

IMPACT OF BEDNET USE, INTERMITTENT PREVENTIVE TREATMENT (IPT), AND ANTENATAL CARE (ANC) DURING PREGNANCY ON THE HEALTH OF NEWBORNS IN THE KASSENA NANKANA DISTRICT OF NORTHERN GHANA

David J. Fryauff¹, Frank Atuguba², Kwadwo A. Koram³, Thomas Anyorigiya², Martin Adjuik², Abraham R. Oduro², Patrick Ansah², Abraham Hodgson², Lucas Amenga Etego², Francis Nkrumah³

¹Naval Medical Research Center, Silver Spring, MD, United States,

²Navrongo Health Research Center, Navrongo, Ghana, ³Noguchi Memorial Institute of Medical Research, Accra, Ghana

Primigravid women and their first-born babies are especially vulnerable to malaria but health practices during pregnancy can give mother and baby better defense against disease and longer malaria-free periods in which to grow and develop. We focused upon infants born of first-time mothers to analyze for protective effects that may result from IPTp coverage, ANC attendance, and/or bednet use during pregnancy. Primigravid mothers gave birth more often to low birth weight (LBW) infants than multigravida mothers (26% vs 16%; $p < 0.0001$). Near equal proportions of LBW girls and boys were born to first-time mothers, but among multigravida mothers, the majority of LBW babies were girls (24% vs. 13%; $p = 0.01$). Primigravid mothers averaged 9 years younger than multigravida mothers but these younger mothers had significantly more education, more ANC attendance, and better IPTp compliance. Despite lower birth weights of infants born to first time mothers, these children had healthier Hb profiles and significantly less mild anemia ($Hb < 11.0$). Relative to primigravid mothers who reported no IPTp during pregnancy (86/551=16%), just one IPTp dose of Fansidar (114/551=21%) was associated with a significantly greater mean birth weight, a significantly lower proportion of LBW newborns, an improved Hb profile, and fewer mild anemias. In contrast to the positive effects seen in primigravid mothers, analysis of a larger sample of multigravida mothers revealed no significant or even marginal improvements in birth weights or hemoglobin associated with one dose of Fansidar. We found no benefit to newborns that could be associated with bednet use during pregnancy in either primigravid or multigravida mothers who reported no IPTp use. Bednet use during pregnancy was associated with some notable differences in mothers: older age, more education, more ANC visits, better IPTp coverage, more frequently reported illness, and a history of miscarriage or the death of a child. Our findings suggest that first time mothers and their babies may benefit from even minimal IPTp use during pregnancy.

FACTORS AFFECTING MALARIA PREVENTION AND TREATMENT DECISIONS FOR CHILDREN IN THE DEMOCRATIC REPUBLIC OF CONGO

Olufunke A. Alaba¹, Gauthier Tshiswaka Kashalala¹, Adefolarin A. Fawole²

¹University of Pretoria, Pretoria, South Africa, ²CentaMed Practitioners, Pretoria, South Africa

Though endowed with natural resources with the country is one of the poorest countries of the world with a Human Development Index of 0.43; ranking 152nd out of 175 countries. The economic, political and war crises in the Democratic Republic of Congo (DRC) has negatively impacted on public health risks for the population. In recognition of the deep crisis, the government of DRC is thereby, promoting expansionary strategies to combat malaria as an important component in the global strategy to fight poverty and improve the standard of living of the people. Malaria is one of the primary causes of mortality and morbidity in the country, especially among pregnant women and young children, accounting for 30% of child mortality. However, insofar the government has come up with various control strategies including appropriate case management in both community and health infrastructures, scaling up the use of insecticide treated nets (ITNs) among others, it is also necessary to think

beyond supply. Specifically, we need to consider how family take decisions on behalf of the children during episodes of malaria and what social, economic and environmental factors affect this behaviour. Therefore, the main objective of this paper is to provide quantitative and qualitative evidence on the importance of individual, household and community characteristics on care seeking decisions during episodes of malaria in children as well as factors affecting malaria prevention methods in the DRC. The analyses will be based on the DRC Multiple Indicator Cluster Survey, (MICS) of 2001 supported by UNICEF. It is expected that the results will shed more light on the demand component associated to the prevention and treatment of malaria in this war-torn country.

EFFICACY AND WASH RESISTANCE OF α -CYPERMETHRIN WHO RECOMMENDED LONG LASTING INSECTICIDE NET AGAINST *ANOPHELES DARLINGI* IN THE PERUVIAN AMAZON

Elvira Zamora¹, Daniel Del Cuadro¹, Hugo Rodriguez², Gregor J. Devine³, Yuri Alegre¹, Angel Rosas²

¹Regional Direction of Health Loreto, Iquitos, Peru, ²Multi-Country Malaria Project PAMAFRO - Organismo Andino de Salud - Convenio Hipolito Unanue, Lima, Peru, ³Rothamsted Research, Harpenden, United Kingdom

The Peruvian Malaria Program has adopted Long Lasting Insecticide Net (LLIN) distribution as important strategy to prevent and control malaria in high risk areas of malaria transmission in the Amazon Region. The Interceptor[®] net, manufactured by BASF, is an α -cypermethrin treated LLIN that received WHOPES interim recommendation after an accelerated wash-resistance and small scale field-testing against susceptible African mosquitoes, mainly *An. gambiae*. It is necessary to confirm the efficacy of this LLIN against *Anopheles darlingi*, the most common and efficient malaria vector in the Peruvian Amazon. The objective of this study was to determine the efficacy and wash resistance of Interceptor[®], under laboratory conditions, against *Anopheles darlingi* in Zungarococha, San Juan, Loreto-Peru. Mosquitoes were collected the night before each bioassay. According to the WHO guidelines for laboratory testing, four different Interceptor[®] nets were tested without washing, and then with standard numbers of washes (1, 5, 10, 15 and 20 times). Susceptible *Anopheles darlingi* mosquitoes were exposed to net samples (25 cm x 25 cm) for 3 minutes using standard WHO cones. 50 mosquitoes per net were used (10 replicates). After exposure they were maintained in clean cups with access to sugar solution. Knock down effect (KD) was measured after 60 minutes post-exposure and mortality after 24 hours. A LLIN meets the WHO criteria when KD>95% and/or mortality>80% after 20 washes. Interceptor[®] net caused moderate KD and mortality of *An. darlingi* before washing (KD = 83.0%; mortality = 49.0%), but after first wash efficacy improved (KD = 92.0%; mortality = 79.0%). Efficacy then decreased according to the number of washes: 5 times (KD = 97.0%; mortality = 76.0%), 10 times (KD = 87.0%; mortality = 68.5%), 15 times (KD = 87.5%; mortality = 64.0%) and 20 times (KD = 80.0%; mortality = 62.5%). In conclusion, Interceptor[®] against susceptible *Anopheles darlingi* from Zungarococha (San Juan) had a moderate efficacy without and with washing, and did not meet the WHO criteria.

ACCEPTABILITY AND INITIAL USE OF LONG-LASTING INSECTICIDE NETS (LLIN) AS MEASURE TO PREVENT AND CONTROL MALARIA IN COLOMBIAN BORDER AREAS

Edison Soto, Andres Cuervo, Angel Rosas, Daniel Vargas, Lina Florez, Luis Valera

Multi-Country Malaria Project PAMAFRO - Organismo Andino de Salud - Convenio Hipolito Unanue, Lima, Peru

With the support of the Global Fund's Multi-Country Malaria Project PAMAFRO, Colombian Malaria Program has incorporated the Long Lasting Insecticide Net (LLIN) distribution as important strategy to prevent and control malaria in 5 departments that share borders with Andean

Region countries (Arauca, Guainía, Nariño, Putumayo and Vichada). From April to October 2007, 41,978 Olyset® LLINs were delivered in 229 localities, covering 10,581 households and 71,105 citizens. This study was undertaken to determine the acceptability and initial use of the LLINs as measure to prevent and control malaria in Colombian border areas. Following the introduction of LLIN (3 to 6 months after), a cross-sectional community-based survey was undertaken in 1,973 households from 90 localities, using a structured questionnaire, to assess the demographic variables relevant to LLIN use, acceptability of LLIN and net adverse reactions. Most of people reported to have slept under the net the previous night (92,1%). General LLIN acceptability was high (96,3%), however some specific characteristics of LLIN did not have high acceptability as the too big mesh size (72,1%), mainly in Amazon departments. There were only temporary mild adverse reactions (3%) after use of LLINs, mainly body itch. In conclusion, LLINs were widely acceptable in the user communities of Colombian border areas and their initial use was very high. We will continue promoting and monitoring appropriate LLIN use having into account the local needs and preferences.

229

THE IMPACT OF ANTIMICROBIAL PEPTIDE KNOCK DOWN BY RNA INTERFERENCE ON LONGEVITY AND REPRODUCTIVE OUTPUT IN *Aedes aegypti*

Raul Ursic Bedoya, Courtney Mckay, Carl Lowenberger
Simon Fraser University, Burnaby, BC, Canada

Aedes aegypti is a major vector of arboviruses in the tropics and has become an important research organism to study insect-virus interactions. Insects, including mosquitoes, have a potent innate immune system to eliminate invading microorganisms. Cellular immunity is assumed by hemocytes, which phagocytose small microorganisms. Humoral defenses are characterized by a large number of potent antimicrobial peptides, reactive intermediates of nitrogen or oxygen and a complex enzymatic cascade yielding in clotting or hemolymph melanization. To evaluate the role of humoral defenses in *Aedes aegypti*, we silenced the expression of three different antimicrobial molecules (cecropin, bomanin and lysozyme) using RNA interference and assessed the effects of the knock down on longevity and reproductive output in response to bacterial infection. Gene silencing was verified by real time PCR. Our results show no significant difference in mortality or reproductive output when cecropin, bomanin or lysozyme are knocked down. The data supports the notion that antimicrobial molecules function as a later component of the insect immune response.

230

HUMAN INSULIN INGESTED BY *ANOPHELES STEPHENSII* MOSQUITO SIGNALS IN THE MIDGUT TO INCREASE OXIDATIVE DAMAGE AND REDUCE LIFESPAN

Nazzy Pakpour, Laura Dickson, Kong Cheung, Shirley Luckhart
University of California Davis, Davis, CA, United States

Plasmodium parasites, the causative agent of malaria, are transmitted through the bites of infected *Anopheles* mosquitoes and take up to two weeks in the mosquito to develop into the infectious sporozoite form. The potential exists to disrupt this development by enhancing the mosquito's innate immune system and/or by shortening the lifespan of the mosquito. The insulin/IGF-1 signaling (IIS) cascade can regulate both innate immunity and lifespan in a variety of organisms including *Caenorhabditis elegans* and *Drosophila melanogaster*. We have previously shown that ingested human insulin leads to the activation of Akt in the mosquito midgut, a downstream signaling molecule of the IIS cascade. We have also shown that mosquitoes fed bloodmeals containing human insulin died at a faster rate than blood fed controls. In mammals, the reduction in lifespan observed following induction of the IIS cascade has been linked to increased oxidative damage that occurs due to increased synthesis of reactive oxygen species and down-regulation of antioxidants.

We show here that *An. stephensi* mosquitoes fed bloodmeals containing human insulin had increased phosphorylation of the transcription factor FOXO, which regulates production of antioxidants, such as manganese superoxide dismutase (MnSOD). Additionally, mosquitoes fed bloodmeals containing human insulin on a weekly basis accumulated higher levels of oxidative damage, as determined by total protein carbonyl content. This increased oxidative damage could be reduced by the inclusion of a cell-permeable SOD mimetic in the bloodmeal. Taken together, our data suggests that the IIS induces the synthesis of reactive oxygen species while down regulating antioxidants, leading to increased oxidative damage and a reduction in lifespan. Therefore, manipulation of the IIS cascade in *Anopheles* mosquitoes could be a valid method for reducing the vectorial capacity of these mosquitoes.

231

USING ENHANCED REPRODUCTION AS A NOVEL DRIVE MECHANISM FOR MOSQUITO POPULATION REPLACEMENT

Anam Javed¹, Jason L. Rasgon², Kendra M. Quicke¹, Michael A. Riehle¹

¹University of Arizona, Tucson, AZ, United States, ²Johns Hopkins University, Baltimore, MD, United States

A promising proposal for controlling mosquito-borne diseases is to replace the wild mosquito populations with ones incapable of transmitting disease, such as dengue or malaria. A key step towards implementing population replacement is to devise a mechanism to drive effector genes into natural mosquito populations. We propose that increased reproduction could effectively drive transgenic mosquitoes through a wild population. In *Aedes aegypti*, the primary vector of dengue fever and dengue hemorrhagic fever (DHF), and other mosquitoes the insulin/ insulin growth factor I signaling (IIS) cascade is a key regulator of many physiological processes, including reproduction. Using RNAi, we knocked down expression of the IIS inhibitor, PTEN, leading to increased insulin signaling. Knockdown of AaegPTEN or its specific splice variant AaegPTEN6 (the splice variant responsible for regulating reproduction in the ovary and fat body) led to a 15-63% increase in egg production and up to a 32% increase in egg viability during the first reproductive cycle. Knockdown of AaegPTEN3, expressed predominantly in the head, had no effect on reproduction. Modeling data demonstrated that as little as a 10% increase in reproduction would lead to population replacement within approximately 100 generations; larger fitness benefits lead to population replacement in correspondingly shorter time periods. Our results suggest that increasing reproduction through the manipulation of the IIS cascade could be a novel strategy for driving transgenic mosquitoes into wild populations.

232

FUNCTIONAL ANALYSIS OF TAK1 AND IAP2 IN THE YELLOW FEVER MOSQUITO, *Aedes aegypti*

Shin-Hong Shiao

National Taiwan University, Taipei, Taiwan

It has long been recognized that insects mount a powerful and efficient immune system to fight against invading pathogens. The insect immune response is of cellular and humoral nature, but it lacks adaptive features. They make use of two distinct signaling pathways: the Toll pathway and IMD (immunodeficiency) pathway to mount diverse antimicrobial activities. Here, we identified two components of the IMD pathway, namely inhibitor of apoptosis 2 (IAP2) and transforming growth factor-activated kinase 1 (TAK1) in the yellow fever mosquito *Aedes aegypti*. The transcripts of both IAP2 and TAK1 were analyzed by Real-Time PCR and were increased after *E. coli* challenge. Silencing of IAP2 and TAK1 significantly decrease the survival rate upon *E. coli* but not *S. aureus* challenge, suggesting that both IAP2 and TAK1 involved in the expression of IMD-dependent antimicrobial peptides. The translational expression of IAP2 was increased after *E. coli* challenge over time. Tissue specific expression analysis were examined