicity. For example, the historical 8-AQs, including pamaquine and plasmocid, were abandoned in part after an unacceptable risk of neurotoxicity was identified in studies sponsored by the WWII era Board for the Coordination of Malarial Studies. Similarly, although dozens of experimental 8-AQs with promising antimalarial activity were developed and tested during the post-WWII period, the most promising of these, pentaquine, was abandoned after similar evidence emerged of its neurotoxicity. Tafenoquine, which is currently pending Phase III trials, is widely anticipated for its potential utility against Plasmodium vivax malaria, and has recently been granted FDA Breakthrough Therapy designation for this indication, which is anticipated to speed its licensing and deployment. However, current literature has failed to rule out the drug's neurotoxicity, and no publications have yet described replication of the thorough WWII and post-WWII era neurohistopathological and clinical testing that first revealed this effect among structurally related 8-AQ drugs. With the goal of placing the development of tafenoquine in proper historical context, this poster reviews prior research into the neurotoxicity of the 8-AQ class, including postulated structure-toxicity relationships and histopathological and clinical features associated with neurotoxicity, and discusses implications of these insights for the drug's development and for broader global malaria control efforts.
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A free online app for planning malaria control and outbreak responses in the Peruvian Amazon

Antonio M. Quispe,1 Josiah L. Kephart1
1Johns Hopkins Bloomberg School of Public Health

After declining for over a decade, the number of cases of malaria in the Peruvian Amazon has quadrupled since 2010. The most plausible explanation for this reemergence is administrative, as a concurrent dengue outbreak has motivated authorities to allocate their resources away from malaria and towards dengue. This situation could be prevented if the surveillance system is able to alert the authorities about these threats simultaneously, providing authorities with rapid data to allocate their resources based on competing priorities at a micro level instead of at a macro level only. This is particularly relevant given that dengue spreads primarily within urban areas while malaria is typically found in rural and peri-urban areas. In an attempt to address this problem, we are developing the Johns Hopkins Surveillance Application (JHSAPP), which is an online application (http://tinyurl.com/JHSAPP) that converts time-series data from the malaria surveillance system into a Gapminder-like graph. The overall goal of this application is to allow public health decision-makers to assess malaria burden trends using several display options (bubble, bar, and line charts), the ability to combine or adjust for different relevant covariates (incident rate, population size, \(P. vivax\) proportion, time, etc.), and with a variety of mathematical transformations (arithmetic, geometric, and logarithmic). Additionally, any JHSAPP user will be able to contrast these trends against the epidemiological thresholds for each reporting level, as well as assess the cost of deploying interventions using a cost-effectiveness algorithm at the subdistrict level to enhance local readiness and response. Given that JHSAPP was developed using Motion Chart, a free interactive online Google Spreadsheet, and R statistical software, data managers will be able to update the system without altering their current reporting protocol. Using these free and open-source tools, JHSAPP may contribute to improving the malaria surveillance system in Peru and potentially other reportable diseases, facilitating improved public health decision making.
Examining the function of conserved positively-charged residues in the N-terminus of the circumsporozoite protein of *Plasmodium berghei*

Daniel R. T. Ragheb, Christine Hopp, Photini Sinnis
Johns Hopkins Bloomberg School of Public Health

The circumsporozoite protein (CSP) is the major surface protein of the sporozoite stage of *Plasmodium*. Although its precise function has not yet been determined, CSP is required for the parasite to efficiently develop into sporozoites and subsequently invade mosquito salivary glands and ultimately the mammalian host liver. Recent work in the lab has revealed that mutant parasites expressing a truncated form of CSP lacking the N-terminus are somewhat compromised in their ability to invade the mosquito salivary glands and rendered unable to establish a liver infection via inoculation in the skin of the host. When these parasites are delivered into the bloodstream, however, their ability to invade the liver is as efficient as wild-type parasites. These data suggest some role for the N-terminus in either interacting with cell surface molecules, the rest of CSP or other parasite proteins to allow the parasite to effectively migrate through the skin and invade a blood vessel. The N-terminus can be divided into a region of high conservation among different species that may be structured (according to *in silico* prediction) followed by a degenerate string of amino acids whose identity remain similar but vary greatly in number. Strewn among these two areas are patches of several positively charged lysine and arginine residues. Given previous identification of the interaction of heparan sulfate proteoglycans and the sporozoite surface, we have undertaken a mutagenic approach to alter these charges to neutral (alanine) or negative (glutamate) in order to understand what purpose they may serve. Relative to wild-type parasites, mutants expressing the alanine variant of CSP were reduced in sporozoite number production from the midgut oocyst, and were unable to enter the hemolymph or salivary glands of the mosquito. The parasite with the glutamate version of CSP, however, was able to invade salivary glands, and even eventually liver cells, albeit with greatly reduced efficiency. In addition, the parasite morphology is distinctive from wild-type despite CSP still being exported to the sporozoite surface. Further studies to investigate how these changes are affecting the parasite are underway.
Use of GPS data loggers to track human movement patterns in an area of effective malaria control in southern Zambia.

Kelly M. Searle,¹ Jailos Lubinda,² Timothy Shields,¹ Harry Hamapumbu,² Frank Curriero,¹ Phil Thuma,² William J. Moss¹
¹Johns Hopkins Bloomberg School of Public Health, ²Macha Research Trust

The role of human movement in malaria transmission dynamics, particularly in pre-elimination settings, has not been fully elucidated. GPS data loggers allow for micro-scale estimates of human movement in rural areas of southern Africa, which can aid in explaining the micro-epidemiology of malaria importation and transmission in these areas. Participants currently enrolled in a longitudinal cohort study of the impact of malaria control measures in a region of declining malaria transmission in southern, Zambia (one of three Southern Africa International Centers of Excellence for Malaria Research sites) were invited to participate in a population movement study using GPS data loggers. Approximately 10 participants at a time were asked to carry the loggers for a one-month period. Data will be collected over 12 months to account for seasonality in movement patterns. Enrollment began in October 2013 and is ongoing, with data collection complete for 36 individuals from October 2013 through March 2014. Data loggers were worn using a Velcro strap or lanyard. Serial numbers of GPS data loggers were matched to participant IDs and geographic position logged every two minutes. Movement data from the GPS loggers were imported into ArcGIS for pre-processing and analysis. The movement tracts were used to determine the cumulative amount of time spent in different areas, derived from the frequency of visits and amount of time spent during each visit to different areas. An intensity map was created to display the cumulative time spent in different areas. An analysis to determine whether time spent inside an area of high malaria risk is due to residence in a hotspot or routine travel to a location of high malaria risk (e.g. daily travel to a place of occupation) was conducted.
The feasibility of achieving and sustaining “malaria-free zones” in southern Zambia

The Southern Africa International Centers of Excellence for Malaria Research (ICEMR)

The Government of Zambia is committed to creating “malaria-free zones” in southern Zambia. Through passive case detection at health care facilities and active case detection through community-based surveys, we have documented a dramatic decline in the burden of malaria in the catchment area of Macha Hospital, Choma District, Southern Province, Zambia from 2008 through 2013. However, residual foci of transmission exist and the potential for repeated importation remains. We identified individuals with subpatent parasitemia and gametocytemia who may be responsible for sustained, low-level transmission and evaluated reactive case detection strategies to identify and treat these individuals using simulation models. Factors associated with sustained insecticide-treated bed net use were evaluated in light of the declining burden of malaria. Parasite bar coding of 24 SNPs should permit the identification of imported parasites. Results of a longitudinal analysis of changes in antibody responses to 500 *Plasmodium falciparum* antigens using a protein microarray should allow detection of residual transmission and document loss of humoral immunity in the absence of exposure.
2014 World Malaria Day

Failure of malaria control efforts in northern Zambia

The Southern Africa International Centers of Excellence for Malaria Research (ICEMR)

Despite distribution of insecticide-treated bed nets, indoor residual spraying and case management with rapid diagnostic tests and artemisinin-based combination therapy, the burden of malaria remains high in northern Zambia. Through passive case detection at health care facilities and active case detection through community-based surveys, we have documented persistently high parasite prevalence in Nchelenge District, Luapula Province, Zambia on the border of Lake Mweru with the Democratic Republic of Congo. Individual and household level risk factors for malaria were assessed and a spatial risk map constructed. Pyrethroid resistance in local *Anopheles funestus* populations likely contributes to failure of current control efforts. Potentially contributing to malaria transmission is population movement from the lakeside to inland as fishing and agricultural seasons alternate. Equally important may be cross-border movement between Nchelenge District, Zambia and Katanga Province in the Democratic Republic of Congo, suggesting the importance of epidemiological and entomological studies of cross-border malaria.
Resurgent malaria in eastern Zimbabwe

The Southern Africa International Centers of Excellence for Malaria Research (ICEMR)

Eastern Zimbabwe has experienced recent large outbreaks of malaria after a history of successful control. Through passive case detection at health care facilities and active case detection through community-based surveys, we have documented seasonal malaria outbreaks in Mutasa District, Manicaland Province, Zimbabwe on the border with Mozambique. We identified individuals with subpatent parasitemia who may be responsible for sustaining transmission during the dry season. Pyrethroid resistance in local Anopheles funestus populations likely contributes to failure of current control efforts. Potentially contributing to malaria transmission is population movement across the border with Mozambique.
Chemokines in Zambian children with Cerebral Malaria

Monique Stins\textsuperscript{1,2}, David Sullivan\textsuperscript{2}, Carlos Pardo\textsuperscript{1} and James Chipeta \textsuperscript{3}

\textsuperscript{1} Division of Neurology, Johns Hopkins School of Medicine, \textsuperscript{2} Malaria Research Institute, Bloomberg School of Public Health Baltimore MD, USA. \textsuperscript{3} University Teaching Hospital, University of Zambia, Lusaka, Zambia

Cerebral malaria (CM) is a clinical syndrome associated with \textit{Plasmodium falciparum} infection that is associated with a high mortality of up to 30\%, particular in children. Neurological symptoms and signs include impaired consciousness, coma, delirium, seizures, and increased intracranial hypertension. It has recently become apparent that in African children, persistent neurologic deficits, including recurrent seizures and learning disabilities occur \underline{after} survival of CM episodes. Trophozoite and schizont stages of \textit{P. falciparum}-infected red blood cells (PRBC) adhere to the brain blood vessel endothelium. As opposed to numerous other neuropathogens, PRBC do NOT cross the blood-brain barrier (BBB) into the central nervous system. In \textit{P. falciparum} CM, the intravascular PRBC are still able to elicit neuronal dysfunction. How this occurs is unclear. Since the BBB endothelium is located at the interface of these events, we hypothesize that activation of BBB endothelium plays a role in conferring neurological dysfunction.

Our published and preliminary data with an \textit{in vitro} human BBB model show that in CM, the BBB endothelium responds with increased transcription and release of large amounts of cyto- and chemokines towards the brain side and that this that may be responsible and/or contribute significantly to the observed neurological dysfunction in CM.

To verify and validate these \textit{in vitro} findings for the human situation and to assess whether the BBB endothelium would be an appropriate target for adjunctive therapeutic treatment and to prevent neurologic sequelae, we initiated a collaboration with the University Hospital of Zambia, Lusaka, Zambia. This research is part of an initial research and capacity building program and funded by the Fogarty Program “Brain Disorders in the Developing World: Research across the lifespan”.

We will initially assess a specific cyto-chemokine profile in the CSF of CM patients. This program will set the basis for an extended future collaboration that will focus on the role of chemokines in CM-mediated neurological dysfunction, how this affects a patient’s life and development. Our long term goal is to prevent the neurologic sequelae in brain diseases, such as CM.
iPhones for household malaria surveys in Sierra Leone

Suzanne Van Hulle
Catholic Relief Services

Catholic Relief Services (CRS) and the Ministry of Health and Sanitation (MoHS) of Sierra Leone (SL) are co-implementing nationwide malaria prevention and treatment activities funded by the Global Fund to fight AIDS, Tuberculosis and Malaria. In order to track progress and impact, CRS and partners led the implementation of a malaria indicator survey (MIS) in early 2013 covering a nationally-representative sample of 6,720 households, inclusive of blood testing to determine prevalence of anemia and malaria. In early 2012, CRS also had the experience of using mobile technology for a Knowledge Attitude and Practices (KAP) study. Fieldworkers used Apple 3GS iPhones for both surveys to collect data via the iFormBuilder platform, a web-based, software-as-services application with a companion app for the mobile devices allowing for timely data collection, monitoring, and analysis.

This was the first time that mobile technology was used for a MIS, and lessons learned include: allowing at least four months to transform paper-based questionnaires into electronic format, giving the program enough time for pre-testing the tool and training data collectors/biomarkers/laboratory technicians, and involving key malaria stakeholders to ensure a nationally-led survey. Global Positioning Systems enabled MoHS to make in-depth analyses on malaria trends based on geographic locations.

Overall the benefits of an electronic versus a paper-based MIS questionnaire outweighed the challenges. The iPhone technology eliminated the need for paper transcribing, allowing for quicker data tabulation, real-time identification of mistakes, faster interviewing through skip patterns, and a close-to-clean dataset by the end of data collection saving time and money. Survey results will be used to set evidence-based targets for all partners’ future malaria activities, especially the next 3 years of GF-supported malaria grants.
Salivary gland subcellular organization and regulatory conservation in *Anopheles* mosquitoes

Michael Wells¹ and Deborah Andrew¹
¹Johns Hopkins School of Medicine

Malaria is spread to humans by Anopheles mosquitoes, in a manner that requires travel through mosquito salivary glands (SGs). In order to develop novel strategies for the prevention of human malaria transmission, we are applying our lab’s previously gained knowledge of *Drosophila* salivary gland development and homeostatic regulation to two mosquito species, *Anopheles gambiae* and *Anopheles stephensi*. We conducted homology searches to identify mosquito homologs of fly SG structural proteins and transcriptional regulators and to assess the extent of SG genetic conservation within Diptera. We were able to identify many conserved structural proteins, organelle markers, and fly SG transcription factors of interest, most of which displayed a very high degree of homology. Using available antibodies against *Drosophila* proteins, we have optimized immunofluorescence confocal microscopy methods in mosquitoes and have begun to characterize adult SG organization at two time points in adulthood. Our work confirms and expands upon previous electron microscopy and histological studies and will support our subsequent studies of TF function in mosquito SGs. Finally, we are developing RNAi- and TALEN-based approaches to creating mosquitoes lacking the homolog of a *Drosophila* SG-specific and required factor, sage, both to better assay SG function during development and as a potential tool for targeted SG destruction for the prevention of malaria transmission.