WORKSHOP

Malaria transmission: current challenges and new tools in the elimination context

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BOOK OF ABSTRACTS

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PLENARY SESSIONS

GEOSPATIAL MODELLING OF CHANGING MALARIA VECTOR POPULATIONS

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A continent-wide geospatial analysis of reductions in malaria infection prevalence in Africa has previously shown that insecticide-treated bednets, indoor residual spraying and artemisinin-based combination therapy are linked to dramatic reductions in malaria prevalence from 2000 to 2015. These interventions do not, however, explain all of the variation seen in infection prevalence and information about mosquito populations was not included in the original analysis.

New research on the role of vectors species, relative abundance of these species and insecticide resistance in malaria vectors will be presented in the context of an Africa-wide geospatial analysis of trends in malaria infection prevalence. Taking this continent-wide approach, we have mapped the changing vector species composition after insecticide-based interventions have been introduced. We have also used these estimates of relative vector species abundance to explore some of the factors linked to residual variation in malaria infection prevalence. Finally, we have started to map insecticide resistance in these vectors in time and space. The preliminary results for pyrethroid resistance in the An. gambiae species complex will be presented and future work to incorporate the potential drivers of selection will be discussed.

CURRENT APPROACHES AND NEW TOOLS TO MEASURE MALARIA TRANSMISSION

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Last decade has witnessed a significant decline in the number of malaria deaths and cases worldwide. This decline has motivated more than 30 countries to commit to the goal of eliminating the disease until 2030. However, the success of such elimination agenda is intimately dependent on how these countries can accurately measure the residual malaria transmission intensity. The total number of official cases, although widely used by public health authorities, does not include the information from asymptomatic individuals, thus, underestimating the actual malaria transmission intensity. The parasite and entomological inoculation rates also show limitations due to the high chance of not finding any infected individual or mosquito in routine surveillance studies. In this scenario, antibody-based estimates of malaria transmission, such as seroprevalence and seroconversion rate, are gaining wide interest as they aim to quantify the exposure of the population to malaria parasites instead of presence of infection. This talk builds from the current concepts of a single-antigen serological analysis to discuss how they can be used to inform malaria elimination using antibody data generated from the new multiplex platforms.

THE ECONOMIC CHALLENGES AND BENEFITS OF ELIMINATING MALARIA

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Both large economic challenges and large economic benefits are associated with malaria elimination. On the one hand, malaria elimination implies strong financial efforts in the short term, which are difficult to harmonize with political cycles and with competing problems and interests. In addition, embarking in malaria elimination activities is risky and policy makers may prefer to invest in safer actions. Malaria elimination is a public good with associated "free riding" issues: while everyone can benefit from it, the individual incentive to contribute can be weak. Malaria elimination is extremely equitable but what happens if the initiatives fail and the burden remains concentrated among the poorest?

On the other hand, malaria elimination leads to long-term benefits that go beyond health. Impacts on tourism, foreign direct investments, workers' productivity, are all likely to be relevant. Improvements of school attainment, in particular, have been found consequent to past malaria elimination campaigns. Improvements of school attainment are a prerequisite for human capital accumulation and for economic development in the long-term. Within the school context, we report encouraging preliminary findings of the short-term impact of a current malaria elimination initiative in a district of Southern Mozambique. We found a 23% decrease in primary school children absenteeism and an improvement of 1.4% in grade levels (all subjects) due to the initiative.

In conclusion, interrupting malaria transmission is associated with a number of economic challenges that add complexity to the already known biological and logistical ones. Importantly, economic long-term benefits are likely to go beyond healthcare savings and involve the improvement of economic growth and development. These benefits should not be neglected by researchers and by policy makers when taking decisions on malaria elimination.

IMPACT OF GENETIC AND BIOECOLOGICAL HETEROGENEITIES OF MOSQUITO VECTORS ON MALARIA TRANSMISSION AND CONTROL IN AFRICA: FUTURE PERSPECTIVES IN CONTEXT OF ELIMINATION AND GROWING URBANIZATION

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One of the main reasons for the highest malaria burden in sub-saharan Africa is the existence of a vectorial system characterized by the highest levels of genetic and bio-ecological heterogeneities due to multiple (and sometimes cryptic) species, intra-specific genetic differences and high ecological/behavioural plasticity. In the last few thousands of years this high genetic and ecological flexibility has allowed the main Afrotropical malaria vector species to co-evolve in close association with humans and with changes in human habits and behaviour, starting from the shift from hunting and foraging to sedentary agriculture. Population genomic data clearly show that this evolutionary process is still ongoing triggered by insecticide-based interventions against malaria vectors as well as against agricultural pests and, likely, also by novel human-made environmental changes such as urbanization and deforestation. I will present relevant examples of this process and discuss how this is affecting future perspectives of vector control in the context of malaria elimination.

BEYOND 2020: PUSHING THE ENVELOPE OF MALARIA ELIMINATION TOOLS

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Preventing malaria transmission from human to mosquito and back to humans remains one of the biggest challenges that must be overcome if we are to succeed in regional malaria elimination and global eradication. Two significant challenges include: (a) identifying and treating individuals with subclinical or asymptomatic malaria infections that are infectious to mosquitoes and (b) developing scalable and potent transmission-blocking interventions. Progress and pitfalls viz. these two challenges will be presented and discussed with respect to two interventions: a prototype saliva-based rapid test for detecting subclinical malaria parasite carriers through a novel parasite biomarker and a mosquito-based universal alanyl aminopeptidase N (AnAPN1) malaria transmission-blocking vaccine that targets a midgut-surface antigen of the Plasmodium parasite's obligate invertebrate vector, the Anopheles mosquito. Several gaps in our knowledge remain at both ends of the discovery to implementation pipeline for both interventions; specifically, the basic biology and operational use. These issues and their potential solutions are clearly instructive and will be explored throughout the presentation.

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MIXED PARASITE INFECTIONS: PERSPECTIVES AND IMPLICATIONS IN CONTEXT OF URBAN MALARIA AND TARGETED MALARIA ELIMINATION PROGRAM IN INDIA

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India is highly endemic to malaria with prevalence of all five species of human malaria parasites of Plasmodium genus. India is set for malaria elimination by 2030. Since cases of mixed *Plasmodium* species infections remain usually undetected but cause huge disease burden, in order to understand the distributional prevalence of both mono- and mixed species infections in India, we collated published data on the differential infection incidences of the five different malaria parasites based on PCR diagnostic assay. About 11% of total cases were due to mixed-species infection. Among several interesting observations on both single and mixed parasitic infections, incidences of *Plasmodium falciparum* mono infection were found to be significantly higher than *P. vivax* mono infection. Also, *P. malariae* seems to be emerging as a potential malaria threat in India. Interestingly, *P. vivax* was found to be dominant mainly in metropolitan cities like Delhi (North India) and Chennai (South India) with no cases of *P. falciparum* and mixed infections. On the other hand, *P. falciparum* had shown its prevalence in the central as well eastern and western parts of India. Such variable distribution of parasites can also be attributed to the distribution of vectors of malaria parasite, thereby resulting in variable patterns of infection. Adding to complexity of malaria, India has nine abundant mosquito vectors responsible for malaria. After correlating the results with the prevalence of vectors what we found is that the place where mono-infection by *P. falciparum* is high (Jharkhand, Chattisgarh-mostly rural) has an abundance of *Anopheles fluviatilis* whereas those which show high mono-infection by *P. vivax* are abundant (Delhi, Chennai-urban areas) in *An. stephensi*. Furthermore, places with lot of diversity of infection like Odisha and Assam have more than two vectors in abundance including *An. dirus, An. minimus* etc. *An. culicifacies* is present almost everywhere except Andaman and Nicobar Island where the most prevalent mosquito vector is *Anopheles sundaicus* making *P. knowlesi* restricted to that part of India. Putting all the facts together, it appears that the dream of achieving malaria elimination in India will not be completely successful without dealing with mixed-species infection and the controlling the vector abundance keeping in mind the complex geography and diversity that India hails.

LOCAL EPIDEMIOLOGY AND SPATIAL ANALYSIS OF MALARIA TRANSMISSION IN THE BRAZILIAN AMAZON

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Malaria is a problem in the Americas, Brazil accounted for 37% of the cases in 2015 and of these 99.5% are located in the Brazilian Amazon. Despite the mobilization of resources from Brazilian National Plan for Malaria Control, there are still too many municipalities with moderate and high transmission levels. Several factors modify the dynamic of malaria transmission. Spatiotemporal statistics in conjunction with the Geographic Information Systems have been used to understand this transmission. The aim was to describe and analyse the epidemiological profile of autochthonous malaria and analyse the spatio-temporal transmission in the municipalities of the Brazilian Amazon, in the period 2010 to 2015. Malaria data were stratified by the annual parasite incidence (API), over the six years and by municipality. Time-series and spatial analysis were performed to analyse the API. We used the method of seasonal decomposition by loess smoothing to capture trend, seasonal and irregular components. LISA indicators, scan statistics were used to explore autocorrelation. Malaria API has declined 61% from 2010 to 2015, and there was a 40% reduction of municipalities with high transmission (API higher than 50). This represents 9.4% of all the municipalities in the study site and 63% of all cases, in 2015. The time-series analyses showed different incidence patterns by region after 2012; some States have minimized the effect of the seasonality in their incidence rates. There is spatial autocorrelation locally and globally. To conclude, the Brazilian National policy to control malaria was useful to achieve the national and millennium development goal for malaria control. However, it is necessary that strategies to control and prevent malaria include the use of these spatial statistics to plan more effective interventions that take into account the epidemiological profile of every municipality or local context since environmental and socioeconomic risk factors presented different dynamics in the Brazilian Amazon. Furthermore, this study presents the reduction of cases during malaria season, indicating that, even in a very suitable environment, malaria can be controlled when epidemiological adjusted interventions are applied.

MALARIA DETERMINING RISK FACTORS AT THE HOUSEHOLD LEVEL IN TWO RURAL VILLAGES OF MAINLAND EQUATORIAL GUINEA

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The reduction of prevalence of malaria infections showing remarkable progress during the last decade, after the introduction of an artemisinin-based combination therapy, however the lack of a consistent malaria control program and the socioeconomic inequalities, Plasmodium infection still is the leading causes of disease in Equatorial Guinea, namely in the rural communities. This study explored the associated risk factors of malaria transmission at the microeconomic level (households) in two rural villages of mainland Equatorial Guinea.

This survey involved 232 individuals living in 69 households located in 2 rural villages of coastal and interior of Equatorial Guinea. Malaria prevalence was measured by PCR and parasitaemia, and household socioeconomic status (SES) was measured based on house characteristics using a 2-Step Cluster Analysis. Logistic regression analysis was performed to investigate the relationship of a diverse set of independent variables on being diagnosed with malaria and on showing high level of parasitaemia.

The prevalence of Plasmodium spp. infection was 69%, with 80% of households having at least one parasitaemic member. The majority of houses have eaves (80%), walls of clay/wood (90%) and zinc roof (99%) and only 10% have basic sanitation facilities. The studied areas showed reduced rates of indoor residual spraying coverage (9%) and long-lasting insecticide-treated net ownership (35%), and none of these preventive tools showed any significant effect on malaria risk in these areas. The risk of malaria infection (PCR positive result) did not shown positive association with SES, but results point to an increased vulnerability to develop high parasitaemia by “Poor” SES group.

This study has contributed to identify the most relevant living conditions associated to a high risk of malaria infection and vulnerability to develop high parasitaemia on two villages of mainland Equatorial Guinea, which may help future malaria control interventions to be implemented in mainland Equatorial Guinea.

LEVERAGING ARTIFICIAL INTELIGENCE TO IMPROVE MALARIA EPIDEMICS' RESPONSE

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As the world advances toward malaria elimination, the elimination management paradigm has to change to address early case detection in more local and remote areas. Remote areas face additional difficulties in both detection and treatment demanding innovative approaches. Malaria elimination needs evidence-based decision-making with real-time access to malaria cases data. Years of endeavour towards malaria elimination have created several databases. Unfortunately, these databases often lack interoperability making the use and the crossing of data difficult. Moreover, the access to early alerts can promote decision-maker's leadership and quick action in launching early interventions particularly in some low-resources settings.

Therefore, a smart, comprehensive, sustainable and integrated information system is required to support this challenge. We propose a collaborative design implementation strategy combining simple elements of gamification, Geographical Information System (GIS) and Artificial Intelligence (AI) to enable early-detection and risk of epidemics alerts (e.g. combining actual patterns of malaria with CDC Heuristic-based alerts), and to direct interventions around detected cases. Both GIS/DHIS2 and AI will enable real-time forecasts helping improve malaria interventions response time, hopefully anticipating and preventing the spread of new cases.

Supporting this idea, new technologies can be combined to further reinforce both the sustainability of data collection and the behavioural change of public health decision-makers in malaria risk management. The use of gamification (e.g., adapting tasks into the form of a game to motivate participation) could be particularly useful to improve data entry quality. Timely reporting of malaria cases is a key stage that is currently weak. It often depends on the professionalism of overloaded and scarce health agents that may benefit from an extra motivation to improve case reporting. Furthermore, gamification can be linked to a more transparent quality supported incentive program.

The system's approach to implementation should both strengthen the available information integration processes and decision-makers' capacity to decide and act by engaging them in a collaborative design process that enables the alignment with the malaria elimination decision processes.

The success of such a system depends mostly on how elimination actions will be improved in real settings. Therefore, co-designed science research methodology could both engage health professionals and use evidence-based knowledge in the design of an innovative system that responds to what public health professionals' real needs.

The system should provide forecast capabilities and use cognitive computing to detect patterns associated with malaria epidemics (e.g. using IBM-Watson discovery advisor functionalities) providing the right context/framework to facilitate quick decision-making. Information visualization is very important. Basic gamification functionalities will be applied collaboratively with users to encourage behaviour change, improved resilience and awareness of the time-lapse from information reception to action.

STORIES FROM THE PAST AND EVERYDAY EXPERIENCES OF MALARIA: PORTUGAL, 1930-1960

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This paper is a short story about malaria in Portugal from 1930 to 1960. It is the story of a time when malaria was not just a tropical disease but also a domestic ailment that hassled rural populations at intervals. It is malaria as perceived and defined by former rural workers that I intend to take as the core element of this reflection on malaria as a broad social, ecological, economic and political event, that exposes shifting and multiple definitions. By recovering personal memories of people who had malaria, I intend to retrieve the physical dimension of the disease, the local appropriation of medical models and resources, and the coexistence of different ways of perceiving the disease. Narratives about experiencing malaria reveal its broader ecology, combining natural environment, personal, social, cultural, political, economic and historic factors. They also reveal the contradictions, misunderstandings and intersecting meanings that framed (and still do) malaria, taking this as fundamental to a broader understanding of the disease's complexities patent in the distance between scientific knowledge, sanitary regulation, institutional norms and the everyday practice of malaria control and treatment on the ground. Thus, personal narratives about 'having malaria' may also inspire a reflection about the estrangement between the 21st century's scientific agendas for malaria research and the compound everyday experience of living and managing this disease. Furthermore, malaria memories add to the medical narratives about this disease, meeting contemporary approaches that call attention to its social burden. History meets the present and calls attention to sometimes overlooked perspectives on health and illness, despite international calls on the importance of taking into account the social determinants of disease.

EFFECTIVENESS OF PRIVATE SECTOR MALARIA CONTROL: THE CASE OF SUGARCANE WORKERS IN SOUTHERN MOZAMBIQUE

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A notable recent increase in foreign direct investment in developing countries has raised the question of the potential for large foreign firms to engage more actively in malaria control and elimination activities. However, a prerequisite to a wilful "scaling up" of private sector involvement in fighting malaria is a clear quantification of the return on investment of malaria control activities, from a private perspective. If fighting malaria is demonstrated to be profitable from an investment standpoint, then the only factor preventing more private sector involvement is dissemination of knowledge regarding strategies for combatting malaria. If, on the other hand, private malaria control activities are shown to be unprofitable from a purely financial standpoint (regardless of their social good components), then a focus on petitioning the private sector to play a more active role might be wasteful, and energy would be better spent on recruiting firms' capital (through taxes), rather than guiding their activities.

In this study, we directly address the question of the return on investment of privately-managed malaria control activities from a purely financial standpoint. Using granular absenteeism, malaria control, demographic, and clinic data from a large sugarcane plantation in southern Mozambique, we quantify the effectiveness of firm-managed indoor residual spraying campaigns, employing discontinuity regression to estimate the odds of absence for malaria among workers while accounting for biases through propensity score matching.

Our results suggest that privately-run malaria control activities result in a significant reduction in the incidence of absence and clinical malaria. However, from the narrow perspective of the balance sheet, private firms engaging in malaria control activities should not expect a significant return on investment. Nonetheless, significant benefits do potentially accrue in the realms of public and labor relations, not to

mention the health of employees. Low return on investment may partially explain private sector hesitance to engage in malaria control activities more directly, while also suggesting that those firms which do engage in malaria control may do so for reasons other than pure financial benefit. From a policy standpoint, the lack of financial profitability in malaria control activities suggests that engaging the private sector more fully in the transition to elimination will not be straightforward. Rather it will require one of the following three approaches: (i) "nudging" the private sector through an emphasis on the non-financial benefits of corporate social responsibility, (ii) incentivizing private sector involvement through subsidies for malaria-related activities, or (iii) coercing private sector involvement through taxes or malaria-related policies.

EVALUATION OF THE OWNERSHIP AND USE OF LONG-LASTING INSECTICIDAL NETS IN RURAL MOZAMBIQUE: A CROSS-SECTIONAL HOUSEHOLD SURVEY SIX MONTHS AFTER THE PILOT OF A NEW DELIVERY CAMPAIGN MODEL

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In 2015 Mozambique piloted a new model of long-lasting insecticidal nets (LLINs) delivery in campaign. Two rural districts were intervened with the new delivery model (Gurue and Sussundenga), and two were considered as control (Alto-Molocue and Machaze) maintaining the old delivery model. The aim of this study is to evaluate the effect of the new delivery model in relation to the old delivery model regarding the coverage of ownership and use of LLINs in the intervention and control districts in Mozambique.

A representative cross-sectional household survey was carried out six month after the campaign implementation. A two-stage cluster sampling method was used, and a total of 1,547 households were randomly and systematically surveyed. The analysis between the district categories (with new or old delivery model), with other categories of the study (campaign bed nets ownership, use and universal coverage achievement) was performed through chi-square test, and odds ratio (OR) and their confidence intervals were calculated for each category analyzed. For all statistical procedures, a 0.05 significance level was adopted for rejecting the null hypothesis.

Of the 760 surveyed households in the intervention districts (Gurue and Sussundenga), 98.8% had at least one LLIN from the campaign; of the 787 surveyed households in the control districts (Alto-Molocue and Machaze), 89.6% had at least one campaign LLIN (OR: 9.7, 95% CI: 5.25 - 22.76). Of these, 95.4% and 86.8% reported that they slept under the campaign LLIN the previous night in the intervention and control districts, respectively (OR: 3.2; 95% CI: 2.12-4.69). Seventy-one percent of the surveyed households achieved universal coverage target in the intervention districts against 59.6% in the control districts (OR: 1.6; 95% CI: 1.33 - 2.03).

Conclusions: The universal coverage campaign piloted with the new delivery model has increased LLINs ownership, use, and progression for reaching universal coverage targets in the community.

HUMAN ANTIBODY RESPONSES TO THE ANOPHELES SALIVARY gSG6-P1 PEPTIDE: A NOVEL IMMUNO-EPIDEMIOLOGICAL BIOMARKER TOOL FOR EVALUATING THE EFFICACY OF MALARIA VECTOR CONTROL METHODS

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In order to contribute to the improvement of the evaluation of the efficacy of vector control strategies, it was developed primarily a study based on the human antibody response (IgG) to the *Anopheles* mosquito saliva (gSG6-P1 Peptide). Subsequently this potential tool was used in the evaluation of the efficacy of the use of long lasting impregnated mosquito nets (LLIN), insecticide treated plastic sheeting (ITPS) and indoor residual spraying (IRS). Longitudinal assessments including parasitological, entomological and immunological component, were carried out in five villages in Balombo municipality of Angola, every two months from February 2008 to December 2009. These assessments included *Anopheles* surveys and children and adults control. The evaluation of human IgG levels was carried out by enzyme-linked immunoenzymatic assay. The use of different vector control methods resulted in considerable decreases in all three entomological (82.4%), parasitological (54.8%) and immunological criteria analyzed. After implementation of the vector control methods the medium values of specific IgG levels were significantly lower in 2009 than in 2008 for all campaigns and in all villages ($p<0.001$). The number of *Anopheles*, positive blood smears, and the levels of anti-saliva IgG Ab were most reduced when LLIN and ITPS were used in combination, compared to the use of one vector control method alone, either ITPS or IRS.

In conclusion, Human IgG response to *Anopheles salivary gSG*-P1 Peptide is proved to be an efficient and reliable indicator for evaluating the biting pressure on human population. It is also a fine marker for comparing the effectiveness of different malaria vector control methods or strategies and may represent

an alternative to classical entomo-parasitological monitoring method used by the National Malaria Control Programs in Africa.

A BIOLUMINESCENCE METHOD FOR IN VITRO SCREENING OF PLASMODIUM TRANSMISSION-BLOCKING COMPOUNDS

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The sporogonic stage of the life cycle of *Plasmodium* spp., the causative agents of malaria, occurs inside the parasite's mosquito vector. There, a process of fertilization, meiosis and mitotic divisions culminates in the generation of large numbers of salivary gland-resident, mammalian-infective sporozoites. Efforts to cultivate *Plasmodium* mosquito stages *in vitro* have proved challenging and only moderately successful. We developed a methodology employing a bioluminescence-based method to monitor the *in vitro* development of sporogonic stages of the rodent malaria parasite, *P. berghei*, aimed at simplifying the *in vitro* screening of much-needed transmission blocking (TB) compounds. Our proof-of-principle assessment of the *in vitro* TB activity of several common anti-malarials identified cycloheximide, thiostrepton and atovaquone as most active against the parasite's sporogonic stages. The TB activity of these compounds was further confirmed by *in vivo* studies, which validated the newly developed *in vitro* approach to TB compound screening. This innovative assay constitutes a fast, easy and affordable method for screening large libraries for compound effect on the development stages of *Plasmodium* parasites in the mosquito, minimizing the need for an *in vivo* model. Furthermore, it is urgent to develop and validate high throughput assays allowing for new libraries of compounds to be tested against not only *P. berghei*, but also *P. vivax* and also *P. falciparum*. In the future, new drug combinations should have not only blood stage activity, but also but also transmission blocking components. Such novel assays could help identify compounds to block transmission in the field and in the clinic.

PREGNANCY-SPECIFIC SEROLOGY TO MONITOR MALARIA TRANSMISSION IN ELIMINATION CONTEXTS

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Malaria elimination depends on reliable surveillance tools able to overcome the logistical challenges due to decrease in infection. Pregnancy-specific antibodies against VAR2CSA have the potential to provide estimation of recent exposure (one pregnancy). We aimed at developing a VAR2CSA serology to estimate trends in malaria transmission in different phases of elimination activities. Antibodies against 46 VAR2CSA-peptides were measured by luminex in plasma samples from 2402 exposed and 101 non-exposed individuals. We first selected those peptides not recognized by Spanish individuals and able to mirror changes of malaria prevalence in Mozambican pregnant women during 2003-2012 (qPCR: 27% in 2003/4, 2% in 2010 and 6% in 2012). Among the 7 peptides selected 2 were related with the expected increase of IgG responses in women who had an infection during pregnancy and seroprevalences were above the prevalence of infection at delivery. Antibodies were not observed to increase with parity and half-lives were below 2 years (average time between pregnancies), suggesting that antibodies were not maintained during successive pregnancies. Moreover, seroprevalences were associated with reductions in exposure in HIV-uninfected pregnant women who received intermittent preventive treatment during pregnancy with mefloquine compared to those who received sulfadoxine–pyrimethamine and reflected the pattern of malaria burden in Benin (Sprev=42%, PCR=41%), Gabon (Sprev=24%, PCR=10%) and

Mozambique (Sprev=15%, PCR=6%) during 2010-2012. Pregnant women living in an area from Tanzania where no malaria infection was observed were seronegative. Finally, Seroprevalences to selected peptides detected similar spatial patterns of infection during pregnancy in a rural area in Mozambique. This sero-surveillance tool could be used in pregnant women attending antenatal care clinics to provide information about changes and monitor the absence of malaria transmission resulting from elimination activities.

MALARIA SEROLOGY IN BLOOD DONORS – PERSISTENCE OF ANTIBODIES IN EX-RESIDENTS FROM ENDEMIC COUNTRIES

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The purpose of this study was to evaluate the influence of length of stay in a malaria endemic country (EC) on the rate of positivity in a screening test for anti-Plasmodium antibodies in blood donors and to analyse the population of positive donors, in view of the donor selection criteria in use in Portugal.

366 blood donors from our Center, who were submitted to the Newmarket Malaria EIA antibody screening test between January 2015 and June 2016, were analysed. They fulfilled the conditions: (1) stay in an EC with high or moderate risk; (2) information about the length of stay in EC, in order to obtain two categories: visitors (stay of less than 6 months) and residents (stay of 6 months or more); (3) information about history of malaria.

A clinical history of malaria was admitted by 47 donors and 319 denied the disease. In those with a history of malaria the rate of positivity was 47% in residents and 50% in visitors, similar in both cases.

In the 319 donors who denied the disease, 21% (38) of the 185 residents were positive and there was no case of positivity in visitors. According to Portuguese law, blood donors with a history of malaria are tested before a possible donation. Those without history of malaria may not be tested, if the recommended suspension period after last stay in an EC has been fulfilled (maximum suspension period of 3 years). Of the 38 positive donors with no previous history of malaria, potential parasite carriers, 3 were within the recommended 3-year suspension period, but 35 donors had returned more than 3 years ago. They could have been approved for donation without performing the test. In most of these 35 donors, the presence of antibodies was maintained for about 40 years after the last exposure to risk. It is demonstrated the importance of length of stay in EC on the rate of positivity, being a fundamental question in the screening of blood donors. The results show that the current selection criteria should be reformulated: ex-residents from EC must be tested, even if has been passed more than 3 years after their last stay.

POSTERS

Poster 1

MAPPING URBAN LAND USE CHANGES IN THE CITIES OF LUANDA AND BISSAU USING TIME STACKS OF LANDSAT SATELLITE IMAGERY

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The increasing urbanization of Africa is associated to significant changes in the distribution and incidence of malaria. However, the precise role that urbanization plays in these changes remains largely unknown. Addressing this question requires data on how urban areas have changed over the years, which is often unavailable from local sources. Satellite remote sensing has emerged as an inexpensive and viable option to delimit urban areas and to monitor rates and patterns of urban expansion.

The specific purpose of this study is to map changes in urban extent and density for two major African cities: Luanda (Angola) and Bissau (Guinea-Bissau). For each city, we collected Landsat (missions 5 and 7) satellite images for three time periods: 1990, 2000 and 2010. For each time period we identified urban areas and extracted their digital numbers for four spectral bands. We also extracted these numbers for 1000 locations randomly distributed across the study area. These data were used to train two supervised machine learning algorithms, Random Forests and Maximum Entropy, in classifying urban and non-urban areas. The predictive accuracy of the methods was evaluated by comparison with data left-out of model training and measured through the Area Under Curve (AUC). An AUC value of 0.5 indicates an accuracy of no better than chance and a value of 1 a perfect prediction. Classification accuracy was very good for all combinations of city, year and algorithm, with the lowest values being of AUC=0.93 (for the Maximum Entropy prediction of Bissau in 1990).

We conclude that remote sensing is a viable option for mapping long-term dynamics of African cities, which could help unravelling the role of urbanization over malaria transmission.

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Poster 2

MALARIA CONTROL STRATEGIES IN ANGOLA- 1980-2015- A HISTORY UNFOLDING

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In recent years, malaria has received its fair-share of attention. Angola was no exception. Having said this, what is the longitudinal situation regarding prevalence, number of deaths and/or control strategies in urban and rural contexts? This poster is a first step to tackle this gap and puts forward a 'sketch' of the health policies against malaria applied in the last 20 years in Angola.

Reports from national programs (PNLM- Programa Nacional Luta contra Malaria), International agencies and/or global partnerships programs in Africa (WHO - World Health Organization, UNICEF- United Nations Children's Fund, and PMI - President's Malaria Initiative) were analyzed alongside biomedical publication on these issues. This data permits to have an overview of the results and efficacy of particular health policies, and grasp the importance of particular actors.

In these past years, millions of US dollars and years of different programs aiming to control malaria were applied in Angola, and some changes regarding the incidence of the disease can be noted. There was decrease on the overall number of deaths of children and adults by malaria (from 10 000 deaths in 1990 to 7 832 deaths in 2015) and parasitaemia in children under five years old (it has decreased from 21.1% in 2005 to 13.5% in 2015). Despite such improvement which went alongside the number of people – especially women and children – owning and sleeping under an insecticide treated bed net and having access to malaria diagnosis and treatment in public health facilities, there is still room for much improvement overall, especially regarding precise diagnosis and treatment of the disease. Being a disease of poverty an array of social-economic factors (housing, malnutrition) plus others related to health systems (misdiagnosis in tandem with overmedication) are named. Increasingly urbanization is brought forward as one of the factors that may influence the stagnation of further progress in health policies. This research is but the first step in portraying and unpacking a complex process. It aims thus to pave the way for news studies on longitudinal perspective regarding societal transformations and malaria control policies and its differential incidence in Angola.

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Poster 3

MOLECULAR EVIDENCE OF POSITIVE SELECTION IN TRANSGLUTAMINASES OF ANOPHELES GAMBIAE

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Malaria is the vector-transmitted disease with most global impact. Studies on the interactions between the mosquito vector and the parasite are of utmost importance for the development of novel tools for control. Transglutaminases (TGs) are enzymes involved in coagulation and wound healing processes. In *Anopheles gambiae*, these are associated with the immune response to the infection by Plasmodium sp. In this study, we analysed 26 sequences of the TG1 gene that translate into transglutaminases. These were obtained from samples collected in Guinea Bissau on individual households, and classified as infected and non-infected mosquitoes. Phylogenetic analysis was performed comparing *An. gambiae s.s.* and *Anopheles coluzzii*, and between infected and non-infected individuals.

High nucleotide diversity was observed, and each sequence was a distinct haplotype. We detected positive selection, with π(a)/π(s) (nonsynonymous substitutions/synonymous substitutions) and dN/dS (rate of nonsynonymous substitutions per nonsynonymous site/rate of synonymous substitutions per synonymous site) ratios higher than one in the first four exons. Neutrality tests showed an associated selective sweep and excess of singletons. When comparing infected and non-infected individuals, we found the highest values of dN/dS in the second and fourth exons, which may indicate positive selection. These findings suggest that TG1 may play a role in pathogen containment, being involved in the response against the infection by *Plasmodium sp.*

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Poster 4

A PALATABLE INSECTICIDE

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Mosquitoes are a key threat to millions of people worldwide because they act as vectors for devastating pathogens and parasites. Mosquito larvae are usually targeted using organophosphates, insect growth regulators, and microbial agents. Indoor residual spraying and insecticide-treated bed nets are also employed. However, these chemicals have negative effects on human health and the environment and induce resistance in a number of vectors. Newer and safer methods of mosquito control are needed. The non-nutritive sweeteners that are approved by FDA for human use has potential for use as a novel, human-safe insecticide.

Poster 5

PLASMODIUM BERGHEI SPOROZOITES DETECTION, DIFFERENTIATION AND QUANTIFICATION DURING SPOROGONIC CYCLE IN ANOPHELES TISSUES USING FLOW CYTOMETER APPROACH.

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Malaria is the biggest worldwide public health problem and cause one to two million deaths per year. In human, It is caused by four different species of Plasmodium parasite. The life cycle of Plasmodium, is complex and occurs in mosquitoes and vertebrates. Understand the live cycle of Plasmodium in the mosquito vector is very important to development strategies to control or eliminate malaria. Aim: The aim of this work is establish a new approach to detect, differentiate and quantify Plasmodium sporozoites during the sporogonic cycle in mosquitos using flow cytometer. Methods: *Anopheles stephensi* mosquitoes infected with *Plamodium bergei* ANKA-GFP were dissected at 8; 12; 14; 16 and 18 days post-infection and 20 midguts collected each day. On day 18 post-infection, 50 salivary glands were collected. Tissues were homogenized to release sporozoites, filtrated and fixed with 4% paraformaldeide. The sporozoites were stained with anti-CSP and Anti-αGal antibodies and Lectin conjugate with Alex-647 and analyzed by flow cytometer. Three independent experiments were performed. Results: We could quantified sporozoytes using a three-parameter (tri-colour) flow cytometry technique based on GFP, Anti-CSP or Anti-αGal and lectins. The methodology was able to detect two populations of sporozoites in midgut based on GFP and negative and positive sporozoites population for lectin and Anti-αGal. Conclusion: The flow cytometer could be used to detect, quantify and differentiate sporozoyte populations from mosquito's tissues.

Funding: FCT for funds to GHTM – UID/Multi/04413/2013.

Poster 6

MOLECULAR DETECTION OF PLASMODIUM IN FIELD COLLECTED ANOPHELES SAMPLES FROM GUINEA BISSAU (1993- 2010)

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Malaria is the most important vector-borne disease in the world and its incidence occurs mainly in Africa. This parasitic disease is caused by several species of the genus *Plasmodium* that are transmitted to humans by mosquitoes of the genus Anopheles. The present work aimed at detecting Plasmodium sp. DNA circulating in *Anopheles* mosquitoes collected in Guinea-Bissau from 1993 to 2010.

Mosquito DNA samples previously identified to species of the *Anopheles gambiae* complex were obtained from collections undertaken at different years in three study sites with different ecology: Antula (urban), Mansoa (semi-rural) and Mandigará (rural). To detect parasite DNA, single step-PCR assay targeting the Plasmodium cytochrome oxidase I (COX I) gene, was performed.

Plasmodium DNA was detected in 12% of the 597 mosquito samples analyzed. The highest prevalence of infection (20%) was found in Antula, 1993, and the lowest (3%) in Mansoa, 2010. The level of Plasmodium sp. detection had a declining trend over the years in urban and semi-rural areas Antula (20-13%) and Mansoa (14-3%), but not in the rural Mandingará (10-12%). Prevalence of Plasmodium infection was 14% in both *Anopheles gambiae s.s.* and *Anopheles coluzzii* and 12% in hybrids between the two species.

The PCR assay used appears to be an efficient method for detecting the Plasmodium DNA in field-collected mosquito samples. Results will be discussed with respect to malaria prevalence and ecological differences between sites.

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Poster 7

PLASMODIUM SHORT N-GLYCANS ARE CRITICAL FOR PARASITE SURVIVAL

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Malaria, which is caused by unicellular parasites of the genus *Plasmodium*, remains a serious health problem worldwide. N-glycosylation, the covalent binding of glycans to specific asparagine residues of target proteins is predicted to be the most abundant post-translational modification of proteins. Interestingly, Plasmodium parasites lack most of the genes coding for the biosynthetic pathway of N-glycosylation (alg genes) and display unusually reduced N-glycans composed only of one or two N-acetylglucosamine (GlcNAc) residues. In this work we investigate the importance of N-glycosylation for Plasmodium parasites. Our attempts to knock-out essential components of the N-glycosylation machinery (stt3 and alg7 genes) repeatedly failed both in P. falciparum and in *P. berghei*, suggesting a critical role for N-glycosylation in parasite survival. Growth inhibition of parasites after short treatment with tunicamycin also supports the critical effect of N-glycosylation in Plasmodium. A conditional knock-out for stt3 gene is currently being developed to provide further evidence for this hypothesis. In summary, our data strongly suggests that despite the peculiar short nature of Plasmodium N-glycans, these are essential for the malaria parasite.

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Poster 8

EPIDEMIOLOGICAL AND CLINICAL-LABORATORY PROFILE OF CHILDREN ADMITTED WITH MALARIA IN THE INFECT-CONTAGIOUS DISEASES SERVICE OF MAPUTO CENTRAL HOSPITAL, FROM MAY TO SEPTEMBER 2014

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Malaria is a major public health problem worldwide, but is more common in Africa in sub-Saharan region. In Mozambique the disease contributes to high mortality rates of 28.8% in all ages, 35.2% in children under one year, 51.2% in group 1-4 years and 48.5% 5-14 years. This study was mainly to characterize the epidemiological, clinical and laboratory profile of children hospitalized with malaria in Diseases Department of Pediatrics Infectious the Maputo Central Hospital from May 1 to September 26, 2014.

This was an observational, descriptive, cross in children aged 1-14 years admitted from 1 May to 26 September 2014 were obtained prospective data using a questionnaire prepared for the study. It was done a survey of caregivers, complemented by the information recorded in the admission process in the Emergency Department. Data were analyzed and summarized in frequency tables and bi-variate statistical analysis was performed seeking to identify relationships between variables.

During the study period, 681 children were hospitalized, of which 384 (56%) were malaria, representing the study sample. There was a predominance of male children (55%) and the age group 1-4 years (51%). Most mothers had the basic level of education (58%). The most common clinical manifestations were fever (100%), vomiting (44%) and anorexia (39%). The most common clinical complications were prostration (26%), multiple seizures (20%) and impaired consciousness (20%). The hyperparasitaemia (40%), thrombocytopenia (15%) and severe anemia (14%) were the most prevalent laboratory findings.

The results of the study suggest that malaria was the leading cause of hospitalization in the Infectious Diseases Service, especially in the 1-4 age group. The profile of children hospitalized reflects reported in the literature for severe malaria.

Poster 9

CLINICAL CASE: PLASMODIUM MALARIAE INFECTION IN A 28 YEAR-OLD FEMALE WITH FEVER, THROMBOCYTOPENIA AND BLOOD SMEAR REPETEDLY NEGATIVE FOR PLASMODIUM SPP.

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Malaria needs to be considered as a differential diagnosis when examining patients who have traveled from endemic regions. Establishing a correct and early malaria diagnosis is a prerequisite for an adequate treatment and to minimize adverse outcomes. However, current blood cell analyzers are not specifically designed to detect malaria-related abnormalities. Therefore, observation of peripheral blood smear (PBS) under light microscopy is still necessary for malaria diagnosis. However, it requires an explicit request from clinicians and has variable accuracy.

A 28-year-old female living in Angola attended to a medical appointment with complaints of fever, chills, myalgia, cough, headaches, and diarrhea without blood or mucus. Several laboratory exams were made: complete blood count (CBC) that revealed thrombocytopenia (131.000/mL); search for *Plasmodium spp* in peripheral blood smear (PBS) – negative – and anti-Dengue IgM, also negative. The diagnosis of community acquired pneumonia (CAP) was made and ciprofloxacin was prescribed. Four days after the beginning of symptoms she traveled to Portugal, where she attended the emergency department. On laboratory exams, she presented low platelet count (62 x 109 /L), elevation of transaminases (AST 97 UI/L, ALT 127 UI/L) and 3,0 g/dL of C-reactive protein. Search for Plasmodium spp and the anti-Dengue IgM again were both negative. It was then assumed to be a viral infection, and symptomatic measures were adopted. A CBC was requested 3 days later, which revealed 8% large unstainned cells (LUC). In order to perform white cell differential count, a PBS was requested. Rare trophozoites, gametocytes and schizonts of *Plasmodium malariae* were then observed. The patient was medicated with atovaquone/proguanil.

Misdiagnosis of imported malaria is not uncommon and even abnormal routine laboratory tests may not trigger malaria smears. However, an increase in LUC percentage can occasionally appear as a consequence of malaria infection. Although PBS remains necessary for malaria diagnosis, low parasitemia can initially be undetectable and, therefore, PBS should be repeated every 12-24 hours for a total of 3 sets.