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ABO POLYMORPHISM AND *PLASMODIUM FALCIPARUM* MALARIA

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Malaria has been a major selective force on red blood cell (RBC) polymorphisms that confer protection to severe disease. Several lines of evidence suggest that the outcome of *Plasmodium falciparum* infection may also be influenced by ABO blood group antigens. Blood type O predominates in malaria endemic regions and has been associated with protection from developing severe and complicated malaria. Although the molecular details of protection has not been fully elucidated, previous studies have demonstrated reduced rosetting in type O RBCs. Based on observations showing enhanced phagocytosis of infected RBCs occurs with other RBC polymorphisms associated with protection, we hypothesized that infected type O RBCs may be more efficiently cleared by the innate immune clearance than type A RBCS. Here we show that primary human macrophages phagocytosed *P. falciparum*- infected type O RBCs more avidly than infected type A RBCs ($p<0.001$). Furthermore, that hemichrome deposition in infected type O RBCs is significantly greater than in infected type A RBCs ($p<0.05$), which may account for enhanced recognition and phagocytosis of type O infected RBCs. Collectively our data suggest that type O individuals may have more proficient clearance of infected RBCs contributing to an overall decrease in parasite burden and a reduction of the number of infected erythrocytes available to bind within the microvascular beds of vital organs. This represents an additional putative mechanism by which blood type O may contribute to protection against severe malaria.

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MOLECULAR CHARACTERISATION OF PYRETHROID RESISTANCE IN *ANOPHELES FUNESTUS*, MALARIA VECTOR IN AFRICA

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A major QTL *rp1* conferring pyrethroid resistance to the malaria vector *Anopheles funestus,* was previously identified. Here we present a fine-scale mapping of *rp1*, the identification and characterisation of the genes conferring this resistance. 650 F6 and F8 individuals from reciprocal crosses between susceptible and resistant strains were genotyped with SNPs and microsatellite markers for QTL mapping. A BAC clone containing *rp1* was sequenced and annotated. Quantitative PCR were carried out to study the expression pattern of the P450s genes and the *in vitro* interaction of the genes differentially expressed with pyrethroids was assessed. *rp1* was the major QTL explaining 85% of the genetic variance to pyrethroid resistance. Two other QTLs of minor effect *rp2* and *rp3* were detected. Fifteen genes were identified in the 120kb BAC clone containing the *rp1* QTL with a cluster of 10 P450 genes among which CYP6P9 and CYP6P4 were duplicated. These two genes were significantly differentially expressed between susceptible and resistant strains. Enzymes from these genes metabolise pyrethroid *in vitro*. Specific mutations associated with resistance were identified in CYP6P9 and CYP6P4. For each gene, two A/G SNPs were identified and genotyped for over 650 specimens. The G/G genotypes confer resistance at 100% and these could be used to design of a diagnostic assay to detect this metabolic resistance. In conclusion, CYP6P9 and CYP6P4 are the main genes conferring pyrethroid resistance in the laboratory strain FUMOZ-R. Further studies will be carried out to estimate their contribution in the pyrethroid resistance in field populations.

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TOXICITY OF HIGHLY SELECTIVE CARBAMATES TOWARDS THE MALARIA MOSQUITO, *ANOPHELES GAMBIAE*

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Insecticide-treated bednets (ITNs) are an important tool for the management *of Anopheles gambiae*, the major vector of malaria in Africa. Pyrethroids are the only insecticides approved for bednet treatments; however, widespread resistance and lack of alternative chemicals undermine the use of ITNs for mosquito control. Our research focus is to develop highly selective insecticides with high mosquito toxicity and low mammalian toxicity that might be used in parallel with current-use pyrethroids. We report the re-engineering of carbamate insecticides to increase selectivity and mitigate resistance development in *An. gambiae*. Based on mosquito acetylcholinesterase (AChE) protein homology modeling, we have synthesized new carbamates that are highly selective to *An. gambiae* AChE. Anticholinesterase activities of each carbamate were evaluated for both human and mosquito AChEs and compared to those of propoxur (WHO standard for mosquito control), and other conventional carbamate insecticides. We demonstrate novel carbamates of greater selectivity (ca. > 8000-fold) towards *An. gambiae* AChE, compared to 3-fold selectivity with propoxur. The new carbamates have increased potency towards mosquitoes (ca. 60-fold) than that of propoxur. We confirm both intrinsic and contact mosquito toxicity of these carbamates and demonstrate comparable toxicities to that of propoxur, and other conventional carbamates. With such high levels of selectivity, potency and toxicity, these novel carbamates provide valuable leads to developing of alternative mosquitocides for use in insecticide treated bednets and indoor residual sprays. Our findings are important in the search for new mosquito selective-insecticides and the possible use of these carbamates in malaria control programs will be discussed.

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COMBINING ORGANOPHOSPHATES AND REPELLENTS ON FABRICS: A PROMISING STRATEGY TO BETTER CONTROL PYRETHROID RESISTANT MOSQUITOES

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With the spread of pyrethroid resistance in most mosquito vector species and the lack of alternative compounds for public health, the search for new strategies that provide better control of resistant populations has become a priority. A new concept was developed in the laboratory by mixing repellents and non pyrethroid insecticides. Here, this concept was studied for personal and community protection under field conditions in Benin and Burkina Faso, West Africa**.** Indeed we studied the efficacy of battle-dress and bed nets impregnated with organophosphate(PM)/ repellent(DEET or KBR) mixtures, respectively against *Aedes aegypti,* the main dengue and yellow fever vector and *Anopheles gambiae*, the main

malaria vector, First, KBR and PM+KBR impregnated battle-dress allowed better protection against *Ae. aegypti* bites than permethrin impregnated battle-dress. Secondly, results showed evidence of synergism between repellents (DEET or KBR) and pyrimiphos-methyl (PM) on nets in field conditions. PM+DEET and PM+KBR treated nets were as effective as a standard pyrethroid (deltamethrin 25mg/m²) against susceptible *An. gambiae* populations and more effective against resistant *An. gambiae* populations. Results also demonstrated that mixtures did select neither *Kdr* allele nor *Ace1*^{R} allele. In conclusion, hese field trials showed that mixing repellents and organophosphates has the potential to be a good alternative strategy to manage the spread of resistance. However, significant improvements remain to be done to improve residual effect of Insecticide-Repellent Treated fabrics.

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DEVELOPMENT OF A NOVEL FORMULATION FOR USE IN INDOOR RESIDUAL SPRAY PROGRAMS

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Fenitrothion wettable powder (WP) is recommended by the World Health Organization (WHO) for Indoor Residual Spraying (IRS) against malaria vectors. However, with the increase in the use of Long Lasting Insecticidal Nets (LLINs) as a low cost and highly effective intervention, IRS has more recently been used in many parts of Africa as a secondary treatment option, or for use in epidemic zones. In some instances, particularly in highly malarious areas, the use of LLINs and IRS together can give very dramatic reductions in incidence. With the focus now turning towards the elimination or eradication rather than control of malaria, the combination of several vector control interventions combined with the administration of effective anti- malarial drugs will become the norm as countries step up their efforts to eliminate this parasite. There is an increasing concern over the development of resistance to pyrethroids, possibly affecting LLIN performance, which are currently all dependent on this insecticide class. To minimize selection pressure the use of pyrethroid-based IRS products is not recommended with LLIN applications. Alternatives to the widespread use of pyrethroid and DDT-based IRS products are clearly needed (kdr resistant insects share a common resistance mechanism to DDT and pyrethroids). To meet this need, a novel Sumithion® IRS formulation is being developed. Laboratory trials to evaluate residual efficacy on a range of representative substrate types against *Anopheles* mosquitoes have been conducted. This data, along with interim results of Phase II hut studies being conducted in Benin are presented.

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HUMAN ANTIBODY RESPONSE TO *ANOPHELES GAMBIAE* SALIVA: A NEW IMMUNO-EPIDEMIOLOGICAL MARKER TO EVALUATE THE EFFECTIVENESS OF INSECTICIDES TREATED NETS (ITNS)?

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In a way to improve malaria control, many efforts are conducted under WHO recommendations to develop new tool/indicator for malaria control, such as for evaluating the anti-vector strategies. Previous studies have shown that the evaluation of human antibody (Ab) response to arthropod salivary proteins represent an epidemiological indicator of exposure to vector bites, and especially our team demonstrated that IgG response to whole saliva of *Anopheles gambiae* in exposed individuals represent a marker of the intensity of *Anopheles* exposure. The objective of the present study was to validate whether this immunological marker based on human anti-saliva IgG Ab levels could be one new indicator to evaluate the effectiveness of ITNs use in malaria control programs. One longitudinal study, concerning individuals (n=108, children and adults) living in malaria endemic area in Angola, was performed from March 2005 to October 2006. The studied cohort was followed for parasitological, clinical, entomological and immunological data, each 6 weeks before and after the well-controlled use of Permanet® mosquito nets (Long Lasting Insecticide Net; installation in Feb. 2006). Seasonal variations of anti-saliva IgG Ab levels to *An. gambiae* saliva were observed before and after the installation of ITNs which appeared to be associated with the exposure to *An. gambiae* (evaluated by the classical entomological methods) and the prevalence/intensity of malaria infection. Moreover, a significant decrease of the anti-saliva IgG response was observed after the ITNs use which was correlated with the decrease of malaria parasitemia, the current and referent criteria showing the effectiveness of these ITNs. In a way to identify new tools for malaria control, we have shown that anti-saliva IgG response in exposed individuals could be not only an immuno-epidemiological marker of exposure to *An. gambiae* bites, but also a potential indicator for evaluating the ITNs effectiveness. Several future studies are needed to confirm this hypothesis in other transmission areas and to identify some immunogenic salivary proteins as higher specific markers. Nevertheless, this study represents a first approach to elaborate such new indicators for evaluating the effectiveness of anti-vector strategies, bases on the evaluation of human Ab response to salivary proteins of arthropod vectors.

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EFFICACY OF INSECTICIDE TREATED MATERIALS (ITMS) FOR DENGUE CONTROL IN LATIN AMERICA AND ASIA: CLUSTER RANDOMIZED CONTROLLED TRIALS IN VENEZUELA AND THAILAND

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Dengue fever is the fastest spreading arboviral disease worldwide. In the absence of a vaccine, *Aedes aegypti* vector control remains the most effective strategy to prevent dengue transmission. Our initial studies in Latin America indicated that insecticide treated materials (ITMs) can impact on dengue vector populations and potentially on dengue virus transmission. Cluster randomized trials are underway in Venezuela (6000 households in 75 clusters) and Thailand (2000 households in 26 clusters) to further clarify the efficacy of ITMs in suppressing dengue vector populations. These trials incorporate several advances on the earlier studies: first, different types of ITMs are being tested alone and in combination and householders may choose the manner of deployment; secondly, spill-over effects of the interventions into neighboring control areas are monitored by including external control sites; thirdly, efficacy of ITMs for dengue vector control is measured on a large scale for the first time in SE Asia. Both study sites had high entomological indices at baseline (Venezuela average pupae per person index = 0.52, average Breteau index = 15.3; Thailand average pupae per person index = 0.22, average Breteau index = 22.4), and the ITM interventions were adopted and maintained by the population in both sites to a similar extent, although their manner of deployment varied (Venezuela: window curtains and jar covers; Thailand: indoor and window curtains). Although the trials are set to complete in early 2009, preliminary data and analyses will be presented and important

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Combining organophosphates and repellents on fabrics : a promising strategy to better control pyrethroid resistant mosquitoes

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