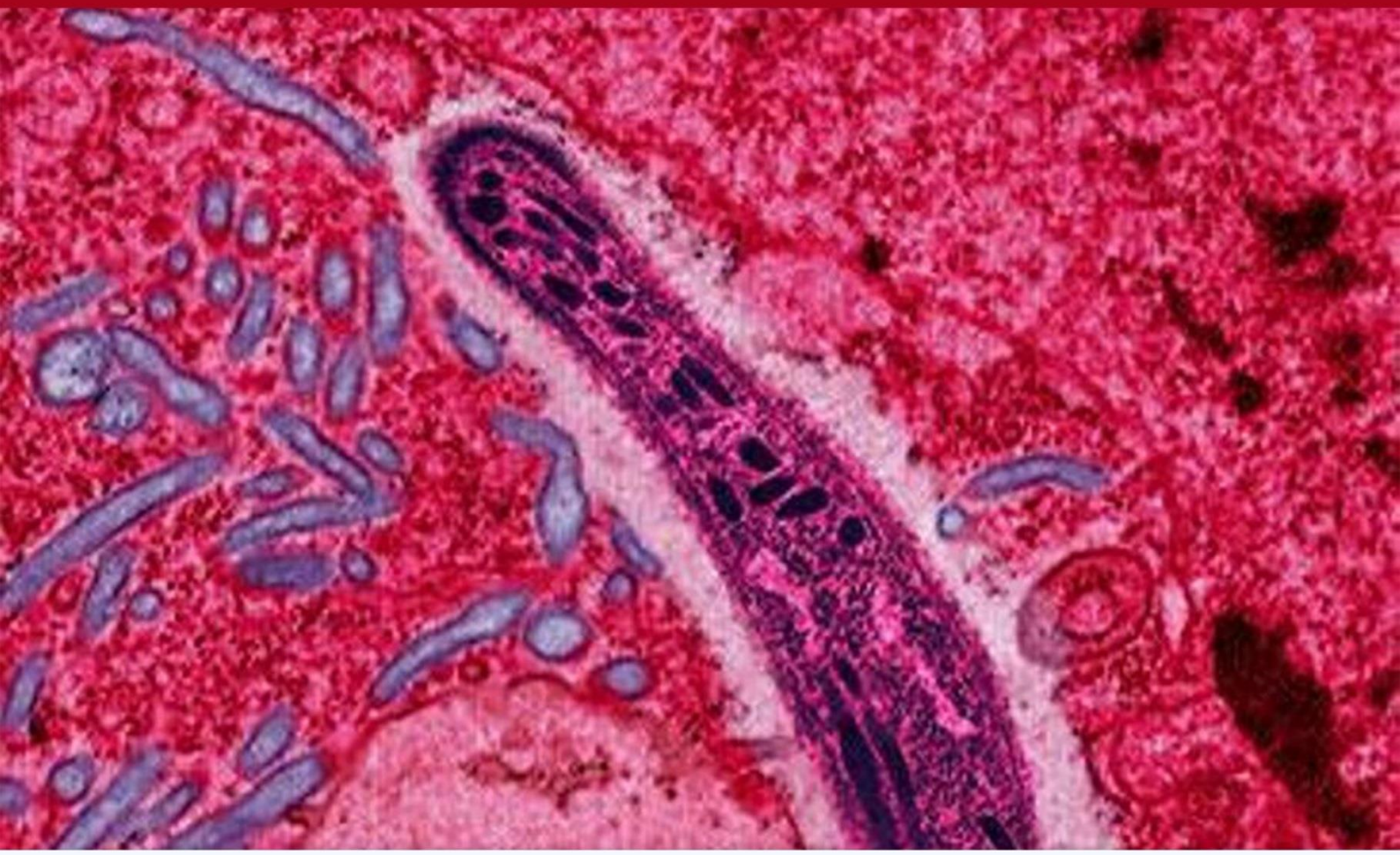
BEATING MALARIA 2015

ABSTRACTS



29TH JUNE - 1ST JULY 2015
LONDON, UK

EuroSciCon

This event has an open abstract session.

Abstracts can be submitted on any subject related to Malaria
The Deadline for abstract submissions for oral presentation is April 10th 2015
Abstracts for *poster presentation only* can be submitted up to two weeks before the event
You can download the instructions for authors at

www.euroscicon.com/ABSTRACTSUBMISSIONS.pdf

This event has CPD accreditation

Hashtag: #Malaria2015

This abstract book will be finalised two weeks before the event
www.regonline.co.uk/malaria2015

Table of Contents

Day 1:

Invited Speakers Abstracts

Novel, potent and selective inhibitors of Plasmodium N-myristoyltransferase: a new antimalarial mode of action

Professor Edward Tate, Department of Chemistry, Imperial College London, UK

Chemical validation of new antimalarial targets is urgently required in view of rising resistance to current drugs. The enzyme N-myristoyltransferase (NMT), catalyses attachment of myristate to protein substrates (N-myristoylation), and chemical biology approaches have been used to demonstrate that NMT is an essential and chemically tractable target in human malaria parasites, both in vitro and in vivo. NMT inhibition cause catastrophic and irreversible failure to assemble critical subcellular parasite organelles, resulting in potent and selective antiparasitic activity in both blood and liver stage. This novel mechanism circumvents clinical resistance, and represents a promising avenue to novel antimalarial drugs.

How genomics is contributing to the fight against artemisinin-resistant malaria

Dr Pedro Cravo, Instituto de Patologia Tropical e Saúde Pública/Universidade Federal de Goiás, Brazil

Arttemisinin (ART) resistance has recently evolved in human malaria parasites. Widespread ART resistance would be catastrophic because there are no effective alternative treatments availble. This talk will focus on the recent contributions in the field of genomics for our understanding of the genetics of resistance to artemisinin in the human malaria parasite Plasmodium falciparum. Specific emphasis will be placed on how genomics has contributed to the recent discovery of a number of molecular markers that are being used in real time to track the appearance and spread of artemisinin resistance in natural parasite populations.

Computational modelling of the interactions between antimalarial drugs and hemozoin crystal faces

Dr Marcus C. Durrant, Northumbria University, London, UK

Several antimalarial drugs are known to act by interfering with the parasite's ability to detoxify iron(III) ferriprotoporphyrin (FePPIX) by crystallization to haemozoin. In this work, a combination of quantum calculations and molecular modelling has been used to investigate the binding of five quinine-like drugs and five chloroquine-like drugs to free FePPIX and to the crystal faces of haemozoin. The results suggest that all 10 drugs have a common mode of action, involving formation of a strong Fe-O bond. We have used this model to initiate a computational search for novel antimalarial drugs.

Towards next generation of medicines to meet the ambitious goal of malaria eradication

Dr Benjamin Blasco, Medicines for Malaria Venture, Switzerland

For new medicines to meet with the ambitious goal of malaria eradication, they must be able to block transmission, prevent relapse of dormant forms and contain or circumvent drug resistance. In the absence of a highly effective vaccine, prophylactic treatments are also needed to protect patient populations. During this presentation, the strategy of Medicines For Malaria Venture (MMV) - a not-for-profit public-private partnership – to tackle these challenges will be presented through the description of current mainstay treatments along with a selection of emerging new clinical molecules from our portfolio.

Role of hemozoin in the pathogenesis of malaria-associated acute respiratory distress syndrome

Professor Philippe Van den Steen, Laboratory of Immunobiology, Rega Institute for Medical Reseach, University of Leuven – KU Leuven, Belgium

Malaria-associated acute respiratory distress syndrome (MA-ARDS) is an often lethal complication which occurs mainly in adult patients. To investigate the pathogenesis and therapeutic options, we developed a mouse model of MA-ARDS with Plasmodium berghei NK65. Similar to postmortem findings in patients, MA-ARDS in our model is characterized by extensive interstitial and alveolar edema, hyaline membrane formation, microhemorrhages, and the presence of abundant inflammatory infiltrates in lungs. These infiltrates mainly consist of monocytes and macrophages, lymphocytes and some neutrophils. Interestingly, we observed abundant accumulation of hemozoin or malaria pigment in the lungs. Further investigations indicated that hemozoin induces pulmonary inflammation and is an important pathogenic factor in MA-ARDS.

Polyamidoamine nanoparticles: A versatile dual tool for malaria therapeutics and prophylaxis

Dr Xavier Fernàndez Busquets, Research Associate, Institute for Bioengineering of Catalonia, Barcelona, Spain

We have developed poly(amidoamine)-derived nanovectors that combine into a single chemical structure drug encapsulating capacity, antimalarial activity, low unspecific toxicity, specific pRBC targeting, optimal in vivo activity, and affordable synthesis cost. Our recent data suggest that the antiparasitic mechanism of PAAs can be based on blocking the erythrocyte invasion of egressed parasites. The ensuing prolonged exposure of the pathogens to the immunitary system might be applied to the design of new malaria vaccination approaches where PAAs could play a dual role as carriers of antimalarial drugs and as vaccination adjuvants. This unexpected synergistic effect combining therapeutics and prophylaxis represents a radically new approach to the treatment of malaria for which we propose the new term theralaxis.

Regulators of Plamsodium falciparum Protein Phosphatases: new means to control malaria

Dr Jamal Khalife, Univ Lille Nord de France, Cente National de la Recherche Scientifique, Lille Cedex, France

Protein phosphatases are enzymes involved in many cellular essential functions. The most important and studied phosphatases are the Protein Phosphatase type 1 (PP1) and type 2A (PP2A). The selective action of these phosphatases, are controlled by their capacity to bind several protein regulators. In P. falciparum, PP1 and PP2A are known to be crucial for parasite (sur)vival. We undertook the characterization of interactors/regulators of both phosphatases. This allowed to characterize three regulators of PfPP1 (PfLRR1, PfI2, PfI3) and one regulator of PP2A (PfPTPA), which have been shown to participate in the regulation of the cell cycle. Genetic approaches showed that these regulators of PP1, which present several important differences with their human homologs, are as essential as PP1 itself for P. falciparum survival. We explored the binding motifs of these regulators to PP1 and showed that peptides derived from these motifs, exhibited anti-plasmodial activity against blood stage parasites in vitro.

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

OVERCOMING DRUG RESISTANCE IN MALARIA BY INHIBITING EFFLUX

Professor David Peyton, Department of Chemistry, Portland State University, Portland, Oregon, U.S.A.

We are developing a class of hybrid molecules made from a chloroquine-like (CQ-like) moiety and a chemosensitizer (reversal agent; RA) against chloroquine resistance (CQR) in malaria. We have shown these molecules to have low-nanomolar *in vitro* IC_{50} values against all strains of malaria tested so far, whether CQ-sensitive or CQ-resistant, often surpassing the activity of even CQ against chloroquine-sensitive strains of *P. falciparum*. Here we report on the progress of a lead compound, PL69/DM1157, and its progress along its preclinical development pathway. This report will cover results from *in vitro* evaluations (including off-target activity), rodent toxicology, and pharmacokinetics, as well as initial initial second-species work. Aspects of the scale-up chemistry for GMP-production will be discussed, as well as the potential for suppressing the evolution of resistance against such a new drug. The advantages of a drug with the projected safety and efficacy profiles as favorable as chloroquine's, but without the associated resistance would be considerable.

NEW ANTIMALARIAL COMPOUNDS FROM A PLANT USED TRADITIONALLY IN BENIN

J. Bero, M.-F. Herent, G. Schmeda-Hirschmann, M. Frédérich, J. Quetin-Leclercq
Université catholique de Louvain, Louvain Drug Research Institute, Pharmacognosy Research Group, 72 Avenue E. Mounier, B1.72.03, B-1200 Brussels, Belgium

Parasitic diseases are still responsible for many health problems. Among them, African trypanosomiasis or sleeping sickness caused by *Trypanosoma brucei*, malaria transmitted by *Plasmodium* species of which the most dangerous is *Plasmodium falciparum* and leishmaniasis. Plant biodiversity and knowledge of traditional healing allow, as it was the case for artemisinin, to open new ways in the field of therapeutic. In the work we will present, we analyzed the activity of several plants from Benin selected by ethnopharmacological and bibliographical studies. These plants are used in traditional medicine as antimalarials. Crude extracts from powders of leaves, twigs, roots or aerial parts were prepared by maceration. These extracts were studied for their antiparasitic activities by *in vitro* tests on *Plasmodium falciparum*, *Trypanosoma brucei brucei* and *Leishmania mexicana mexicana*. In addition, cytotoxicities were analysed to determine the selectivity of crude extracts. The extracts of *Keetia leucantha* were selected for further investigations. So we tested the *in vivo* antimalarial activity of the dichloromethane extract of twigs

which showed 40.7 % inhibition in mice infected by *Plasmodium berghei* at 100 mg/kg/day and the total aqueous extract which had a 30.8% inhibition at 200 mg / kg / day.
Known antiparasitic compounds were identified and quantified by LC-MS in the dichloromethane extract. As they could not account for the total activity observed, we isolated by bioguided fractionation several triterpenic esters, vanillin derivatives, a sterol and a coumarin. The structure determination of isolated compounds was performed by NMR studies and high resolution mass spectrometry. The isolated compounds were then studied *in vitro* and *in vivo* for their antiplasmodial activities. Several of them showed a sub-micromolar antiplasmodial activity, including some triterpenic esters which are about 10 times more active than ursolic acid.

References
Bero J., Ganfon H,. Jonville M.C., Frédérich M., Gbaguidi F., DeMol P., Moudachirou M., Quetin-Leclercq. J. *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J. Ethnopharmacol., 2009, 122, 439-444.
Bero J., Hannaert V., Chataigné G., Hérent M.F., Quetin-Leclercq J. *In vitro* antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. J. Ethnopharmacol., 2011, 137, 998-1002.
Bero, J., Hérent, M-F., Schmeda-Hirschmann, G., Frédérich, M., Quetin-Leclercq, J., 2013. *In vivo* antimalarial activity of *Keetia leucantha* twigs extracts and *in vitro* antiplasmodial effect of their constituents. Journal of Ethnopharmacology, 149(1), 176-183

RECYCLING CLASSICAL DRUGS: TOWARD LOW-COST ANTIMALARIALS

Ana Gomes[1,2], Bianca Pérez[1], Cátia Teixeira[2], José R. B. Gomes[2], Paula Gomes[1]*
[1]*UCIBIO/REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre 687, P-4169-007 Porto, Portugal*
[2]*CICECO, Universidade de Aveiro, Campus Universitário de Santiago 3810-193 Aveiro, Portugal*
*pgomes@fc.up.pt

Developing new drug products is a costly and time-consuming process, which is a delicate issue when addressing diseases of poverty; a low-cost reduced-risk strategy towards sustainable drug development may pass by performing simple chemical modifications on classical drugs (recycling), or by identifying new therapeutic uses for shelved drugs (rescuing), or for drugs approved to treat a specific disease while being potentially relevant against other therapeutic targets (repurposing) [1]. For the past few years, our group has been working on the recycling of classical antimalarials chloroquine (**1**) and mepacrine (**2**), whose originally thrilling antimalarial properties gave place to disappointing news about, respectively, emergence of parasite resistance and toxicity issues; starting from the heteroaromatic cores of these two antimalarial classics, we performed simple chemical reactions to obtain new derivatives, whose promising antimalarial properties will be presented [2-4].

Acknowledgements
This work was co-funded by the European Union through FEDER (ref. FCOMP-01-0124-FEDER-020963) and by Portuguese National Funds through FCT (ref. PTDC/QUI-QUI/116864/2010). CT and IF thank FCT for post-doctoral grants SFRH/BPD/62967/2009 and SFRH/BPD/86173/2012, respectively.

References
[1] Teixeira, C. *et al. Chem. Rev.* **2014,** *114*, 11164-220
[2] Pérez, B. *et al. ChemMedChem* **2012**, *7*, 1537-40
[3] Pérez, B. *et al. J. Med. Chem*. **2013**, *56*, 556-67
[4] Gomes, A. *et al. ChemMedChem* **2014**, *9*, 305-10

EPIDEMIOLOGICAL AND PROPHYLACTIC DATA OF IMPORTED MALARIA IN THE MILITARY HOSPITAL OF INSTRUCTION MOHAMMED THE FIFTH OF RABAT, MOROCCO.

El-Houcine Sebbar, Hafida Naoui, K. Sbai Idrissi, Badre Eddine Lmimouni
Service of Medical Parasitology & Mycology, the Military Hospital of Instruction Mohammed the fifth, PO Box number 1018, Rabat, Morocco.

Introduction: For his many interventions in sub-saharian Africa, the Moroccan army has gained considerable experience in management of imported malaria. Chemoprophylaxis is stratified according to drug sensitivity of *Plasmodium* in the host country. The use of impregnated mosquito nets is a key strategy in the army. The aim of our study is to investigate the epidemiology of imported malaria and the prophylactic and therapeutic approach.

Materials and methods: This is a prospective study which took place from 1st January 2005 to December 31 2009 at the Military Hospital of Instruction Mohammed the fifth in Rabat. All military personnel with a

search request of *Plasmodium* in the blood are included. Data are collected in real time on a standardized form.

Results: During the study period, 658 applications corresponding to 612 patients are included and 57 patients (9,31%) were diagnosed positive for *Plasmodium* spp. All patients were male and the average age is 33,6 years. The average stay is 182 days. *Plasmodium falciparum* is responsible for most processes, only in 56,14% of cases (N = 32), and in combination with other *Plasmodium* species in 14,03% of cases (N = 8). 87,7% (N = 50) had taken chemoprophylaxis. As for physical protection, it was applied in only 59,64% of cases (34 cases).

Conclusion: This study allowed us to describe the epidemiological characteristics of imported malaria in the Military Hospital of Instruction Mohammed the fifth of Rabat, and identify a number of problems on disease prevention.

Keywords: Imported malaria, Army, *Plasmodium falciparum*, Epidemiology, Chemoprophylaxis.

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

EXPLORING NEW HYDRAZINE AND HYDRAZIDE QUINOXALINE 1,4-DI-N-OXIDE DERIVATIVES AS POTENTIAL ANTIMALARIALS

M. Quiliano[1,2], A. Pabón[3], C. Barea[1], S. Galiano[1,2], I. Aldana[1,2*]
[1]Department of Organic and Pharmaceutical Chemistry, Facultad de Farmacia. Universidad de Navarra, c/Irunlarrea 1, 31008 Pamplona, Spain. Email*: ialdana@unav.es
[2]Institute of Tropical Health (ISTUN), Universidad de Navarra, c/Irunlarrea 1, 31008 Pamplona, Spain.
[3]Malaria Group, Universidad de Antioquia, Medellín, Colombia.

Malaria is one of the world's most important tropical parasitic diseases. Mortality due to malaria is estimated to be over 1 million deaths annually and this situation is worsened by the spread of drug-resistant strains of the parasite. In the present study, a series of 19 quinoxaline 1,4-di-N-oxide derivatives were designed, synthesized and evaluated in vitro against infectious pathogens Plasmodium falciparum (3D7 strain chloroquine-sensitive and FCR-3 strain chloroquine-resistant) and Leishmania infantum. Among them, 14 novel compounds correspond to hydrazine and hydrazide derivatives. Their cytotoxicity and selectivity were also evaluated against HEGP-2 cells. The in silico ADMET properties were calculated for all compounds. One hydrazine derivative was found to inhibit 50% of Plasmodium growth at 0.24 μM. Hydrazine and hydrazide quinoxaline 1,4-di-N-oxide derivatives constitute a new class of antimalarial compounds. It can potentially serve as templates for future drug-optimization and drug-development efforts for use as therapeutic agents in developing countries.

NEW QUINOXALINE DERIVATIVES OF CHALCONE AS ANTI-PLASMODIUM FALCIPARUM AGENTS: SYNTHESIS, BIOLOGY EVALUATION AND STRUCTURE-ACTIVITY RELATIONSHIP

S. Galiano [1,2], A. Gil [1], A. Pabón [3], A. Burguete [1,4], S. Pérez-Silanes [1,2], E. Deharo [5], A. Monge [2] and I. Aldana [1,2]

[1] Department of Organic and Pharmaceutical Chemistry. University of Navarra, Irunlarrea, s/n, 31008-Pamplona, Spain. [2] Institute of Tropical Health, University of Navarra, Pamplona, Spain. [3] Group of Malaria. Universidad de Antioquía, Medellín, Colombia. [4] Instituto de Química Avanzada de Cataluña, Consejo Superior de Investigaciones Científicas, Barcelona, Spain; [5] PHARMA-DEV, UMR 152 IRD-UPS, Université Paul Sabatier, Toulouse, France. e-mail sgaliano@unav.es

Malaria is one the most important devastating parasitic disease with an estimated 219 million cases and 660,000 deaths in 2010 in 104 endemic countries1. Five species of Plasmodium parasites are the responsibles of malaria, but Plasmodium falciparum is the most dangerous form of the malaria parasite and it is responsible for a very high percentage of clinical attacks and Plasmodium vivax is the most widespread2.

The appearance and spreading resistance of Plasmodium falciparum to the existing anti-malarial drugs is one of the biggest obstacles in the fight against malaria. Novel antimalarial drugs, structurally diverse, with rapid efficacy, minimal toxicity and low cost are urgently needed to avoid cross-resistances.

Based on the demonstrated antiplasmodial activity of the quinoxaline derivatives3 and the chalcone compounds4 and with the aim of establishing the structural requirements in order to optimize the activity, new series of quinoxaline and quinoxaline 1,4-di-N-oxide analogs of chalcone and other compounds derived from them have been developed and evaluated against the FCR-3 cloroquine-resistant Plasmodium

falciparum strain (Fig. 1)5. The SAR study demonstrated the importance of an enone moiety linked to the quinoxaline ring for obtaining better antimalarial activity. Compounds 1a and 2a showed the best antiplasmodial activity.

Acknowledgements: This work has been carried out with the financial support of the PIUNA project from University of Navarra. A.G is indebted to the Navarra Government for a grant.

1. World Malaria Report 2012.
http://www.who.int/malaria/publications/world_malaria_report_2012/en.
2. R.W. Snow, et al., Nature 2005, 434, 214.
3. C. Barea, et al.,Molecules 2013, 18, 4718.
4. K.V. Sashidhara, et al., Bioorg. Med.Chem. 2012, 20, 2971.
5. A. Gil, et al., Molecules 2014, 19, 2166.

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW AMINOQUINOLINETHANOLS AS ANTIMALARIAL DRUGS VIA AN ENANTIOSELECTIVE AMINOHYDROXYLATION PATHWAY

G. Bentzinger*, A. Dassonville-Klimpt, C. Demailly-Mullié, P. Sonnet
Laboratoire LG2A, CNRS FRE 3517, UFR de pharmacie, 1 rue des Louvels, Université Picardie Jules Verne, 80037 Amiens cedex 1 FRANCE
*:guillaume.bentzinger@u-picardie.fr

Malaria, due to protozoa belonging to the genus Plasmodium, is the 5th most lethal infection in the world.[1] The emergence and spread of multiple drug resistances among strains of these parasites pose a serious public health problem. New antimalarial drugs are therefore needed and this is why our team is involved in the design and synthesis of new antimalarial compounds. Mefloquine and its derivatives remain very attractive targets for the synthesis of more potent and less toxic analogues. Recently, we have described the asymmetric synthesis and biological activity of aminoquinolinemethanols.[2] In this series, the most active molecule is the aminoquinolinemethanol with a S configuration and a pentyl group (IC50 = 6.98 nM on the chloroquine-resistant W2, strain). To further understand the structure-activity relationships in these molecules, we next endeavored to study the influence of the position of the amino group and the alcohol group on the antimalarial activity. We present here the synthesis and biological evaluation of new aminoquinolinethanols, analogues to the most active aminoquinolinemethanols described previously.[2]

References:
[1] WHO. World Malaria Report. WHO: Geneva, Switzerland, 2009.
[2] a) A. Jonet, A. Dassonville-Klimpt, S. Da Nascimento, J.-M. Leger, J. Guillon, P. Sonnet, Tetrahedron : Asymmetry 2011, 22, 138, b) C. Mullié, A. Jonet, C. Degrouas, N. Taudon, P. Sonnet, Malaria Journal 2012, 11, 65, c) C. Mullié, N. Taudon, C. Degrouas. A. Jonet, A. Pascual, P. Agnamey, P. Sonnet, Malaria Journal 2014, 13, 407.

Day 2:

Invited Speakers Abstracts

In-depth immunoprofiling as tool to establish immune correlates of protection

Dr. Elke Bergmann-Leitner, Chief, Flow Cytometry Center, MVDB/USMMRP/WRAIR, Silver Spring, MD, USA
Immune correlates of protection against Plasmodia have been elusive, slowing the rational design of an effective vaccine. Repeated exposure to Plasmodium does not yield protection against subsequent infection, but a form of immune-tolerance. Therefore, “natural immunity” to malaria does not provide a model to study protection. Immune correlates of protection against malaria are likely complex and identifying correlates or surrogate markers of protection requires immunoprofiling with unprecedented depth. Samples from trials of malaria vaccines with measureable sterile protection were analyzed and provide a first glimpse of the landscape of antigen-specificity and types of immune responses associated with protective, sterile immunity.

Systematic analysis of malaria-related research investments awarded to UK institutions 1997-2013: where's the money gone?

Dr Michael Head, Network Manager, Infectious Disease Research Network, Based at the UCL Farr Institute, London, United Kingdom

Across this seventeen year time period, there has been approximately £490m of research investment into malaria research, across 621 separate studies (this compares with £609m for HIV, and £226 million for tuberculosis, for example). This talk would go into more detail about the breakdown of the direction of spend for malaria research in the UK, as well as quantifying the relative levels of investment against the burden of disease. It would also highlight gaps in the UK research portfolio and discuss potential priority areas for funders to consider.

Development and evaluation of a direct on blood PCR-NALFIA system (DIAGMAL): bringing molecular diagnostics to the field

Dr Henk Schallig, Royal Tropical Institute / Koninklijk Institut voor de Tropen (KIT) - Parasitology Unit, Amsterdam, The Netherlands

Molecular tools allow for specific/sensitive malaria diagnosis, but current formats, like PCR with gel-electrophoresis, are difficult to implement in resource poor settings. Therefore, a simple, fast, sensitive/specific molecular diagnostic platform, direct on blood PCR combined with nucleic acid lateral flow immunoassay to detect amplified PCR products of Pan-Plasmodium and human GAPDH (internal control) was developed. This test format circumvents DNA extraction and complex read-out systems. The diagnostic was favorably evaluated under laboratory conditions, multi-country ring trial and two malaria endemic countries, which brings molecular diagnostics for malaria closer to field use in disease endemic countries where resources are often limited.

Beating Malaria: A ten-year retrospective report from Nigeria

Mr. Emmanuel Amadi, Department of Medical Microbiology, College of Medicine, Enugu State University of Science and Technology, Nigeria
E.C. Amadi, T.N Nwagu, B.A.F, Ngwu and M.N. Ugwuanyi.

In endemic regions of malaria, the actual incidence and mortality rates are unknown due to incomplete reporting. Major factor against this is the irritating sampling technique (finger prick, etc) which are repulsing to the volunteers - that then becomes reluctant - necessitating need for a more accurate estimate through clinical malaria. Medical records of patients in two different hospitals in Enugu (Eastern Nigeria) between January – December of 2005 to 2009 and 2010 to 2014 were critically studied. Ages, sexes, occupations, locations, and monthly plus yearly incidences of diagnosed cases of malaria were analyzed. 27,100 records were examined in the first hospital and 24,180 in the second hospital. The result of the first hospital revealed a yearly statistically significant increase in diagnosed cases of malaria from 2005 to 2009 (38%, 395, 42%, 43% and 42%, in yearly ascending order). Prevalence rates were highest during the drier periods of the 5 year. > 35 years age-group has the highest incidence (56.25%) and lowest incidence in 1-14 years (8.75%). Females (52.2%) were diagnosed more of malaria than the males (47.8%). Farmers and cattle herdsmen (30% and 25%, respectively) were most affected while students and office workers (11.25% and 12.5%, respectively) has the lowest incidence. The second hospital (2010 – 201`4) revealed a slight but very high percentage decline down the years (98.5%, 96.9%, 57%, 95.6% and 96.3%). Prevalence rate was also highest during the drier period of the year. Females (56.4%) again were more affected than the men (43.6%). Farmers (25%) were also most affected, but cattle herdsmen (7.5%) surprisingly were least affected here. In reverse, 01 – 14 age-group (45.1%) were most affected in this case; > 35 years were next (27.8%) and 30 – 34 years were least affected (12%). In conclusion, besides evidences of still serious lag in malaria combat and control, some niches apparently serves as "reservoirs" of the disease in form of the mosquitoes vectors and Plasmodium sp etiologic agent that seeds other parts of the country/world of the disease and hinder the global efforts.. Plasmodium-parasitaemia-negative is advocated for cure, and not mere physical fitness.

The role of visiting friends and relatives (VFRS) in imported malaria

Assistant Professor Paola Di Carlo, Department of Sciences for Health Promotion and Mother-Child Care, University of Palermo, Italy

With the integration of immigrants in their host countries, a new, special group of travellers - Visiting Friends and Relatives (VFRs) - has emerged over time. The term VFRs refers in particular to immigrants who move to high-income countries from countries where socio-economic status is low, and regularly return to their country of origin, sometimes for fairly long periods of time, to visit their friends and relatives or, where women are concerned, also to give birth and spend the post-partum period with their family. Precisely because they are "special tourists", VFRs are considered to be at higher risk than other regular travellers of contracting diseases like tuberculosis, HIV and malaria. In fact, because they still feel ethnically integrated in their country of origin, many of them do not take adequate preventive measures to reduce risks to their health.Although many studies have been conducted on this particular group of

travellers, we still lack sufficient information, in our country especially, that enables us to undertake suitable prevention initiatives. Malaria is the most frequently contracted infection among VFRs, with a significant difference ($p<0.001$) compared to more recent immigrants and other categories of travellers. More than 75% of imported malaria cases occur in immigrants, and almost all of them are in the Visiting Friends and Relatives category. In more than 90% of cases the infection is caused by P. falciparum and regards individuals from sub-Saharan African countries. It is important to note that almost all the women and children with imported malaria belong to the VFRs category.

Oral Presentation Abstracts

SEX HORMONES DOWN REGULATE THE IMMUNE RESPONSE IN *P. berghei* ANKA INFECTED MICE.

M Legorreta-Herrera, NA Mosqueda-Romo, KE Nava-Castro, AL Morales-Rodríguez, FO Buendía-González and J Morales-Montor.
Laboratorio de Inmunología Molecular, Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Batalla 5 de mayo esq. Fuerte de Loreto, Iztapalapa 09230, México, D.F. México

Pathology and mortality in malaria is different between females and males, this sexual dimorphism may have important implications for vaccines and drug' effects. However, little is known about the mechanisms mediating these sex differences. Since the main differences between sexes are dictated by sex hormones and these molecules are mainly produced by gonades, in this work we studied the effect of gonadectomy on the immune response to *P. berghei* ANKA in CBA/Ca mice. We evaluated parasitaemia, splenic index, the cells involved in the immune response such as $CD3^+$, $CD4^+$, $CD8^+$, NK^+, B^+ and macrophages in the spleens of female and male mice infected with *P. berghei* ANKA. In addition, we measured antibody and cytokine levels in blood. Gonadectomy increased T^+ and B^+ splenic cells in both sexes but increased Mac-3^+ cells only in male mice. Interestingly, gonadectomy decreased the NK^+ cell population only in male mice. Female mice developed higher antibody levels than males. Contrary to our expectations, gonadectomy increased the synthesis of IgG1, IgG2b, IgG3 and total IgG in female mice, indicating negative regulation of antibody production by female sex hormones. Gonadectomy increased the production of Tumour Necrosis Factor alpha (TNF-α) and Interleukin-6 (IL-6) only in female mice, suggesting that female sex hormones have anti-inflammatory properties. This work, demonstrates that sex hormones supress the immune response and should be considered when designing malaria vaccines.

This work was supported by DGAPA, UNAM Grant IN216914

SERUM SELENIUM CONCENTRATION IN PATIENTS WITH *VIVAX* AND *FALCIPARUM MALARIA* BY ATOMIC ABSORPTION SPECTROSCOPY

Mohsin Ali Baloch[1], Saira Baloch[2]
[1]Faculty of Pharmacy, University of Sindh, Jamshoro, Pakistan
[2] Medical Research Centre, Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan

Abstract
Malaria is one of the most serious tropical diseases in the world and has been a health risk to humans for many generations. It is very widespread disease, covering many areas of Europe, North America, South America, Asia and Africa. It is also a major public health problem in Pakistan. Selenium is an essential antioxidant trace mineral for the human body. In the present study, Selenium concentration was determined in the serum of Patients with *vivax* and *falciparum* malaria (n=50) with comparison to healthy control subjects (n=50). Selenium was determined using Atomic Absorption Spectroscopy (AAS, Model Varian A-20). The serum concentration of selenium determined to be in *vivax* 42.92±1.21, in *falciparum* 41.72±1.1 malaria were lower as compared to the 49.01±1.01in healthy control subjects. It is concluded that selenium supplementation should be recommended in the therapies used for the management in patients with *viavx* and *falciparum malaria*.

Key words: *Vivax, Falciparum,* Selenium, Atomic Absorption Spectrometry
amohsin1710@gmail.com

ANTIPLASMODIAL POTENTIAL OF *THLASPI ARVENSE* (BRASSICAEAE) USED IN TRADITIONAL MEDICINE

N.S. Walter[1], U. Bagai[1*]
[1]*Parasitology Laboratory, Department of Zoology, Panjab University, Chandigarh, 160014, India.*

Abstract
Traditionally used medicinal plants are rich sources of new drugs against malaria and other infectious diseases. *Thlaspi arvense* (field pennycress) is used in traditional herbal medicine as febrifuge, blood tonic and blood purifier. The present study has been designed to explore its *in vitro* and *in vivo* antimalarial efficacy. Phytochemical screening of extract revealed the presence of diterpenes, triterpenes, steroids, anthraquinones and phytosterols. Ethanolic whole plant extract of *Thlaspi arvense* (EWETA) was found to inhibit schizont maturation of both chloroquine-sensitive (MRC-2) and resistant (RKL-9) strains of *Plasmodium falciparum* with IC50 <5µg/ml and =5µg/ml respectively. The extract revealed no signs of toxicity against both *HeLa* cells and normal fibroblasts with CC50>1000µg/ml. Selectivity index of EWETA was calculated to be >200 and =200 for MRC-2 and RKL-9 strains of the parasite respectively with both cell lines. The extract also exhibited considerable repository and curative against *Plasmodium berghei* (NK-65) *in vivo*. In case of repository activity, maximum chemo-suppression of 91.75% was observed on day 7 at a concentration of 500mg/kg in comparison to positive control (78.78%). However, maximum curative efficacy in established infection (91.93%) was observed at an intermediate dose of 100mg/kg. Mean survival time of 27.33±1.63 days was also observed at this concentration, which was extremely statistically significant ($p<0.0005$) in comparison to infected control which died by day 9 post inoculation. Based on WHO recommendations EWETA can be classified as highly active antimalarial against chloroquine-sensitive (MRC-2) strain and possesses promising antimalarial efficacy against chloroquine-resistant (RKL-9) strain. High selectivity index (SI>10) also establishes field pennycress as an active antimalarial. Considerable preventive potential at a dose of 500mg/kg indicates that higher doses of the extract are more effective in preventing parasite development in the host. However, intermediate dose is more effective in curing established infection as well as non-toxic to host leading to enhancement of mean survival time of mice. Hence, present study provides scientific evidence for the traditional usage of this plant as an antipyretic agent in the Himalayan region. Further studies are being carried out to isolate the active components responsible for its antiplasmodial activity.

*Corresponding author: Dr. Upma Bagai, Associate Professor, Department of Zoology, Panjab University, Chandigarh, India. E. mail: bagaiupma@gmail.com

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

WHY DO WE HAVE A LOT OF ANTIPLASMODIAL SUBSTANCES BUT ONLY A FEW ANTIMALARIAL DRUGS?

F. Echeverri [1], Y.Upegui [2], S.M. Robledo [2]
[1] Instituto de Química, Facultad de Ciencias Exactas y Naturales
[2] PECET, Instituto de Investigaciones Médicas, Facultad de Medicina,
Universidad de Antioquia, Calle 67 No. 53–108 , Medellín 050010, Colombia
feche@une.net.co

The search of new antimalarial substances is an urgent public health necessity, since more than of half world people is under treat. In the other hands, in the last 50 years only a few new drugs have been launched in the pharmaceutical market against malaria.

Traditional medicine is an excellent tool to found new molecules and to develop more and best drugs. However, this search and develop of natural molecules is neither easy nor cheap process, due to a number of factors including the follows:

1. A great diversity in the bioassays and their reproducibility (protozoal stage, dose, evaluation of the antiplasmodial activity etc.)
2. The use of different *Plasmodium* strains, some of them drug-susceptible
3. High cytotoxicity or dose applied
4. The lack of pure compounds instead of crude extracts
5. The absence of *in vivo* assays or animal model disease
6. In the case of animal model malaria the therapeutic scheme

7. Non-availability of raw material to provide enough active molecules for clinical testing.

Besides, there is so much confusion since molecules assayed *in vitro* have been more frequently reported in scientific journals as antimalarial.

In this work these issues are discussed and a selection of the more promissory compounds from literature is presented, like a source of old molecules to be analyzed again in animal malarial model disease.

EVALUATION OF A NOVEL MICROCIP TYPE REAL-TIME POLYMERASE CHAIN REACTION SYSTEM FOR MALARIA

Mun Sub Byoun, Mi-Ae Jang, Chae Seung Lim, Sung Woo Kim
Department of Laboratory Medicine, Korea University College of Medicine, Seoul, Korea

Corresponding author: Chae Seung Lim, MD, PhD
Department of Laboratory Medicine, Korea University Medical School, Korea University Guro Hospital, Guro 2 Dong, GuroGu, Seoul 152-703, Republic of Korea.
Telephone: +82-2-2626-3245
Fax: +82-2-2626-1465
E-mail address: malarim@korea.ac.kr

Background: Malaria is worldwide health concern and remains to be one of the most difficult infectious diseases to control. Early diagnosis and treatment are essential for reducing its morbidity and mortality. Therefore, rapid and accurate diagnosis of malaria is required. We evaluated a novel ultrafast microchip-type real-time PCR, NBS LabChip shortened the PCR time (27 min for 45 cycles) (NanoBioSys, Seoul, Republic of Korea) for the identification of malaria from clinical isolates.

Methods: Blood samples collected from 307 malaria suspected cases were examined by microscopy, real-time PCR with the NBS LabChip (NBS LabChip G2-3), and a conventional tube-type real-time PCR system. The diagnostic accuracy of the NBS LabChip system and the agreement between the two assays were evaluated.

Results: Among the 307 malaria suspected cases, 151 patients were confirmed to have *P.vivax* by microscopy. The NBS LabChip detected *P. vivax* in 150 of 156 microscopically positive samples (96.1%; 95% confidence internal (CI), 91.4-98.4%), whereas conventional real-time PCR detected *P. vivax* in 151 of 156 samples (96.8%; 95% CI, 92.3-98.8%). There were no significant differences in the sensitivity and specificity between the NBS LabChip and a conventional tube-type real-time PCR system ($p>0.05$), although the NBS LabChip shortened the PCR time (27 min for 45 cycles).

Conclusions: The NBS LabChip G2-3 system is an ultrafast, sensitive, specific diagnostic tool for malaria and can be used as a confirmatory test.

TRES CANTOS ANTIMALARIAL SET-CONFIRMATION OF THE TRANSMISSION BLOCKING POTENTIAL OF SELECTED HITS IN THE STANDARD MEMBRANE FEEDING ASSAY (SMFA)

S.Lozano, C.Gonzalez, J. Rodrigues, M. Almela and E.Herreros(*)
Sonia.a.lozano@gsk.com
(*)esperanza.a.herreros@gsk.com

Discovery of transmission blocking drugs is essential for efforts to eradicate malaria. Such anti-malarial molecules target the mature sexual gametocyte stages of the Plasmodium parasite in the human host interfering with transmission to the mosquito vector and limiting the spread of the disease. The Standard Membrane Feeding assay is the current gold standard to determine transmission blocking (TrB) potential of anti-malarial compounds. Drug-treated mature gametocytes are fed via membrane to mosquitoes and the final assay read-out is measured by counting the mosquito midgut oocyst numbers. With the goal of obtaining molecules with TrB capacity, the Tres Cantos Antimalarial Set (TCAMS) with approximately 13,533 molecules (1) was screened against stage V gametocytes using an In-vitro HTS ATP assay which measures ATP intracellular levels as a surrogate of gametocyte viability (2). 56 hits with activity in asexual and sexual stages showing a high degree of chemical novelty were selected and further six molecules displaying a wide range of gametocytocidal activity and representing great structural diversity within the drug-like space were selected for evaluation of transmission blocking potential in the SMFA (3). A complete block in P. falciparum transmission as measured by oocyst intensity and prevalence in Anopheles stephensi mosquitoes was observed with all six selected molecules. The study therefore reinforces the value of the SMFA as a gold standard for identifying hits with gametocytocidal activity and importantly provides new starting points for malaria drug discovery.

Day 3:

Invited Speakers Abstracts

Symbiotc Control of Malaria Vectors

Associate Professor Guido Favia, School of Biosciences & Veterinary Medicine, University of Camerino, Camerino, Italy

Bacterial symbiosis is prevalent in insects that are efficient disease vectors, and numerous studies have targeted the basic mechanisms of the host–symbiont relationships to develop ways to control vector borne diseases. 'Symbiotic control' is a multifaceted approach that uses symbionts to control insect pests or reduce vector competence. Three such approaches currently at the cutting edge are: the disruption of microbial symbionts required by insect pests; the manipulation of symbionts to express anti-pathogen molecules within the host; and the introduction of endogenous microbes affecting life-span and vector capacity of the new hosts. Implication in malaria control will be discussed.

Syatematic Sampling Approach Reveals Fewer Falsified First Line Antimalarials than Previously Reported

Dr Harparkash Kaur, LSHTM, London, United Kingdom

Malaria is curable provided patients have timely access to efficacious drugs, namely artemisinin based combination therapies (ACTs), recommended as first line treatment by the World Health Organisation. However, reports indicate that up to 35% of 2,296 antimalarial drugs from 21 malaria endemic countries were of poor quality.

To investigate this we purchased (using three sampling approaches) and tested over 10,000 ACTs from 6 countries. Our reassuring findings exposed only 1% fakes in 2 countries.

Data presented will illustrate that a representative sampling approach is essential to accurately quantify the ineffective drugs which jeopardise treatment of a life threatening disease.

Plasmodium infection of Anopheles species from the Amazon: Development of experimental models of neglected vectors when compared to other malaria vectors

Dr. Paulo F. P. Pimenta, Centro de Pesquisas René Rachou Fundação Oswaldo Cruz – Brazil

In the Americas, areas with a high risk of malaria transmission are mainly located in the Amazon Forest, which extends across nine countries. The establishment of experi¬mental mosquito infections with Plasmodium parasites can provide interesting models for studying malaria in the Amazonian scenario is important. The knowledge of susceptibility of Amazon anopheline populations to Plasmodium infections is necessary to better understand their vector capacity. In this presentation, we present data and review the literature on malaria transmission from the perspective of its Amazon vectors.

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

TREATMENT OF ARTEMISININ RESISTANT MALARIA CASES WITH NOVEL FORMULATION OF TRADITIONAL MEDICINE IN INDIA

Prakash U. Tahiliani
Prime Ever Ayurvedic Research Laboratories (PEARL), Navsari, Gujarat, 396445, India.
putahiliani@gmail.com

Development of resistance by malaria parasite against anti-malarial drugs has been a big challenge in the treatment of malaria. In the present study, one drug prepared by me, here termed as 'Novel Formulation of Traditional Medicine (NFTM)' has been tried in 60 cases of malaria, most of them resistant to Artemisinins

& ACTs, during the period of April 2009 to June 2013. All patients were given 3 days' indoor treatment with NFTM. Pulse rate & Temperature was monitored 6 hourly and Blood smear examined for malaria parasite at 12 hours, 24 hours, 30 hours, Day-5, Day-30 & Day-60. Fever Clearance Time (FCT) observed was 30 to 48 hours along with normalization of pulse rate. Parasite Clearance Time (PCT) was 12 to 30 hours. Fever clearance & Parasite clearance was observed in 98% cases of P. falciparum & 94% cases of P. vivax.

It was observed with NFTM drug trial that (1) Need of symptomatic medication was much lesser as compared to that with other anti-malarials, (2) Blood count was regained, (3) Appetite returned to normal early, (4) Convalescence period was significantly shorter, (5) Long term recurrence was seen in 9% cases. (6) There was no intolerance to the drug and no adverse effect.

It is recommended to further investigate the potentiality of this drug for the treatment of malaria.

A NEW APPROACH TO MALARIA DIAGNOSIS USING INFRARED SPECTROSCOPY

Bayden R. Wood[1], Phil Heraud[1], David Perez-Guita[1], Aazam Khoshmanesh[1], Patcharee Jearanaikoon[2], Matthew W. A. Dixon[3], Leann Tilley[3] and Don McNaughton[1]

1. *Centre for Biospectroscopy, School of Chemistry, Monash University, 3800, Victoria, Australia*
2. *Department of Clinical Chemistry, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand*
3. *Department of Biochemistry and Molecular Biology and Bio21 Molecular, Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria 3010 Australia*

Malaria is one of the most deadly diseases resulting in over 600,000 fatalities per annum.(*1*) Accurate and early diagnosis followed by the immediate treatment of the infection is essential in reducing mortality(*2*). New technologies to diagnose malaria must be cost effective and have high sensitivity to enable the detection of premature parasitic forms in peripheral blood. During the course of its life the malaria parasite transgresses through several developmental stages including a sexual and an asexual reproductive pathway. The detection of the rings and gametocytes at low parasitemia in peripheral blood is critical for early diagnosis and treatment. Here we show that Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy in combination with a partial least squares regression modeling has the required sensitivity and ease of sample preparation to become a laboratory standard for malaria detection and most importantly quantification. The absolute quantification limit was found to be 0.001% (50 parasites/uL of blood) for cultured ring stage and gametocyte parasites in a suspension of normal erythrocytes. The absolute detection limit was found to be 0.00001% for laboratory cultured parasites.(*3*) The method is simple, quick and only requires the whole blood to be spun down and the plasma and white cells removed. The red blood cells are then fixed in methanol and a 5 µL aliquot of packed cells is placed on the diamond window of the ATR-FTIR spectrometer, rapidly dried and a spectrum recorded in approximately 20 seconds.

In December 2014 we commenced a pilot trial in North East Thailand investigating the potential of the technology to diagnose malaria in a field/clinical setting. Samples were collected from two independent clinics and analysed with two independent ATR-FTIR spectrometers. The results were combined and a Partial Least Squares Discriminant Analysis (PLS-DA) model developed and tested on a totally independent test set. Although the sample number was small 28 negatives and 30 positives (including patients infected with *P. falciparum*, *P. vivax* and a mixture of both) the replicate number was high as three aliquots for each sample were analysed. The percentage of correctly diagnosed spectra based on comparison with antibody capture rapid diagnostic test (RDT) was 97 %. The high sensitivity, low cost, ease of use, portability and robustness of the ATR-FTIR technique could see it become a standard diagnostic tool in both the clinic and remote field locations.

1 C. J. L. Murray, L. C. Rosenfeld, S. S. Lim, K. G. Andrews, K. J. Foreman, D. Haring, N. Fullman, M. Naghavi, R. Lozano, A. D. Lopez, Global malaria mortality between 1980 and 2010: a systematic analysis. *The Lancet* **379**, 413-431 (2011).

2 K. Chotivanich, K. Silamut, N. P. J. Day, Laboratory diagnosis of malaria infection-A short review of methods. *New Zealand Journal of Medical Laboratory Science* **61**, 4 (2007).

3 Khoshmanesh, M. W. Dixon, S. Kenny, L. Tilley, D. McNaughton, B. R. Wood, Detection and Quantification of Early-Stage Malaria Parasites in Laboratory Infected Erythrocytes by Attenuated Total Reflectance Infrared Spectroscopy and Multivariate Analysis. *Analytical chemistry* **86**, 4379-4386 (2014).

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

DETECTION OF PLASMODIUM SPP. IN BLOOD SAMPLES FROM ANGOLA

MA Santana-Morales[1-4], AR Aragao[1], P Freitas[1], Quispe MA[2], G Bonnet[1], P Berzosa[3-4], B Valladares[1-4], E Martinez-Carretero[1-4]

[1] University Institute of Tropical Diseases and Public Health of the Canary Islands University of La Laguna, Tenerife-Spain.

[2] University San Antonio Abad, Cuzco-Peru.

[3] National Centre of Tropical Medicine, Institute of Health Carlos III, Madrid-Spain.

[4] The Spanish Tropical Diseases Research Network (RICET in Spanish), Madrid-Spain. msanmor@ull.edu.es

According to the Angolan National Malaria Control Programme, malaria remains a major problem in Angola. The entire population is at risk of infection, and according to Ministry of Health estimates, Plasmodium falciparum accounts for 92% of infections, followed by P. vivax in about 7% of cases (COSEP 2011). Other study showed that the majority of cases of malaria are caused by P. falciparum (87%); P. vivax, P. ovale, and P. malariae represented 7%, 3%, and 3% of cases, respectively (PMI 2014). This situation could be changing and the infection caused by species of Plasmodium different to P. falciparum can be gaining higher presence in some parts of Africa.

The aim of this study was to investigate the presence of Plasmodium in two Angola region (Luanda y Caxito) and determine the proportion of the different species detected.

This study was conducted in Luanda and Caxito during April-June in 2009. A sample of 434 outpatients (age range, 5 to 70 years) was randomly selected from health facility posts. One capillary blood specimen is taken from each patient on filter paper (Whatman C 3MM) for molecular assay. Blood samples were assayed by SnM-PCR, according to the protocol of Rubio et al. 1999, for detection of Plasmodium species.

The presence of infection in Luanda was 5,5% of the total samples, where the three species of Plasmodium (P. falciparum, P. vivax and P. ovale) were identified. P. falciparum showed the highest infection rate with 54.5% of the positives samples. In the case of the other species, the infection rate was 36.4% of P. vivax and 9.1% of P. ovale.

In Caxito, 18% of the samples were positives for Plasmodium. In this area, the three species of Plasmodium (P. falciparum, P. vivax and P. ovale) were identified and mix infection too. The infection rate in the different species was 45.2% of P. falciparum, 38.1% of P. vivax, 12% of P. ovale and 4.7% of mix infection (P.f.-P.v.).

In this study, Plasmodium frequency in Caxito was three times higher than in Luanda. In both regions, the three Plasmodium species (P. falciparum, P. vivax and P. ovale) were detected in the study population.