

Book of Abstracts

6th PAMCA

Annual Conference & Exhibition

Theme:

Strengthening surveillance systems for vector-borne disease elimination in Africa

Hilton Hotel, Yaoundé, Cameroon 23rd - 25th September 2019









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THE 6th PAMCA Annual Conference & Exhibition

Theme:

"Strengthening surveillance systems for vector-borne disease elimination in Africa"

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WELCOME ADDRESS



Prof. Charles Mbogo | PAMCA President

It is with great pleasure that I extend a warm welcome to all participants to the 6th Pan-African Mosquito Control Association (PAMCA) that will take place here in Yaounde, Cameroon, between 23rd and 25th September 2019. The PAMCA conference and exhibition is an international forum where scientists, national program officials, NGOs and industry representatives meet to discuss the latest updates in vector control, to disseminate their research findings, to build

collaborations, networks and partnerships. The conference and exhibition has been held in several African countries including Kenya, Tanzania, Nigeria, Burkina Faso, Zimbabwe and now Cameroon. I note with pride that this conference has become a truly international event and has helped bring together all stakeholders working to eliminate vector borne diseases in Africa.

This year, the PAMCA Cameroon Chapter, in collaboration with the PAMCA Secretariat is hosting the conference. I am happy that many representatives here come from all corners of the continent and beyond. A record 350 delegates are expected to attend this conference. More would have liked to attend noting from the numerous communications handled by the Secretariat but due to budgetary constraints we were unable to extend the sponsorship requested. We hope to be able to source for more funding in future to support more participants. PAMCA has continuously grown not only in total number of delegates and exhibitors but also in its stature and significance with regards to the practice of mosquito research and vector control implementation in Africa and worldwide. With its comprehensive oral and poster presentations as well as high-level plenary sessions discussing the latest developments and issues in vector control, the three-day event truly serves as an important platform for networking, knowledge sharing, partnering, and for ultimately shaping the vector control agenda on the continent.

The 6th PAMCA Conference theme, "*Strengthening Vector Surveillance Systems for Malaria Elimination in Africa*", fully captures the current focus of the Association in the face of the heterogeneity in malaria transmission, the growing threat of insecticide resistance, and the arrival of new vector control tools on the market that will require a strong surveillance system to target the limited resources where they are needed. One key highlight of this conference is the **Women in Vector Control forum** which organized a workshop for the past two days deliberating on the challenges women face in their workplace and careers. During this conference they will hold a symposium to update us on their conclusions. This initiative is a deliberate effort of the Association to create a safe space for women in their workplace and allow them to play their rightful role in the control of vector borne diseases.

This year we have continued to partner with Target Malaria to organize a pre-conference training course on gene drive technology in which over 25 young scientists from different disciplines across Africa were competitively selected to undergo a three-day fully sponsored training on the gene drive technology.

Last year the Pan African Mosquito Control Association in partnership with the Bill & Melinda Gates Foundation (BMGF) and in collaboration with the Wellcome Sanger Institute (UK)initiated a new program that brings together different African institutions to accelerate research on the genomics of malaria vectors in the continent. This initiative is supporting local capacities in selected African institutions for collection, curation, analysis and interpretation of essential genetic data on diversities and gene flow in *Anopheles* mosquitoes in Africa. Recent efforts such as the *Anopheles gambiae* 1000 genome consortium have made contributions to this process but genetic diversity in the other important vectors have not been elucidated. Remaining challenges include the lack of essential information of the genetic diversity and gene-flow trends in *An. funestus*, *An. colluzzi* and *An. arabiensis*, which dominate malaria transmission in Africa. In this conference, you will get updates on this initiative from some of the 9 funded candidates.

I would like to thank all the members of the Local Organizing Committee (LOC), the International Organizing Committee (IOC) and the Scientific Committee, organizers of the pre-conference workshops on women in vector control and gene drive technology, and all who have worked tirelessly to make this conference a success. A lot of organizations have contributed financially, materially or in human resources. I wish to thank all the conference Gold, Silver and Bronze partners. PAMCA wishes to extend its gratitude to all of them. I also would like to extend a special thank you to the Chair of the local organizing committee Dr Antonio Nkondjio for all his work organizing this conference. Others whom I am not able to thank individually should know that their services were also a great contribution to the success of this meeting.

I believe that you will be truly inspired, and will leave with a renewed energy and focus. If you are not already a registered member of PAMCA, I encourage you to sign up to join our network. No man is an island and together we can truly impact and eliminate vector borne diseases in Africa.

I hope you enjoy every minute of this conference! We are confident that the event will truly be fruitful and memorable for everyone, and we now look forward to welcoming you all!



Dr. Antonio-Nkondjio Christophe | Chair, Local Organizing Committee

We are pleased to welcome you in Cameroon also call Africa in miniature. Delegates presenters, scientists and sponsors it is an immense honor for us to welcome you to the 6th edition of the Pan Africa Mosquito Control Association (PAMCA) annual conference and exhibition which is organized for the first time in Central Africa. The main theme for this year is "Strengthening surveillance systems for vector borne diseases elimination in Africa". Vector borne diseases account for 17% of infectious diseases, and are responsible

for more than one million deaths each year worldwide. Africa alone accounts for 40% of the burden caused by these diseases. Eliminating insect transmitted diseases could make Africa become economically prosperous. Today we are in a position to work for Africa and make significant changes, let us take advantage of this situation to contribute to Africa prosperity and inspire the next generation.

This conference is the lieu for delegates to address challenges affecting the elimination of vector borne diseases in Africa which stand at many levels (vector control (insecticide resistance, change of behavior...), case management ...) and advocate for support to strengthen capacities of the personnel and the health system and control interventions.

The conference is well designed to enable us properly assess past successes and failures, and to look for sustainable actions and innovative interventions that could drive us through the elimination of vector borne diseases in Africa. No actors should be put aside in the fight and elimination of vector borne diseases, communities, scientist, partners, stakeholders all need to work together to achieve this goal that we cherish.

Let me introduce you Yaoundé the host city of the conference also known as the city of 7 mountains which charms we believe will inspire you and make your stay in Cameroon a pleasant and wonderful one. We hope the 6th PAMCA annual conference in Yaoundé will be one of the most fruitful in terms of collaborations, new ideas, new networks, renew efforts of partners to support the fight and elimination of vector borne diseases.

Enjoy your stay in Yaoundé

KEYNOTE SPEAKER



Prof Wondji Charles

Charles Wondji is a Professor of vector genetics at the Liverpool School of Tropical medicine in United Kingdom and a Wellcome Trust Senior Research Fellow in Biomedical Sciences. He is currently the head of the LSTM research Unit at the Centre for Research in Infectious Diseases in Cameroon. He uses genetic and genomic tools to better characterise mosquito populations and help control vectors of diseases such as malaria, dengue or Zika. His research aims at understanding the genetic basis of insecticide resistance by detecting

molecular resistance markers using genomic tools and designing suitable molecular assays to track resistance in field populations. He is also defining patterns of gene flow and selective sweeps to predict the evolution and spread of resistance while assessing the fitness cost of resistance and its impact on control interventions using experimental huts trials in Africa. He is member of the WHO prequalification team in vector control.

Abstract of talk

Title: Molecular basis of Insecticide Resistance in Major Malaria Vectors

Charles S. Wondji^{1,2}

¹Liverpool School of Tropical Medicine, Liverpool, United Kingdom

²Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon

Malaria control relies heavily on insecticide-based interventions such as insecticide-treated nets or indoor residual spraying although the continued success of these tools is threatened by the growing spread of insecticide resistance. Unless such resistance is properly managed, the recent gains in reducing malaria transmission through these tools could be lost.

Recent studies have revealed a complex genomic evolution of resistance in malaria vectors in Africa which is likely to impact the design of IRM. Target site resistance mechanisms notably knockdown resistance (kdr) markers are spreading extensively and in some regions are now nearly fixed. There are also extensive reports of metabolic resistance to insecticides notably to pyrethroid insecticides in major malaria vectors (An. gambiae and An. *funestus*) with evidences that it is reducing the efficacy of pyrethroid-based interventions. Transcriptomic and genomic analyses have revealed that cytochrome P450 gene are playing a major role beside glutathione S-transferase genes. Whole genome sequencing has detected extensive genomic signatures of selective sweep around the major resistance genes in resistant populations and in some cases clear evidences that the selection was caused by the scale up of insecticide-treated nets. Furthermore, a complex evolution of metabolic resistance has been associated with the selection of several evolutionary features such as copy number variation (CNV), structural variations including indels or transposons and variations in cis-acting regulatory elements including transcription factor binding sites. Significantly, DNA-based markers of metabolic resistance are now being detected in major vectors including An. funestus and An. gambiae.

The recent improved elucidation of molecular basis of insecticide resistance should help to better tract resistance, assess its impact on control interventions and malaria transmission and improve resistance management.

PLENARY SPEAKERS



Prof. Yeya Touré

Yeya Tiemoko Touré, was born in 1952 in Gao (Mali). He has a Doctorat d'Etat ès Sciences (1985) in life sciences (vector population ecological genetics) from Université de Droit, d'Economie et des Sciences d'Aix-Marseille III, France. He was Professor of cell biology and genetics and medical entomologist at the Faculty of Medicine and odonto-stomatology (FMOS) in Bamako, Mali from 1981 to 2017. As a professor and researcher, he has conducted and managed several research grants, trained

several trainees at MSc and PhD levels and published more than 90 articles and book chapters on the subjects of vector biology, ecology and genetics, vector control and resistance to insecticides, malaria and lymphatic filariasis epidemiology and transmission and research management. He was the Director General of the National Research Council (CNRST) from 1987 to 1991. He has created and managed the Malaria Research and Training Centre (MRTC) from 1991 to 2001 in Bamako in partnership with the USA-NIH. From 2001 to 2014, he has managed the vector biology and control research program of the World Health Organization Special Program for Research and Training in Tropical Diseases (TDR) in Geneva (Switzerland). He retired from World Health organization (WHO) in July 2014. He served as member of several scientific advisory committees for WHO and for institutions in Africa, Europe and USA. He is a fellow of the World Academy of Sciences (TWAS) since 1997 and a member of the Malian Academy of Sciences since 2016. He retired from the University of Sciences Techniques and Technologies of Bamako (USTTB) in January 2018. His honours include: Médaille d'argent du Mérite National du Mali avec effigie Abeille (1995) and UNESCO-TWAS Scientific Research Prize (27 November 2018).

Abstract of talk

Title: Issues and challenges for vector control contribution to malaria elimination in Africa

Prof Yeya Tiemoko Touré

The World Malaria Report 2018 indicated that in 2017 most malaria burden was observed in the WHO African Region (200 million or 92%) and progress in global malaria control has stalled. Also, in sub Saharan Africa, access to core malaria vector control tools (insecticide-treated nets: ITNs and indoor residual sprayings: IRS) remains low (50% coverage for ITNs and 3% for IRS) and vector resistance to insecticide was widespread. However, despite this situation, globally, there is a strong indication that malaria elimination is within reach, as the number of countries with less than 100 indigenous cases increased from 15 in 2010 to 26 in 2017. Consequently, it is important to focus continuing to support both countries with a high burden of malaria and those accelerating towards elimination.

The key vector control issues included: (i) continued rise of malaria in high burden countries; (ii) insufficient coverage by ITNs and IRS; (iii) continued spread of resistance of

vectors to insecticides; and (iv) insufficient resources (human, financial, infrastructure..). Therefore, the key challenges are: (i) halting the rise of malaria in high burden countries; (ii) improving the impact of integrated vector control interventions and surveillance; (iii) ensuring adequate coverage by ITNs and IRS; (iv) improving insecticide resistance monitoring and management; and (v) securing sufficient resources.

In order to overcome the challenges and achieve continuing progress in malaria elimination, vector control requires: (i) integrated approach, tailored responses, high coverage and secured resources; (ii) strengthened integrated vector surveillance; (iii) strengthened multi-sectoral collaboration, coordinated country response and community engagement; and (iv) fostered research for the development and testing for public health impact of new and innovative vector control tools and strategies.

Keywords:

Malaria, lymphatic filariasis, epidemiology and transmission, vector biology, ecology, genetics, resistance to insecticides and control



Dr Fontenille Didier

I am basically a medical entomologist. After 17 years working on malaria and arboviruses vector biology, genetics and control in Africa (Madagascar, Senegal, Cameroon) I have directed the CNRS-IRD-Montpellier University Research Unit MIVEGEC, IRD, Montpellier, France (165 scientists in 11 countries) : <u>www.</u> <u>mivegec.ird.fr</u>, from 2005 to 2014. During this period in France I initiated research works on potential arboviruses and malaria vectors in Europe.

I was the first director of the CNEV (French national reference center on Vectors), France : <u>www.cnev.fr/</u> from its beginning in 2011 to 2014.

I have been the initiator and Coordinator (13) or co-PI of about 40 scientific projects and training networks, in Africa, Europe and Asia between 1990 and 2018 (ANR, EU, WHO, NIH, etc.).

I have been a member of several European Networks (VBornet, BioMalPar / Evimalar, Infravec, EDEN, ESOVE), and an expert in medical entomology for several international bodies (WHO, ECDC, EU).

From October 2014 to August 2019, I have been the director of the Pasteur Institute of Cambodia (IPC, http://www.pasteur-kh.org/), leading a staff of > 240 people. IPC is conducting research and expertise works on infectious diseases and health concerns, like Tuberculosis, antimicrobial resistance, HIV, Hepatitis, malaria, dengue, Japanese encephalitis, Rabies,

Since September 2019, I am back to MIVEGEC, IRD, Montpellier, France, where I work as a expert on vector borne diseases and emerging tropical diseases

I continue to supervise graduate students. I have more than 335 publications and book chapters, mainly in the field of mosquitoes and mosquito borne diseases.

Abstract of talk

Title: Lessons of the fight against mosquito vectors in different ecological settings worldwide

Didier Fontenille

Mosquitoes transmit hundreds of different species of parasites and viruses to humans and animals.

Some, such as dengue fever viruses, are transmitted by similar cycles around the world, i.e. mainly from man to man, via less than 5 to 10 species of mosquitoes such as *Aedes aegypti* and *Ae. albopictus* in «anthropogenic» biotopes. The main human *Plasmodiums* pecies (*P. falciparum* and *P. vivax*) are also transmitted from human to human, but the vectors, which are generally wild anopheles vectors from savannah and forest, probably belong to more than 150 species depending on the world's regions and ecosystems. Biology (i.e. behavior, vector capacity and competence, distribution, susceptibility to pesticides, ...) of

these anopheline vectors is dramatically different. Moreover, some zoonotic viruses, such as the fearsome Japanese encephalitis virus in Asia or the Mayaro virus in South America, can be transmitted from wild or domestic vertebrates to humans by many species of wild mosquitoes (*Culex, Haemagogus*), whose biology is poorly known.

Vector control, when relevant, must take into account these specificities from global to local, and the fight against mosquito vectors is above all about ecology

Through several examples we will see how the vector control tools, historical or modern, such as destruction of larval development sites, IRS, impregnated mosquito nets, traps, SIT, genetically modified mosquitoes, spatial repellents... are based on a good knowledge of the ecology of the vectors.



Prof. Hilary Ranson

Professor Hilary Ranson is a vector biologist whose research focuses on the control of mosquito borne disease. Her research group at the Liverpool School of Tropical Medicine is developing and validating molecular and bioassay tools to monitor insecticide resistance in African malaria vectors. In addition, with partners in multiple countries, she is investigating the impact of insecticide resistance on malaria control and evaluating alternative products and strategies to overcome resistance.

Professor Ranson is coordinator of MIRA, a Wellcome Trust Collaborative Award to understand the performance of insecticide treated nets under contemporary malaria transmission settings and is the lead scientist of a major research capacity strengthening programme, the 'Partnership for Increasing the Impact for Vector Control', funded by the UK government.

She is member of the WHO Vector Control Advisory Committee.

Abstract of talk

Title: Improving the Efficacy of Malaria Prevention in an Insecticide Resistant Africa

Hilary Ranson, Tom Churcher, Heather Ferguson, Federica Guglielmo, Hyacinthe K Toe, Caroline Jones, Steve W Lindsay, Natalie Lissenden, Philip J McCall, Jason Matthiopoulos, Greg Murray, Sagnon N'Fale, Antoine Sanou, Alfred B Tiono, David P Towers, Eve Worrall, Jean-Baptiste Yaro

Impressive reductions in malaria have occurred throughout sub-Saharan Africa in the 21st century, but progress has not been geographically uniform: despite high coverage of WHO recommended interventions for prevention and treatment, malaria persists in some high-burden countries. We have been undertaking a multidisciplinary study recording the human, mosquito and health systems factors that are collectively contributing to the intolerably high levels of malaria transmission in Burkina Faso. Entomological data from this region highlight the huge challenges imposed by high levels of pyrethroid resistance and extensive outdoor biting in the vector population. By combining this entomological data with data on human sleeping patterns we highlight the coverage gaps that remain even if universal coverage with bednets is achieved, and present findings from ethnographic studies identifying opportunities and challenges afforded by a deeper understanding of the activities different sectors of society are conducting outside the home. Preliminary modelling outputs from the study, showing the potential impact of the addition of other interventions in this region will be presented.



Dr. Carnevale Pierre

He is Director of Research "Exceptional Class". He has a special Diploma in Ecology Option Entomology (Marseille University); a PhD from the university of Orsay France and Habilitation for Directing Research (HDR) from the Montpellier University. He has over 200 publications published in refereed journals; authors and coauthors of several books. He was for several years member of WHO experts groups: International Health Regulation (IHR), Vector Biology Control (VBC), Technical Expert Group (TEG) on

insecticide resistance and vector control for malaria control programme of the Global Malaria Control (GMP); Panel Expert Environmental Management for Vector Control (PEEM). He also held position as an expert in the French Ministry of Cooperation, Interdepartmental Agency for mosquito control" (EID) External Scientific Advisory Committee (ESAC) of the Innovative Vector Control Consortium group (IVCC) of the B & M Gates Foundation. He chaired several expert groups Biocide (and repellents) Experts group AFSSAPS Agency of the French Ministry of Health; the Scientific and Technical Council (STC) of ADEGE (French agency for mosquito control and management of natural areas without any more mosquitoes).

He acted as head of Medical Entomology Laboratory of the National Public Health Laboratory of ORSTOM in Brazzaville, Bobo-Dioulasso and Yaoundé.

Since 2003 he has retired from IRD. Since then he has conducted several research programmes in Angola: Head /coordination of the Malaria Control Programme (MCP) of the Sonamet Angolese Private Society in Lobito

Guinea Conakry: entomological and epidemiological surveys, evaluation of risks and measures to be taken in remote mountain mines for scheduled settlement (Medical) Programme International SOS;

China: IHR WHO surveys of several airports evaluation for "International Airport" label;

VietNam: evaluation of ITN programmes at the request of the Tropical Institute of Anvers;

Nigeria, Gabon: surveys for the evaluation of malaria risks and measures to be taken for off shore and on shore "oil and gas companies" workers;

Haïti, surveys at the request of PAHO, to evaluate the malaria situation and actions to be taken after the earthquake.

Abstract of talk

Title: The Balombo (Benguela Province, Angola) Project: a village scale malaria vector control programme with a long term comprehensive evaluation.

Pierre Carnevale¹ Vincent Foumane², Jean Claude Toto² ; Maria Dos Santos³ Filomeno Fortes⁴, and Sylvie Manguin⁵

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Since 2007 a village scale malaria vector control (VC) programme was implemented by MCP team, its collaborators and the NMCP in 8 villages around Balombo (Benguela Province, Angola) to evaluate and compare the efficacy of 4 methods: the classical LLIN PermaNet® 2 (deltamethrin, 55 mg a.i./m²), Inside Residual Spray (IRS) (lambdacyhalothrin, target dosage 25 mg a.i./m2) and the recently developed Insecticide Treated Plastic Sheetings (ITPS) presented in 2 models: the "Wall lining" (deltamethrin dosage 170 mg a.i//m²) and the "Zero Fly®" (deltamethrin, concentration 360 mg a.i. /m²). ITPS was used alone or in association with LLIN or after the fading of IRS. The average amount of insecticide/house was greatly different according to the method of VC used: # 2 gr a.i./house with LLIN or IRS; # 5 gr a.i./house with LLIN alone and > 10 gr a.i./house with the association "P2" + "ZF" or ZF alone. 2 paired villages randomly received one of these 4 methods of VC.

The project involved 3 phases (" ϕ "): ϕ 1: 2007-2011: base line data (2007-2008), full coverage in VC (end 2008), first 3 years comprehensive evaluation (entomology, parasitology, immunology) in the 8 villages; ϕ 2: 2011-2015: social studies and sustainability issues with parasitological regular Cross Sectional Surveys in 4 villages (1/method of VC); ϕ 3: 2015-2018: comparison of parasitological situations between "initial villages" and some other villages without any MCP interventions.

 \oint 1: From 2007 to 2011: 202 catching session with CDC Light Traps were implemented in the 8 selected villages representing a total of 1880 "trap-nights". 1152 Anopheles were caught belonging to 10 species: the main one was An.funestus (44.5%) then An.marshalli (21.1%); An.maculipalpis (17.9%); An.ziemanni (6.5%) and An.gambiae s.l. (4.8%) (mainly An.gambiae with few An.coluzzii and some An.arabiensis), some An.pharoensis (1.9%); An.nili (1%); An.coustani (0.9%); An.tenebrosus (0.9%) and An.hancocki (0.6%).

The analysis focused on the main vectors ("MV") An.funestus and An.gambiae.

Comparing the situation "before/after" vector control it appeared that the "average density of main vectors/trap" decreased by around 71% with similar drop for each VC method (-63% with LLIN; -69% with LLIN + ZF; - 82% with WL; - 75% with IRS then ITPS). The average infectivity of MV was reduced by 66, 4% (with comparable efficacy for each VC method) and the average Yearly "Entomological Inoculation Rate" (EIR) was reduced by 90% with comparable efficacy whatever the method of VC was showing that increasing by 5 and more the amount of insecticide /house did not increase the entomological efficacy (and could increase the risk of selective insecticide pressure).

On the other hand 188 Cross Sectional field parasitological Surveys (CSS) were done collecting a total of 21817 thick films where asexual Plasmodial infections (P+) were noticed 5433 times i.e. an overall Plasmodic indice (PI) of 24.9% almost similar in the 4 "vector control" methods conditions. P.falciparum was largely predominant with very few P.malariae alone and some mixed infections P.falciparum + P.malariae. Gametocytes of P.falciparum (G+) were observed in 555 thick films i.e. an overall gametocytic indice (GI) of 2.5% almost similar in the paired villages.

Comparing the situation "before/after" VC it appeared that the Plasmodic Indice (PI) of children < 15 years sharply and significantly decreased (from # 41% to # 12% i.e. overall reduction 71%) with the 4 methods of VC: - 65% with LLIN; - 74% with LLIN+ZF; - 72% with WL alone and – 72% with IRS then ITPS and during these 5 years the trends of decrease were the same for the 4 VC methods. Interestingly it also appeared a significant drop of parasitaemia with each VC showing the potential of malaria morbidity reduction obtained with VC which also induced a significant reduction of gametocyte indice (overall reduction 65%) with LLIN (--53%); LLIN + ZF (-67%); WL alone (- 67%) and IRS (-77%) and therefore of the potential of infectivity for vectors. Checking the evolution each year of the average plasmodial prevalence (PI) it appeared that it dropped from > 50% the year before any intervention to < 5% in 3 years and the parasitological impacts appeared similar with every VC implemented. At that stage it was considered that VC was a great success in Balombo villages.

 $\frac{1}{222}$ 2011-2015: during the field surveys we often noticed LLIN and ITPS outside houses, used/misused (see pictures) and a special study was devoted to the sustainability of these tools implemented in a full coverage at the end of 2008 and we systematically checked the presence or not of LLIN or ITPS in the house of each patient during each CSS. It appeared that in "LLIN alone village" 50% of nets distributed were withdrawn the 3rd year and 20% only remained the 5th year, being in poor shape. In "WL alone village" 30% of houses had still these treated sheetings the 3rd and 4th year and 20% the 4th and 5th year.

LLIN were kept longer than ZF in village which received this association.

On the other hand 5275 thick films were done between 2011 and 2015 and 141 found with Plasmodium i.e. a general Plasmodic Indice of 2.7% which confirms the remarkable long lasting efficacy of VC implemented in these villages moreover other measures undertaken by the National Authorities.

ф3: 2015-2018:

But in 2015 less than 2% of people had kept their "protection" and at the same simple a National Malaria Outbreak blowed up in Angola; Plasmodial Prevalence increased sharply: from 5% in 2015 to # 20% in 2016 and 30% in 2018 while being lower than in "other villages" where PI reached > 50 or even 60% with heavy parasite load.

It was concluded that ITPS could usefully replace IRS being easier to implement (people pinned themselves their WL in their houses) and as efficient as IRS but they were often withdrawn and misused; LLIN were well accepted and used for several years; the

sustainability is an issue requiring IEC. Nevertheless classical vector control at village scale level can be done and it actually reduced significantly the transmission and the Plasmodium prevalence and parasitaemia which is a favorable point for VC at National Level as scheduled by the NMCP.

We want to thank the National Authorities of Angola, with the Public Health Department of Balombo and Benguela, the population of villages, the Head of Sonamet and SubSea7 Companies for their support.



Dr. Fredros Okumu

He is director of Science at Ifakara Health Institute; associate professor of Public Health at the University of Witwatersrand, South Africa; a visiting researcher at the Federal University of Minas Gerais, Brazil; and an honorary research fellow at the Institute of Biodiversity, Animal Health and Comparative Medicine at the University of Glasgow. Since 2008, Dr Okumu has been studying human-mosquito interactions and developing new techniques to complement existing malaria interventions and accelerate efforts

towards elimination. His other interests include quantitative ecology of residual malaria vectors; mathematical simulations to predict effectiveness of interventions, improved housing for marginalised communities, and prevention of child malnutrition.

He was awarded by the American Society of Tropical Medicine & Hygiene the Young Investigator Award in 2009, a Welcome Trust Intermediate Research Fellowship in Public Health and Tropical Medicine (2014–2019), and a Howard Hughes-Gates International Research Scholarship (2018–2023). He is currently a co-chair of the Malaria Eradication Research Agenda consultative group on Tools for Elimination, an associate editor of the journal, Parasites & Vectors, and co-Chair of theVector Control Working Group on New Tools for Malaria Vector Control at the World Health Organization. Dr Okumu has also participated in various international expert panels on a wide range of subjects including, genetically modified mosquitoes and ivermectin for vector control, and the NEPAD Agency of the African Union's agenda on biotechnology in Africa. He was inducted in 2016 as a Young Affiliate of the African Academy of Sciences and named among the 2016 Top 100 Global Thinkers by the US-based Foreign Policy Magazine.

He originally trained as a Public Health Officer in the College of Health Sciences at the Moi University in Kenya. He later earned a Master's degree in Applied Parasitology from the University of Nairobi, Kenya, and a second Master's degree in Geo-information Science, Earth Observation, and Environmental Modeling from Lund University, Sweden. In 2012, Dr Okumu earned a Doctor of Philosophy degree in Infectious Tropical Diseases from London School of Hygiene and Tropical Medicine, and is currently working towards a Master of Business Administration in International Health Management at the Swiss Tropical and Public Health Institute, University of Basel, Switzerland.

The multiplicity of malaria transmission and opportunities for sustainable control

Fredros Okumu, Ifakara Health Institute, Tanzania

Malaria transmission is a simple process typically occasioned by an infectious Anopheles bite. The associated events are theoretically well-understood, and could be disrupted by simply preventing human-mosquito interactions. Yet, in practice, malaria transmission remains extraordinarily complicated and heterogenous. Across Africa, it is a complex web of different vector species, pathogen species, human behaviors, environmental determinants, intervention choices and political pressures, among other factors. Though most progress accrued against malaria in the last two decades was due to scale-up of insecticide-treated nets and house spraying with residual insecticides, these interventions cause differential impact on the malaria transmission complex. This talk will discuss the multiplicity of factors in malaria transmission and the dominance of certain Anopheles species. Special attention will be put on pyrethroid resistant Anopheles funestus, which now dominates malaria transmission across east and southern Africa, despite occurring in lower densities than other vectors such as An. arabiensis. The talk will also provide an analysis of how different vector species respond to current interventions, and what opportunities exist to sustain or expand the gains so far accrued. Specific examples will be provided from east and southern Africa. In conclusion, there will be an outline of possible pathways for accelerating current efforts towards malaria elimination in Africa. In summary, all malaria control efforts must recognize the multiplicity of the disease, and deploy targeted interventions that target the most dominant vector species.



<u>Mr. Brian Gitta</u>

He invented Matibabu, a medically non-invasive device that detects malaria in two minutes. As CEO of thinkIT Limited, Brian aims to use this and other science-based innovations to help people adapt to survive climate change — which has been found to be a major driver of malaria in Africa by recent, peer-reviewed research from the Massachusetts Institute of Technology (MIT). The next step for spreading the Matibabu technology, which has the capacity to save hundreds of thousands or even millions of lives, is to get it

approval for use in various countries. In addition to Brian's work at thinkIT, he is a teaching fellow at Young Disaster Resilience Leadership, a program that helps young people address conflict and disaster challenges in schools, communities, and at home. He previously served as a software developer at United Nations Population Fund and worked in business management at Merck Group. Brian holds a bachelor's degree from Makerere University and was awarded a United Nations Empowerment Award.

Abstract of talk

In general, the lack of low cost diagnostics for malaria results in late diagnosis of the disease in many communities (contributing to high morbidity and mortality from severe forms of malaria), and over-treatment of malaria where syndromic management is used due to lack of point-of-care diagnostics (contributing to wastage of money on treatment of non-malarial illness especially since the new recommended Artemisinin-based therapies are expensive. Matibabu addresses malaria disease management through cost-effective early diagnosis of malaria, reducing the amount of medication, duration of treatment and infection rate. We also address malaria surveillance management through cost-effective real-time data collection on the results from the malaria diagnostics, reducing the resources misused as malaria fighting bodies misallocate the resources or hit barriers during the planning of interventions within the malaria control programs.

CONFERENCE PROGRAMME

Day 1: Monday 23rd September 2019

Time				
07:30 - 08:30	REGISTRAT	ION		
08:30 - 11:00	Opening Cere Main hall (BO	mony DUMA ABC)		
08:30 - 08:50		Moderator	Welcoming Minister of Health and his delegation	
08:50 - 09:00	Opening	LOC Chair	Welcome address	
09:00 - 09:15	ceremony	OCEAC Head of regional organization	Address on sub-regional strategic plans	
09:15 - 09:25		Executive Director	Opening remarks	
09:25 - 09:35		PAMCA President	Opening remarks	
09:35 - 09:45		Group Entertainment	Cultural animation	
09:45 - 10:05		Minister	Awards of distinction to senior scientists	
10:05 - 10:15		Minister	Official opening of the conference	
10:15 - 10:45		Keynote Speaker Prof. Charles Wondji	Insecticide resistance in malaria vectors in Africa: Benefit and challenges of using new molecular tools	
10:45 - 11:00	Group photo			
11:00 - 11:30	0	COFFEE BREAK (Ex	hibition and Poster session)	
11:30 - 13:00	Plenary session 1 Chairs: Prof. Rose Leke & Dr. Silas Majambere <i>Main hall (BOUMA ABC)</i>			
Time	Abstract N°	Presenter	Title	
11:30 - 11:50	P001	Prof. Yeya Touré	Issues and challenges for vector control contribution to malaria elimination in Africa	
11:50 - 12:10	P002	Dr. Didier Fontenille	Lessons of the fight against vector borne diseases in different ecological settings worldwide	
12:10 - 12:30	P003	Prof. Hilary Ranson	What can bednets achieve in an insecticide resistant Africa?: Lessons from a multidisciplinary study to assess the impact of malaria control in a high burden setting	

12:30 - 13:00	Q&A				
13:00 - 14:00	I	LUNCH BREAK (Ex	hibition and Poster session)		
Parallel sess	Parallel session 1: Harnessing research capacity in Africa to empower national programs Chairs: Dr. Mamadou Coulibaly & Dr. Awono Ambene <i>Main hall (BOUMA ABC)</i>				
14:00 - 14:10	ABS-19-0057	Agapitus Kato	Decision support to integration of larval source management in the national IVM malaria control strategy: Piloting a community-based larviciding model in Nakasongola district, Uganda		
14:10 - 14:20	ABS-19-0118	Jessy Marlene Goupeyou-Youmsi	Susceptibility of <i>Anopheles</i> to <i>Plasmodium falciparum</i> and <i>Plasmodium vivax</i> infections in Madagascar		
14:20 - 14:30	ABS-19-0131	Omo-Imafidon S Evbodaghe	Factors influencing the translation of malaria research findings to policies and practice in Nigeria		
14:30 - 14:40	ABS-19-0164	Elodie Vajda	Patterns of gene flow correlate with the expression profile of detoxification genes associated with pyrethroid resistance in the malaria vector <i>Anopheles funestus</i> in East Africa.		
14:40 - 14:50	ABS-19-0256	Mutale Chisanga	Managing a reference laboratory to support programmatic entomological surveillance		
14:50 - 15:00	ABS-19-0058	Aurelie Prisca Yougang	Nationwide distribution of insecticides resistance in <i>Aedes albopictus</i> (diptera: culicidae) in Cameroon, Central Africa		
15:00 - 15:10	ABS-19-0196	Efundem Agboraw	Methods and challenges for systematically reviewing the costs and cost-effectiveness of vector control tools and strategies		
15:10 - 15:40	Q&A				
15:40-16:10	(COFFEE BREAK (Ex	hibition and Poster session)		
	Parallel session Chair: Dr.	2: Mosquito genomic Abdoulaye Diabaté & Workshop Hall (BE	cs: progress and challenges c Dr. Didier Fontenille <i>TE ABC</i>)		
14:00 - 14:10	ABS-19-0237	Nsa Dada	The mosquito microbiome and insecticide resistance		

14:10 - 14:20	ABS-19-0092	Alistair Miles	Insights into the evolution and spread of insecticide resistance from whole genome sequencing of 1,142 <i>Anopheles</i> <i>gambiae</i> mosquitoes from 13 countries
14:20 - 14:30	ABS-19-0072	Kouamo Mangoua Mersimine Flore	Functional validation of the role of Anopheles funestus epsilon glutathione-s-transferases in insecticide resistance
14:30 - 14:40	ABS-19-0183	David Weetman	Duplications, selection and introgression drive the spread of resistance to organophosphates in West African <i>Anopheles gambiae</i>
14:40 - 14:50	ABS-19-0235	Tony Nolan	Improving gene drives towards complete suppression of <i>Anopheles</i> <i>gambiae</i> populations
14:50 - 15:00	ABS-19-0227	Keith Hayes	Assessing risks associated with genetic control methods for malaria vectors
15:00 - 15:10	ABS-19-0035	Thabo Mashatola	Progress on the development of sex- separation techniques for sterile insect technique applications against malaria vectors <i>Anopheles arabiensis</i>
15:10 - 15:40	Q&A		
15:40 - 16:10	(COFFEE BREAK (Ex	hibition and Poster session)
16:10 - 18:00	Symposium 1: Guidance on stakeholder engagement principles to inform the development of area-wide vector control methods <i>Main hall (BOUMA ABC)</i> Principal organizer: Ms. Lina Finda, Co-organizer: Dr. Delphine Thizy		
	Parallel session 3: Vector biology and control Chairs: Dr. Ndo Cyrille & Mr. Elijah Juma <i>Workshop Hall (BETE ABC)</i>		
16:10 - 16:20	ABS-19-0003	Dickson Lwetoijera	Autodissemination of pyriproxyfen suppresses stable populations of <i>Anopheles arabiensis</i> under semi- controlled settings
16:20 - 16:30	ABS-19-0028	Alia Armel Roland	Study of the efficacy of <i>Bacillus</i> <i>thuringiensis israelensis</i> for the control of <i>Anopheles gambiae</i> s.l resistant to pyrethroid

16:30 - 16:40	ABS-19-0082	Caroline Kiiru	Flourishing in germs: deciphering the role of bacteria in development of the malaria vector, <i>Anopheles gambiae</i>
16:40 - 16:50	ABS-19-0095	Kenyssony Valera	A good spray: entomological surveillance results from a cluster randomized trial to evaluate the impact of a third-generation indoor residual spray product on malaria transmission in Mozambique
16:50 – 17:00	ABS-19-0108	Theresia Nkya	Evaluating the feasibility and impact of community-based winter larviciding on malaria transmission in southern of Africa countries aiming for malaria elimination
17:00 - 17:10	ABS-19-0110	Etienne Fondjo	Insecticide susceptibility status of <i>Anopheles gambiae</i> s.l. in four sentinel sites in Cameroon
17:10 – 17:20	ABS-19-0022	Delenasaw Yewhalaw	Characterization of resistance mechanisms and bio-efficacy of pyrethroid-PBO nets against pyrethroid resistant populations of <i>Anopheles</i> <i>gambiae</i> s.l in selected malaria sentinel sites in Ethiopia
17:20 - 18:00	Q&A		
18:00 - 20:00	Welcome reception/cocktail sponsored by Sumitomo (Hilton Hotel)		

Plenary session 2 Chairs: Prof. Wilfred Mbacham & Dr. Carnevale Pierre Main hall (BOUMA ABC) Time Abstract N° Presenter Title 08:30 - 08:50P004 Dr. Fredros New tools in the fight against vector borne Okumu diseases 08:50 - 09:10Dr. Pierre P005 A successful vector control programme in Carnevale rural area of Balombo (Angola) with the newly developed tool insecticide treated plastic sheeting 09:10 - 09:30How do we close the gap between the P006 Mr. Brian Gitta communities and their rightful access to healthcare? 09:30 - 09:50P007 Neglected tropical diseases in Central Dr Bonventure Africa Savadogo 09:50 - 10:30Q&A COFFEE BREAK (Exhibition and Poster session) 10:30 - 11:0011:00 - 13:00Symposium 2: Strengthening the role of women in the control of vector borne diseases Main hall (BOUMA ABC) Principal organizer: Dr. Damaris Matoke, Co-organizer: Ms. Emma Orefuwa Symposium 3: Target Malaria insectary and field entomology preparedness 11:00 - 13:00towards the 1st releases of genetically-modified malaria mosquito Anopheles gambiae s.l. Workshop Hall (BETE ABC) Principal organizer: Dr. Frederic Tripet, Co-organizers: Dr. Abdoulave Diabate, Dr. Mamadou Coulibaly, Dr. Jonathan Kayondo & Dr. Andy **McKemey** 13:00 - 14:00LUNCH BREAK (Exhibition and Poster session) Parallel session 4: Innovations in vector surveillance and control Chairs: Prof. Charles Wondji & Prof. Timoleon Tchuinkam Main hall (BOUMA ABC)

Day 2: Tuesday 24th September 2019

14:00 - 14:10	ABS-19-0001	Carmène Sandra Ngadjeu	Influence of house characteristics, on mosquito distribution and malaria transmission in the city of Yaoundé
14:10 - 14:20	ABS-19-0145	Piameu Michael	The LabDisk diagnostic tool: contribution to vector surveillance toward malaria elimination in Cameroun
14:20 - 14:30	ABS-19-0149	John Lucas	SumiShield 50WG: meeting the challenge of insecticide resistance
14:30 - 14:40	ABS-19-0073	Michael Coleman	Quality Assurance in IRS – New Tools
14:40 - 14:50	ABS-19-0083	Yacouba Poumachu	Towards development of genetic sexing strain for sex separation and female elimination in the malaria vector <i>Anopheles</i> <i>arabiensis</i> (Diptera: Culicidea) for use in sterile insect technique program
14:50 - 15:00	ABS-19-0039	Elijah Juma	Effect of sublethal pesticides exposure on the gut microbiota of <i>Culex pipiens</i> L. (Diptera: Culicidae)
15:00 - 15:10	ABS-19-0245	Elias Miyituma	Host preference and feeding patterns of primary malaria vectors, <i>Anopheles</i> <i>arabiensis</i> and <i>Anopheles gambiae</i> s.s. in sites with or without Indoor Residual Spraying in Rwanda.
15:10 - 15:30	Q&A		
15:30 - 16:00	C	OFFEE BREAK	(Exhibition and Poster session)
Parallel	session 5: Adop Chairs: Pro	ting the One Hea of. Josiane Etang Workshop Hall (.	alth approach – breaking down silos & Dr. Fredros Okumu BETE ABC)
14:00 - 14:10	ABS-19-0084	Ellie Sherrard- Smith	The potential of mosquito habitat management for malaria control across Africa
14:10 - 14:20	ABS-19-0127	Tovi Lehmann	Windborne long-distance migration of African arbovirus mosquito vectors: the case for one health paradigm
14:20 - 14:30	ABS-19-0077	Fatou Fofana	The prevalence of schistosomiasis in Minna community, Jarra central district the Gambia, 2014 and 2016

14:30 - 14:40	ABS-19-0240	Rousseau Djouaka	Linking agriculture to malaria transmission under a one health approach: Does vegetable farming contribute to insecticide resistance selection in the malaria vector <i>Anopheles coluzzii</i> ?
14:40 - 14:50	ABS-19-0200	Miriam Karuitha	Arboviruses transmission dynamics along the Kenyan coast
14:50 – 15:00	ABS-19-0261	Magang Kemta Melaine Eugenie	Targeting vector control of animal trypanosomiasis by identifying drug- resistant trypanosomes in tsetse flies
15:00 - 15:10	ABS-19-0081	Francis Wat'senga	Increasing intensity of pyrethroid resistance in <i>Anopheles gambiae</i> s.l. and the implication for vector control in Democratic Republic of Congo
15:20 - 15:40	Q&A		
15:40 - 16:00	COFFEE BREAK (Exhibition and Poster session)		
16:00 - 18:00	Symposium 4: Vector control innovations to drive progress in malaria and other mosquito-borne disease control <i>Main hall (BOUMA ABC)</i> Principal organizer: Dr. Allison Tatarsky, Co-organizers: Dr. Derric Nimmo; Dr. Fredros Okumu		
16:00 - 18:00	Symposium 5: Enhancing entomological capacity in Africa for effective vector-borne disease control: prospects, challenges, and opportunities <i>Workshop Hall (BETE ABC)</i> Principal Organizer: Mr. Elijah Juma, Co-organizer: Dr. Kevin O. Opondo		
18:30 - 21:00	Quiz night sponsored by Vestergaard (Cascades du Mfoundi)		

Day 3: Wednesday 25th September 2019

	Plenary session 3 Chairs: Prof. Same Ekobo & Prof. Jude Bigoga Main hall (BOUMA ABC)				
Time	Abstract N°	Presenter	Title		
08:00 - 09:00	PAMCA Symposium				
09:00 - 10:00	Industry update				
10:00 - 10:30	COFFEE BREAK (Exhibition and Poster session)				
10:30 - 12:00	Symposium 6: Introduction to the PIIVec: Partnership for increasing the impact of vector control <i>Main hall (BOUMA ABC)</i>				
	Principal organizer: Prof. Charles Wondji, Co-organizer: Prof. Philip J McCall				
12:00 - 13:00	LUNCH BREAK (Exhibition and Poster session)				
P	Parallel session 6: Innovation in vector surveillance and control Chairs: Prof. Samuel Wanji & Prof. Giseli Foko Workshon Hall (BETE ABC)				
10:30 - 10:40	ABS-19-0089	Michelle Stanton	The operational feasibility of using drones to identify <i>Anopheles</i> mosquito breeding sites in Malawi		
10:40 - 10:50	ABS-19-0093	Cédric Pennetier	The REACT randomised controlled trial to assess whether addition of complementary vector control strategies to long-lasting insecticidal mosquito nets provides additional protection against clinical malaria in areas with pyrethroid-resistant vectors in rural Burkina Faso and Ivory Coast		
10:50 - 11:00	ABS-19-0163	Emmanuel N'dille	Overexpression of two members of D7 Salivary genes family is associated with pyrethroid resistance in the malaria Vector <i>Anopheles funestus</i> s.s. but not in <i>Anopheles</i> <i>gambiae</i> in Cameroon		
11:00 - 11:10	ABS-19-0182	Francesco Baldini	Age and species prediction of field malaria mosquitoes through deep learning of mid- infrared spectra		

11:10 - 11:20	ABS-19-0216	Givemore Munhenga	A five-year (2014 -2018) entomological surveillance in prospective of a pilot sterile insect technique feasibility study in Mamfene, Kwazulu-Natal, South Africa		
11:20 – 11:30	ABS-19-0088	Nanwintoum Severin Somda Bimbile	Insects to feed insects: larval diets based on insects for mass-rearing a major vector of malaria, <i>Anopheles arabiensis</i> , Patton (Diptera: Culicidae).		
11:30 - 11:40	ABS-19-0275	Litula Litula	Entomological Surveillance for Malaria Elimination in Namibia		
11:40 – 11:50	ABS-19-0271	A. Sanou	Biting and Resting Behaviours of Malaria Vectors in Rural Burkina Faso Following Scaling Up of Llins.		
11:50 - 12:00	Q&A				
12:00 - 13:00	12:00 – 13:00 LUNCH BREAK (Exhibition and Poster session)				
	Parallel	session 7: Vector	or biology and control		
	Chairs: Dr. F	Rousseau Djoua	ka & Dr. Damaris Matoke		
		Main hall (BC	DUMA ABC)		
13:00 - 13:10	ABS-19-0167	Konan Yao Lucien	Resistance status of <i>Aedes aegypti</i> to insecticides in Abidjan, Cote d'Ivoire		
13:10 - 13:20	ABS-19-0178	Arthur D Djibougou	Population patterns of sandflies from two Leishmania circulating areas in the western Burkina Faso: Bobo-Dioulasso and Larama		
13:20 - 13:30	ABS-19-0186	Rosemary Bateta	Phylogeography and population structure of the tsetse fly <i>Glossina pallidipes</i> in Kenya and the Serengeti ecosystem		
13:30 - 13:40	ABS-19-0193	Athanase	Bionomics variations and genetic diversity of		
12.40 12.50		Badolo	Aedes degypti populations from Burkina Faso		
13:40 - 13:30	ABS-19-0166	Badolo Basile Kamgang	Potential of Aedes albopictus and <i>Aedes</i> <i>aegypti</i> (Diptera: Culicidae) to transmit yellow fever virus in urban areas in Central Africa		
13:40 - 13:30	ABS-19-0166 Q&A	Badolo Basile Kamgang	Potential of Aedes albopictus and <i>Aedes</i> <i>aegypti</i> (Diptera: Culicidae) to transmit yellow fever virus in urban areas in Central Africa		
Parallel session 8: Vector biology and control					
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Chairs: Prof. Hilary Ranson & Dr. Theresia Nkya					
Workshop Hall (BETE ABC)					
13:00 - 13:10	ABS-19-0154	Jennifer Stevenson	Primary and secondary outdoor vectors contributing to malaria transmission in low and high transmission settings in Zambia		
13:10 - 13:20	ABS-19-0141	Edmond Kopya	Exploring the impact of glutathione S-transferase (GST)-based metabolic resistance to insecticide on vector competence of Anopheles funestus for Plasmodium falciparum		
13:20 - 13:30	ABS-19-0268	Josiane Etang	Factors governing deltamethrin resistance in <i>Anopheles gambiae</i> s.l. from North Cameroon		
13:30 - 13:40	ABS-19-0190	Sarah Moore	Non-inferiority testing of insecticide treated nets (ITNs): comparison of results from experimental huts and Ifakara ambient chamber tests		
13:40 – 13:50	ABS-19-0211	Antonio Nkondjio Christophe	Review of malaria situation in Cameroon: challenges and prospects for disease elimination		
13:50 - 14:05	Q&A				
14:05 - 14:30	C	OFFEE BREA	K (Exhibition and Poster session)		
Booster Talk: My thesis in 3 minutes Main hall (BOUMA ABC)					
Jury: Prof. Flobert Njlokou & Dr. Prosper Chaki					
14:30 – 14:33	ABS-19-0191	John P Masalu	Potential benefits of combining transfluthrin- treated sisal products and long-lasting insecticidal nets for controlling indoor-biting malaria vectors		
14:33 - 14:36	ABS-19-0136	Hien Aristide	2018 insecticide resistance status of <i>Anopheles gambiae</i> s.l. in Burkina Faso		
14:36 – 14:39	ABS-19-0079	Amelie Wamba Ndongmo Regine	Investigating the role of CYP325A in pyrethroid resistance in <i>Anopheles funestus</i> , a major malaria vector across Africa		

14:39 – 14:42	ABS-19-0086	Harun Njoroge	The origin and spatiotemporal history of swept mutations associated with pyrethroid resistance in <i>Anopheles gambiae</i> population in East and Central Africa
14:42 – 14:45	ABS-19-0109	Mgeni Mohamed	Evaluation of passive emanator treated with transfluthrin and BG sentinel trap as the push-pull for the control of outdoor biting mosquitoes
14:45 – 14:48	ABS-19-0133	Leonard Dandalo	The biting patterns of anopheles mosquitoes from three high malaria burden districts in Malawi
14:48 – 14:51	ABS-19-0173	Dominique Mieguim Ngninpogni	Study of malaria vectors biology in Okola, South Cameroon an area with high prevalence of human malaria.
14:51 – 14:54	ABS-19-0234	Magellan Tchouakui	Investigation of the influence of a glutathione S-transferase metabolic resistance to pyrethroids/DDT on mating competitiveness in males <i>Anopheles funestus</i> , African malaria vector
14:54 – 14:57	ABS-19-0184	Armel Tedjou	Assessment of the entomological risk of arbovirus outbreaks in Yaoundé, the capital city of Cameroon based on Stegomyia and pupae indices
14:57 – 15:00	ABS-19-0272	Kaminsi Nenkam Hélène Gaelle	Investigating the influence of larval nutrition on the life traits and vector competence of Anopheles coluzzii major vector of malaria in Cameroon
15:00 - 15:30	Closing ceremony		

SYMPOSIA

SYMPOSIUM 1:

TITLE: GUIDANCE ON STAKEHOLDER ENGAGEMENT PRINCIPLES TO IN-FORM THE DEVELOPMENT OF AREA-WIDE VECTOR CONTROL METHODS

Principal organizer: Lina Finda, Ifakara Health Institute

Co-organizer: Delphine Thizy, Target Malaria, Imperial College London

Chair: Fredros Okumu, Ifakara Health Institute

Speakers:

- Delphine Thizy, Target Malaria, Imperial College London, dthizy@ic.ac.uk
- Elinor Chemonges Wanyama, Ugandan Virus Research Institute, Chemonges ewchemonges@uvri.go.ug
- Lina Finda, Ifakara Health Institute, lfinda@ihi.or.tz
- Damaris Matoke-Muhia, KEMRI, dmatoke@kemri.org

Description:

Recent innovations in the field of vector control – such as genetic approaches for example – have contributed to a renewed interest on stakeholder engagement and the participation of the public and potential beneficiaries in their design, development and implementation. Reports (National Academies of Sciences Engineering and Medicine, 2016, guidance documents (WHO/TDR and FNIH, 2014) highlight the importance of stakeholder engagement but offer very little practical guidance on how this engagement is to be done. Researchers got together in the last years to review those questions and some literature is emerging from this discussion, hoping to contribute to a better integration of stakeholder engagement into vector control (Bartumeus et al., 2019; Hartley et al., 2019; Thizy et al., 2019).

This symposium proposes to bring key contributors and experts involved in this process and to present perspectives from different countries and experience on how stakeholder engagement can be carried out for the research and development phases of innovative vector control. This symposium also hopes to be a first opportunity to gather challenges from the audience on this topic to feed into the broader reflection that experts are having on principles and best practices for engagement.

Draft programme:

5 min - Introduction to the topic – Fredros Okumu

20 min - Brief presentation of the guidance publication - Delphine Thizy

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45 min (15min x3) - Case studies on stakeholder engagement for innovative vector control

- Elinor Chemonges Wanyama: Ugandan experience
- Lina Finda: Tanzanian experience
- Damaris Matoke: Kenyan experience

15 min: Discussion – what are the challenges and learning that can be applied to other vector control projects – question to the audience

5min: Closing remarks from the chair - Fredros Okumu

References:

Bartumeus, F., Costa, G. B., Eritja, R., Kelly, A. H., Finda, M., Lezaun, J., ... Vaughan, M. (2019). Sustainable innovation in vector control requires strong partnerships with communities. *PLOS Neglected Tropical Diseases*, *13*(4), e0007204. https://doi.org/10.1371/journal.pntd.0007204

Hartley, S., Thizy, D., Ledingham, K., Coulibaly, M., Diabaté, A., Dicko, B., ... Paré Toé, L. (2019). Knowledge engagement in gene drive research for malaria control. *PLOS Neglected Tropical Diseases*, *13*(4), e0007233. https://doi.org/10.1371/journal.pntd.0007233

National Academies of Sciences Engineering and Medicine. (2016). *Gene Drives on the Horizon*. https://doi.org/10.17226/23405 Thizy, D., Emerson, C., Gibbs, J., Hartley, S., Kapiriri, L., Lavery, J., ... Robinson, B.

(2019). Guidance on stakeholder engagement practices to inform the development of areawide vector control methods. *PLOS Neglected Tropical Diseases*, *13*(4), e0007286. https:// doi.org/10.1371/journal.pntd.0007286

WHO/TDR and FNIH. (2014). Guidance Framework for testing genetically modified mosquitoes, 159. Retrieved from

http://r4d.dfid.gov.uk/Output/197475/Default.aspx

SYMPOSIUM 2:

WIVC symposium

TITLE: STRENGTHENING THE ROLE OF WOMEN IN THE CONTROL OF VECTOR BORNE DISEASES

Introduction:

Women are often key agents of change in programs to combat public health challenges, including Vector-Borne Diseases (VBDs). Advancing their participation in disease management and promoting their leadership involvement in the workplace can contribute to better control of VBDs. The Pan-African Mosquito Control Association (PAMCA) is an African professional body that brings together actors in the field of vectors and vector-borne disease control. PAMCA exists to provide a platform for capacity building, knowledge sharing and collaboration for concerted vector control initiatives in the African continent. The association identifies the need to build capacity in women leaders in vector control in Africa. Despite mentoring being reported to contribute immensely to career development, regrettably, it's not readily available to many scientists and other vector control specialists in Africa. A clear and practical understanding of participation, sustainable engagement and leadership for optimizing the potential of women in the control of VBDs is key priority to the association.

Symposia objectives are to:

- 1. Provide a platform for open dialogue on challenges for women involvement in VBD control and sharing experiences
- 2. Promote enhanced involvement and advance leadership of women in vector control
- 3. Highlight workshop outcomes and way forward

Programme:

Chair: Damaris Matoke-Muhia Co-chair: Emma Orefuwa

Time	Activity	Speaker
14:00 - 14:05	Introduction to symposium	Dr. Damaris Matoke
14:05 - 14:20	Women in vector control: The experiences, challenges and success	Dr. Theresia Nkya
14:20 - 14:35	Gender and leadership in global partnerships in vector control	Dr. Chioma Nkasiobi Amajoh Dr. Sylvie Kwedi
14:35 - 14:50	<i>Fireside Chat</i> 10 years of PAMCA: "A woman's perspective "	MS. Emma Orefuwa Dr. Silas Majambere
14:50 - 15:05	Summary of WIVC workshop: Outcomes & way forward	Dr. Damaris Matoke
15:10 - 15:40	Panel Discussion: 'Men as Allies: – the role men in women's leadership development'	Dr. Neil Lobo Ms. Emma Orefuwa
15:40-16:00	Q&A with audience	ALL

SYMPOSIUM 3:

TITLE: TARGET MALARIA INSECTARY AND FIELD ENTOMOLOGY PREPAREDNESS TOWARDS THE 1ST RELEASES OF GENETICALLY-MODIFIED MALARIA MOSQUITO *ANOPHELES GAMBIAE* S.L.

Abstract: Every year Malaria affects approximately 200 million and kills approximately 435 thousand people worldwide, most of them African children under the age of five years (WHO, 2017). In many countries, progress in malaria control has been threatened by the rapid spread of resistance to antimalarial drugs and insecticides. Target Malaria, is a not-for-profit research consortium that aims to develop and share new genetic vector control tools for integrated malaria control strategies. The consortium brings together researchers from 14 institutions worldwide to develop high impact, well-organised and large-scale malaria control campaigns involving genetic modification of mosquitoes.

This symposium will focus on Target Malaria's insectary and field entomology preparedness in 3 African partner countries endemic for malaria, namely, Burkina Faso, Mali and Uganda. These include: the construction of compliant Arthropod Containment Level 2 facilities in each country; the development of optimized rearing protocols for maintenance of colour variants and genetically-modified strains; the establishment of extensive longitudinal baseline entomological surveys; technical and analytical improvements on mark release recapture studies; and the development of medium-throughput assays for post-release GM mosquitoes monitoring.

Alongside extensive stake-holder engagement activities, these Insectary and Field Entomology activities play a crucial role in the stepwise preparedness approach advocated by regulators that will pave the way to the 1st release of GM *Anopheles gambiae* s.l.

Organizer: Frederic Tripet¹

Co-organizers: Abdoulaye Diabate², Mamadou Coulibaly³, Jonathan Kayondo⁴, Andy McKemey⁵

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4 Uganda Virology Research Institute, Entebbe, Uganda, jkayondo@gmail.com

5 Imperial College, UK, a.mckemey@imperial.ac.uk

Sessions:

1. Construction of compliant Arthropod Containment Level 2 facilities in African partner countries

Presenter:

Jonathan Kayondo, Target Malaria country PI, Uganda Virology Research Institute, Entebbe, Uganda, jkayondo@gmail.com

2. Development of optimized rearing protocols for maintenance of colour variants and genetically-modified *Anopheles gambiae* s.l. strains

Presenter:

Moussa Namountougou, Target Malaria Insectary Lead, Institut de Recherche des Sciences de la Santé, Bobo Dioulasso, Burkina Faso, namountougou_d@yahoo.fr

3. Establishment of extensive longitudinal baseline entomological surveys Presenter:

Amadou Guindo, Target Malaria Field Entomology Lead, Malaria Research and Training Centre, University of Bamako, Mali, amadoug@icermali.org

4. Technical and analytical improvements on mark release recapture studies Presenter:

Frederic Tripet, Director Centre for Applied Entomology and Parasitology, Keele University, UK, f.tripet@keele.ac.uk

5. Development of medium-throughput assays for post-release GM mosquitoes monitoring

Presenter:

Florian Noulin, Centre for Applied Entomology and Parasitology, Keele University, UK, f.noulin@keele.ac.uk

12 minutes per sessions, 30 minutes question-answers session at end

SYMPOSIUM 4:

TITLE: VECTOR CONTROL INNOVATIONS TO DRIVE PROGRESS IN MALARIA AND OTHER MOSQUITO-BORNE DISEASE CONTROL

Principal organizer: Allison Tatarsky, RBM VCWG New Tools, New Challenges / UCSF Malaria Elimination Initiative, <u>Allison.Tatarsky@ucsf.edu</u>

Co-organizers: Derric Nimmo, Innovative Vector Control Consortium; Fredros Okumu, Ifakara Health Institute

Speakers:

- 1. **Derric Nimmo**; Innovative Vector Control Consortium; <u>Derric.Nimmo@ivcc.com</u>; Overview of the vector control pipeline and rationale for an IVM approach
- 2. Ingrid Etoke; Innovative Vector Control Consortium; <u>Ingrid.Etoke@ivcc.com</u>; New nets: project scale up and progress to date
- **3.** Andrew Saibu; Innovative Vector Control Consortium, NGenIRS Project: early results from PMI VectorLink rollout of 3rd generation IRS insecticides, Fludora Fusion and SumiShield.
- 4. Jane Bonds; Bonds Consulting Group; jasbonds@gmail.com; Area wide spraying using swarming as an indicator
- 5. Silas Majambere; Mosquito Consulting; <u>simajambere@gmail.com</u>; Precision larviciding with drone technology and new longer lasting larvicides for Aedes and Anopheles control
- 6. Senegal Representative (TBD); Ivermectin: results from a Senegal trial and snapshot of BOHEMIA, a new multi-country ivermectin trial
- 7. Emmanuel Kaindoa; Ifakara Health Institute; <u>ekaindoa@ihi.or.tz</u>; Spatial repellents: community-wide effect of transfluthrin-treated eave ribbons
- 8. Rohani Ahmad; Institute for Medical Research, Malaysia; <u>rohania@imr.gov.my</u>; Outdoor residual spraying (ORS): impact in Asia for Aedes and Anopheles control and potential for impact in Africa

In line with the sub-theme on innovation in vector surveillance and control, this symposium will focus on innovation in vector control tools and approaches under development and evaluation across sub-Saharan Africa and globally for both Anopheles and Aedes control. The symposium will also include early findings from the scale up of new tools and describe the decision-making processes for malaria programs when selecting and scaling these new tools. We propose a 120 minute session with seven presentations followed by a moderated panel discussion with all speakers. The first presentation will set the scene for the vector control pipeline, followed by two presentations on new nets and new IRS insecticides. The

next presentation will focus on exploiting mosquito biology for control and will include two parts: the first on area wide spraying using swarming as an indicator and the second on precision larviciding. The following three presentations will focus on three novel vector control tools and approaches including ivermectin (also reflecting the sub-theme of Adopting the one health approach), spatial repellents, and outdoor residual spraying.

This symposium will deliver the latest research on an ever-expanding toolbox of vector control tools and approaches with two objectives: 1) to disseminate important findings to PAMCA membership that can inform future research and implementation of vector control across the African continent, and 2) make visible to national malaria programs the diverse and high impact tools that can be integrated into countries' vector control response now or in the future. Topics covered in this symposium are of great importance to malaria burden reduction and elimination efforts and control of other mosquito-borne diseases. A vibrant moderated panel discussion with audience Q&A following the presentations will ensure cross-fertilization of ideas and explore further application of these innovations.

SYMPOSIUM 5:

TITLE: ENHANCING ENTOMOLOGICAL CAPACITY IN AFRICA FOR EFFECTIVE VECTOR-BORNE DISEASE CONTROL: PROSPECTS, CHALLENGES, AND OPPORTUNITIES

Organizer: Elijah Juma -Ph.D. candidate, Medical Entomology, the University of Illinois at Urbana-Champaign, IL, USA

Co-organizer: Kevin O. Opondo, Ph.D. – Postdoc Fellow, Medical Research Council Unit, London School of Hygiene and Tropical Medicine, The Gambia

Summary: Vector-borne diseases (VBD) like malaria, dengue, chikungunya, and Zika remain a major public health challenge worldwide, including sub-Saharan Africa (SSA). The threat posed by the VBDs is further aggravated by rapid urbanization, global commerce, increasing incidences of insecticide resistance, changing climate favoring vector range expansion and lack of capacity to respond timely and effectively to increased disease transmission.

Currently, there are only a handful of entomologists supporting vector-borne disease control programs in SSA. An entomological capacity mapping survey conducted by PAMCA in 2018 highlighted an acute shortage of medical entomologists as well as limited institutional capacity and constraints in resources to tackle the growing needs of VBDs in SSA. Of the few entomologists, approximately 95% of them work on malaria-transmitting mosquitoes leaving the rest of the VBDs, particularly arboviral infections, with very few entomologists. Part of the problem is structural, given that most of vector-borne disease control initiatives rely on donor funding, and with most of the funding going to research and control of malaria.

Several factors have contributed to this low entomological capacity, including; lack of standardized training curricular for entomologists, weak or lack of proper collaboration framework between training institutions and national vector-borne disease control units. There are also limited training opportunities for entomologists, poor career prospects upon graduation, and limited funding opportunities to undertake research. The lack of political will at the national level to recognize VBDs as important public health threats and, therefore, create appropriate structures at the ministries of health (MoH) to mobilize resources for research and implementation of the agreed action plans, is also an ongoing challenge.

This symposium seeks to bring together entomologists in the industry, academia and public sector, students, early-career entomologists, and other allied professionals to deliberate on strategies to enhance the institutional and entomological capacity in SSA to effectively respond to the growing challenge of vector-borne diseases.

The objectives of the symposium are:

- I. Discuss the gaps in research & training, structural and policy frameworks in entomology and human resource capacity to tackle vector-borne diseases.
- II. Discuss alternative, sustainable approaches to enhance entomological capacity by adopting a decentralized model of capacity building at regional and district level, down to community level the experience E8 experience
- III. Deliberate on the approaches to enhance entomological research capacity in SSA: The Burkina Faso African Centre of Excellence in innovative technologies for vector-borne disease control in SSA
- IV. Provide an overview of the entomological capacity training fellowship hosted and run by the SADC Malaria Elimination 8 Initiative, it's objectives and rationale

Presenters:

Objective I: Fredros Okumu, Ph.D. - Director of Science, Ifakara Health Institute, Tanzania

Objective II: **Chadwick Sikaala, Ph.D.** – Entomologist and Vector Control Specialist, SADC – Malaria Elimination Eight Secretariat, Widhhoek, Namibia

Objective III: **Abdoulaye Diabate, Ph.D.** - Head of medical entomology, The Institut de Recherche en Sciences de la Sante/ Centre Muraz, Bobo-Dioulasso, Burkina Faso

Objective IV: Lesley-Anne van Wyk – Consultant: Programme Coordination Support, SADC Malaria Elimination 8 Initiative Secretariat

Objective I

Title: The African entomologist has a duty to train and a duty to nurture

Presenter: Fredros Okumu, Ph.D.

Director of Science, Ifakara Health Institute, Tanzania

A significant proportion of Africa's leading public health entomologists were trained abroad or by western scientists. This is true for the old and young experts alike. One reason for this is that African health researchers and institutions are not paying adequate attention to the growth of essential human resources necessary to address priority needs of the continent. Yet, without skilled personnel to set appropriate agenda and to implement key programs, adequate control of vector-borne diseases will remain elusive. In this session, we will discuss why it is our duty to train, and why we must aspire to achieve the highest possible quality. We will also discuss options for integrating across Africa, and for improving partnerships within and beyond the continent to ensure the requisite training is achieved. Lastly, we will examine opportunities for African research and academic institutions to promote idea generation and innovation among early-career public health entomologists

Objective II

Title: Alternative approach to address the challenges of entomologists in the E8 region

Presenter: Chadwick H. Sikaala, Ph.D.

Entomologists and Vector Control Specialist, SADC – Malaria Elimination Eight Secretariat, Windhoek, Namibia

The importance of developing entomological surveillance skills within the context of malaria control and elimination can never be over-emphasized. Across sub-Sahara Africa, malaria control and elimination, are faced with challenges of having national operation research agendas that incorporate epidemiological and entomological approaches to facilitate the choice of optimal tools. While research institutions and partners have over the past bridged this gap, their remains challenges of sustainability within the programmes. Designing alternative approaches that involve capacitating regional and district level person to conduct basic entomology surveillance through the communities they serve would be, among other initiatives, approaches to addressing the challenges of sustainability. These personnel while conducting their routine work can be capacitated to conduct entomological surveillance so they transfer the skills to the communities. Community members can be trained on how to set collection tools, basic morphological identification, preservation of samples and basic data management; trained health facility, district, and regional or provincial level staff can oversee and supervise these activities while the central level provide more technical assistance, trainings, quality assurance, advanced operational research such as insecticide resistance resource and resource mobilization.

Although resources remain challenging, programmes can leverage on existing partnerships, including research and academia, for support while advocating for more domestic funding. With increased domestic funding and decentralized vector control and entomological surveillance, programmes may afford to sustain basic operations to inform programmatic decision making for optimal application of current and future vector control tools.

Objective III

Title: Towards the establishment of a Pan-African Centre of Excellence in innovative technologies for vector-borne disease control

Presenter: Diabate Abdoulaye, Ph.D.

Head of medical entomology, The Institut de Recherche en Sciences de la Sante/ Centre Muraz, Bobo-Dioulasso, Burkina Faso

Vector-borne diseases, such as malaria, dengue, vellow fever and trypanosomiasis, account for a significant proportion of the global burden of infectious disease. Nearly half of the world's population is infected with at least one type of vector-borne pathogen. In the past three decades, there has been dramatic global re-emergence of epidemic vectorborne diseases. Many of the diseases that were effectively controlled in the middle part of 20th century have re-emerged, and new pathogens have also emerged, both of which are causing major epidemics of disease. In Burkina Faso, despite substantial efforts devoted to controlling these infectious diseases, malaria statistics are on the rise and the country is now being severely hit by dengue outbreaks. Vector-borne diseases significantly reduce agricultural productivity, disrupt ecosystems and profoundly restrict socioeconomic status and development in many countries. Increased global trade, rapid population growth, urbanization, and deforestation are among factors that explain the distribution of these diseases. In order to successfully anticipate outbreaks and intervene with preventive actions, it is imperative to have a health workforce that is specialized in dealing with vector-borne diseases but also to invest in research towards new intervention tools as the current ones have reached their fundamental protective limits. The current proposal seeks to create an African centre of excellence focalized on emerging innovative technologies with a global aim of increasing and improving quality research and training on the disease vectors, build entrepreneurship and impart value to intellectual property by fostering implementation of innovation. The centre will build on the experience and expertise of 2 major players in the health ecosystem in Burkina Faso, namely IRSS and the UNB to 1) train a critical mass of next-generation African scientists in the genetic engineering process in relation to disease vectors, and 2) increase participation and contribution of African countries to the global health response through leadership in research and evidence generation. Aided by our national, regional and international networks, we will seize opportunities to create an enabling environment that will lift the centre to be world-class champion in capacity building and innovation in Africa.

Objective IV

Title: The E8 Entomology Fellowship program: early lessons from implementation of a dynamic career development fellowship for junior to mid-career vector control technicians

Presenter: L. van Wyk, N. Mwendera and C. Sikaala

SADC Malaria Elimination Eight Initiative Secretariat

Background: The SADC Malaria Elimination Eight (E8) Entomology Fellowship program was established with the aim to strengthen capacity for entomology and its application within E8 country national malaria control and elimination programs, to complement other interventions within malaria elimination strategies, and enhance programmatic decision-making. The program was designed to bridge entomological capacity gaps identified by malaria control and elimination programs.

Description: In partnership with the University of California San Francisco (UCSF) Institute for Global Health Sciences Global Health Group (GHG), the E8 launched the inaugural year of the Entomology Fellowship in May 2018 to June 2019 as a tailored program for skills-building to meet specific member country needs. The first year of the Fellowship program consisted of: intensive training courses divided up into three in-residence training programs at selected academic and research institutions, Capstone Projects during which each Fellow completed a related operational research assignment with direct relevance or application to the country's malaria elimination strategy, mentorship and professional networking opportunities. The target group for fellowship candidates was junior to midcareer vector control practitioners employed within the structures of the national malaria control program.

Lessons learned and Next steps: Preliminary evaluation results show that the program meets a gap for capacity development within the E8 member states and malaria programs, fellows, mentors and academic and research institutions express demand for the intervention to continue and be scaled up. The significance of early findings points to the need for capacity development initiatives to address the lack of capacity to respond in timely and effective ways to resurgence and increased disease transmission by malaria vectors. This abstract refers to work-in-progress. Final results will be available by the end of October 2019.

Keywords: malaria elimination, entomological surveillance, regional cooperation, capacity development

Title: Evaluation of Indoor Residual Spraying (IRS) Efficacy In Zambezi Region, Namibia.

Author: M. M. Lifasi and Chadwick Sikaala

Affiliation: Southern Africa Malaria Elimination Eight (E8) Entomology Fellowship

Background: This work was conducted to evaluate effectiveness of an IRS program in Zambezi region of Namibia focusing on coverage verification, residual efficacy and insecticide susceptibility of vector population.

Method: The study was conducted between December 2018 and April 2019. In total 1546 structures were sampled and physically verified for IRS coverage. Residual efficacy of different IRS insecticides on different substrates (Mud, modern - cement and grass thatched) was assessed using the WHO standard cone bioassays procedures. Standard WHO bioassays, 4% DDT and 0.05% Deltamethrin were performed on wild-collected adult anopheline mosquitoes reared from larval collections. All mosquito were morphologically identified to genus level.

Results: Data showed the IRS coverage of 54% in sleeping structures. The least and highest IRS coverage recorded during this study 39 % and 67% respectively against official national figures of 62 % and 100%.

DDT show residual efficacy on both mud and thatch grass with a mortality of 98% on mud surface by week 16. Deltamethrin was not efficacious as DDT with mortalities of 49%, 59% 70% for mud, modern surfaces and thatch grass respectively during the same period. Anopheles gambiae s.l and An. funestus s.l were both susceptible to 4% DDT with mortalities of 99% each. Suspected resistance to 0.05% Deltamethrin was observed in An. gambiae s.l population (97% mortality). Morphological identifications showed that 91% (n =157) were An. gambiae s.l., 6% (n = 10) were An. funestus s.l 6% and 3% (n= 6) could not be identified.

Discussion and Conclusion: Results of this study showed that National IRS coverage records might be overstated in sleeping structures. DDT has a longer residual efficacy on both mud and grass thatched surfaces compared to Deltamethrin. Anopheles gambiae s.l and An. funestus s.l poulations from this area are still susceptible to DDT but show reduced susceptibility to Deltamethrin. Further studies are required to understand this low IRS uptake by the community.

Key words: IRS, Residual Efficacy, Anopheles gambiae, Anopheles funestus

Title: Insecticides susceptibility status of malaria vectors from Chiredzi district, Masvingo province, Zimbabwe

Authors: Regis Mavhiya, Joel Mbedzi and Chadwick Sikaala

Affiliation: Southern Africa Malaria Elimination Eight (E8) Entomology Fellowship

Background: Zimbabwe relies on IRS and LLINs for vector control with DDT and Deltamethrin being the insecticides of choice. Despite a marked increase in IRS coverage in Chiredzi, malaria cases remain comparatively high. Factors responsible for this sustained malaria transmission are largely unknown. We hypothesize that Chiredzi vector populations' insecticide resistance might be playing a role in the failure to reduce the malaria burden. To test this hypothesis, a study was conducted to assess the susceptibility status of malaria vectors in Chiredzi district (Masvingo province) to DDT and Deltamethrin and ascertain the vector distribution in two selected sentinel sites.

Methods: Mosquito larvae and pupae were collected from sentinel site in Chiredzi between October 2018 and April 2019 and reared into adults. Using standard WHO procedures phenotypic insecticides resistance in emerging adults was carried using 4% DDT and 0.05% deltamethrin impregnated papers. An observational checklist was also used to determine species distribution in the areas. Morphological identification to genus level was determined using Gilles and Coetzee (1987) key.

Results: Insecticide susceptibility results of populations from Rupangwana showed a 24hr post exposure mortalities of 99% (n=100 specimens) and 100% (n=100 specimens) for DDT and Deltamethrin respectively. Populations from Mkwasine indicated 100% susceptibility to both DDT (n=100 specimens) and Deltamethrin (n =100 specimens). A total of 600 specimens were collected from the two sentinel sites. After morphological identification, all specimens were identified as belonging to the *Anopheles gambiea* complex.

Discussion and Conclusion: These results showed that mosquito populations from Rupangwana and Mkwasine are still susceptible to both DDT and Deltamethrin in contradiction of earlier studies. This shows that insecticide resistance is a locality-specific and transient phenomenon which needs constant monitoring. In conclusion, this study means that the current insecticides can safely be used for IRS activities in the district. However, this is a short research and is limited to a small locality and more studies need to be conducted to prove this assertion.

SYMPOSIUM 6:

TITLE: INTRODUCTION TO THE PIIVEC: PARTNERSHIP FOR INCREASING THE IMPACT OF VECTOR CONTROL

Principal organizer: Charles Wondji

Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon & Liverpool School Tropical Medicine (LSTM) UK

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Co-organizer: Philip J McCall

Liverpool School Tropical Medicine (LSTM) UK

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Description:

Until eliminated, vector-borne diseases will continue to present new challenges that must be met with novel intervention tools and strategies, a task that requires a new generation of skilled African researchers who will develop sustainable solutions and partnerships with their international collaborators. Until recently, vector control's importance in reducing transmission and reaching elimination targets was greatly undervalued, and recruitment fell, eventually leading to the current shortage of appropriately skilled researchers. Addressing this need, the Partnership for Increasing the Impact of Vector Control (PIIVeC) was established, bringing together leading research institutes and national disease control programmes to develop evidence-based solutions for integrated vector control in countries with exceptionally high burdens of vector-borne disease: Burkina Faso, Cameroon and Malawi,

PIIVeC aims to increase autonomy of key institutions and reinforce links between researchers and policy-makers in each country. Research capacity strengthening in diverse fields is driven by a cohort of post-doctoral African Research Career Development Fellows (RCDF).

The symposium will introduce PIIVeC structure and strategy, highlighting the range of diseases and approaches covered and how its outputs will inform integrated vector management strategies to reduce disease burden and increase resilience for responding to disease outbreaks.

Justification:

PIIVeC is an international collaboration promoting capacity strengthening and evidencebased policy change to combat vector-borne disease in Africa. This symposium highlights research being conducted by young African scientists, addressing contemporary challenges, including surveillance and control of *Aedes*-borne arboviruses, residual malaria transmission, and evaluation of next generation vector control tools. Speakers will examine common challenges faced by research institutes to sustainably deliver relevant evidence and consider approaches for improving research uptake and policy change, and challenges and opportunities for integrated technical working groups for vector control. This symposium



is directly linked to PAMCA's theme of "Harnessing Africa's research capacity to empower national programmes" by showcasing innovative research, and also by describing how links with implementers and policy makers can stimulate robust evidence supply and demand pathways. Secondarily, we address the theme "Vector biology and control" through the individuals talks presented by the RCDFs.

Speakers:

Hilary Ranson

Liverpool School Tropical Medicine (LSTM) UK Introduction to PIIVEC

Elizabeth Bandason

Malaria Alert Centre of the College of Medicine, University of Malawi in Blantyre, Malawi. The effect of disengagement on efficacy of vector control interventions

Huguette Simo

Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon Assessing the epidemiological risk of Chikungunya Dengue and Zika virus outbreaks in Cameroon

Hyacinthe Toé

Institut National de Santé Publique (INSP), Burkina Faso Peri-domestic ecology and behaviour of *Aedes* sp. mosquitoes: a west African evidence base for effective control of urban arboviruses

Evelyn Olanga

Malaria Alert Centre at the Malawi College of Medicine, Blantyre Malawi Characterising vector behaviours and the risk of malaria infections in communities in southern Malawi

Lassane Koala

Institut de Recherche en Sciences de la Santé (IRSS) Bobo Dioulasso, Burkina Faso Development of efficient traps and targets for the xenomonitoring of the population of Simulium damnosum s.l. in the south-west region of Burkina Faso.

Tito Tresor Melachio

Centre for Research in Infectious Diseases (CRID), Yaoundé, Cameroon Microgeographic structure, vector control and population dynamics of Glossina palpalis palpalis: Impact on Human and animal trypanosomiases in the Campo focus, southern Cameroon

Jessica Amegee

Liverpool School Tropical Medicine (LSTM) UK Strengthening institutional capacity to conduct vector control research: common gaps and priority actions

Chikondi Mwendera

Liverpool School Tropical Medicine (LSTM) UK

Establishment of technical vector control advisory groups (TVCAGs): experiences from three countries, Cameroon, Burkina Faso and Malawi, within the PIIVEC programme

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ORAL ABSTRACTS

Parallel session 1:

Harnessing research capacity in Africa to empower national programs

Title: Decision Support to Integration of Larval Source Management in the National Ivm Malaria Control Strategy: *Piloting A Community-Based Larviciding Model In Nakasongola District, Uganda.*

Authors: Kato A. B., Shady M. A., Nambatya G., Okedi L. M. O., Okia M., Omujal F., Egitat G., Kigongo S., Onek D., Mukwaya L., Tarek A-El-Tayeb

Background: Large populations could be protected from malaria by controlling aquatic stages of mosquitoes if cost-effective and scalable implementation systems can be designed.

Methodology: A large-scale larviciding trial was conducted in Lwabyata Village, Nakasongola District in Uganda, employing modestly paid community members, known as Village Health Technicians (VHTs) and Community-Owned Resource Persons (CORPs), fielding a Bio-larvicide called Sunlight Active Formulation Extract (SAFE).

Results: We were able to demonstrate the applicability of mosquito larviciding for malaria control to village level. The population of both aquatic and adult stages of Anopheline and Culicine mosquitoes reduced. Analysis of variance showed significant difference ($P \ge 0.05$) between the treatment and control sites. Likewise, malaria cases showed reducing trends by the end of the study period (y = -149.9x + 659.5; $R^2 = 0.67502$), suggesting that larviciding could substantially contribute to malaria reduction. Much as the community exhibited moderate to low levels of awareness about larviciding, their attitudes and opinions were significantly positive, indicating a strong willingness to accept larviciding as a new tool.

Conclusions & recommendations: Larviciding can be an effective tool in reducing malaria transmission, especially when integrated with other methods. Further, longer-term trials with additional larvicides will however help make the choice of the most cost-effective larvicides to be integrated in the Ugandan IVM policy.

Title: Susceptibility of *Anopheles* to *Plasmodium Falciparum* and *Plasmodium Vivax* Infections in Madagascar

Authors: <u>GOUPEYOU-YOUMSI J. M.</u>^{1,2,3}, PUCHOT N.², TCHIOFFO-TSAPI M.², GIROD R.¹, VIGANWOMAS I.¹, PAUL R.², RANDRIANARIVELOJOSIA M.¹, NDIATH M. O.¹ & BOURGOUIN C.²

1 Institut Pasteur de Madagascar, Antananarivo, Madagascar 2 Institut Pasteur, Paris, France

3 University of Malawi College of Medicine, Blantyre, Malawi

Background: Unlike the *Anopheles–P.falciparum* pair, very few studies focused on the *Anopheles–P.vivax* interactions in Africa. In an epidemiological context such as Madagascar, marked by the presence of several species of malaria parasites and vectors, understanding the vector–parasite interactions constitutes a malaria post-elimination challenge. The aim of this study was to assess the susceptibility of *Anopheles* vectors to *P. falciparum* and *P. vivax* infections in Madagascar.

Methods: Wild *An. gambiae* s.l. were produced as F0 from larval collections from different breeding sites in the commune of Andriba, Madagascar. Using membrane feeding assays, female mosquitoes aged 3-10 days were infected with the blood of *P. falciparum* gametocyte carriers and any *P. vivax* carriers. Eight days after feeding, mosquitoes were dissected and midguts were stained in mercurochrome for microscopic examination and enumeration of oocyst, as a semi-quantitative assessment of susceptibility using a logistic regression model. Members of the *An. gambiae* complex were identified by PCR.

Results: All mosquitoes from the *An. gambiae* complex belonged to the species *An. arabiensis*. Compared to a colonized strain, wild *An. arabiensis* was roughly equally susceptible to infections by *P. falciparum* (odds ratio 1.45, 95% confidence interval 0.30– 4.32) and *P. vivax* (OR 0.28, 95% CI 0.01– 7.73). It had a marginally higher oocyst load for *P. falciparum* infections and increasing gametocyte density increased oocyst load and increasing gametocyte density had decreased oocyst load (OR 0.29, 95% CI 0.15–0.55).

Conclusions: Our study shows the complexity of interactions *An.arabiensis–P.falciparum* and *An.arabiensis–P.vivax*. This could have a direct consequence in malaria transmission, emphasizing the great significance of interrupting or reducing the human-to-mosquito *Plasmodium* transmission. Thereby, malaria control interventions should be designed for better targeting the transmission reservoirs.

Title: Factors Influencing the Translation of Malaria Research Findings to Policies and Practice in Nigeria

Authors: Evbodaghe Omo-Imafidon S.¹, Adeogun Adedapo O.², Olawumi Kehinde O³, Oluwaseun Akinyemi O.⁴,

¹Department of Health Policy and Management, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.

²*Public Health and Epidemiology Department, Nigerian Institute of Medical Research, Yaba, Lagos/Department of Biological Sciences, Lead City University, Ibadan, Oyo State, Nigeria.*

³ Department of Health Policy and Management, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.

⁴Department of Health Policy and Management, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Background: Nigeria currently accounts for the highest single national prevalence of malaria in the world. Despite all efforts at combating this scourge, little achievements have been recorded. Indicator surveys have driven preparedness but gaps in translating these research into policy appears to be challenged by several factors, some of which have not been properly elucidated. This study therefore, assess, some of the factors influencing the translation of malaria research evidence to policy and practice in Nigeria.

Method: Descriptive cross-sectional study was conducted across 10 States in Nigeria. A total of 18 professionals were selected across the Federal and State Ministries of Health, academic staff that are involved in malaria operation research and consultants from the private sector. Key Informants Interviews were conducted on the respondents by purposive and snow balling techniques. Analyses was done using NVIVO version 8 software.

Results: The results from this study indicate that malaria research in Nigeria is at an advanced stage but gaps exist in molecular studies, implementations and operations research. Strong collaboration currently exist between program officers and policy maker but interaction between researchers and policy makers is weak. Communication, collaboration, presence of technical support and increased appreciation for evidence were the enabling factors in the research to policy process while misplaced priority, opportunity cost, political will, personnel issues, funding, inadequate facilities, corruption and lack of continuity were the barriers. On the other hand, political will, assess to donor funds, staff commitment were the promoting factors of the policy implementation while poor planning, lack of commitment, poor local funding, shortage of personnel and lack of synergy were the limiting factors.

Conclusion: Communication channels between malaria researchers and policy makers to enhance the research to policy process, increased support for malaria molecular and operations researches and improved funding of malaria interventions is hereby needed for malaria operational effectiveness in Nigeria

Title: Piloting of An Entomological Surveillance Planning Tool to Improve Entomological Intelligence for Evidence-Based Vector Control Decision-Making Towards Malaria Elimination.

Authors: Elodie Vajda*, Allison Tatarsky, Neil F. Lobo

Practical, programmatic guidance on entomological data use and its integration with epidemiological, rainfall, and intervention data for evidence-based malaria vector control is lacking. An Entomological Surveillance Planning Tool (ESPT) was developed to provide national malaria programs with a decision framework for entomological surveillance planning, analysis of entomological data, and for measuring human behavior associated to vector behavior.

The ESPT was piloted in three countries across Mesoamerica, southern Africa, and southeast Asia (2018/2019). Preliminary entomological endpoints are presented here. In Country A, program questions were 1) Are LLINs effective based on both human and vector behaviors? 2) Could IRS be effective based on both vector and human behaviors? Findings from an ESPT-guided plan included 1) Anopheles primarily bite outdoors, early in the evening, 2) human behavior dictates exposure to mosquito bites, and varies seasonally, 3) LLINs use higher in locality X than in locality Y, 4) Anopheles do not rest indoors, and 5) preliminary evidence for insecticide resistance for IRS insecticides in use. With this information, the program can make decisions about insecticides and targeting for IRS, heighten resistance monitoring around a planned pyrethroid LLIN distribution campaign, and promote the benefit of the physical barrier of LLINs.

Country B asked if IRS was effective given vector bionomics and human behaviors measured. Susceptibility testing data led to the country deciding to pilot a new insecticide, and to the planning of piloting new outdoor vector control tools.

In Country C, an ESPT-based focus investigation was conducted to identify the origin of malaria cases in high-risk groups, and found that cases were coming from outside the peridomestic area, where vector control interventions were not in place.

We are now tracking decisions made by programs around vector control interventions, and how entomological data generated through the ESPT pilots informed these decisions.

Title: Managing a Reference Laboratory to Support Programmatic Entomological Surveillance

Authors: Mutale Chisanga¹, Alice Mungo¹, Limonty Simubali¹, Twig Mudenda¹, Gift Mwaanga¹, Jennifer Stevenson^{1, 2}

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- 2. The W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, Maryland, 21205, USA.

Background: As Zambia is working towards achieving malaria elimination, entomological investigations are being conducted to better understand malaria vector behaviors, to identify and design appropriate tools to control and eliminate these vectors. In view of this, Macha Research Trust set up a reference laboratory 3 years ago to which other institutions would outsource entomological services to support country-wide entomological surveillance. It is in this regard that the MRT reference laboratory is supporting programmatic operations in Zambia.

Description: Running a reference laboratory at MRT to support entomological surveillance in Zambia has been manageable due to the following attributes;

- 1) Turnaround time- we deliver results back to our client in the timeframe agreed.
- 2) **Quality services** we are diligent and accurate in sample handling and processing. Services being provided are; analysis of mosquitoes for species identification using both morphological and molecular methods, blood meal analysis, and infectivity.
- 3) Affordability- as a non-profit organization we aim to support entomological research studies at cost.
- 4) Good communication system- our clients are able to communicate freely with us in case of any changes or inquiries. Regular updates of progress during sample processing are provided.

Lessons learned:So far we have managed to support four institutions at an affordable cost and have processed about 15,000 samples. Leveraging existing infrastructure and delivering good laboratory services, our client demand has extended to other reference services that require support in entomological investigations in field and semi-field activities.

Challenges identified are as follows; balancing client demand, processing damaged samples and inappropriate packaging of samples by clients. With the aid of a good communication system with our clients, these challenges are being addressed.

Conclusions/Next steps

Maintaining quality services through high laboratory standards and client engagement, MRT is successfully running a reference laboratory to support entomological studies.

Title: Nationwide Distribution of Insecticides Resistance

Author: Aurelie Prisca Yougang

Background & objective: the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is an invasive species that can be found on all continents. It has been described in Central Africa for the first time in Cameroon in 2000 and a few years later in most countries of the the subregion. This species is considered to be one of the main epidemic vector dengue virus, chikungunya virus and zika virus worldwide. In the absence of effective treatment and vaccines, vector control remains the cornerstone to prevent transmission of these viruses. The aim of this study is to establish the current level of susceptibility of Cameroonian populations of *A. albopictus*.

Materials & Method: For this purpose, immature stages of *Aedes* were sampled between March and July 2017 in fourteen towns in Cameroon. The immature stages collected were transported to the insectary and reared until to adult stage. Adult mosquitoes were morphologically identified and pooled in a breeding cage and further reared until generation 1 (G1) or subsequent G2 and G3. Bioassay was carried out according WHO recommendations using non blood fed mosquitoes aged between 2 to 4 days for adult and synergist assays and late-third or earlyfourth instars of larvae for larval assays.

Results & Discussion: Analysis showed that *A. albopictus*, are resistant to 0.05% deltamethrin, 0.25% permethrin, 0.1% propoxur and 4% DDT. A probable resistance to 0.1% bendiocarb and a full susceptibility to 1% fenitrothion was observed. The mortality rate was increased after preexposure to synergists such as: piperonyl butoxide and diethyl maleate. Concerning larval tests, all the populations tested were susceptible to temephos.

Conclusion & recommendation: These findings are useful to planning vector control programme against arbovirus vectors in Cameroon and can be used as baseline in Africa where data on *Aedes* resistance is very scarce.

Title: Methods and Challenges for Systematically Reviewing the Costs and Cost-Effectiveness of Vector Control Tools and Strategies.

Presenter: Efundem Agboraw

Authors: Kathryn Shuford, Lesong Conteh, Efundem Agboraw, Mara Kont and Edith Patouillard

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Organization and position: Liverpool School of Tropical Medicine, World Health Organization [Global Malaria Programme]

Background: Data on the cost and cost-effectiveness (CE) of disease control interventions are needed to support national strategic planning, including the production of resource needs estimates, health impact analyses, prioritization and financing of strategies. For malaria, the most recent systematic review was published in 2011 with a focus on evidence generated between 1990 and 2010. As the coverage of malaria control interventions increases, elimination strategies develop, and new tools are approaching, a new evidence base is required.

Methods: Published and grey literatures in English-, French- or Spanish-language on the cost or CE of malaria control strategies were searched for the period 2005-2018. Main outcomes were the unit costs and CE of malaria-related health outcomes associated with World Health Organization (WHO) currently recommended interventions. To reduce potential bias and error in study selection, three reviewers independently evaluated studies, with a fourth reviewer to resolve any conflicts. Only studies meeting all inclusion criteria were retained for the review. To ensure consistency, each reviewer worked independently from the same data extraction template on an online review software, Covidence.

Results: After applying all exclusion criteria, 128 studies were finally retained for data extraction and QA. Predominance of studies in SSA settings, where most of the burden of malaria lies with 11 LLIN studies, 1 IRS study and 9 other vector control study. Challenges in the review included the clear definition of the review boundaries to ensure quality and rigor, the lack of flexibility of the review management software, the application of inclusion and exclusion criteria on studies, the variety in costing methods used and the development of a data repository that was user friendly. A positive aspect of the review should be noted, which is the more explicit way recent studies have reported on essential costing and economic evaluation information.

Parallel session 2:

Mosquito genomics: progress and challenges

Title: Progress on the Development of Sex-Separation Techniques for Sterile Insect Technique Applications Against Malaria Vectors *Anopheles Arabiensis*

Authors: Thabo Mashatola, Cyrille Ndo, Lizette L. Koekemoer, Leonard C. Dandalo, Oliver Wood, Lerato Malakoane, Yacouba Poumachu, Leanne N. Lobb, Maria Kaiser, Kostas Bourtzis And Givemore Munhenga

Introduction: The feasibility of the sterile insect technique (SIT) against *Anopheles arabiensis* is currently under investigation. A critical requirement of a mosquito SIT programme is a sex-separation system that eliminates females prior to field release of sterile males only. Existing mosquito sex-separation strategies have not worked in *An. arabiensis*. This work describes investigations into pupal sexual dimorphism, exclusive blood feeding behaviour of females and classical genetics to accomplish female elimination in *An. arabiensis*.

Methods: Pupal sexual dimorphism was investigated by measuring and recording pupal cephalothorax sizes, the efficiency of an endectocide (ivermectin) added to a blood meal source was assessed by recording adult female mortality post feeding, and a genetic sexing strain (GSS) was developed by introgressing a local *An. arabiensis* wild-type population with an existing GSS. Additionally, due to concerns regarding dieldrin use, alternative, more environmentally acceptable insecticides with a similar mode of action to dieldrin (i.e. target the GABA receptor) were used to treat larvae and mortality and sex ratio recorded. Furthermore, exploitation of a temperature-sensitive lethal (*tsl*) marker to develop a *tsl*-based GSS are currently underway. Ethyl methanesulfonate (EMS) is used to induce, isolate and characterize an *An. arabiensis tsl* strain.

Results and discussion: *Anopheles arabiensis* pupal sizes overlap greatly, rendering pupal sexual dimorphism impractical. Elimination of females using ivermectin shows great potential, however, its practicality in mass-rearing settings still requires testing. The established dieldrin-based GSS is showing positive developmental attributes and genetic stability. Lindane and picrotoxin eliminated 90% females in the laboratory, suggesting their potential to replace dieldrin. An *An. arabiensis tsl* strain has been successfully established and characterised, however, a visible selectable marker closely linked to *tsl* is required to trace the *tsl* loci.

Conclusion: These findings are useful towards selection of a reliable sex-separation method applicable in subsequent small-scale SIT pilot field releases.

Title: Functional validation of the role of An. funestus epsilon glutathione-stransferases in insecticide resistance

Authors: Kouamo Mangoua, Mersimine Flore

Background: Insecticide resistance is a huge threat to the effectiveness of insecticidebased control interventions for malaria control. Management of resistance cannot be done without prior knowledge of the underlying mechanisms. Although metabolic resistance plays a major role in conferring resistance in the major vector *Anopheles funestus;* the contribution of carboxylesterases to this resistance remains uncharacterized. Here, we characterized the carboxylesterases in *An. funestus*.

Methodology: Carboxylesterase amino acid sequences of *An. funestus s.s, An. gambiae, An. Coluzzii; An. albimanus* and *An. minimus* were downloaded from vector base. Sequences were aligned using Clustal Omega. The structural features of the carboxylesterases were revealed using the Swiss-Model, pymol 1.1. RNA was extracted from field mosquitoes, cDNA synthesized and carboxyl AFUN002514 was amplified. Genetic diversity were analyzed after sequencing.

Results: Thirty carboxylesterase genes were identified on 17 scaffolds. Carboxylesterases in *An. funestus* share a similarity above 30%. Many carboxylesterases in *An. funestus* have one domain compared to *An. albinamus*. Fifteen genes were classified as α -estrases, 5 as β -esterases and 10 non classified esterases. Structurally, carboxylesterases have GxSxG –xEG/xDG-HxDD as catalytic triad, the EDCLY roof forming disulfide bridge and HGG participate in forming oxyanion hole. The RNAseq results showed high expression profile of some carboxylesterases in permethrin resistance field mosquitoes. qRT-PCR results revealed carboxylesterase over-expressed 25 times in Benin and Cameroon and 9 times in Ghana. The analysis of carboxyl AFUN002514 showed amino acid changes out of catalytic site but two of them were conserved across countries; Alanine to Valine in 327 and Serine to Alanine in 388. The overall of haplotype and nucleotide diversity was 0.00024 and 0.00309 respectively.

Conclusion: This work annotated the carboxylesterases in *An. funestus* and lays a foundation for future study of carboxylesterases involved in insecticide resistance.

Title: Insights into the Evolution and Spread of Insecticide Resistance from Whole Genome Sequencing of 1,142 *Anopheles Gambiae* Mosquitoes from 13 Countries

Background: The Anopheles gambiae 1000 Genomes Project (Ag1000G) is studying natural genetic variation in mosquito populations and creating an open access resource of whole genome sequencing data to inform research and surveillance. Here we report on analyses of insecticide resistance using data from phase 2 of the Ag1000G project.

Methods: We sequenced whole genomes of 1,142 mosquitoes (655 An. gambiae, 283 An. coluzzii, 204 admixed gambiae/coluzzii ancestry) wild-caught from 13 countries. Data were processed to discover genetic variants, and were filtered and curated to retain only high confidence variant calls. We then performed a range of population genetic analyses to generate new insights into insecticide resistance, including genome-wide analyses to investigate the molecular profile of resistance in different populations, and gene-specific analyses to learn more about the spread of known forms of resistance such as target-site and metabolic resistance to pyrethroids.

Results: We give an overview of results in three main areas. First, we characterise the molecular profile of resistance in each of 16 mosquito populations, highlighting both known and novel mechanisms of resistance. Second, we describe the geographical distribution of copy number variation at cytochrome P450 genes, which is likely to underpin metabolic resistance to pyrethroids and should be counteracted by "next-generation" LLINs including the PBO synergist. Thirdly we give an update on the distribution and spread of target-site pyrethroid resistance, including the evolution of secondary mutations associated with known *kdr* alleles.

Conclusions: Molecular data are potentially useful for mosquito population surveillance, particularly as new tools for vector control are introduced, including new LLINs and new IRS formulations. Data from the Ag1000G phase 2 resource provide a new foundation for investigating mechanisms and spread of resistance, and for further surveillance to inform optimal intervention strategies and monitor the efficacy and impact of the introduction of new vector control tools.

Selection and Introgression Drive Spread Title: Duplications, the of **Organophosphates** West African Anopheles Resistance to in Gambiae

Authors: Xavier Grau-Bove, Edi Constant, Eric Lucas, Dimita Pipini, Arjen van T' Hof, Martin J. Donnelly, **David Weetman**

LSTM, Liverpool, United Kingdom

The organophosphate pirimiphos methyl is the most important insecticide for indoor residual spraying-based malaria control across Africa. However, organophosphate-resistant Anopheles are increasingly common in West Africa, and in some locations a mutation (G119S) in the insecticide target site, acetylcholinesterase (Ace-1) has also been increasing sharply in frequency. Here, from whole genome sequence-based GWAS we establish a clear association between pirimiphos-methyl resistance and copy number of mutated Ace1 genes in An. coluzzii from rice fields in southern Cote d'Ivoire. We establish repeatability of this association in multiple pirimiphos-methyl populations from West Africa. The mutation appears to have emerged and duplicated once but subsequent divergent pathways of duplication have resulted in heterogeneous and homogeneous patterns of copy number variation, both under strong selection. Our results highlight the importance of CNV scanning in in GWAS studies and provide a strongly predictive marker system for monitoring operationally-crucial insecticide resistance in An. gambiae.

Title: Assessing Risks Associated with Genetic Control Methods for Malaria Vectors

Authors: K. R. Hayes¹ ¹CSIRO Data61, Castray Esplanade, Hobart, Tasmania, 7000, Australia

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Novel genetic-biocontrol methods, particularly those involving gene drive mechanisms, are being discussed as a possible cost-effective way to suppress populations of malaria vectors. Risk assessments for the release of genetically modified organisms typically use qualitative methods. The advent of gene drive, however, has led some agencies to question the adequacy of these approaches. The United States National Academies of Science Engineering and Medicine (NASEM) and the Australian Academy of Sciences (AAS) for example have recently recommended probabilistic risk assessments in this context. This presentation will describe how to conduct probabilistic risk assessment for novel genetic control techniques, highlighting how expert opinion and data are incorporated into the assessment, and the important role for databases such as VectorBase. The presentation will draw on examples of risk assessments recently completed by the CSIRO DEERA team for technologies that are pre-cursors to gene-drive based control of malaria vectors in sub-Saharan Africa.
Title: Improving Gene Drives Towards Complete Suppression of *Anopheles Gambiae* **Populations**

Authors: Tony Nolan*†, Kyros Kyrou†, Andrew Hammond†, Xenia Karlsson†, Andrea Beaghton†, Andrea Crisanti†

*Liverpool School of Tropical Medicine, UK. † Imperial College London, UK.

CRISPR-Cas9 nuclease-based gene drives rely on inducing chromosomal breaks that are repaired in the germline in ways that lead to a bias in their inheritance. The first generation of gene drives showed a strong bias in inheritance but their ability to invade a population was compromised by unintended fitness costs due to ectopic nuclease expression and high levels of resistant mutations that limited the potential of the first generation of gene drives to spread.

We show that regulatory sequences designed to better contain nuclease expression to the germline can confer improved fecundity over previous versions and these gene drives generate drastically lower rates of target site resistance. We show that this effect is explained by reduced rates of end-joining repair of DNA breaks at the target site caused by deposited nuclease in the embryo. These results have important implications for the prospect of making gene drives less prone to resistance.

These new regulatory sequences were used to make an improved CRISPR–Cas9 gene drive construct targeting a highly conserved sequence in the gene *doublesex*. The drive spread rapidly in caged mosquito populations, reaching 100% prevalence within 7–11 generations while progressively reducing egg production to the point of total population collapse. Owing to functional constraint of the target sequence, no selection of alleles resistant to the gene drive occurred in these laboratory experiments.

Title: The mosquito microbiome and insecticide resistance

Authors: Nsa Dada^{1,2,3}, Diana Omoke Nyanting'a^{3,4}, Ezekiel Mugendi⁴, Eric Ochomo³, Mathew Kipsum³, Samson Otieno³, Edward Esalimba³, Juan C. Lol⁵, Ana Cristina Benedict⁵, Francisco López⁵, Kelly Liebman^{2,6}, Jesus Pinto^{7,} Mili Sheth⁸, Nicole Dzuris¹, Norma Padilla⁵ and Audrey Lenhart¹

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Background: A deeper understanding of the mechanisms underlying insecticide resistance would support mitigation of its threat to mosquito control. Following evidence of microbiota-mediated insecticide resistance in agricultural pests, we hypothesize that insecticide exposure might alter the mosquito microbiota and thus contribute to insecticide resistance.

Methods: Using whole metagenome sequencing in mosquitoes for the first time, we screened for differences in the microbiome between historical samples of Peruvian Anopheles albimanus that were either resistant (n=30) or susceptible (n=10) to the organophosphorus insecticide (OP), fenitrothion. To test for consistency, we further characterized the bacterial communities of more malaria vectors in relation to their pyrethroid resistance phenotypes using 16S rRNA amplicon sequencing. We focused on F_1 progeny of field-collected mosquitoes from locations with low intensity (Southern Guatemala; An. albimanus, n=135) and high intensity (Western Kenya; An. gambiae s.s., n=100) pyrethroid resistance—mosquitoes were tested for permethrin and **alphacypermethrin (only Guatemalan population) resistance.**

Results: In the Peruvian population, we found significant differences (p<0.01) in bacterial composition and putative enzymes between resistant and susceptible mosquitoes, with more bacterial taxa associated with OP-degradation and more hydrolases (the principal



class of enzymes involved in OP-degradation) in resistant vs susceptible mosquitoes. In oyrethroid-tested mosquitoes, we found significant differences in bacterial composition between insecticide-exposed and non-exposed mosquitoes overall (p<0.01), and between resistant and susceptible mosquitoes in Kenya (p<0.001), where pyrethroid intensity was high. Bacterial taxa associated with pyrethroid degradation were more abundant in pyrethroid-exposed mosquitoes compared to those that were not exposed, particularly in resistant mosquitoes.

Conclusions: These results show that insecticide exposure alters mosquito bacterial communities, and demonstrate how insecticide pressure might select for insecticide metabolizing bacteria/bacterial components that could be contributing to insecticide resistance in mosquitoes. These bacteria/their components could be exploited for monitoring insecticide resistance development in mosquito populations.

Parallel session 3:

Vector biology and control

Title: Autodissemination of pyriproxyfen suppresses stable populations of Anopheles arabiensis under semi-controlled settings

Author: Dickson Lwetoijera

Background: Autodissemination of pyriproxyfen (PPF), i.e. co-opting adult female mosquitoes to transfer the insect growth regulator, pyriproxyfen (PPF) to their aquatic habitats has been demonstrated for *Aedes* and *Anopheles* mosquitoes. This approach, could potentially enable high coverage of aquatic mosquito habitats, including those hard to locate or reach via conventional larviciding. This study demonstrated impacts of autodissemination in crashing a stable and self-sustaining population of the malaria vector, *Anopheles arabiensis* under semi-field conditions in Tanzania.

Methods: Self-propagating populations of *An. arabiensis* were established inside large semi-field cages. Larvae fed on naturally occurring food in 20 aquatic habitats in two study chambers (9.6×9.6 m each), while emerging adults fed on tethered cattle. The mosquito population was monitored using emergence traps and human landing catches, each time returning captured adults into the chambers. Once the population was stable (after 23 filial generations), PPF dissemination devices (i.e. four clay pots each treated with 0.2- 0.3 g PPF) were introduced into one of the chambers (treatment) and their impact monitored in parallel with untreated chamber (control).

Results: Daily adult emergence was similar between control and treatment chambers, with average (\pm S.E) of 14.22 \pm 0.70 and 12.62 \pm 0.74 mosquitoes/trap, respectively, before treatment. Three months post-treatment, mean number of adult *An. arabiensis* emerging from the habitats was 5.22 \pm 0.42 in control and 0.14 \pm 0.04 in treatment chambers. This was equivalent to >97% suppression in treatment chamber without re-treatment of the clay pots. Similarly, the number of mosquitoes attempting to bite volunteers inside the treatment chamber decreased to zero, six months post-exposure (i.e. 100% suppression). In contrast, biting rates in control rose to 53.75 \pm 3.07 per volunteer over the same period.

Conclusion: These findings demonstrate effective suppression of stable populations of malaria vectors using a small number of simple autodissemination devices, from which adult mosquitoes propagated pyriproxyfen to contaminate aquatic habitats in the system. This is the first proof that autodissemination can amplify treatment coverage and deplete malaria vector populations. Field trials are necessary to validate these results, and assess impact of autodissemination as a complementary malaria intervention.

Title: Study of the Efficacy of *Bacillus thuringiensis israelensis* for the Control of *Anopheles gambiae sl* Resistant to Pyrethroid.

Author: Roland ALIA*

* Center of Entomological Research of Cotonou (Benin)

Background: The vector control is the main strategy recommended by the world health organisation for fighting vector-borne diseases. In this study, the efficacy of *Bacillus thuringiensis* used today to replace chemical insecticides to circumvent vector resistance, was tested in natural conditions.

Methods: To determine the optimal dose applicable under natural conditions, three different doses $(1g / m^2 - 1.5g / m^2 \text{ and } 2g / m^2)$ of the commercial Vectobac GR 3.33% AI (200 ITU / mg) formulation of *Bti* were separately tested in laboratory on stage II larvae of resistant *Anopheles gambiae sl* population to measure adult emergence inhibition rates. This dose is the one that emergence inhibition rate is the highest in adults. The persistence of the product was then determined under natural conditions through the measurement of larval density reduction after application of the optimal dose.

Results: After laboratory assessments, the dose $2g/m^2$ showed the best inhibition emergence rate in adults. The linear regression model used considering the $1g / m^2$ dose as a reference showed that the $2g / m^2$ dose inhibited 1 / 76.71 = 0.01 - relative risk = 76.71 [69.41; 84,77] — times the emergence of adults than doses $1g / m^2$ and $1.5 g / m^2$. Under natural conditions, the larval density reduction observed on day 1 with evolved larvae stage (L3-4) was greater (99.8%) than that was observed (73.5%) with young larvae stage (L1-2). According to the mixed linear regression model, the persistence of the product was about 3 days on evolved larvae stage.

Conclusion:

The formulation of *Bti* tested in this study is not significantly effective to be considered at indicated doses in the perspective of large scale use against mosquitoes in Benin.

Title: Flourishing in Germs: Deciphering the Role of Bacteria in Development of the Malaria Vector *Anopheles Gambiae*

Authors: Caroline W. Kiuru¹ and Elena A. Levashina¹

¹Vector Biology Unit, Max Planck Institute for Infection Biology, Berlin, Germany

Background: In Africa, *Anopheles gambiae* s.l. mosquito is the major malaria vector. Life history traits, such as development and reproduction, play an important role in the vector's capacity to transmit malaria parasites. Here, we investigated the role of environmental factors in mosquito development. Like all living beings, mosquitoes acquire from the environment a wide range of microbes, mainly bacteria. Accumulating evidence suggest that these microbes regulate diverse physiological processes throughout mosquito life cycle. Of great significance is the role the gut microbiota plays in larval development, as development is arrested in sterile larvae. Indeed, arrest in development of sterile larvae can be restored by association with multiple bacterial species, indicating that the bacterial factors required for development are produced by many bacteria species.

Methods: Using a systematic screen of a collection of viable *E. coli* single-gene knockouts (Keio collection), we identify bacterial factors and processes that regulate mosquito development.

Results: Our results suggest two possible functions of bacteria in mosquito development: (1) metabolic function, particularly provision of aromatic amino acids and (2) signaling function that could be achieved through metabolites or mediated by bacterial flagella.

Conclusions: Understanding the essential developmental requirements is crucial for a better understanding of the environmental factors that regulate mosquito growth and should provide new insights into the role of environmental microbiota in structuring of mosquito populations.

Title: A Good Spray: Entomological Surveillance Results from a Cluster Randomized Trial to Evaluate the Impact of a Third Generation Indoor Residual Spray Product on Malaria Transmission in Mozambique

Authors: Joseph Wagman¹, Aklilu Seyoum⁹, Stephen Magesa⁸, <u>Kenyssony Varela⁸</u>, Rodaly Muthoni⁸, Christelle Gogue¹, Kenzie Tynuv¹, Carlos Chaccour^{2,3}, Francisco Saute², Rose Zulliger⁴, Abuchahama Saifodine⁵, Candrinho Baltazar⁶, Jason Richardson⁷, Christen Fornadel⁷, Laurence Slutsker¹, Molly Robertson¹

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The Mopeia District of Zambezia Province in Mozambique has a high malaria burden despite high levels of household coverage with long-lasting insecticidal nets (LLINs). In this context, a two-year, two-armed cluster-randomized trial (CRT) was conducted to evaluate the impact of indoor residual spraying (IRS) with a third-generation IRS product when combined with a 2017 mass LLIN distribution campaign. One study arm received pyrethroid-impregnated LLINs and the other arm received two annual rounds (late 2016 and again in late 2017) of IRS with a microencapsulated formulation of pirimiphos-methyl (Actellic®300CS) in addition to LLINs. After the 2017 mass distribution campaign, LLIN use measured in an active cohort of over 1500 children was consistently greater than 85% for 15 months across both study arms. Based on entomological surveillance, the primary vector species throughout all of Mopeia was Anopheles funestus s.s., though An. gambiae s.s. and An. arabiensis were also collected at much lower densities. Field-collected An. funestus populations demonstrated emerging resistance to multiple pyrethroids and to bendiocarb (between 80% – 90% mortality in standard WHO tube tests) but were 100% susceptible to pirimiphos-methyl. During the six months after each IRS campaign, monthly An. funestus densities were substantially reduced at sentinel sites in IRS clusters compared to non-IRS clusters: 63% fewer specimens were collected in indoor light traps after the 2016 campaign, and 85% fewer after the 2017 campaign. Similar reductions in the numbers of An. funestus landing on human landing collectors were also recorded from houses in IRS clusters, both indoors and outdoors, following each spray campaign. Taken together with concurrent significant reductions in malaria infection and case incidence rates in the IRS clusters during the trial, these vector surveillance results provide compelling evidence of the additional impact of combining a third generation IRS campaign with a universal LLIN coverage campaign in an area of high transmission, moderate pyrethroid resistance, and high LLIN usage rates (>85%).

Title: Evaluating the feasibility and impact of community-based winter larviciding on malaria transmission in southern of Africa countries aiming for malaria elimination.

Authors: Theresia Nkya*, Ulrike Fillinger, Florence Soroses, Godira Segoea, Makhoselive Dlamili, Emmanuel Chanda, Birkinesh Ameneshewa, Cliofford Mutero.

Background: Long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS), has permitted a decrease in malaria transmission and malaria burden. To further reduce vector densities from the low levels currently achieved with LLINs and/or IRS to lower levels that may lead to faster and sustainable elimination of parasite transmission, additional interventions will need to be augmented to these already existing tools. These additional interventions are to be implemented under integrated vector management (IVM) approach as promoted by the World Health Organization. The AFRO II Demo project aims at demonstration of effectiveness of diversified, environmentally sound and sustainable interventions, and strengthening national capacity for innovative implementation of IVM for disease prevention and control in the WHO AFRO Region.

Description: The component two of the AFRO II demo project is aimed at evaluating the feasibility and impact of community-based winter larviciding on malaria transmission in Namibia, Botswana and Swaziland. It is hypothesised that, fortnightly winter larviciding (targeted 4 months per year prior to IRS) with *Bacillus thuringiensis israelensis (Bti)* in addition to IRS at the start of a rainy season will reduce habitat positivity (*Anopheles* larvae present) and *Anopheles* adult densities indoors and outdoors by 70% as compared to areas/ times when only IRS is implemented. The project is being implemented in 12 study areas in each country for the duration of three years.

Lessons learned: Initial project evaluation showed the importance for the country buy-in of the project and implementation by the national programs for the success of the intervention. In addition, some methods that were not being used before project implementation, such as participatory mapping in community-based larviciding, had been adopted.

Next step: Evaluate effectiveness in reducing habitat positivity and adult mosquito densities indoors and outdoors in intervention areas. The roll out of the community-based winter-larviciding intervention, where applicable.

Title: Insecticide Susceptibility Status of An. Gambiae S.I. In Four Sentinel Sites in Cameroon

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Background: Information about insecticide resistance is required for decisions about deployment of vector control strategies and insecticide resistance management. Accordingly, susceptibility of *Anopheles gambiae* s.l. to deltamethrin, permethrin, alpha-cypermethrin, pirimiphos methyl, clothianidin, and bendiocarb was assessed in four sentinel sites (Simatou, Gounougou, Mangoum, and Nyabessang) in 2018 in Cameroon. The objective of the study was to determine the level of resistance to different insecticides used in public health and provide data to inform insecticide resistance management.

Methods: Bioassays were carried out using WHO susceptibility kits and protocol. Adult female *An. gambiae* s.l. between 2 and 5 days old that emerged from collected larvae were tested for susceptibility using WHO papers impregnated with standard doses of 0.05% deltamethrin, 0.75% permethrin, 0.05% alpha-cypermethrin, 0.25% pirimiphos methyl, 0.1% bendiocarb and 0.06% clothianidin (13.2 mg/m2). When resistance was confirmed, resistance intensity was further assessed using 5x and 10x the standard doses of the insecticide tested. Synergist assays were also conducted using WHO papers impregnated with 4% PBO (piperonyl butoxide) to assess the implication of P450 enzymes in the vectors' resistance mechanisms.

Results: *An. gambiae* s.l. was resistant to pyrethroids in all sites with less than 52% mortality for deltamethrin, below 17% for permethrin, and less than 79% for alpha-cypermethrin. Pirimiphos-methyl resistance was detected in three of the four sites (Simatou, Mangoum, and Nyabessang). An. gambiae s.l. was susceptible to clothianidin and bendiocarb in Gounougou and Simatou. The intensity of pyrethroid resistance ranged from moderate (mortality \geq 98% at 10x the diagnostic concentration) to high (mortality \leq 98% at 10x) and was either low (mortality \geq 98% at 5x) or moderate for organophosphate. Pre-exposure to PBO partially restored susceptibility to the pyrethroids tested, indicating the involvement of P450 monooxygenases as a resistance mechanism.

Conclusion: These results reinforced the need to develop a resistance management plan to ensure effective malaria vector control in Cameroon.

Title: Characterization of Resistance Mechanisms and Bio-Efficacy of Pyrethroid-Pbo Nets Against Pyrethroid Resistant Populations Of *An. Gambiae* S.I in Selected Malaria Sentinel Sites in Ethiopia

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Long-lasting insecticidal treated nets (LLINs) and indoor residual spraying (IRS) are the main vector control interventions in Ethiopia. The high levels of resistance of *Anopheles gambiaes*.1 to pyrethroidsobserved in many malaria endemic areas of the country highlight the need to further investigate the resistance mechanisms involved and continuous close monitoring which possibly help for informed decision in planning vector control. Therefore, this study aimed at monitoring insecticide resistance in populations of *An. gambiaes.l.* in selected malaria surveillance sentinel and other sites, characterize mechanisms conferring resistance to pyrethroid insecticides and determine the bio-efficacy of pyrethroid-only LLINs (MAGnet, Yarkool andPermaNet 2.0) and pyrethroid- PBO nets (PermaNet® 3.0) against pyrethroid resistant populations of *An. gambiaes.l.* to generate data for evidence-based policy making for optimal malaria vector control, insecticide choice and insecticide resistance management.

The susceptibility status of populations of An. gambiaes.l. to alphacypermethrin, permethrin, and deltamethrin was assessed following standard WHO susceptibility test procedure in twelve sites selected from five out of nine regional states (Oromyia,Amhara, SNNPR,Gambella and BenshangulGumuz) of Ethiopia. Of the twelve selected sites, eight (Goro, Asendabo, Angergutin, Pawi, Abobo, Wondo Genet, Halaba and Efratana Gidim) were national malaria surveillance sentinel sites. Other selected sites included Jimma, Serbo, Harbu andLokha Abaya. Bioassays were also conducted using piperonylbutoxide (PBO), a synergist to detect the involvement of elevated detoxifying oxidase P450 enzymes (or metabolic resistance). Both insecticide susceptibility tests and synergist assays were carried out using 3-5 days old unfed female An. gambiaes.1. mosquitoes reared from field collected larvae. Each insecticide with and without pre-exposure to PBO was tested in four replicates using 20-25 adult female mosquitoes for each test; a control in two replicates each with 20-25 female mosquitoes was run in parallel. Moreover, the bio-efficacy of pyrethroid-only nets (MAGnet, Yarokoland PermaNet 2.0) and a PBO-pyrethroid net (PermaNet® 3.0) was evaluated against wild resistant An. gambiaes.l. populations following WHO cone bioassay procedure.

The results of WHO susceptibility tests showed that there was high resistance in populations of *An. gambiaes.*l. from all sites to alphacypermethrin (mortality 44.4% to 68.6%), permethrin (mortality 8% to 39%) and deltamethrin (mortality 15% to 52%). With

1 hour pre-exposure to PBO, mosquito mortality rates in all sites increased significantly for alphacypermethrin (mortality 100%), permethrin (mortality 84%-95%) and deltamethrin (mortality 94%-100%). However, mortality rates in all mosquito populations exposed to permethrin and in few deltamethrin exposed mosquito populations following 1 hour pre-exposure to PBO were still below resistance threshold(<98% mortality). Overall, analysis of bio-efficacy of LLINs (MAGnet, PermaNet 2.0 and PermaNet 3.0) by net type and section against pyrethroid resistant populations of *An. gambiaes*.1. indicated that there was reduced susceptibility in all the twelve populations An. *gambiaes*.1. to the three pyrethroid-only nets (MAGnet, Yarokol,PermaNet 2.0) and (PermaNet 3.0 side) tested (mortality 4.4%-83.8%). Bio-efficacy of the roof of pyrethroid-PBO net(PermaNet 3.0) was however significantly higher (p < 0.001) than the pyrethroid-only nets and PermaNet 3.0 side against all the twelve mosquito populations.

This study once again demonstrates widespread pyrethroid resistance in *An. gambiaes.l.* in different areas of Ethiopia. The significant increase in mortality rates in mosquitoes exposed to alphacypermethrin, permethrin and deltamethrin after pre-exposure to PBO showed the partial or full involvement of detoxifying enzymes (cytochrome P450 monoxygenases), a mechanism which largely confers resistance to pyrethroid insecticides; previously reported *kdr* mutation from this mosquito population may also be involved. The evaluation of bioefficacy of LLINs indicated that both pyrethroid-only nets and Pyrethroid-PBO net side performed less as compared to Pyrethroid-PBO net roof, where mosquitoes typically approach a net. These results suggest that synergists such as PBO should be considered in vector control products such as LLINs so that tools with the highest efficacy are used in areas with pyrethroid resistant mosquito populations.

Key words: Bio-efficacy, Insecticide resistance, synergist, piperonylbutoxide, malaria, Ethiopia

Parallel session 4:

Innovations in vector surveillance and control

Title: Influence of House Characteristics, on Mosquito Distribution and Malaria Transmission in the City of Yaoundé

Authors: Carmene S Ngadjeu^{1,2}, Patricia Doumbe-Belisse^{1,2}, Abdou Talipouo^{1,2}, Landre Djamouko-Djonkam^{1,3}, Edmond Kopya^{1,2}, Roland Bamou^{1,3}, Parfait Awono-Ambene¹, Sevilor Kekeunou², Wilson Toussile⁴, Charles S Wondji⁵, Christophe Antonio-Nkondjio^{1,5*}

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Background: Improving houses characteristics is known to limit the contact between human and mosquito and reduce malaria transmission risk. In the present study, the influence of house characteristics on mosquito distribution in the city of Yaoundé was assessed.

Methods: The study was conducted from March 2017 to June 2018 in 32 districts of the city of Yaoundé. Mosquito collections were performed in 462 houses using CDC lights traps. House characteristics including construction materials, presence of ceilings, eaves, vegetation, breeding sites, windows and the number of people in houses, usage of LLINs were recorded. Mosquitoes collected were identified morphologically down to the genus and species level using morphological identification keys. All anopheline collected were analysed by ELISA to detect their infectivity. Bivariate and multivariate regression analyses were undertaken to assess the influence of houses characteristics on mosquito distribution.

Results: A total of 168,039 mosquitoes were collected; Culex emerged as the predominant species (96.48%), followed by *An. gambiae* s.l. (2.49%). Characteristics like: number of inhabitants/houses, the type of house, the presence of holes on walls, the presence of screens on windows and presence of vegetation were found to significantly influence indoor mosquito densities (p < 0.05). Indoor biting *An. gambiae* s.l. densities were found to be affected by the type of walls, presence of ceiling and vegetation (p < 0.05). The usage of LLINs in houses was not found to affect mosquito distribution. Out of the 3,644 Anophelines tested using ELISA CSP, 87 were found infected. The proportion of mosquitoes infected did not vary significantly according to house characteristics.

Conclusion: The study indicated several house characteristics favoring mosquitoes' entry in houses. Promoting better housing should also be integrated in strategies to improve malaria control in the city of Yaoundé.

Keywords: Malaria, houses characteristics, Culicines, Anophelines, An. gambiae s.l, Yaoundé.

Title: The LabDisk diagnostic tool: contribution to vector surveillance toward malaria elimination in Cameroon.

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Malaria is a life-threatening disease (435,000 deaths in the world in 2017). Prevention of this disease is best achieved by vector control which, today, heavilyrelies on the use of insecticidesthrough Indoor Residual Spraying andLong-LastingInsecticidal Nets. However, the increase of resistance to insecticidesis jeopardizingtheprogress towards its elimination. Monitoring mosquito vector populations is therefore needed for effective interventions. Several individual methods are used for this task; however, there are many obstacles to the uptake of thecurrent protocols, including thelimited resources in malaria endemic countries.

To address the challenges encountered the diagnostic of malaria vectors, a fully automated and cost-effective diagnostic platform (LabDisk) is being developed in the framework of the DMC-MALVEC project. The platform consists of a disposablecentrifugal-microfluidic cartridge, in which completely integrated biochemical protocols for nucleic acid or protein analyses can be performed. Thedevice automatically controls fluid processing, heating and signal acquisition via fluorescence or luminescence. The novel multiplex diagnostic platform will rapidly screen large numbers of mosquitoes (pooled or individual) at low cost and provide reliable results (species ID, infection status, insecticide resistance profile), with minimum laboratory equipment. Twelve to 15 markers/traits will be measured simultaneously in each mosquito population, with a test time of less than1hour.

Four African countries(Ethiopia, Zambia, Ivory-Coast and Cameroon) are involved in the validation of the LabDisk diagnostic tool. In Cameroon mosquito populations have be sampled in three study sites (Ekie, Nkolbisson and Nkolondom) around the Yaounde city. Sub samples of collected mosquitoes will be analysed by conventional technics(PCR, TAQMAN, ELISA...) versus the LabDisk diagnostic platform.

The novel LabDisk System is expected to improve vector surveillance toward elimination of malaria in Cameroon.

Key Words: Malaria Elimination; Mosquitosurveillance; LabDisk, Cameroon.

Title: Sumishield 50Wg: Meeting the Challenge of Insecticide Resistance

Author: John Lucas¹

¹Sumitomo Chemical Company

Introduction: SumiShield 50WG, containing the neonicotinoid clothianidin, was prequalified by WHO in October 2017 - making this the first WHO recommended new mode of action chemistry for IRS in 40 years. Without this and other new chemistries that are now in the pipeline the future of IRS was in doubt. Since then this product has been deployed extensively throughout Africa - programs now have a choice of effective chemistries allowing for the implementation of rotational strategies.

Methods: This presentation will share recent lab and field efficacy data

Results: The product has been well received due to its good efficacy and ease of use. Studies indicate excellent and long residual activity of SumiShield and widespread field susceptibility against a range of *Anopheles* species.

Conclusions: While results are very encouraging for the continued future of IRS, we need to ensure IRS products with different modes of action are developed and rotated to help preserve their effectiveness.

Title: Quality Assurance in IRS – New Tools

Authors: Deb R, Kok P, Shaw A, Coleman M

Background: Indoor residual spraying (IRS) is one of the core strategies used to control insect transmitting diseases of public health importance such as malaria and visceral leishmaniasis. To ensure the strategy remains effective within an operational setting, the World Health Organisation (WHO) have set process, performance and impact indicators which should be monitored during each round IRS. Quality assurance (QA) forms a key component of performance monitoring; whereby the method to quantify insecticide deposited is conducting high performance liquid chromatography on filter papers affixed to walls prior to IRS. This coupled with WHO Cone Bioassays to determine efficacy, aims to provide comprehensive information on the quality of IRS. However, these methods require expensive equipment, skilled staff and an extensive supply of live insects.

Methods: The use of electromagnetic waves to real-time monitor the levels of compounds present in solutions is well established in a variety of industries. More specifically, microwave sensing has been used successfully for applications such as: fluid level measurement, material moisture content, monitoring biogas plants, verification of vegetable oil types, monitoring glucose in diabetic patients and non-invasive body fluid monitoring.

Results: This technology has been adapted to detect alpha-cypermethrin at different concentrations to monitor IRS quality, efficacy and residual decay rates, and miniaturised to form a hand-held field friendly QA tool. In many IRS programmes QA is a secondary priority - this technology aims to eliminate issues associated with performing IRS monitoring effectively.

Conclusion: Here we present details of the initial development and evaluation in an IRS programme.

Title: Towards Development of Genetic Sexing Strain for Sex Separation And Female Elimination in the Malaria Vector *Anopheles Arabiensis* (Diptera: Culicidea) For Use in Sterile Insect Technique Program

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Traditional Anopheles mosquito control methods become inefficient because of lack of efficient drugs and vaccine against malaria but also in the context of expansion of vector resistance to insecticides. Therefore, new or complementary techniques, base on vector population suppression such as novel genetic strategies like sterile insect technique (SIT) are urgently needed to combat major mosquito vector species. SIT rely on male-only releases as female mosquitoes bite and transmit pathogens. However, the lack of efficient and robust sex separation methods presents a major bottleneck to the successful application of these techniques as the currently available sex separation methods are inadequate for safe and bio-secure male-only releases in large scale operational programs. Here, we aim to develop the Genetic Sexing Strain (GSS) in *An. arabiensis*, by sex linkage of the wild allele conferring temperature resistance to male.

Wild type pupae were irradiated one day before emergence (at 20-22 hrs old) at 30 Xy and 40 Gy. Emerged adults were crossed to homozygous thermosensitive virgin females in 1: 2 ratios. Each individual F2 male was backcrossed with 5 F0 susceptible virgin females. L1 larvae from each isofamily were screened for temperature resistant male at 41°C during 3 hours. The effects of irradiation on adult emergence, pupal survival, adult's longevity, female fecundity, hatch rate and sex ratio were followed.

Irradiation of pupae, for two doses tested, had no effect on adult emergence. Survival curves of adults irradiated were slightly lower than that of control. The mean number of eggs laid by females as well as egg hatch rate per treatment didn't varied significantly. There was no significant difference in adult emergence rate between both treatments and control. From 750 isofamilies exposed to 41°C for 3hrs, 11 have shows mortalities range between 0.06



and 90 %. The use of temperature at the F1 stage allowed selecting genetic sexing strain with 80 % of irradiated males on average after eight generations.

A good male rate production makes this GSS suitable for any control method based on mass production of *An. arabiensis*. However cytogenetics investigations of this strain are warranted prior to it used in large-scale operational SIT programmes.

Keys words: An. arabiensis, sterile insect technique, genetic sexing strain, translocation.

Title: Effect of sublethal pesticides exposure on the gut microbiota of *Culex pipiens* **L. (Diptera: Culicidae**)

Authors: Elijah Juma

Pesticide contaminants are common in aquatic environments and can modify the microbial community composition of the aquatic habitats. However, their role in shaping the mosquito gut microbiota is not well understood. We used a microcosm approach to investigate the effect of exposure to sub-lethal concentrations of three commonly used pesticides – atrazine, permethrin, and malathion – on the gut microbiota of juveniles, and newly emerged adults of Cx. pipiens L. mosquitoes. Illumina MiSeq sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene was used to compare the microbial community composition of larvae and adults of Cx. pipiens L. and the water samples from microcosms treated with each of the pesticides, separately. Bacterial community composition was significantly different between larvae and adult stages and water samples, but not by pesticide treatment. Bacterial OTU richness was highest in larval samples receiving malathion treatments. intermediate in the water and larval samples from the remaining treatments, and lowest in adult samples regardless of the treatment. This study provides insights into how larval exposure to sub-lethal concentrations of pesticides affects the gut microbiota of Cx. pipiens L. mosquitoes. It provides a basis for future studies investigating how pesticides interact with mosquito gut microbiota and the potential implications for mosquito-borne disease control.

Title: Host Preference and Feeding Patterns of Primary Malaria Vectors, *Anopheles Arabiensis* And *Anopheles Gambiae* S.s. In Sites With or Without Indoor Residual Spraying in Rwanda.

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Background: Blood-meal source analysis provides insight into host preference of disease transmitting insects and their efficiency in pathogen transmission. In this study, blood meal source of *Anopheles gambiae* s.l. mosquitoes known to be the dominant malaria vectors in Rwanda was investigated, the composition of sibling species identified and the potential impact of indoor residual spraying (IRS) on species composition described.

Method: Pyrethrum spraying catch method was used to collect mosquitoes resting inside houses from IRS and non-IRS sites. Blood fed *An. gambiae* s.l. collected from July to December 2018 were analyzed using direct enzyme linked- immunosorbent assay and sibling species identified by polymerase chain reaction (PCR).

Results: Overall 225 blood fed *An. gambiae* s.l. were identified by PCR; 55.1 % were *An. gambiae* s.s., 39.1% *An. arabiensis* and 5.8% of samples failed to amplify. Species composition in IRS sites was 16.1% *An. gambiae* s.s. and 83.9% *An. arabiensis*. In the non-IRS sites, the proportion was 93.2% *An. gambiae* s.s. and 6.8% *An. Arabiensis*. Single-source blood meal in IRS sites was 75% human and 10% bovine for *An. gambiae* s.s; whereas 26.8% and 65.9% of blood meals taken by *An. arabiensis* were from humans and bovines, respectively. Other identified sources of blood meals were either goat, mixtures from hosts, or unidentified animals.

Conclusion: The dominant malaria vector in IRS sites is *An. arabiensis* with greater preference for feeding on cattle than on humans. *An. gambiae* s.s. was found to be the primary vector in non-IRS sites and fed mainly on humans. The dominance of *An. arabiensis* in IRS sites is likely related to selection with *An. arabiensis* less affected by IRS due to its zoophagic and exophagic behaviors. Regular treatment of cattle with effective insecticide could provide additional protection against human malaria in areas where *An. arabiensis* is becoming the dominant malaria vector.

Parallel session 5:

Adopting the One Health approach – breaking down silos

Title: The Potential of Mosquito Habitat Management for Malaria Control Across Africa

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Background: Malaria transmission is dictated by mosquito vector ecology. Reducing land available for aquatic stages of the mosquito lifecycle has been demonstrated to play a role in local disease elimination in previously endemic countries. The World Health Organisation advise that larval source management (LSM) should be considered only when breeding sites are few, fixed and findable though it is unclear what effect partial clearing of water bodies inhabited by juvenile mosquitoes has on transmission.

Methods: We use a mathematical modelling approach to explore how LSM influences malaria control across Sub-Saharan Africa (SSA). We adjust a previously established transmission model by simulating the effects of temporarily removing larval habitat by reducing adult mosquito carrying capacity. This simulates reduced emergence of adult mosquitoes. The model is calibrated using detailed entomological data from a randomised control trial (RCT) of *Bacillus*-based larvicide in Western Kenya and is subsequently shown to be predictive of epidemiological outcomes. Using the fitted model, we then explore scenarios where LSM, or alternative strategies reducing adult mosquito emergence, are minimally deployed across SSA.

Results: Our model estimates reductions in adult mosquito emergence of 2% across Africa would result in 42.7 million malaria cases being averted over a 10-year period, which is comparable to model forecasts of the reduction in malaria cases due to a 2% increase in LLIN coverage (73.1 million). The predicted impact of reduced adult mosquito emergence ranged across Districts, from no effect to over 1 million cases averted across 10-years (Kano, Nigeria).

Discussion: Community engagement programmes encouraging environmental management of water bodies to render them less hospitable to mosquitoes, could have substantial epidemiological impact even if associated with relatively minor changes in adult emergence. This method was successful in eradicating malaria in large parts of the world before the advent of chemical approaches (DDT) in the 1940s.

Title: Windborne Long-Distance Migration of African Arbovirus Mosquito Vectors: The Case for One Health Paradigm

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As exemplified with malaria and dengue, mosquitoes are a highly efficient engine of disease transmission. For over a century, mosquito control has been the most effective target for control of vector borne diseases. Among other arboviruses, Rift Valley Fever (RVF) outbreaks have been attributed to long distance migration of infected mosquitoes, although direct evidence for it remained elusive. We sampled insects flying between 40 and 290 m above ground level in four Malian villages over three years using sticky nets mounted on tethered helium-filled balloons. Approximately 3,000 mosquitoes were collected among half a million insects. Control nets confirmed that the insects were captured at altitude. Over forty mosquito species, including members of the genus Anopheles, Aedes, and *Culex* were intercepted, representing secondary and primary vectors of RVF, West Nile, Yellow Fever, Zika, O'Nyong-Nyong among others, were identified. Importantly, females accounted for >80% of all mosquitoes. Of these, 90% had taken a blood meal before their migration, implying that pathogens will be transported by migrating females. Simulated trajectories of mosquito flights indicated mean nightly displacements of up to 300 km for 9-hour flight durations. Annually, the estimated number of mosquitoes at altitude crossing a 100 km line perpendicular to the winds ranged among species between 60,000 (A. gambiae s.s.) to 300,000,000 (Culex perexiguus). The importance of such samples for pathogen discovery affecting human and livestock health falls into perspective when considering that mosquitoes represent a mere 0.5% of the total insects collected. The abundance of these other taxa is at least two orders of magnitude larger than those of mosquitoes. These other insects include known pests (including vectors of plant diseases) and biocontrol agents of crops affecting food security while others probably provide vital services needed for ecosystem stability. These results provide grounds for an African network of aerial and ground sampling that will survey changes in the spread of disease agents, vectors, pests, and biocontrol agents on local, regional, and continental scales and inform on changing risks including changes to biodiversity. Such network can also contribute to capacity building in the sciences including medical, veterinary, and agricultural entomology, public health, microbiology, meteorology, modeling, ecology, population genetics.

Title: The Prevalence of schistosomiasis in Minna community, Jarra Central District the Gambia, 2014 and 2016

Author: Fatou Fofana RHPEO, LRR

Background: Schistosomiasis is an acute and chronic disease caused by parasitic worm Schistosoma. Transmission is by contact with water infested by infected water snail. The control of this neglected tropical disease under surveillance is based on mass drug administration, health education and the environmental sanitation. In 2014, two cases of schistosomiasis confirmed cases were reported by the laboratory personnel to the Public Health Office all from the same community Minna. Minna is a small community in Jarra Central District, lower river region of the Gambia. It has a total population of 161(GBoS, 2013). We conducted an investigation to confirm the prevalence of the disease in the community. After two years a similar investigation was also conducted to compare and determine the prevalence.

Method: In 2014, we screened any community member who volunteered and tested at the laboratory. Urine samples were collected from the community and tested for Schistosoma. We also conducted environment assessment to determine the environmental factors in relation to the problem. The case definition of schistosomiasis was any person from Minna with abdominal pain, fever and blood in urine. The people that were positive for the test were administered with Praziquantel and health education was emphasized to them on the mode of transmission and the preventive measures, this was conducted routinely even at the RCH clinics.

In 2016, we reviewed the records of the laboratory to determine the total schistosomiasis suspected cases and the confirmed cases, in order to compare the prevalence.

Results: In 2014, out of the 112 suspected cases that were tested in a population of 161. 43 were positive, 69 negative, resulting to a prevalence rate of 26.7%. In 2016 out of 112 samples collected and tested 22 were positive, prevalence rate of 19.6%. All these positive cases were found to be within the age 6-15 years. During the environmental assessment we found out that there was a small water pond in the community where the children of school going age plays.

Conclusion: Despite the numerous interventions done in the community, Schistosomiasis remain to be a public health problem in Minna. We therefore advocate for routine mass drug administration and strengthen the health education in schools to enhance behavioral change. In addition we recommend that Schistosomiasis surveillance to be strengthened.

Title: Linking Agriculture to Malaria Transmission Under a One Health Approach: Does Vegetable Farming Contribute to Insecticide Resistance Selection in the Malaria Vector *Anopheles coluzzii* ?

Authors: Armand Defo Talom^{1,2*}, Michele Agnes Essoung^{1,2}, Adam Gbankoto⁴, Genevieve Tchigossou^{3,4}, Romaric Akoton^{3,4}, Bio Bangana A. Sahabi⁷ Seun Michael Atoyebi⁸;, Apollin Fotso², Rudi Vespoor⁹; Manuele Tamo³, Timoleon Tchuinkam¹, Jo Lines⁶, Charles Wondji^{5,10}, Rousseau Djouaka^{4*}

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Background: Vegetable production has been identified as a cropping system that could contribute to the evolution of insecticide resistance in malaria vectors, however good support for this remains scarce. For a better design of sustainable resistance management programs, this research aims to identify the potential contribution of vegetable production to the selection of insecticide resistance in malaria vectors in Benin.

Methods: A knowledge,Attitude and Practice (KAP) study was undertaken with 81 farmers from the vegetable production sites at Houeyiho and Seme in the southern Benin. Information on the most commonly used insecticides, their mode of usage, and frequency of application were gathered. For these identified insecticides, the susceptibility profile of anopheles mosquitoes was tested. The presence of insecticide residues and heavy metals in surveyed breeding sites were also assessed.

Results: LambdaCyhalothrin constituted the main insecticide used in surveyed vegetable farms. *Anopheles coluzzii* was the main species found in the 4 surveyed vegetable farms and high resistance to λ -Cyhalothrin was recorded in all populations of this malaria vector in selected vegetable farms. However, a very low number of the examined breeding sites were found to be contaminated by λ -Cyhalothrin residues. Interestingly, a positive correlation was recorded between the presence of copper in breeding sites and λ -cyhalothrin resistance of anopheles mosquitoes that emerged from these breeding sites.

Conclusion: λ -Cyhalothrin is used widely, and probably misused, by vegetables farmers in Benin. This insecticide quickly degrades in the environment hence less likely to contribute to resistance selection in *An. coluzzii* populations. The presence of copper in most breeding

sites highlights the possible contribution of non-insecticidal compounds in the evolution of insecticide resistance in malaria vectors.

Keys words: Anopheles coluzzii; insecticide resistance; λ -Cyhalothrin; vegetables farm; copper

Title: Arboviruses Transmission Dynamics Along the Kenyan Coast

Authors: Karuitha M¹, Lutomiah J², Bargul J^{1, 3}, Sang R^{2, 3} and Mbogo C⁴

Introduction: Arboviruses are believed to have originated in tropical Africa. Although much of their distribution and epidemiology remains unknown, confirmed arboviral outbreaks have continued to impact heavily on health, agriculture and socio-economic activities in African countries including Kenya. This study was conducted to establish arboviruses transmission dynamics in coastal Kenya.

Method: Mosquito eggs, larvae and adults were collected in Arabuko sokoke forest, Haller Park, Gede and Bamburi in coastal Kenya. The immatures were reared to adult stage and identified to their respective species. Pools of 25 mosquitoes were screened for arboviruses infection by cell culture. Viral RNA was extracted from positive cultures and amplified by PCR. Purified amplicons are awaiting sequencing and querying in the Barcode of Life Database (BOLD) and GenBank for virus identification.

Results: A total of 5,028 mosquitoes identified to 19 species were processed in 638 pools. Out of these, 6 pools were positive for Alphaviruses and 23 positive for Flaviviruses. An overall infection rate of 0.045 was recorded across the study sites with Haller Park recording the highest infection rate (0.034) followed by Bamburi (0.008) and Gede (0.003). No arboviruses were isolated in mosquitoes from Arabuko Sokoke forest. *Aedes aegypti* recorded the highest infection rates with 0.17 followed by *Ae. tricholabis* and *Ae. vittatus* (0.006) each, *Anopheles funestus* (0.005), *Cule. pipiens, Cx. vansomereni* (0.003) each and *Ae. hirsutus, Cx. annulioris*, and *Filcabia mediolineata* 0.002 each.

Conclusion: The study established in the absence of outbreaks, active transmission of arboviruses still occurs in peri urban regions. Although the specific viruses have not been identified, the findings suggest, these sites may act as sources of arboviruses which spill over to urban areas causing outbreaks. Interventions should therefore be established to prevent human-vector contact in peri-urban areas and ensure effective vector control measures are implemented.

Title: Targeting Vector Control of Animal Trypanosomiasis by Identifying Drug-Resistant Trypanosomes in Tsetse Flies

Authors: AuthorsMagang K. Eugenie¹, Mewamba M. Estelle¹, Farikou Oumarou², Kamga N. Mitteran¹, Simo Gustave¹

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Background: African Animal Trypanosomiasis (AAT) is a vector-borne disease caused by the protozoa *Trypanosoma*. Pan African Tsetse and Trypanosomiasis Eradication Compain (PATTEC) is a continental initiative aiming to eradicate tsetse and trypanosomiases countries. Despite the wishes of African head of states, the implementation of PATTEC initiatives has not been achieved for many reasons like the misuse of trypanocides that has led to the emergence of drug resistance which further hampers the eradication process. Studies undertaken on drug-resistant trypanosomes in animals enabled to understand some aspect of drug resistance. However, the transmission of drug-resistant trypanosomes is still not well understood for the designing of efficient control against drug-resistant trypanosome in tsetse flies from Yoko with the ultimate goal of boosting and designing targeted vector control strategies against AAT.

Method: 215 tsetses were caught in two villages of Yoko. DNA was extracted from whole tsetse using CethylTrimethyl Ammonium Bromide (CTAB). Molecular identification of tsetse and trypanosome species was performed. From tsetse found with trypanosome infections, *DpnII*PCR-RFLP was used to detect diminazene aceturate resistant *Trypanosoma congolense*.

Results: Of the 215 tsetse flies caught, 49.8% (107/215) belonged to *Glossina fusca congolensis*, 33% (71/215) to *Glossina fusca fusca* and 17.2% (37/215) to *Glossina palpalis palpalis*. The overall trypanosome infection rate was 12.56% (27/215) including 6.51% T. *congolense* savannah, 4.65% *T. congolense* forest and 1.39% (3/215) *T. vivax*. Diminazene resistant strains of *T. congolense* were found in three tsetse flies.

Conclusion: This study report for the first time that drug resistance can be evaluated in trypanosomes infecting tsetse flies. Mapping with accuracy the regions where the transmission of drug-resistant trypanosomes occurred will enable to implement vector control in such areas in order to reduce the spread of resistant trypanosomes.

Key words: AAT, diminazene aceturate, resistance, PCR-RFLP, Yoko

Title: Increasing Intensity of Pyrethroid Resistance in An. Gambiae S.l. and the Implication for Vector Control in Democratic Republic of Congo

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Background: Malaria vector control in DRC relies on the distribution and use of pyrethroid long lasting insecticidal nets (LLINs). Within the country, mass distribution campaigns have been coordinated in 3-year cycles at the provincial level, involving different brands of LLIN that contain either permethrin, deltamethrin or alpha-cypermethrin. Under the National Malaria Control Program (NMCP) Strategic Plan 2016–2020, the DRC seeks to ensure that at least 80% of persons at risk of malaria sleep under an LLIN. However, pyrethroid resistance in malaria vectors is a major threat to the effectiveness of these LLINs.

To evaluate the impact of LLIN use on malaria vectors (seasonal vector distribution, behavior, species composition and resistance status) and make decisions regarding the choice of LLIN insecticides, the USAID President's Malaria Initiative (PMI) has been supporting entomological monitoring activities in DRC, under the PMI VectorLink project. Activities, including pyrethroid resistance and intensity testing as well as bio-efficacy of LLINs, are being implemented through the National Institute of Biomedical Research (INRB).

Methods: VectorLink, in collaboration with the NMCP and INRB, conducted nationwide insecticide resistance monitoring in 11 sites (across 11 provinces) in 2017 and 2018 using the WHO tube test. Intensity assays were conducted in 7 sites using the CDC bottle bioassay in 2017 and 11 sites using the WHO intensity assay in 2018. *An. gambiae* s.l. larvae were collected from the field and reared to adults in field insectaries before 2-5 day old females were tested. In 2017, used pyrethroid nets containing permethrin (Olyset®: 5 years old), deltamethrin (Yorkool®: 1 year old), and alpha-cypermethrin (Duranet®: 6 months old) were collected from Inongo (Mai-Ndombe Province) and tested using WHO cone bioassays with insectary reared susceptible *An. gambiae* Kisumu and wild pyrethroid resistant *An. gambiae* s.l from Kinshasa. The efficacy of the used nets was compared with new nets of each brand.

Results: Results in 2017 showed widespread permethrin resistance (mortality rate < 90%) at all 11 sites, but deltamethrin resistance was only present in 4 of 11 sites, and alphacypermethrin resistance in just 2 of 11 sites. Resistance intensity to permethrin was high in all sites in 2017, while deltamethrin resistance intensity was low or moderate using the CDC bottle bioassay. In 2018, the number of sites with deltamethrin resistance increased to 9 of 11 and there was alpha-cypermethrin resistance in all 11 sites. The resistance intensity using WHO tube tests to permethrin was high in Kinshasa, Lodja and Kalemie and moderate in 5 other locations. The intensity of resistance was generally moderate for all 3 pyrethroids, although in some sites survivors were recorded at 10 times the diagnostic doses of permethrin and deltamethrin, indicating high intensity resistance. All used and new permethrin and deltamethrin LLINs produced mortality rates inferior to the WHO bio-efficacy threshold of 80% mortality at 24h after exposure for both insectary and wild *An. gambiae* s.l. The biggest difference in mortality was for the used 6 months old Duranet, with mortality at 47% for wild *An. gambiae* s.l. and 100% for *An. gambiae* Kisumu.

Conclusions: Pyrethroid resistance is now widespread in DRC to all 3 pyrethroids used on LLINs, with an apparent increase in deltamethrin and alpha-cypermethrin resistance between 2017 and 2018. Moderate and high intensity resistance was recorded in several provinces and has the potential to compromise the effectiveness of pyrethroid LLINs. Next generation nets (PBO or bi-treated nets) should be seriously considered for distribution in DRC if evidence is generated showing greater performance compared to pyrethroid insecticides.

Parallel session 6:

Innovation in vector surveillance and control



Title: The Operational Feasibility of Using Drones to Identify Anopheles Mosquito Breeding Sites in Malawi

Authors: Stanton, M.C.¹², Hoek Spaans, R.², Kalonde, P.³, Jones, C.M.²³

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Background: In Malawi, despite significant advances in malaria control, progress made using approaches such as the distribution of long-lasting impregnated nets is showing signs of slowing down. Supplementary approaches that target high transmission areas are therefore needed, such as manipulating potential mosquito breeding sites to prevent mosquitoes developing i.e. larval source management. This study assessed whether drone imagery can feasibly be used to identify these breeding sites.

Methods: Initial field studies were conducted in June 2018 in Kasungu district, Malawi to evaluate drone usage in the dry season. The factors under consideration included (1) type of drone (fixed wing vs quadcopter), (2) sensors required (standard vs multispectral camera) and (3) the optimal approach for identifying breeding sites within the imagery (manual vs classification algorithms). Two drones were used to capture aerial images of five reservoirs within the area. Simultaneously, larval sampling was conducted along the accessible shoreline and the GPS coordinates, vegetation type and information on identified larvae were recorded. Machine learning techniques were then applied to classify the images according to breeding site suitability, and their accuracy was assessed by comparing the classified image to the larval sampling data.

Results: Mosquito larvae were identified in all five of the surveyed reservoirs with *Anopheles* larvae being found in 35% of the sampling sites (44/124). Of the two drones used to capture imagery, the quadcopter was more user-friendly and robust in comparison to the more expensive fixed wing drone, despite being able to cover a smaller area in a single flight. Image classification is ongoing with results due to be completed by August 2019.

Conclusions: An initial assessment of drone feasibility indicates their potential for identify potential breeding sites without the need for manual surveys. Further work is planned to establish how best to integrate this technology into vector control programmes.

Title: The React Randomised Controlled Trial to Assess Whether Addition of Complementary Vector Control Strategies to Long-Lasting Insecticidal Mosquito Nets Provides Additional Protection Against Clinical Malaria in Areas With Pyrethroid-Resistant Vectors in Rural Burkina Faso and Ivory Coast

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The fight against malaria faces now the challenge of the emergence and expansion of the resistance to both curative (drugs) and preventive (vector control) tools. Vector control which relies primarily on mass distribution of long lasting pyrethroid-treated nets contributes to the reduction of malaria transmission. The resistance of Anopheles vectors to pyrethroids, the only insecticide class recommended to treat bed nets, is threatening the considerable progresses made over the last decade.

The REACT project aims to assess whether addition of complementary vector control strategies to longlasting insecticidal mosquito nets provides additional protection against clinical malaria in areas with pyrethroid-resistant vectors in rural Burkina Faso and Ivory Coast. The tested strategies are 1) Indoor residual sprayings of insecticide; 2) intensive communication for human behavioural changes; 3) larviciding with natural toxins of *Bacillus thurengiensis israelensis*; 4) Use of Ivermectin both in human and cattle.

These tools are at late-stage of development or are already available within the arsenal of vector control tools in order to complement the current massive LNs distribution scaled up by national malaria control programs (NMCPs). Nevertheless as far as we know, except for Indoor residual sprayings of insecticide, there is no epidemiological data evidencing the benefit to use such complementary tools in high resistance areas. The main results of this 5 arms randomised control trial will be presented.

Title: Overexpression of Two Members of D7 Salivary Genes Family is Associated With Pyrethroid Resistance in the Malaria Vector Anopheles Funestus S.s. but not in Anopheles Gambiae in Cameroon

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D7 family proteins are among the most expressed salivary proteins in mosquitoes. They facilitate blood meal intake of the mosquito by scavenging host amines that induce vasoconstriction, platelet aggregation and pain. Despite this important role, little information is available on the impact of insecticide resistance on the regulation of D7 proteins and consequently on the blood feeding success. In this study, real-time quantitative polymerase chain reaction (qPCR)qPCR) analyses were performed to investigate how pyrethroid resistance could influence the expression of genes encoding D7 family proteins in Anopheles. gambiae and Anopheles. funestus s.s. mosquitoes from Elon in the Central Cameroon. Out of 328 collected mosquitoes, 256 were identified as An. funestus sl and 64 as An. gambiae sl. Within the An. funestus group, An. funestus s.s. was the most abundant species (95.95%) with An. rivulorum, An. parensis and An. rivulorum-like also detected. All An. gambiae s.l mosquitoes were identified as An. gambiae. High levels of pyrethroid resistance were observed in both An. gambiae and An. funestus mosquitoes. RT- qPCR analyses revealed a significant overexpression of two genes encoding D7 proteins, D7r3 and D7r4, in pyrethroids resistant An. funestus. However, no association was observed between the polymorphism of these genes and their overexpression. In contrast, overall D7 salivary genes were under-expressed in pyrethroid resistant An. gambiae. This study provides preliminary evidences that pyrethroid resistance could influence blood meal intake through over-expression of D7 proteins although future studies will help establishing potential impact on vectorial capacity.

Keywords: D7 salivary proteins; gene expression; insecticide resistance; pyrethroids; *Anopheles gambiae*; *Anopheles funestus*; malaria disease

Title: Age and Species Prediction of Field Malaria Mosquitoes Through Deep Learning of Mid-Infrared Spectra

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To improve and assess the effectiveness of vector control strategies it is crucial to determine the distribution of vector species and their age structures as these parameters influence the intensity of disease transmission. Nevertheless, a major limitation in vector surveillance is the lack of appropriate methods to measure these traits, as current approaches are expensive, time consuming or not accurate. We developed a rapid and cost effective tool based on midinfrared spectroscopy (MIRS) and machine learning analysis to simultaneously determine species and age in malaria vectors. By measuring the amount of light absorbed by the mosquito cuticle through MIRS, we obtained information on its biochemical composition, and consequently on mosquito traits such as species and age. We then used machine learning algorithms to disentangle the complexity of the MIR spectra and predict these traits. Using laboratory mosquito colonies, we used MIRS to measure 2 mosquito species (*Anopheles gambiae* and *An. arabiensis*) over several ages; with this approach we could predict species with more than 80% accuracy and accurately reconstruct the age structures of mosquito populations.

Here we have further developed this approach for its application in wild mosquito populations. We have measured three species in the *Anopheles gambiae* s.l. complex (*An. gambiae, An. coluzzii* and *An. arabiensis*) over the first 2 weeks upon emergence into female adults by MIRS. To account for both ecological and genetic variation, we used both colonies and wild collected mosquitoes in Tanzania and Burkina Faso, that were reared under laboratory and semi-field conditions. Here we will present the age and species prediction model of this dataset based on convolutional neural networks. Initial results suggest that MIRS is a robust method for the simultaneous prediction of these cuticular associated traits. This approach is easy-to-use, cost-effective and high-throughput. Once fully validated, we envision that this technology can revolutionize vector surveillance programmes.
Title: Insects To Feed Insects: Larval Diets Based on Insects for Mass-Rearing a Major Vector of Malaria, *Anopheles Arabiensis*, Patton (*Diptera: Culicidae*).

Authors: Nanwintoum Séverin Bimbilé Somda, Hamidou Maiga, Wadaka Mamai, Hanano Yamada, Antoine Sanon, Abdoulaye Diabaté, Kounbobr Roch Dabiré, Jérémie Gilles, Jeremy Bouyer,

Background: Efficiency of novel genetic control strategies requires cost-effective production of mosquitoes. To provide more affordable and suitable mass production of *Anopheles arabiensis*, we investigated the use of edible insects as alternative larval diet ingredients. Ten different insect meal (IM) of black soldier fly (BSF), yellow mealworm (YM) or house fly (HF) were assessed on mosquito life history traits.

Methods: The IM were evaluated either alone and/or in replacement of the bovine liver powder in the reference IAEA diet. Furthermore, optimal combinations between tuna meal (TM), brewer's yeast (BY) and IM were figured out through an augmented simplex design. Finally, five more promising mixtures were assessed on both larva and adult mosquito life history traits.

Results: *An. arabiensis* larvae developed successfully from L1 to L4 but did not pupate, when reared in pure IM diets. Interestingly, when used individually as BLP replacement in the IAEA diet, eight IM led to enhanced or similar effects on the larval development parameters. Three-component mixture design experiment, considering TM, BY and BSF, revealed promising mixtures, most of which included only two ingredients such as $\frac{1}{2}TM+\frac{1}{2}BSF$, $\frac{1}{3}TM + \frac{2}{3}BSF$. Further assessment of the five most promising mixtures on mosquito development parameters including fecundity, longevity showed enhanced or similar results compared to the control. Based on all parameters, including diet cost reduction, the mixtures 50%TM+35%BSF+15%BY and $\frac{1}{2}TM+\frac{1}{2}BSF$ can be recommended for *An. Arabiensis* mass rearing.

Conclusions: Our study provided effective *An arabiensis* larval diets, more than 10-fold cheaper than the reference IAEA diet, highlighting the possibility to use insects to feed insects. More broadly, these results could be applicable to the species of the *An gmabiae* complex knowing their similarity in larval nutrition.

Title: A five-year (2014 -2018) entomological surveillance in prospective of a pilot sterile insect technique feasibility study in Mamfene, KwaZulu-Natal, South Africa

Authors: Givemore Munhenga^{1,2}, Basil D Brooke^{1,2}, Lizette L Koekemoer¹, Eunice Jamesboy^{1,2}, Michael Samuel¹, Leonard C Dandalo*1*, Oliver R Wood¹, Leanne N R Lobb¹, Miriam Mwamba² and Maria Kaiser^{1,2}

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Background: As part of South Africa's malaria elimination strategy, complementary vector control approaches are being investigated. One such approach is the use of the sterile insect technique (SIT). Central to the successful implementation of an SIT programme is having comprehensive information on the size and spatial distribution of mosquito populations in the targeted area. The aims of this study were to conduct regular entomological surveillance to gather baseline information on composition and relative abundance of anopheline species in an area targeted for a pilot SIT intervention.

Methods: Mosquito samples were collected twice weekly, over a 5 year period (January 2014 to December 2018), from clay pots permanently stationed at various sampling points in the proposed SIT intervention and control sites. Collected mosquitoes were identified morphologically and subsequently to species-specific level using PCR. The feeding status and blood-meal source was determined for all female mosquitoes collected. Additionally, females were tested for *Plasmodium falciparum* sporozoites.

Results: Over 8, 000 anophelines were collected over 5 years. Of these 70.8% and 13.8% were identified as members of the *Anopheles gambiae* complex or *Anopheles funestus* group, respectively. *Anopheles arabiensis*, the most abundant species constituted 81.5% of the *An. gambiae* complex. Mosquito density varied between seasons, highest in summer and lowest during winter. *Plasmodium falciparum* infection rates for *An. arabiensis* were 0.7% and 0.5% in 2014 and 2015, respectively. No *P. falciparum* infected mosquitoes were found during 2016 through to 2018.

Conclusions: This study has highlighted the importance of long-term malaria vector surveillance and has provided valuable information on the population dynamics of *An. arabiensis* in the Mamfene region of KZN. Importantly *An. arabiensis* was incriminated as a malaria vector in South Africa during this study. The perennial presence of a fairly isolated population of *An. arabiensis* that declines in density during the dry winter months, makes this site suitable for a small pilot study to test the feasibility of the Sterile Insect Technique as a malaria vector control strategy.

Title: Biting and Resting Behaviours of Malaria Vectors in Rural Burkina Faso Following Scaling Up of Llins.

Authors: A. Sanou, W. M. Guelbéogo, L. Nelli, K. H. Toé, S. Zongo, P. Ouédraogo, F. Cissé, N. Sagnon, J. Matthiopoulos, H. M. Ferguson.

Background: Long Lasting Insecticide-Treated Nets (LLINs) and Indoor Residual Spraying are the most common and successful methods for malaria vector control in Africa. However, studies in areas of sub-Saharan Africa with high LLIN coverage suggest vectors are adapting their biting and resting behaviours minimising their contact with these tools. In combination with growing insecticide resistance, such vectors behavioural shifts could significantly reduce the efficacy of LLINs. Changes in major malaria vector behaviours have not been yet documented in Burkina Faso. Here we investigated spatial and temporal variation in malaria vector abundance, biting and resting behaviour in Burkina Faso in the 2 years following a mass LLIN distribution.

Methods: A longitudinal mosquito vector surveillance was initiated (September 2016 November 2018) within 12 villages in south-western Burkina Faso. Host-seeking and resting malaria vectors were collected in and outside of houses on two days per month from 19h to 06h using respectively Human Landing Catch and Resting bucket.

Results: A total of 47,654 mosquitoes males and females were collected, with most being from the *Anopheles gambiae* s.l. species complex. (96.74%). Within this complex, *An. coluzzii* (53.82%) and *An. gambiae* (45.9%) were the dominant species. Controlling for seasonality and spatial variation, there was an overall decline in malaria vector abundance across the study period (z=-2.49, p=0.02). Contrary to expectation, the *An. gambiae s.l.* community was relatively exophagic (~54% biting outdoors) with no evidence of long-term trend. Malaria vector biting time peaked between 00h-02h but showed some variation between villages (df =11, F =2.46, p=0.009) and earlier biting in dry season, (F=3.81, p<0.0001). Exophily was also common in *An. gambiae* (~60% resting outdoors) while *An. coluzzii* was more endophilic (~35% resting outdoors).

Conclusion: This study revealed a long-term decline in abundance but no evidence of longterm shift in biting location and time over the study period. While this degree of outdoor biting in this area was higher than anticipated, the late-night timing of outdoor bites indicates that majority could still be prevented through use of LLINs. However, further investigation and analysis of the behaviour and population dynamics of these vector populations is still needed to improve control strategies.

Parallel session 7:

Vector biology and control

Title: Potential of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) to transmit yellow fever virus in urban areas in Central Africa

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Background: Yellow Fever (YF) remains a major public health issue in Sub-Saharan Africa and South America, despite the availability of an effective vaccine. In Africa, most YF outbreaks are reported in West Africa. However, urban outbreaks occurred in 2016 in both Angola and the

Democratic Republic of Congo (DRC), and imported cases were reported outside Africa. In Central Africa, Cameroon and the Republic of the Congo host a high proportion of nonvaccinated populations increasing the risk of urban outbreaks. The main vector is *Aedes aegypti* and possibly by *Aedes albopictus*, both being anthropophilic and domestic mosquitoes. Here, we provide evidence that both *Ae. aegypti* and *Ae. albopictus* in Cameroon and the Republic of the Congo are competent for Yellow Fever virus (YFV) with higher rates (infection, disseminated infection, and transmission) for *Ae. aegypti*. We conclude that these *Aedes* species increase the risk of YF transmission and urge Public Health authorities to intensify their efforts to control domestic vectors, and extend vaccine coverage to prevent major YFV outbreak.

Keywords: Aedes aegypti, Aedes albopictus, yellow fever virus, vector competence, Central Africa

Title: Attract-And-Kill Strategy for Mosquito Larval Control: Effect of Oviposition Cues on the Efficacy of the Larvicides Temephos and *Bacillus Thuringiensis Israelensis* on Malaria and Lymphatic Filariasis Vectors.

Mosquito females detect breeding sites using volatile chemical signals emanating from these sites. These cues can be exploited for inducing the mosquitoes to lay eggs in sites that contain selected toxicants so that mosquito offspring gets killed in the immature stage. The aim of the present study was to assess whether mosquitoes can be lured to lay eggs in insecticide-treated sites baited with attractive semiochemicals.

In this study, the effect of the oviposition attractant mixed with the synthetic insecticide temephos or the natural biocide *Bacillus thuringens israliensis* (*Bti*) on oviposition choice and larval development of the malaria vector *Anopheles gambiae* was determined. Studies were done in a screened semi-field habitat and in a natural mosquito habitat in Tanzania.

The frequency of eggs laid in pots containing temephos was lower than in pots containing attractant only or Bti. Pots with attractant + temephos produced significantly fewer larvae than those with attractant only. There was no difference in larval numbers between pots containing attractant + Bti and attractant only. All mosquito larvae in sites treated with either temephos or Bti, died before reaching the pupal stage. Sites treated with attractant alone were more productive for larvae than sites treated with temephos or Bti alone. It is concluded that the attract-and-kill method has high potential to be implemented as a novel tool for mosquito vector control.

Title: Population Patterns of Sandflies From Two *Leishmania* Circulating Areas In The Western Burkina Faso : Bobo-Dioulasso and Larama

Authors: Djibougou A.^{1,2}, Hien A.¹, Ouari A.², Somé A.F.¹, Soma D.D.¹, Namountougou M.⁴, Diabaté A.¹, Fournet F.³, Sangaré I.⁴, Diagbouga P.S.⁵, Hien H.^{1,2}, Dabiré K.R.¹

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Background: Leishmaniasis is the third vector parasitic disease after malaria and lymphatic filariasis. Bobo-Dioulasso was identified as canine reservoir of leishmaniasis whereas in Larama a rural village, its transmission remains active. However, no entomological data were collected to support this transmission features. This study aimed to gather basic information focusing specifically vector species, their blood meal preference and infection status.

Methods: A cross-sectional entomological surveys targeting sandflies were conducted from July to September, 2018 in two hotpots areas of leishmaniasis transmission in Burkina Faso (Larama and Bobo-Dioulosso) using both CDC traps and indoor spraying of pyrethrum insecticides. Morphological identification was performed according the key of Abonnec (1971). Bloodmeals origin from fed females was identified by ELISA technique. The PCR kit STAT-NAT[®] *Leishmania spp* has been used to assess the infectivity status sand flies

Results: A total of 976 sandflies were collected with 944 specimens in Larama and 32 in Bobo-Dioulasso. *Sergentomya* and *Phlebotomus genus* were found in high abundance at respectively 94.05% and 5.95%. Three species belonging *Phlebotomus* genus were identified such as *Phlebotomus dubosqi*, *Phlebotomus bergeroti*, and *Phlebotomus rodhaini* whilst eighteen species were identified within *Sergentomyia* genus with *Sergentomyia schwetzi* being the most frequent (43.75%). 31 bloodfed females were screened for blood meal origin from which *Sergentomiya* 38.70% took human blood meal *vs* 6, 45% of *Phlebotomus*, 38,70% from animals (Sergentomiya) and 3. 22% as mixed human-animals blood meals. Among 438 females tested in PCR for *Leishmania spp*. infection, no infected female was detected.

Conclusion: Our study allowed to identify sandflies species from urban and rural settings of leishmaniasis. However, we failed to detect pathogens within vectors even during active circulation of the pathogens that raises the necessity to develop more sensitive diagnostic tools to monitor leishamiasis surveillance in the field.

Keywords: Sandflies, Sergentomya, Phlebotomus, Leishmania spp, Burkina

Title: Phylogeography and Population Structure of the Tsetse Fly *Glossina Pallidipes* in Kenya and the Serengeti Ecosystem

Authors: Bateta R, Saarman NP, Okeyo WA, Dion K, Mireji PO, Okoth S, Malele 1, Murilla G, Aksoy S and Caccone A

Background and Objective: Glossina *pallidipes* is the main vector of animal African trypanosomiasis and a potential vector of human African trypanosomiasis in eastern Africa. Vector control efforts have succeeded in reducing infection rates, but recent resurgence in tsetse fly densities raises concerns that vector control programs require improved strategic planning over larger geographic and temporal scales. Knowledge of population structure and dispersal patterns can provide the required information to improve planning.

Materials and Methods: We investigated the phylogeography and population structure of *G. pallidipes* in Kenya and northern Tanzania using 11 microsatellite loci genotyped in 600 samples.

Results and Discussion: Our results indicate distinct genetic clusters east and west of the Great Rift Valley, and divergence in the west between the northwest and the Serengeti ecosystem in the southwest. Estimates of genetic differentiation and first-generation migration indicated high genetic connectivity within genetic clusters even across large geographic distances of more than 300 km in the east, but only occasional migration among clusters. Patterns of connectivity imply a major role for river basins in facilitating gene flow in *G. pallidipes*, and confirm isolation by distance among genetic clusters but not within clusters. Estimates of Ne and results from Approximate Bayesian Computation further support recent *G. pallidipes* population reductions in the Serengeti ecosystem and the northwest during the last century, but also suggest that the full extent of differences in genetic diversity and population dynamics between the east and the western clusters was established over evolutionary time periods (estimated at ~13 million years).

Conclusion and Recommendation: Findings suggest the need to treat eastern Kenya, the Serengeti ecosystem, and northwestern Kenya as independent management units, and to specifically monitor to detect migration events across the geographic boundaries that demarcate genetic clusters to allow rapid and targeted responses to reinvasion after local eradication.

Title: Bionomics Variations and Genetic Diversity of Aedes Aegypti Populations from Burkina Faso.

Author: Badolo Athanase¹ et al

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Burkina Faso experienced two severe consecutive outbreaks of dengue, in 2016 and 2017 in Ouagadougou and elsewhere in the country. The lack of information on *Aedes aegypti* populations has impaired an efficient response to dengue outbreaks. We have collected three-year data on the bionomics of *Aedes aegypti* including biting and resting behavior, insecticide resistance and mechanisms involved, morphological variations and genetic diversity of the populations based on 12 microsatellites analysis.

We recorded an increasing density from urban to rural consistent with the abundance and productivity of breeding sites. *Ae. aegypti* exhibited more outdoor than indoor biting and, although mixed human-animal blood meals were detected, it showed a strong preference for human hosts. Morphological identification showed continuous white scales pattern inconsistent with the *aegypti/formosus* subdivision. The analysis of genetic variations in *Aedes aegypti populations using 12 microsatellites diversity did not reveal any structure* in the populations of *Aedes aegypti*, which is much more consistent with a single species of *Aedes aegypti*. We recorded highly resistant to pyrethroid insecticides; supported by high frequency of the 1534C kdr mutation, at a less extend by 1014I kdr mutation and by metabolic resistance. The populations are still susceptible to organophosphate and moderately resistant to carbamate insecticides. While these data are important for *Aedes aegypti* populations control in Burkina Faso, more investigation coupled with current genome sequencing projects could bring more light and give a clearer image of *Aedes aegypti* bionomic and genetic links.

Parallel session 8:

Vector biology and control

Title: Primary And Secondary Outdoor Vectors Contributing To Malaria Transmission In Low And High Transmission Settings In Zambia

Authors: Jennifer C. Stevenson^{1,2}, Christine Jones^{1,} Ilinca I. Ciubotariu^{1,} Limonty Simubali², Twig Mudenda², Mbanga Muleba³, and Douglas E. Norris¹ for the Southern Africa International Centers of Excellence for Malaria Research (ICEMR)

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Background: Zambia aims to eliminate malaria by 2021. However, despite impressive gains over the past decade, malaria persists. This may be, in part, due to suboptimal coverage of indoor-targeted insecticide-based vector control and/or the persistence or emergence of vectors with altered behaviors that allow them to evade insecticidal interventions. To determine whether outdoor caught mosquitoes may be maintaining transmission, entomological studies were carried out in both low and high transmission settings in Zambia.

Methods: In Macha, a pre-elimination setting in southern Zambia, cross-sectional, longitudinal and transect studies were carried out from 2016 to 2019 comparing indoor and outdoor light traps collections of mosquitoes, both within homesteads and where people congregate in the evenings. In the high transmission site of Nchelenge District, northern Zambia, comparisons of catches were made from light traps set outdoors baited with a lure or set next to animals or people, to inform future surveillance of outdoor vectors. All mosquitoes were identified morphologically and molecularly, and their relative abundance and level of infectivity assessed.

Results: Outdoor collections in Macha were far more diverse and abundant than indoors. *Anopheles squamosus* and *An. rufipes* made up more than 50% of outdoor collections. Indoors, the primary vector, *An. arabiensis*, dominated (83%). Malaria sporozoites were found in molecularly confirmed *An. squamosus*, *An. coustani* and *An. rufipes* outdoors and in *An. arabiensis* indoors. In Nchelenge, 86% were found to be *An. funestus s.s.* whilst a further 12% were lesser known anophelines. All infective mosquitoes (5/524) were *An. funestus s.s.* caught outdoors. Questionnaires revealed potential human exposure to bites between 17:00 and 22:00 in the evening.

Conclusion: These data demonstrate potential exposure to vectors outdoors in both pre-elimination and holoendemic malaria sites in Zambia. These findings highlight the importance of outdoor mosquito surveillance and the necessity for combining indoor and outdoor vector control.

Title: Exploring the Impact of Glutathione S-Transferase (Gst)-Based Metabolic Resistance to Insecticide on Vector Competence of Anopheles funestus for Plasmodium falciparum

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Background: Malaria control heavily relies on insecticide-based interventions against mosquito vectors. However, the increasing spread of insecticide resistance is a major threat. The extent to which such resistance, notably metabolic resistance, interferes with the development of the *Plasmodium* parasite and its impact on overall malaria transmission remains poorly characterized. Here, we investigated whether glutathione S-transferase-based resistance could influence *Plasmodium falciparum* development in *Anopheles funestus*.

Methods: Anopheles funestus females were infected with *P. falciparum* gametocytes and midguts were dissected at day 7 post infection for detection/quantification of oocysts. Infection parameters were compared between individual with different L119F-GSTe2 genotypes and the polymorphism of the GSTe2 gene was analysed in infected and uninfected mosquito groups.

Results: Overall, 403 mosquitoes were dissected and genotyped. The frequency of the L119F-GSTe2 resistance allele was significantly higher in non-infected (55.88%) compared to infected (40.99%) mosquitoes (Fisher's exact test, P<0.0001). Prevalence of infection was significantly higher in heterozygous and homozygous susceptible genotypes (P<0.001). However, homozygous resistant and heterozygous mosquitoes exhibited significantly higher infection intensity (P<0.01). No association was observed between the GSTe2 polymorphism and the infection status of mosquitoes.

Conclusion: Altogether, these results suggest that GSTe2-based metabolic resistance may affect the vectorial competence of resistant *An. funestus* mosquitoes to *P. falciparum* infection, by increasing its permissiveness to *Plasmodium* infection.

Keywords: Malaria, Insecticide resistance, Anopheles funestus, Plasmodium falciparum, metabolic resistance, GSTe2

Title: Factors Governing Deltamethrin Resistance in Anopheles gambiae s.l. from North Cameroon

Authors: Josiane Etang, Stanislas Elysée Mandeng, Jude D. Bigoga, Etienne Fondjo, Herman Parfait Awono-Ambene

The effectiveness of insecticide-based malaria vector control in Africa is threatened by the spread and intensification of pyrethroid resistance in targeted mosquito vector populations. The present study aimed at investigating the determinants of deltamethrin resistance in *An. gambiae s.l.* from North Cameroon.

Mosquito larvae were collected from 24 settings of the Garoua, Pitoa and Mayo Oulo Health Districts (HDs) from 2011 to 2015. Two to five days old female *An. gambiae s.l.* emerging from larval collections were tested for deltamethrin resistance using the World Health Organization's standard protocol. Sub samples of test mosquitoes were identified to species using PCR-RFLP and genotyped for knockdown resistance alleles (*Kdr* 1014F and 1014S) using Hot Ligation Oligonucleotide Assay (HOLA). Multiple factor analysis was performed on mortality rates post exposure to deltamethrin, species distribution (*An. arabiensis, An. coluzzii, An. gambiae*) and *Kdr* L1014F allelic frequencies in field mosquito samples over the 5 years study period.

Deltamethrin resistance frequencies increased significantly between 2011 and 2015, with mosquito mortality rates declining from 97-70% to 72-50% in the three HDs (JT= 5638, P< 0.001). Resistance was mostly found in urban settings compared with semi-urban and rural settings (JT= 5282, P< 0.0001). A strong positive linear correlation was observed between mortality rates to deltamethrin and occurrence of *An. arabiensis* conversely to a strong negative correlation was found between the mortality rates and the proportions of *An. coluzzii*, and the proportion of *An. gambiae*. Also, there was a negative correlation between mortality rates and the frequencies of *Kdr* L104F allele in *An. coluzzii* and *An. arabiensis* respectively.

These data suggest that the increase in the frequencies *An. coluzzii*, *An. gambiae* and the *Kdr* L1014 alleles led to the increase of deltamethrin resistance in *An. gambiae s.l.*, stressing the urgent need for vector surveillance and insecticide resistance management strategies in Cameroon.

Keywords: *Anopheles gambiae s.l.*; *Kdr* alleles; Insecticide resistance; Malaria vector control; North Cameroon.

Title: Non-Inferiority testing of Insecticide Treated Nets (ITNs): comparison of results from experimental huts and Ifakara Ambient Chamber tests

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Candidate Insecticide Treated Nets (ITNs) with a claim of improved control of vector borne disease must demonstrate both non-inferiority to the first-in-class product and superiority to the current standard of care on both mortality and feeding inhibition endpoints. Experimental hut trials with free-flying mosquitoes are currently the gold-standard method for evaluating ITNs. However, due to variability in mosquito densities at field sites it may be necessary to carry out large studies to get enough power to measure the non-inferiority of ITNs. Therefore, the WHO has encouraged exploration of other potential alternative test methods including the I-ACT (Ifakara ambient chamber test). In this study, I-ACT and experimental huts were compared for non-inferiority studies.

Three net products were evaluated in fully randomized, double blinded equivalence field studies to compare the performance of the investigational interventions to active comparators following WHO guidelines. Study 1: an alpha-cypermethrin + PBO LN in comparison to alpha-cypermethrin only ITN. Study 2: alpha-cypermethrin and pyriproxyfen ITN against alpha-cypermethrin only ITN. Study 3: deltamethrin incorporated polyethylene ITN compared deltamethrin-coated ITN. The nets were first evaluated in experimental huts and then the same nets were subsequently evaluated in the I-ACT using both pyrethroid susceptible and pyrethroid resistant mosquitoes.

In each experimental hut study, products performed very similarly to active comparators with wide 95% confidence intervals. However, in the I-ACT where 30 mosquitoes of each strain are used each night, confidence intervals were smaller and the studies were powered to detect non-inferiority at the 10% effect difference. Using the I-ACT, after 20 nights of data collection superiority of the PBO product was seen against the CYP450 resistant mosquito strain. A difference in mosquito fertility between the susceptible and resistant strains with the pyriproxyfen net was also observed and for the pyrethroid only net the incorporated product showed superiority over the coated product after 20 washes against the pyrethroid resistant strain. The I-ACT assay is useful for measuring small effect differences between new products needed for non-inferiority studies using both insecticide susceptible and resistant strains.

Title: Review of Malaria Situation in Cameroon: Challenges and Prospects for Disease Elimination

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Malaria still has a devastating impact on public health and welfare in Cameroon. Despite the increasing number of studies conducted on disease prevalence, transmission pattern or treatment, there is up to date, not enough studies summarising findings from previous works in order to identify gaps in knowledge and areas of interest where further evidences are needed to drive malaria elimination efforts. The present study seeks to address these gaps by providing a review of studies conducted so far on malaria in Cameroon since the 1940s to date. Over 190 scientific publications were consulted for this purpose.

Although there have been increased scale up of vector control interventions which significantly reduced the global burden of malaria across the country from 41% in 2000 to an average prevalence of 24% in 2017, the situation is not yet under control. There is a high variability in disease endemicity between epidemiological settings with prevalence estimates varying from 7 to 85% in children aged 6 months to 15 years. Four *Plasmodium* species have been recorded across the country including *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax*. Several primates *Plasmodium* are also circulating in Cameroon. Malaria treatment is affected by the rapid expansion of drug resistance in *Plasmodium falciparum*. Several mutations in the *Plasmodium falciparum* chloroquine resistance (*Pfcrt*) and *P. falciparum* multidrug resistance 1 (*Pfmdr1*) genes conferring resistance to either4-amino-quinoleine, mefloquine, halofanthrine and quinine have been documented. Mutations in the *Pfdhfr and Pfdhps* genes involved in sulfadoxine pyrimethamine are also on the rise. No mutation associated with artemisinine resistance has been recorded. Sixteen anopheline species contribute to malaria transmission with six recognized as major vectors:

An. gambiae, An. coluzzii, An. arabiensis, An. funestus, An. nili and An. moucheti. Studies conducted so far, indicated rapid expansion of DDT, pyrethroid and carbamate resistance in An. gambiae, An. coluzzii, An. arabiensis and An. funestus threatening the performance of LLINs.

This review highlights the complex situation of malaria in Cameroon and the need to urgently implement and reinforce integrated control strategies in different epidemiological settings, as part of the substantial efforts towards malaria elimination in the country.

Keywords: Malaria, *Plasmodium*, vector control, drug resistance, insecticide resistance, *Anopheles*, Cameroon

Title: Potential Benefits of Combining Transfluthrin-Treated Sisal Products and Long-Lasting Insecticidal Nets for Controlling Indoor-Biting Malaria Vectors

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Background: Transfluthrin vapour prevents mosquito bites by disrupting their host-seeking behaviors.

Aim: We measured the additional benefits of combining transfluthrin-treated sisal decorations and long lasting insecticidal nets (LLINs) with an aim of extending protection against early evening, indoor-biting malaria vectors when LLINs are ineffective.

Methods: We investigated the indoor protective efficacy of locally made sisal decorative baskets (0.28 m²), treated with 2.5 ml and 5.0 ml transfluthrin in terms of mosquito density, exposure to bites and 24 hour mortality. Experiments were conducted in experimental huts, located in Lupiro village, Ulanga district, south-eastern Tanzania. Human landing catches (HLC) were used to measure exposure to bites between 1900-2300 hrs. Each morning, at 0600 hrs, mosquitoes were collected inside huts and in exit traps and monitored for 24 hours mortality.

Results: Sisal decorative baskets (0.28 m²) treated with 2.5 ml and 5.0 ml transfluthrin, deterred three-quarters of *Anopheles arabiensis* mosquitoes from entering huts (relative rate, RR = 0.26, 95% confidence interval, CI: 0.20-0.34, p< 0.001 and RR= 0.29, 95% CI: 0.22-0.37, p< 0.001 respectively). Both treatments induced a 10 fold increase in 24 hour mortality of *An. arabiensis* mosquitoes (odds ratio, OR= 12.26, 95% CI: 7.70-19.51, p< 0.001 and OR = 18.42, 95% CI: 11.36-29.90, p< 0.001 respectively).

Conclusion: Sisal decorative items, treated with spatial repellents, provide additional household and personal protection against indoor biting malaria and nuisance mosquitoes in the early evening, when conventional indoor vector control tools such as, LLINs are not in use. We recommend that future studies to investigate the epidemiological relevance of combining LLINs and transfluthrin decorated baskets in terms of their effect on reduction in malaria prevalence.

Title: Determining species of field-collected mosquitoes using near infra-red spectroscopy

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Tracking changes in vector populations—such as species abundance, age distribution and infection-constitutes a direct measure of the efficacy of vector control interventions. Near infra-red spectroscopy (NIRS) is a rapid and non-destructive scanning technique that has been shown to be able to determine the species of morphologically indistinguishable mosquitoes. In this study, we evaluate the potential of NIRS to determine the species of laboratory-reared mosquitoes using machine learning (ML) models calibrated using colony mosquitoes. Mosquitoes of different species (Anopheles arabiensis, Anopheles coluzzii, Anopheles gambiae) were collected in three different field locations in Burkina Faso and reared to adult under laboratory conditions alongside colony mosquitoes. Following emergence colony mosquitoes were killed and scanned daily using NIRS. Maternal generation (F0) and offspring generation (F1) reared from field collected mosquitoes were killed and scanned 4 days post-emergence and the species determined by PCR. ML models were constructed to predict mosquito species from their spectra. NIRS was able to differentiate the three species of colony mosquitoes of constant age, including the previously untested An. coluzzii, with misclassification rates below 7% in all cases. Species could also be determined in colony mosquitoes where the age of the mosquito varied and was unknown though the accuracy was lower. Colony mosquitoes could predict the species of laboratory reared F0 and F1 mosquitoes with some loss of accuracy depending in species, location and generation. The work indicates that NIRS is able to identify the three most epidemiologically important species in the Anopheles gambiae complex with relatively high accuracy. The precision of the method diminishes with the increased realism investigated here (location of the population, age of mosquito) though NIRS still appears to a promising tool for mosquito surveillance that needs to be further validated in the field.

Key words: Anopheles, near-infrared spectroscopy

The Origin and Spatiotemporal History of Swept Mutations Associated With Pyrethroid Resistance in *Anopheles gambiae* Population in East and Central Africa

Authors: Harun Njoroge Ng'ang'a, Arjen Van't Hof, Ambrose Oruni, Eric Lucas, Charles Mbogo and Martin James Donnelly

Background: Insecticides continue to play a major role in malaria control. World health organization recommends periodic monitoring of insecticide resistance to inform or guide public health organization on implementation of current and new vector control tools and management of insecticide resistance. This recommendation calls for more research geared towards development of markers for surveillance of insecticide resistance. The study aimed to develop better markers for surveillance of pyrethroid resistance in *Anopheles gambiae*

Method: Novel markers of pyrethroid resistance were screened within a genomic region encompassing the CY6P cluster in chromosome 2R of *Anopheles gambiae* population from Uganda. The identified structural and point mutations were genotyped in recent and archived samples collected between 2004 and 2018 in different countries in Africa to determine their origin and spatiotemporal history. Field collected samples and a cross of mutants and susceptible wildtype lab strains were phenotyped for pyrethroid resistance and genotyped for the mutations to establish their association with pyrethroid resistance.

Result: A point mutation in CYP6P4, an insertion of a partial Zanzibar transposable element and duplication of the CYP6AA1 gene were identified in a swept region encompassing the CYP6 cluster of *An. gambiae* senso stricto. Spatiotemporal analysis of the frequency of the mutations suggest they originated in Western Kenya or Uganda and later spread westwards to DRC and recently to Tanzania but they haven't spread to coastal Kenya, Cameroon and countries in west Africa. The mutations are strongly associated with survival following deltamethrin exposure of field collected mosquitoes and offspring that inherited them.

Conclusion: The mutations can be exploited as markers for monitoring temporal changes and spread of pyrethroid resistance in *Anopheles gambiae* senso stricto in east and central Africa.

Title: Evaluation of Passive Emanator Treated With Transfluthrin and BG Sentinel Trap as the Push-Pull for the Control of Outdoor Biting Mosquitoes

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Introduction: *Aedes aegypti*, the vector of several Arboviral diseases, is primarily controlled through larval source reduction and space spraying. These measures are costly and logistically difficult to implement, suggesting that new tools need to be evaluated. Previous studies have demonstrated that volatile pyrethroids such as transfluthrin "Push" and odor baited trap "Pull" have shown to reduce house entry for indoor biting mosquitoes. Therefore, this study investigate new tool that provide long term protection using spatial repellent and odor baited trap to control for the outdoor biting mosquitoes.

Method: We developed a long-lasting transfluthrin passive push and evaluated its efficacy to reduce the human landing rate of Aedes aegypti mosquitoes over six months (at month 0, 3 and 6) in a semi-field system. The pushes (each treated with 3g of transfluthrin) were placed 6 m apart and human volunteers sat between them, performing Human Landing Catches (HLC). For this experiment, the BG sentinel baited with BG lure and CO2 was used as the pull. The BG sentinel trap was placed 10m away from the volunteer.

Results: Significant fewer Aedes aegypti mosquitoes were collected in the compartment treated with push compared to the control over six months (OR=0.595% C.I (0.52-0.58) P=0.001). There was no significant difference in personal protection when push used alone or in combination with pull (OR=0.99 (95% CI [0.77-1.10], P=0.30).

Conclusion: The use of push devices provides protection for up to six months. The combination of push and pull did not result into much higher protection. While the BG-Sentinel has failed to work synergistically with push in this situation, it might be useful as a removal trap.

Title: The Biting Patterns of *Anopheles* Mosquitoes from three High Malaria Burden Districts in Malawi

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Background: Understanding the biting behavior of the vectors of malaria is essential for selection, monitoring and evaluation of vector control interventions. This study assessed the biting patterns of malaria vectors in three high malaria burden districts of Malawi as a baseline for monitoring the impact of indoor residual spraying and long-lasting insecticidal nets.

Methods: Anopheles human-biting patterns were monitored in Salima, Nkhotakota and Nkhata Bay Districts every three months from July to December 2018 using human landing catches recorded hourly between 5:00 pm and 11:00 am. Adult mosquitoes were morphologically identified to the species level. Enzyme-linked immunosorbent assays were performed to assess infections by *Plasmodium falciparum (Pf)*.

Results: Of 1,054 *Anopheles* mosquitoes collected, *An. coustani* was the most abundant species (43%) followed by *An. funestus* (36%) and *An. gambiae* s.l. (21%).

The indoor vs outdoor human biting rates expressed as the number of bites/person/night were 5.8 vs 2.0 for *An. funestus*, 2.1 vs 2.5 for *An. gambiae* s.l., and 4.8 vs 4.7 for *An. coustani*. Peak biting time for *An. funestus* and *An. gambiae* s.l. was from 00:00 - 6:00 am in all districts. There was no distinct peak biting time for *An. coustani*. The proportion of morning/daytime biting (6:00 - 11:00 am) was 19.3% indoors and 1.9% outdoors for *An. funestus*, 5.8% indoors and 0.4% outdoors *for An. gambiae* s.l., and 3.1% indoors and 1.5% outdoors for *An. coustani*.

Plasmodium falciparum sporozoite infection rates were 5.0% in *An. funestus* and 0.9% in *An. gambiae* s.l. No *Pf* infection was detected in *An. coustani*.

Conclusion: An. funestus is the primary vector of malaria based upon high biting rates and

a high sporozoite rate. This species fed primarily indoors at night although a substantial proportion of indoor biting occurred between 6:00 and 11:00 am. Additional vector control tools may be required to address indoor, late morning biting by *An. funestus*.

Title: Population Patterns of Sandflies From Two *Leishmania* circulating areas in the Western Burkina Faso : Bobo-Dioulasso and Larama

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Background: Leishmaniasis is the third vector parasitic disease after malaria and lymphatic filariasis. Bobo-Dioulasso was identified as canine reservoir of leishmaniasis whereas in Larama a rural village, its transmission remains active. However, no entomological data were collected to support this transmission features. This study aimed to gather basic information focusing specifically vector species, their blood meal preference and infection status.

Methods: A cross-sectional entomological surveys targeting sandflies were conducted from July to September, 2018 in two hotpots areas of leishmaniasis transmission in Burkina Faso (Larama and Bobo-Dioulosso) using both CDC traps and indoor spraying of pyrethrum insecticides. Morphological identification was performed according the key of Abonnec (1971). Bloodmeals origin from fed females was identified by ELISA technique. The PCR kit STAT-NAT[®] *Leishmania spp* has been used to assess the infectivity status sand flies

Results: A total of 976 sandflies were collected with 944 specimens in Larama and 32 in Bobo-Dioulasso. *Sergentomya* and *Phlebotomus genus* were found in high abundance at respectively 94.05% and 5.95%. Three species belonging *Phlebotomus* genus were identified such as *Phlebotomus dubosqi*, *Phlebotomus bergeroti*, and *Phlebotomus rodhaini* whilst eighteen species were identified within *Sergentomyia* genus with *Sergentomyia schwetzi* being the most frequent (43.75%). 31 bloodfed females were screened for blood meal origin from which *Sergentomiya* 38.70% took human blood meal *vs* 6, 45% of *Phlebotomus*, 38,70% from animals (Sergentomiya) and 3. 22% as mixed human-animals blood meals. Among 438 females tested in PCR for *Leishmania spp*. infection, no infected female was detected.

Conclusion: Our study allowed to identify sandflies species from urban and rural settings of leishmaniasis. However, we failed to detect pathogens within vectors even during active circulation of the pathogens that raises the necessity to develop more sensitive diagnostic tools to monitor leishamiasis surveillance in the field.

Keywords: Sandflies, Sergentomya, Phlebotomus, Leishmania spp, Burkina

Title: Investigation of the Influence of a glutathione S-transferase Metabolic Resistance to Pyrethroids/DDT on Mating Competitiveness in Males *Anopheles funestus*, African Malaria Vector

Authors: Magellan Tchouakui, Billy Tene Fossog, Brigitte Vanessa Ngannang, Doumani Djonabaye, Williams Tchapga, Flobert Njiokou, Charles S. Wondji.

Background: Metabolic resistance is a serious challenge to current insecticide-based interventions. The extent to which it affects natural populations of mosquitoes including their reproduction ability remains uncharacterised. Here, we investigated the potential impact of the glutathione S-transferase L119F-GSTe2 resistance on the mating competitiveness of male *Anopheles funestus*, in Cameroon.

Methods: Swarms and indoor resting collections took place in March, 2018 in Tibati, Cameroon. WHO tube and cone assays were performed on F₁ mosquitoes from indoor collected females to assess the susceptibility profile of malaria vectors. Mosquitoes mated and unmated males collected in the swarms were genotyped for the L119F metabolic marker to assess its association with mating male competitiveness.

Results: Susceptibility and synergist assays, showed that this population was multiple resistant to pyrethroids, DDT and carbamates, likely driven by metabolic resistance mechanisms. Cone assays revealed a reduced efficacy of standard pyrethroid-nets (Olyset and PermaNet 2.0) with low mortality (<25%) whereas synergist PBO-Nets (Olyset Plus and PermaNet 3.0) retained greater efficacy with higher mortality (>80%). The L119F-GSTe2 mutation, conferring pyrethroid/DDT resistance, was detected in this *An. funestus* population at a frequency of 28.8%. In addition, a total of 15 mating swarms were identified and 21 *An. funestus* couples were isolated from those swarms. A comparative genotyping of the L119F-GSTe2 mutation between mated and unmated males revealed that heterozygote males were less able to mate than homozygote susceptible (OR=7.2, P<0.0001). Surprisingly, heterozygote mosquitoes were also less able to mate than homozygote resistant (OR=4.2, P=0.010) suggesting the presence of a heterozygote disadvantage effect. Overall, mosquitoes bearing the susceptible allele were significantly more able to mate than those with resistant allele (OR=2.1, P=0.03).

Conclusion: This study showed that *An. funestus* swarms can be easily detected and provides preliminary evidences that metabolic resistance potentially exerts a fitness cost on mating competiveness in resistant mosquitoes.

Title: Assessment of the entomological risk of arbovirus outbreaks in Yaoundé, the capital city of Cameroon based on Stegomyia and pupae indices

Authors: Armel Tedjou^{1,2,} Basile Kamgang^{1*}, Aurélie P. Yougang^{1,2,} Theodel A. Wilson-Bahun^{1,3,} Flobert Njiokou^{2,} Charles Wondji^{1,4.}

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Background & Objective: Dengue, Zika, yellow fever and chikungunya viruses are increasingly reported in several region in the world including Africa. In Cameroon where both epidemic vectors *Aedes aegypti* and *Ae. albopictus* are well established, little information are available on the transmission dynamic of these viruses. To be prepare for future outbreak, we undertook this study aiming to assess the spatial distribution of *Ae. aegypti* and *Ae. albopictus* in Yaoundé and level of infestation of both species based on Stegomyia indices.

Materials & Method: Entomological survey was conducted in April 2018 in 447 houses of 29 randomly selected neighborhoods of 7 boroughs in Yaoundé, where households were hand-picked with a distance ranged from 100 to 150m. In each household, the number if inhabitants were recorded, water holding containers were inspected for *Aedes* immature stages and environmental characteristics were recorded for each container with water. *Aedes aegypti* and *Ae. albopictus* were identified, in view to study their prevalence and their habitat preferences. Traditional Stegomyia indices were calculated in addition to pupae-based indices.

Results & Discussion: In total, 10801 Aedes larvae were collected in 954 containers habitats, with 84.95% Ae. albopictus and 15.05% Ae. aegypti. Analysis revealed that *Ae. albopictus* bred mainly in used tires while *Ae. aegypti* it's rather in discarded tanks. *Aedes albopictus* was the most prevalent species in almost all the neighborhoods (24/29). Overall, Stegomyia indices estimated for Ae. albopictus were higher than those of *Ae. aegypti*. For example, House Index and Pupae Index were higher for *Ae. albopictus* (38.26 and 446.31%) than *Ae. aegypti* (25.73 and 56.82%). For each species, indices calculated varied according to neighborhood suggesting the risk of outbreak is not the same in different neighborhoods.

Conclusion and recommendation: Our analyses suggest a higher risk of outbreaks of arboviral related diseases in Cameroon and recommended actions like good waste management system to reduce vector densities.

Title: Investigating the Influence of Larval Nutrition on the Life Traits and Vector Competence of *Anopheles coluzzii* Major Vector of Malaria in Cameroon

Authors: Kaminsi Nenkam H.G^{1*}, Ndo C^{2,3,4}, Kopya E¹, Awono-Ambene P², Njiokou F^{1,3}.

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Background: In some mosquito species, the conditions experienced by larvae during development have been shown to influence the life traits and the development of the pathogens which they transmit. If mosquitoes are not reared under ideal conditions, vector competence experiments in the laboratory may not accurately reflect vector-pathogens relationships in nature. In this work, the influence of larval nutrition on the life traits and vector competence of *Anopheles coluzzii* (Ngousso strain), has been studied.

Methods: Larvae were reared to a fixed densities but the daily amount of food available for each larva varied across experiments. Thus, five food amount (diets) were used: 0.025mg/larva/day (d1), 0.05mg/larva/day (d2), 0.1mg/larva/day (d3), 0.2mg/larva/day (d4) and 0.3mg/larva/day (d5). For each diet, three replicates were made and the following parameters were assessed: larval mortality rate, larval development time, larval pupation rate, emergence rate, adult longevity, and adult body size. Moreover, the influence of larval nutrition on *Plasmodium falciparum* development was assessed by comparing oocyst load in midgut after experimental infection of females with *P. falciparum* gametocytes taken from the blood of children aged between 5 and 11 years.

Results: Larval nutrition deprivation resulted in increased development time, decreased pupation and emergence rates and smaller adult female body size. Moreover, larval nutrition has strongly influenced blood intake in adults and reproductive success. The fecundity, the prevalence of the infection and the oocyst load in midguts were higher in mosquitoes that were abundantly fed at the larval stage.

Conclusion: These results suggest that larval nutritional rearing condition is a major factor in laboratory estimates *An. coluzzii* vector competence. This observation emphasizes the importance of considering larval development factors in all studies aiming to understand the interactions between this vectors and *Plasmodium* parasites.

Key words: Anopheles coluzzii, Plasmodium falciparum, diet, life trait, vector competence.

POSTER PRESENTATION

Day 1: Monday 23rd September 2019

Vector biology and control

Title: Larvicidal activity of Bacillus subtilis isolated from soil sample within Abubakar Tafawa Balewa, University Bauchi against Anopheles mosquito larvae.

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Background: For decades, the use of chemical insecticides in the control of mosquito has become a great problem in the world due to the development of resistance by the mosquito and environmental pollution.

Aim/Objective: This study focused on the larvicidal activity of Bacillus subtils against mosquito larvae.

Methods: Soil samples and mosquito larvae were collected within Abubakar Tafawa Balewa University (ATBU), Bauchi-Nigeria. The soil samples were inoculated into a nutrient medium. The species *Bacillus subtilis* was identified by microscopic and biochemical characteristic. The bacterial isolates were used as an inoculum. Different concentration $(200\mu l, 300\mu l, 400\mu l, 1500\mu l \& 2000\mu l)$ of the bacterial inoculum were added into beakers containing 100 mls of distilled waters with each concentration having five replicates. Water without inoculum was used as control. Twenty mosquito larvae were placed into each beaker containing the inoculum and mortality rate were recorded at 24, 48 and 72 hours.

Results: The highest percentage of mortality rates were recorded after 72hrs at 80%, 90%, 100%, 90%, 100%, 100%, which correspond to the concentration of the bacterial inoculum. The median lethal concentration (LC_{50}) of the inoculum recorded after 24hrs, 48hrs, and 72hrs are 3905µl, 261µl, and 68µl respectively.

Conclusion: The results shows that as the time of exposure increases, the median lethal concentration (LC_{50}) decreases. It was recommended that biological control should be used as a means of larvicidal control due to its non-toxic and eco-friendly characteristics. This research shows that Bacillus subtilis is an efficient agent of mosquito control as these could not only improve bio-efficacy to Bacillus subtilis, but also reduce the possibilities of development of resistance in mosquito population.

Keywords: Bacillus subtilis, inoculum, Anopheles gambiae s.l, larvicidal, Bio-efficacy

Title: Innovative Communication Strategies for Enhancing Effective Community Participation in Integrated Vector Management in Malindi, Kenya

Authors: Lydiah W. Kibe^{1†}, Joseph Mwangangi¹, Clifford Metero², Charles M. Mbogo¹.

¹Kenya Medical Research Institute (KEMRI); ²International Centre of Insect Physiology and Ecology (ICIPE)

Background: Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 1 million deaths annually. A key element in integrated vector management is that of community empowerment, social mobilization and inter sectoral collaboration. Integrated vector management involves making rational decisions on use of vector tools and disease control in relation to local conditions, community support, awareness and action.

Methodology: A community based integrated vector management was carried out in Malindi along the Kenyan coast. An easy to follow curriculum was developed which included components on basic entomological skills and communication skills. Lay persons known as 'mosquito scouts' were trained for 3 weeks and supportive supervision provided during field work. Each mosquito scout was assigned an area of 1km². Their activities included searching for mosquito breeding areas, communicating mosquito information to residents and community based groups and stakeholders.

Results and Discussion: A total of 62 mosquito scouts, 11 community groups affiliated to PUMMA (Punguza Mbu Malindi) and 11 primary schools were mobilized and trained in mosquito control activities. Communication strategies included awareness campaigns such as Annual Mosquito Field Events, neighborhood campaigns, door to door campaigns and school mosquito clubs. The strategies motivated and enabled communities to understand more about mosquitoes and control measures and voluntarily took part in vector management activities. The mosquito doctors". This made it easy to disseminate and communicate to the household owners on mosquito breeding areas and measures to be taken to control the larval habitats identified. These approaches are suitable and applicable to areas with low knowledge of mosquito biology and resource constraints thus ensuring sustainability and ownership by the communities.

Title: Insecticide Resistance and Resting Behaviour of Anopheles Gambiae S.l. in Northern Ghana

Authors: Majidah Hamid-Adiamoh, Davis Nwakanma, Umberto D'alessandro, Gordon A. Awandare, Alfred Amambua-Ngwa And Yaw A. Afrane

Background & Objective: Selection pressure from continued exposure to insecticides seems to be driving development of resistance and changes in resting behavior of malaria vectors. These have been implicated to contribute significantly to residual transmission in several endemic settings. We examined the association between insecticide resistance and concurrent indoor and outdoor resting behavior within members of *an. Gambiae s.L.* In northern ghana.

Materials & Method: live adult mosquitoes were collected indoors and outdoors from two communities. F^1 progenies were reared from a subset of fed mosquitoes and exposed to dichloro diphenyl trichloroethane (ddt), deltamethrin, malathion and bendiocarb using who insecticide susceptibility tests to determine phenotypic resistance. The specific mutation markers to the four insecticides were subsequently analyzed using molecular assays.

Results & Discussion: A statistically significant difference was observed in susceptibility to ddt and deltamethrin between indoor and outdoor mosquito populations with a 24-hour post-exposure mortality of 0% (indoor) and 9% (outdoor) for ddt; 5% (indoor) and 2.5% (Outdoor) for deltamethrin [ddt: p = 0.006. Deltamethrin: p=0.02]. Mosquitoes were also found with suspected resistance to bendiocarb but the difference in mortality between indoor (90%) and outdoor (95%) populations was not statistically significant (p = 0.16). However, mosquitoes were fully susceptible to malathion.

Frequencies of voltage-gated sodium channel (*vgsc*)-1014f and *vgsc*-1575y mutations were significantly higher in ddt and deltamethrin-resistant outdoor than the indoor population (p =0.008). However, glutathione-s-transferase epsilon 2 (*gste2*)-114t was more significantly abundant in indoor deltamethrin-resistant mosquitoes than the outdoor mosquitoes (p = 0.01). *Vgsc*-1014f associated strongly with deltamethrin resistance (or =5.46, P= 0.001).

Conclusion & Recommendation: We found differences in levels of both phenotypic and genotypic resistance to insecticides within *an. Gambiae s.L.* Populations resting concurrently indoors and outdoors in our study. Continued monitoring of vector behavior with evaluation of intra-species behavioral variations is recommended.

Title: Assessment of Performances in Field Conditions of Actellic 300 CS Indoor Residual Spraying (IRS) on Malaria Transmission in Benin, West Africa.

Authors: Rock Aïkpon^{*}, Filémon Tokponon, Razaki Ossè, Marius Allossogbé and Martin Akogbéto

Background: IRS is one of the key vector control interventions for malaria control in Benin after LLINs. The National Malaria Control Program (NMCP) has chosen Pirimiphos Methyl (PM) (Actellic 300 CS) for IRS based on the resistance profile of local vectors that showed a good susceptibility to this insecticide. Here, we assess the entomological impact of Actellic 300 CS IRS on malaria transmission. Entomological parameters of malaria transmission in the control area (without IRS) were compared with those on intervention sites.

Methods: Mosquito collections were carried out in three districts in the Atacora-Donga region. Two districts were treated with (Actellic 300 CS) (Toukountouna and Kouandé), the untreated district (Copargo) serving as control.

Results: A significant reduction (94.25%) in human biting rate was recorded in treated districts where an inhabitant received less than 1 bite of *An. gambiae* per night during the four months following IRS campaign. In the meantime, the entomological inoculation rate (EIR) dramatically declined in the treated area (99.24% reduction). We also noted a significant reduction in vectors longevity and an increase in induced exophily by Actellic 300 CS on *An. gambiae*. However, no significant impact was found on the blood feeding rate. Moreover, the residual activity of Actellic 300 CS was four months against six months of high transmission.

Conclusion: Actellic 300 CS was found to be effective for IRS in Benin. However, the four months of residual activity of Actellic 300CS appear too short to cover the whole high transmission period, which is a limitation that remains to be addressed.

Keywords: IRS, Actellic 300 CS, performances, Malaria, Benin.

Title: Competence of the secondary vectors An. coustani, An. squamosus and An. rufipes for Plasmodium falciparum as measured by direct membrane feeding assays

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Background: In West Africa, *An. rufipes, An. squamosus* and *An. coustani* have been reported to be possible secondary malaria vectors but their intrinsic competence for *P. falciparum* has not been experimentally examined.

Methods: We infected F1 females of wild-caught *An. rufipes*, *An. squamosus* and *An. coustani* from South Western Burkina Faso with two sympatric field isolates of *Plasmodium falciparum*, using direct membrane feeding assays. Individuals from a laboratory colony of *Anopheles coluzzii* were used as controls. From day ten post-infection, the head and thorax of dead mosquitoes were individually stored at -20°C and sporozoite dissemination was assessed using qPCR.

Results: An. coluzzii and An. rufipes showed similar competence for P. falciparum sporozoites $(69 \pm 14\% \text{ and } 62.5 \pm 23.7 \%, \text{ respectively})$. However, An. coustani and An. squamosus were significantly less permissive for the development of P. falciparum sporozoites $(11 \pm 14\% \text{ and } 35 \pm 22 \%, \text{ respectively})$. Finally, mosquito longevity in laboratory conditions significantly varied among vector species, with best survivorship observed in An. coluzzii (mean longevity: $26 \pm 1.4 \text{ days}$), followed by An. rufipes ($20.5 \pm 2.15 \text{ days}$), An. squamosus ($16.2 \pm 0.5 \text{ days}$) and An. coustani ($16 \pm 1.1 \text{ days}$).

Conclusions: An. rufipes, An. squamosus and An. coustani were efficiently infected with *P. falciparum* gametocytes in laboratory conditions. The mechanisms leading to lower competence in An. squamosus and An. coustani are not yet known, but mosquito immune system response or the quantity of the blood meal could be involved. Because An. rufipes displayed relatively long lifespan and high competence for *P. falciparum*, it has the potential to ensure robust transmission, provided that it can feed on humans in natural conditions.

Keywords: secondary vectors, An. coustani, An. squamosus, An. rufipes, Competence, Plasmodium falciparum

Title: Influence of mosquito age on parasite development and the transmission potential of human malaria

Author: Lefevre Thierry

Background & Objective: Mosquito competence for *Plasmodium falciparum*, mosquito survival and the parasite's extrinsic incubation period (EIP) play key roles in determining the intensity of malaria transmission. How mosquito age influences each of these three traits is currently poorly understood.

Materials & Method: *Anopheles coluzzii*, a major African malaria vector, belonging to three different age classes (4 day-old, 8 day-old, and 12 day-old) was infected with sympatric field isolates of *Plasmodium falciparum*, using direct membrane feeding assays. Through a series of experiments, the effects of mosquito age on (i) mosquito competence, (ii) mosquito survival, (iii) and the parasite EIP was then examined.

Results & Discussion: 12 day-old mosquitoes displayed reduced competence for *P. falciparum* compared to younger mosquitoes (4 and 8 day-old). Old mosquitoes showed both reduced infection prevalence and intensity. As expected, old mosquitoes died at a faster rate than young mosquitoes but interestingly this pattern was affected by *P. falciparum* infection, with infected 12 day-old mosquitoes surviving better than uninfected 4 and 8 day-old mosquitoes. This suggests that infection may act as a "fountain of youth" in old mosquitoes only. Although we predicted that parasites should speed up their EIP when infecting old mosquitoes, we found that the sporogonic cycle of *P. falciparum* tends to be slower in old- compared to young- mosquitoes.

Conclusion & Recommendation: Variation in mosquito age could shape malaria dynamics. The collected results will be combined into an epidemiological model to predict the contribution of mosquito age variation to overall malaria transmission potential. Control tools reducing mosquito lifespan could result in the selection of parasite genotypes with shorter EIP, hence threatening the efficacy of these tools.

Title: Molecular basis of DDT and permethrin resistance in an *Anopheles funestus* population from Benin

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Background: Insecticide resistance in *Anopheles* mosquitoes is threatening the success of malaria control programmes. In order to implement suitable insecticide resistance management strategies, it is necessary to understand the underlying mechanisms involved. To achieve this, the molecular basis of permethrin and DDT resistance in the principal malaria vector, *Anopheles funestus* from Kpome (Benin), was investigated.

Methods: Females *Anopheles funestus* were collected from Kpome, in the southern Benin. Susceptibility assays to various insecticides was assessed. A microarray-based genomewide transcription and qRT-PCR analysis were performed to detect the sets of genes differentially expressed in relation to observed resistance in *An. funestus*. The role of the knockdown resistance mutation in DDT and permethrin resistance was also investigated

Results: Metabolic resistance mechanisms through over-expression of cytochrome P450 and glutathione S-transferase genes (GSTs) are the major contributor to DDT and permethrin resistance in *Anopheles funestus* from Kpome. The *GSTe2* gene was the most upregulated detoxification gene in both DDT- [fold-change (FC: 16.0)] and permethrin-resistant (FC: 18.1) mosquitoes. *CYP6P9a* and *CYP6P9b* genes that have been previously associated with pyrethroid resistance were also significantly overexpressed with FC 5.4 and 4.8, respectively, in a permethrin resistant population. Noticeably, the GSTs, *GSTd1-5* and *GSTd3*, were more upregulated in DDT-resistant than in permethrin resistant *Anopheles funestus*. The absence of the L1014F or L1014S *kdr* mutations in the voltage-gated sodium channel gene coupled with the lack of directional selection at the gene further supported that knockdown resistance plays little role in this resistance.

Conclusions: The major role played by metabolic resistance to pyrethroids in this *An. funestus* population in Benin suggests that using novel control tools combining the P450 synergist piperonyl butoxide (PBO), such as PBO-based bednets, could help manage the growing pyrethroid resistance in this malaria vector in Benin.

Keywords: Anopheles funestus, Insecticide resistance, Permethrin, DDT, Kpome, Resistance mechanisms

Title: Feeding Preferences of *Anopheles* Species Under Prolonged Use of Insecticide-Treated Bed Nets in Kamuli District, Uganda

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Background:The blood-feeding patterns are crucial in incriminating disease vectors as well as facilitating the design and consolidation of effective vector control interventions in an area. This study aimed to determine the feeding preferences of *Anopheles gambiae s.l.* and *An. funestus* group in relation to the epidemiology of malaria in the highly endemic Kamuli district, Uganda.

Methods: A total of 187 indoor resting blood-fed *Anopheles gambiae sensu lato* and *An. funestus* mosquitoes collected from 48 households were tested by direct enzyme-linked immunosorbent assay (ELISA) for blood meal host identification in Kamuli district. Of these, 73 mid guts came from 24 households in villages with 69% of households using insecticide-treated bed nets/ITNs, while 114 mid guts were from 24 households in non-ITN villages. The blood feeding patterns and Anthropophilic index of *Anopheles gambiae s.l.* and *An. funestus* mosquitoes in both zones were determined.

Results: Blood meal hosts were identified in only 10.96% (n = 8) and 14.91% (n = 17) of the *Anopheles* blood meals from the intervention and non-intervention zones, respectively, by the ELISA method. Other blood meals could not be clearly identified. Eight (100%) blood meals in the intervention zone were from humans, while in the non-intervention zone, 15 (88.24%), one (5.88%) and one (5.88%) of the identified blood meals were obtained from humans, cattle and goat, respectively. Findings from this study demonstrate that *An. gambiae s.l.* and *An. funestus* mosquitoes in Kamuli district are anthropophilic, with nearly all the mosquitoes collected from both zones feeding on humans during every blood meal (p > 0.05).

Conclusion: The high vector-human contacts implicate these species as important in the transmission of *Plasmodium* species and other infections. This study suggests that the use of insecticide-treated bed nets is effective for controlling malaria vectors inside houses in Kamuli district, evoking universal coverage of houses in the area.

Key words: Anopheles mosquitoes, Anthropophily, ELISA, ITNs
Title: Paraquat-mediated oxidative stress in *Anopheles gambiae* mosquitoes is regulated by an endoplasmic reticulum (ER) stress response

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Paraquat is a potent superoxide $(O_{a}(-)-inducing agent that is capable of inducing an$ oxidative imbalance in the mosquito midgut. This oxidative imbalance can super-stress the malaria parasite, leading to arrested development in the mosquito midgut and reduced transmission. While several studies have explored the effect of paraguat on malaria parasites, a fundamental understanding of the mosquito response to this compound remains unknown. Here, we quantified the mosquito midgut proteomic response to a paraquat-laced sugar meal and found that An. gambiae midguts were enriched in proteins that are indicative of cells under endoplasmic reticulum (ER) stress. We also carried out gRT-PCR analyses for nine prominent thioredoxin (Trx) and glutathione (GSH)-dependent genes in mosquito midguts post P. falciparum blood meal ingestion to evaluate the concordance between transcripts and proteins under different oxidative stress conditions. Our data revealed an absence of significant upregulation in the Trx and GSH-dependent genes following infected blood meal ingestion. These data suggest that the intrinsic tolerance of the mosquito midgut to paraquat-mediated oxidative stress is through an ER stress response. These data indicate that mosquitoes have at least two divergent pathways of managing the oxidative stress that is induced by exogenous compounds, and outlines the potential application of paraquatlike drugs to act selectively against malaria parasite development in mosquito midguts, thereby blocking mosquito-to-human transmission.

Title: Annual Estimates of Entomological Indicators in Zambia During Three Years of Indoor Residual Spraying With Pirimiphos Methyl

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Background: Indoor residual spraying (IRS) is an important vector control strategy for the National Malaria Elimination Program in Zambia. Due to pyrethroid resistance, Zambia has used an organophosphate—pirimiphos-methyl (Actellic 300CS)—for IRS since 2012.

Description: The U.S. President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) Project conducted IRS annually in 2015, 2016, and 2017 with pirimiphosmethyl in Luapula, Northern, Muchinga, and Eastern provinces in Zambia. Following each IRS campaign, monthly vector surveillance using three standard collection methods, monthly evaluation of residual efficacy of pirimiphos-methyl by cone bioassays, and yearly insecticide susceptibility testing by WHO tube assays were performed. Entomological monitoring was conducted in seven districts at two sentinel sites, one sprayed and one unsprayed per district.

Lessons learned: *Anopheles funestus* s.l. was the predominant malaria vector identified during the three years of surveillance. The average number of mosquitoes per collection effort in the sprayed sites was 4.35, 8.87, and 6.44 for *An. funestus* s.l. and 0.36, 0.42 and 0.44 for *An. gambiae* s.l. in 2015, 2016, and 2017, respectively. In the sprayed sites, the indoor density of *An. funestus* s.l. one month after IRS fell by 17% in 2015, 28% in 2016, and 56% in 2017, while concomitant increases of 73, 60, and 47% were observed at matched unsprayed sentinel sites. Vector densities returned to pre-spray levels at five to six months after IRS indicating a waning effect of the insecticide sprayed. The overall residual efficacy of pirimiphos-methyl was four to five months. Local vectors were susceptible to pirimiphos-methyl during all three years of surveillance.

Conclusions/Next steps: IRS with primiphos-methyl was effective at reducing mosquito populations in this area of Zambia, but the duration of the effect was short relative to the transmission season. Alternatives such as use of longer lasting insecticides, twice per year spray, or use of next generation LLINs may need to be considered.

Title: Evidences of the low implication of mosquitoes in the transmission of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer.

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Background & Objective: Buruli ulcer (BU) continues to be a serious public health threat in wet tropical regions and the mode of transmission of its etiological agent,Mycobacteriumulcerans (MU), remains poorly understood. In this study,mosquito species collected in endemic villages in Benin were screened for the presence of MU. In addition, the ability of mosquitoes larvae to pick up MU from their environment and remain colonized through the larval developmental stages to the adult stage was investigated.

Methodology & Principal findings: 7,218 adults and larvae mosquitoes were sampled from endemic and nonendemic villages and screened for MU-DNA targets (IS2404, IS2606, and KR-B) using qPCR. MU was not detected in any of the field collected samples. Additional studies of artificially infected larvae of *Anopheles kisumu* with MU strains revealed that mosquitos' larvae are able to ingest and host MU during L1, L2, L3, and L4 developmental stages. However, we noticed an absence of these bacteria at both pupae and adult stages, certainly revealing the low ability of infected or colonized mosquitoes to vertically transmit MU to their offspring.

Conclusion & Significance: The overall findings highlight the low implication of mosquitoes as biological vectors in the transmission cycle of MU from the risk environments to humans.

Keywords: Buruli ulcer, *Mycobacterium ulcerans*, mosquitoes, vertical transmission, Benin.

Title: Characterization of resistance mechanisms and bio-efficacy of pyrethroid-PBO nets against pyrethroid resistant populations of An. gambiae s.l in selected malaria sentinel sites in Ethiopia

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Long-lasting insecticidal treated nets (LLINs) and indoor residual spraying (IRS) are the main vector control interventions in Ethiopia. The high levels of resistance of *Anopheles gambiaes*.1 to pyrethroidsobserved in many malaria endemic areas of the country highlight the need to further investigate the resistance mechanisms involved and continuous close monitoring which possibly help for informed decision in planning vector control. Therefore, this study aimed at monitoring insecticide resistance in populations of *An. gambiaes.l.* in selected malaria surveillance sentinel and other sites, characterize mechanisms conferring resistance to pyrethroid insecticides and determine the bio-efficacy of pyrethroid-only LLINs (MAGnet, Yarkool andPermaNet 2.0) and pyrethroid-PBO nets (PermaNet® 3.0) against pyrethroid resistant populations of *An. gambiaes.l.* to generate data for evidence-based policy making for optimal malaria vector control, insecticide choice and insecticide resistance management.

The susceptibility status of populations of An. gambiaes.l. to alphacypermethrin, permethrin, and deltamethrin was assessed following standard WHO susceptibility test procedure in twelve sites selected from five out of nine regional states (Oromyia,Amhara, SNNPR, Gambella and BenshangulGumuz) of Ethiopia. Of the twelve selected sites, eight (Goro, Asendabo, Angergutin, Pawi, Abobo, Wondo Genet, Halaba and Efratana Gidim) were national malaria surveillance sentinel sites. Other selected sites included Jimma, Serbo, Harbu andLokha Abaya. Bioassays were also conducted using piperonylbutoxide (PBO), a synergist to detect the involvement of elevated detoxifying oxidase P450 enzymes (or metabolic resistance). Both insecticide susceptibility tests and synergist assays were carried out using 3-5 days old unfed female An. gambiaes.l. mosquitoes reared from field collected larvae. Each insecticide with and without pre-exposure to PBO was tested in four replicates using 20-25 adult female mosquitoes for each test; a control in two replicates each with 20-25 female mosquitoes was run in parallel. Moreover, the bio-efficacy of pyrethroid-only nets (MAGnet, Yarokoland PermaNet 2.0) and a PBO-pyrethroid net (PermaNet® 3.0) was evaluated against wild resistant An. gambiaes.l. populations following WHO cone bioassay procedure.

The results of WHO susceptibility tests showed that there was high resistance in

populations of *An. gambiaes*.1. from all sites to alphacypermethrin (mortality 44.4% to 68.6%), permethrin (mortality 8% to 39%) and deltamethrin (mortality 15% to 52%). With 1 hour pre-exposure to PBO, mosquito mortality rates in all sites increased significantly for alphacypermethrin (mortality 100%), permethrin (mortality 84%-95%) and deltamethrin (mortality 94%-100%). However, mortality rates in all mosquito populations exposed to permethrin and in few deltamethrin exposed mosquito populations following 1 hour pre-exposure to PBO were still below resistance threshold(<98% mortality). Overall, analysis of bio-efficacy of LLINs (MAGnet, PermaNet 2.0 and PermaNet 3.0) by net type and section against pyrethroid resistant populations An. *gambiaes*.1. to the three pyrethroid-only nets (MAGnet, Yarokol,PermaNet 2.0) and (PermaNet 3.0 side) tested (mortality 4.4%-83.8%). Bio-efficacy of the roof ofpyrethroid-PBO net(PermaNet 3.0) was however significantly higher (p < 0.001) than the pyrethroid-only nets and PermaNet 3.0 side against all the twelve mosquito populations.

This study once again demonstrates widespread pyrethroid resistance in *An. gambiaes.l.* in different areas of Ethiopia. The significant increase in mortality rates in mosquitoes exposed to alphacypermethrin, permethrin and deltamethrin after pre-exposure to PBO showed the partial or full involvement of detoxifying enzymes (cytochrome P450 monoxygenases), a mechanism which largely confers resistance to pyrethroid insecticides; previously reported *kdr* mutation from this mosquito population may also be involved. The evaluation of bioefficacy of LLINs indicated that both pyrethroid-only nets and Pyrethroid-PBO net side performed less as compared to Pyrethroid-PBO net roof, where mosquitoes typically approach a net. These results suggest that synergists such as PBO should be considered in vector control products such as LLINs so that tools with the highest efficacy are used in areas with pyrethroid resistant mosquito populations.

Key words: Bio-efficacy, Insecticide resistance, synergist, piperonylbutoxide, malaria, Ethiopia

Title: High malaria transmission sustained by *Anopheles gambiae* s.l. occurring both indoor and outdoor in the city of Yaoundé, Cameroon

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Background: Malaria remains a major public health problem in Cameroon. However, despite reports on the adaptation of anopheline species to urban habitats, there is still not enough information on malaria transmission pattern in urban settings. In the frame of a larval control trial in the city of Yaoundé, we conducted baseline surveys to assess malaria transmission dynamics.

Methods: Adult mosquitoes were collected using CDC light traps and human landing catches from March 2017 to March 2018 in 30 districts of Yaoundé, Cameroon. Mosquitoes were identified up to the species level using PCR. The TaqMan method and ELISA were used to determine mosquito infection status to Plasmodium. Bioassays were conducted to assess female *Anopheles gambiae* susceptibility to insecticides.

Results: A total of 218,991 mosquitoes were collected. The main malaria vectors were *An*. *gambiae* s.l. (n=6154) and *An*. *funestus* s.l. (n=229). Of the 1476 *An*. *gambiae* s.l. processed by PCR, 92.19% were *An*. *coluzzii* and 7.81% *An*. *gambiae*. *An*. *funestus* s.l. was composed of 93.01% (173/186) *An*. *funestus* and 4.84% (13/186) *An*. *leesoni*. The average biting rate of anopheline was significantly high outdoor than indoor (P=0.013). Seasonal variation in mosquito abundance and biting rate was recorded. The infection rate by *Plasmodium falciparum* was 2.13% (104/4893 mosquitoes processed). The annual entomological inoculation rate was found to vary from 0 to 92 infective bites/man/year (ib/m/y). Malaria transmission risk was high outdoor (66.65 ib/m/y) compared to indoor (31.14 ib/m/y). *An*. *gambiae* s.l. was found highly resistant to DDT, permethrin and deltamethrin. High prevalence of the West Africa kdr allele 1014F was recorded and this was not found to influence *An*. *gambiae* s.l. infection status.

Conclusions: The study suggests high malaria transmission occurring in the city of Yaoundé and call for immediate actions to improve control strategies.

Keywords: Malaria, urbanization, Anopheles, transmission, Yaoundé, Cameroon

Title: Malaria Transmission around the Memve'ele Hydroelectric Dam in South Cameroon: A Combined Retrospective and Prospective Study, 2000–2016

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Background: Dam constructions are considered a great concern for public health. The current study aimed to investigate malaria transmission in the Nyabessan village around the Memve'ele dam in South Cameroon.

Materials and method: Adult mosquitoes were captured by human landing catches in Nyabessan before and during dam construction in 2000–2006 and 2014–2016 respectively, as well as in the Olama village, which was selected as a control. Malaria vectors were morphologically identified and analyzed for *Plasmodium falciparum* circumsporozoite protein detection and molecular identification of *Anopheles (A.) gambiae* species.

Results: Overall, ten malaria vector species were identified among 12,189 *Anopheles* specimens from Nyabessan (N = 6127) and Olama (N = 6062), including *A. gambiae* Giles (1902), *A. coluzzii* Coetzee (2013), *A. moucheti* Evans (1925), *A. ovengensis* Awono (2004), *A. nili* Theobald (1903), *A. paludis* Theobald (1900), *A. zieanni*, *A. marshallii* Theobald (1903), *A. coustani* Laveran (1900), and *A. obscurus* Grünberg (1905). In Nyabessan, *A. moucheti* and *A. ovengensis* were the main vector species before dam construction (16–50 bites/person/night-b/p/n, 0.26–0.71 infective bites/person/night-ib/p/n) that experienced a reduction of their role in disease transmission in 2016 (3–35 b/p/n, 0–0.5 ib/p/n) (p < 0.005). By contrast, the role of *A. gambiae* s.l. and *A. paludis* increased (11–38 b/p/n, 0.75–

1.2 ib/p/n) (p < 0.01). In Olama, *A. moucheti* remained the main malaria vector species throughout the study period (p = 0.5).

Conclusion: These findings highlight the need for strong vector-borne disease surveillance and control system around the Memve'ele dam.

Keywords: malaria vectors; *Plasmodium* transmission; dam construction; Memve'ele; Cameroon

Title: Gametocyte clearance in Children, from western Kenya, with uncomplicated *Plasmodium falciparum* malaria after Artemether-Lumefantrine or Dihydroartemisinin-Piperaquine Treatment.

Background: The efficacy and safety of Artemether-lumefantrine(AL) and Dihydroartemisinin-piperaquine (DP) against asexual parasite population has been documented. However, the effect of these antimalarials on sexual parasites is still unclear. Gametocyte clearance following treatment is essential for malaria control and elimination efforts therefore the study sought to determine gametocyte clearance after AL or DP treatment.

Methods: Children from Busia, western Kenya with uncomplicated *Plasmodium falciparum* malaria were assigned randomly to AL or DP treatment. A total of 334 dried blood spot samples were collected for up to 5 weeks after treatment during the peak malaria transmission season in 2016. *Plasmodium falciparum* gametocytes were detected by *Pfs25* qRT-PCR and gametocytes prevalence, density and duration of gametocyte carriage were determined.

Results: At baseline all the 334 children had positive asexual parasite by microscopy, 12% (40/334) had detectable gametocyte by microscopy and 83.7% (253/302) children had gametocytes by RT-qPCR. The prevalence of gametocytes decreased from 84.6% (125/148) to 7.04% (5/71) at day 42 in AL group and from 82.4 % (127/154) to 14.5% (11/74) in DP group. The duration of gametocyte carriage as estimated by qRT-PCR was slightly shorter in AL group (4.5days) than in DP group (5.1days) but not significant (p=0.301). Submicroscopic gametocytes persisted in some patients up to day 28 despite a significant number of patients being negative for asexual parasites by microscopy on day 7.

Conclusion: The study indicates that AL may clear *Plasmodium falciparum* gemetocytes slightly faster than DP. However, there is need to interpret the findings in the context of transmission intensity since submicroscopic gametocyte densities persisted after treatment with AL and DP. This highlights the limitation of interventions that aim to reduce malaria transmission by use of antimalarial drugs therefore a gametocidal drug in combination to ACTs will be useful in blocking malaria transmission more efficiently.

Title: Involvement of *Anopheles nili* in *Plasmodium falciparum* transmission in North Benin

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Background: Several studies carried out in Benin have shown the involvement of *Anopheles gambiae s.l.*, the *Anopheles funestus* group in malaria transmission, but none of them reported the contribution of the *Anopheles nili* group to the transmission of this disease. The current study investigated the question through an entomological cross-sectional survey performed in Northern Benin.

Methods: Mosquito samplings were performed in September and October 2017 in villages of Bambaba and Wodara (Kérou district) and, Péhunco 2 and Béké (Péhunco district). The collections were carried out indoors and outdoors using human landing catches to assess the human biting rate (HBR) and pyrethrum spray catches to evaluate the blood feeding rate and the blood meal origin using the ELISA test. All collected mosquitoes were morphologically identified and, the PCR technique was used for molecular identification of sibling species of *An. gambiae s.l., An. funestus* group and *An. nili* group sporozoite index (SI) was also assessed by the ELISA test.

Results: Overall, *An. gambiae s.l., An. funestus* and *An. nili* groups were the three vectors found in the study area. A significantly higher HBR was recorded in *An. nili* group (5 bites/human/night) compared to *An. funestus* group (0.656 bites/human/night) in the Kérou district (p<0.0001). *Anopheles gambiae s.l.* displayed the highest HBR (26.19 bites/human/ night) in the same district. The entomological inoculation rate (EIR) was 1.875 infected bites/human/month in *An. nili* group against 13.05 infected bites/human/month in *An. gambiae s.l.* and 0.938 infected bites/human/month in *An. funestus* group in Kérou. PCR results showed that *An. nili s.s.* and *An. funestus s.s.* were the only species of the *An. nili* and *An. funestus* groups, respectively.

Conclusion: This study provides useful information on the contribution of *An. nili* group as secondary vector to malaria transmission in Northern Benin. This will aid to better plan malaria vector control interventions.

Keywords: Malaria, EIR, Anopheles nili, contribution, Benin

Title: Larvicidal activity of Bacillus subtilis isolated from soil sample within Abubakar Tafawa Balewa, University Bauchi against Anopheles mosquito larvae.

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Background: For decades, the use of chemical insecticides in the control of mosquito has become a great problem in the world due to the development of resistance by the mosquito and environmental pollution.

Aim/Objective: This study focused on the larvicidal activity of Bacillus subtils against mosquito larvae.

Methods: Soil samples and mosquito larvae were collected within Abubakar Tafawa Balewa University (ATBU), Bauchi-Nigeria. The soil samples were inoculated into a nutrient medium. The species *Bacillus subtilis* was identified by microscopic and biochemical characteristic. The bacterial isolates were used as an inoculum. Different concentration $(200\mu l, 300\mu l, 400\mu l, 1500\mu l \& 2000\mu l)$ of the bacterial inoculum were added into beakers containing 100 mls of distilled waters with each concentration having five replicates. Water without inoculum was used as control. Twenty mosquito larvae were placed into each beaker containing the inoculum and mortality rate were recorded at 24, 48 and 72 hours.

Results: The highest percentage of mortality rates were recorded after 72hrs at 80%, 90%, 100%, 90%, 100%, 100%, which correspond to the concentration of the bacterial inoculum. The median lethal concentration (LC_{50}) of the inoculum recorded after 24hrs, 48hrs, and 72hrs are 3905µl, 261µl, and 68µl respectively.

Conclusion: The results shows that as the time of exposure increases, the median lethal concentration (LC_{50}) decreases. It was recommended that biological control should be used as a means of larvicidal control due to its non-toxic and eco-friendly characteristics. This research shows that Bacillus subtilis is an efficient agent of mosquito control as these could not only improve bio-efficacy to Bacillus subtilis, but also reduce the possibilities of development of resistance in mosquito population.

Keywords: Bacillus subtilis, inoculum, Anopheles gambiae s.l, larvicidal, Bio-efficacy

Title: Assessment of population dynamics and biting trends of *Aedes aegypti* in northern Benin : Public health implications.

Authors: Rock Aïkpon*, Mark Brettenny, Yllias Lawani

Background: Aedes aegypti represents a major vector of arboviruses, which include Zika viruses, dengue, chikungunya, yellow fever among others. Presence and abundance of this vector is associated with the risk of arboviral diseases transmission. Little is known about biting behaviour of *Aedes* mosquitoes in Benin. This study aimed to assess population dynamics and biting trends of *Aedes aegypti* mosquito in northern Benin.

Methods: Abundance and biting cycle of *Aedes aegypti* were determined indoors *Vs* outdoors through monthly human landing catches.

Results: A total of 485 adult *Aedes aegypti* mosquitoes were collected. The probability of exposure to *Aedes aegypti* bites was highest outdoors and in afternoon hours. This is enormous risk factors for potentiel outbreaks of arboviral diseases.

Conclusion: Findings of our study serve valuable informations about potentiel threat of the emergence of arboviral diseases in northen Benin. Therefore, it will be important to implement urgently vector control strategies to fight against *Aedes* mosquitoes in order to prevent any occurrence of arbovirus.

Keywords: Aedes aegypti, population dynamics, biting, Benin.

Title: The spread of malaria in savannah area in Benin: The contribution of *Anopheles gambiae* and *Anopheles funestus* in the transmission.

Authors: Rock Aïkpon, Albert Salako, Razaki Ossè and Martin Akogbéto.

Background: The role of the *Anopheles complex* and *Anopheles funestus* Giles in the malaria transmission was investigated in a Savannah area in Benin. This study shows the part of their contribution in malaria transmission in the study area.

Methods: Mosquitoes were collected, using human landing catches. All the anopheline mosquitoes were assessed for species identify and sporozoite infection status.

Results: Most of the anopheline mosquitoes collected were members of the *An. gambiae* complex (80.90%) and *An. funestus* group (14.36%). *An. gambiae* and *An. coluzzii* were found in sympatry but with a seasonal variation. All of the females of the *An. funestus* group investigated were identified as *An. funestus s.s.* In spite of being the major malaria vector as far as abundance is concerned, sporozoite prevalence was three times higher with *An. funestus* than *An. gambiae*.

Conclusion: This study documented useful informations on the relative contribution of *Anopheles funestus* and *Anopheles gambiae* to the perennial malaria transmission in savannah area in Benin.

Keywords: Malaria, contribution, Anopheles gambiae, Anopheles funestus

Title: transfluthrin devices **Optimizing** treated for deterrence of mosquitoes from approaching and entering permethrin treated tent.

Authors: David Oullo*, James Mutunga, Sheila Ogoma, Thomas Gilbreath and Wesley McCardle. US Army Medical Research Directorate-Africa; Kisumu Kenya.

Long lasting insecticidal nets and indoor residual spraying are among the widely used strategies for mosquito control. However, despite proven efficacy, distribution and usage it still possesses logistical and practical challenges in temporary shelters that do not have substrates for spraying insecticides. Therefore, these tools may not be feasible vector control interventions for refugee camps and temporary military installations in remote areas. Furthermore current strategies target endophagic and endophilic mosquitoes but not outdoor biting vectors. Since there is a need for novel strategies that are field expedient in areas with no structural substrate to spray, this study investigated the spatial protection of transfluthrin-treated devices against mosquito bites in a semi-field environment. All combinations of permethrin treated/untreated tent, transfluthrin-treated burlap strips, transfluthrin-treated Personal Insect Repellent Kits (PIRK) devices at distances of 0m, 1m, and 3m from the tents were tested using a 4x4 Latin Square design for five nights each, with a two night period between treatments. Starved, female An. gambiae were released inside the semi-field and collected using human landing catches simultaneously inside and outside the tent. All the treatments significantly reduced mosquito biting inside both treated and untreated tents, with an 85% reduction in treated tents combined with transfluthrin devices. Further studies are warranted to estimate how prolonged exposure to doses of volatile pyrethroids might impact insecticide resistance in natural vector populations and warrant further monitoring and study.

Title: Survival of 8 LLINs types 6, 12, 24 and 36 months after a mass distribution campaign in rural and urban settings in Senegal

Authors: Mbaye DIOUF¹, Roger Clément TINE², Demba Anta DIONE³, Olivier BRIET⁴, Babacar Thiendella FAYE², Isma SOW⁵, Abdoulaye KONATE¹, Abdoulaye Kane Dia¹, El Hadji DIOUF¹, El Hadji Amadou NIANG¹, Lassana KONATE¹, Ousmane FAYE¹.

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Background: Long Lasting Insecticidal Nets (LLINs) are one of the core components of global malaria prevention and control. The lifespan of LLINs varies widely depending on the population, and randomized studies are required to compare net types in households under different field condition. This study evaluated the survival of 8 types of llins distributed in Senegal.

Methods: 12,608 LLINs were distributed in 5 regions each stratified by rural and urban setting. As part of the longitudinal follow-up, 2222 nets were randomly sampled and monitored from 6 to 36 months with over of 250 types selected in all areas. Using random effects for households Bayesian model were allowed to estimate independent survival by net type and by area (rural/urban) with a coefficient of variation superior to 0 in IC95%. Attrition rates and median survival time were also calculated for each net type.

Results: Three net types had a proportion of survival above 80% after 24 months: Interceptor[®] 87.8% (95%CI 80-93.4); conical PermaNet® 2.0 86.9% (95%CI 79.3-92.4) and LifeNet 85.6% (95%CI 75-93). At 36 months, conical PermaNet® 2.0 keeps that good survival rate, 79.5% (95%CI 65.9-88.8). The odds of survival was 2.5 times higher in rural settings than in urban settings (OR 2.5; 95%CI 1.7-3.7). The attrition due to redistributed nets showed that the two conical net types (PermaNet® 2.0 and Interceptor[®]) were more retained in households and their median survival time was well above three years. Despite this good retention, Interceptor[®] had a weak physical integrity and its median survival due to wear and tear was below to three years.

Conclusions: Differences in survival among LLINs types maybe driven by brand, shapes or environmental setting. It appears that net acceptability can reduce loss due to removal nets and increase effective lifespan.

Keywords: LLINs, Survival rates, Median survival time, Rural, Urban, Senegal.

Title: Evaluation of *Plasmodium falciparum* circumsporozoite protein ELISA protocols for sporozoite detection in mosquito homogenates

Authors: Nobert Mudare¹, Aramu Makuwaza^{1.4}, Xuxa Gara¹, Charmaine Matimba¹, Brenda Makonyere, Trust Nyakunu, Tinashe Chidziva, Hieronymo Masendu², Joel Muatcho², Antony Chisada³, Wietske Mushonga¹, Joseph Mberikunashe³, Susan Mutambu⁵, Sungano Mharakurwa¹.

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Determination of malaria sporozoite rates in the salivary glands of *Anopheles* mosquitoes is essential for estimating the number of infective mosquitoes, and consequently, the entomological inoculation rate (EIR). EIR is a key indicator for evaluating the risk of malaria transmission. Although the enzyme-linked immunosorbent assay specific for detecting the circumsporozoite protein (CSP-ELISA) is routinely used in the field, it is prone to false positives. The CSP-ELISA boiling method is used to minimize the false positives. The current study compared the performance of the CSP ELISA with and without boiling in detecting *Plasmodium falciparum* in the salivary glands of wild Anopheles mosquitoes, using PCR as the reference. Female Anopheles mosquitoes (n=1864) were collected from 10 of 18 designated national malaria vector sentinel surveillance sites and dissected to separate head and thorax from abdominal segments. The head and thorax sections were crushed into homogenate and subjected to CSP - ELISA following the Robert A Wirtz 2016 protocol. The homogenate was then further boiled for 10 minutes for removal of false positives. Part of the same homogenate for each mosquito sample was subjected to PCR for detection of Plasmodium falciparum infection. The performance of the CSP ELISA was compared against PCR assay. The prevalence of the Plasmodium falciparum sporozoites for unboiled CSP ELISA, boiled CSP ELISA and PCR was 4.2 % (n=78/1842), 0.6 % (n=11/1842) and 5.6 % (n=103), respectively. Compared to PCR, the sensitivity and specificity of boiled CSP ELISA in detecting Plasmodium falciparum was 2.9 %(95% CI: 0.47037, 0.74661) and 99.5 %((95% CI: 0.47037, 0.74661), respectively. Also compared to PCR, the sensitivity and specificity of unboiled CSP ELISA was 9.71 %(95%CI: 0.49946, 0.55767) and 96.0 %(95%CI: 0.49946, 0.55767). The ROC area for boiled CSP ELISA and unboiled CSP ELISA was 0.6085 and 0.5286 respectively, meaning that the CSP ELISA diagnostic accuracy is poor. The chi-square test for difference in performance of the boiled CSP ELISA at 5% level of significance (χ^2 =8.5, df =1, α =0.05, p=0.002), showing that boiled CSP ELISA performance is very poor in informing Plasmodium falciparum infective rates in mosquito homogenates. The chi square test for difference in performance of unboiled CSP ELISA was also done at 5% level of significance (($\chi 2=7.6$, df =1, $\alpha=0.05$, p=0.006).CSP-ELISA has high specificity and a false sporozoite positive rate of 4% without boiling and 0.5% with boiling step, which is instrumental for reliable incrimination of emerging vectors. However, both unboiled and boiled CSP ELISA showed low detection

sensitivity relative to PCR, which may underestimate the true infective sporozoite rate in Anopheline populations.

Keywords: *Plasmodium falciparum* sporozoite, Anopheles mosquitoes, Head thorax homogenate, DHFR FM4 PCR, CSP-ELISA.

Title: Comparing the relationship between resistance and *Plasmodium falciparum* infection rate in 14 sentinel sites (Zimbabwe)

Authors: Brenda Makonyere^{1,4}, Nobert Mudare¹, Aramu Makuwaza^{1,7}, Trust Nyakunu^{1,4}, Tinashe Chidziva^{1,5}, Simbarashe Mashiri^{1,6}, Hieronymo Masendu², Joel Mouatcho², Wietske Mushonga¹, Sungano Mharakurwa¹.

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Malaria remains a life-threatening disease in most parts of Africa including Zimbabwe. The mainstay national malaria control programme interventions include prompt case management and vector control through indoor residual spraying and provision of longlasting insecticidal nets. The effectiveness of vector control interventions can be affected by the development of resistance in mosquitoes under selection pressure from insecticide use. The current study examined the association between the knockdown resistance (Kdr) genotype and Plasmodium falciparum infection rate among wild An. gambiae sl vector mosquitoes.1472 adult mosquitoes collected from 14 designated national sentinel surveillance sites were morphologically identified by entomologists, followed by confirmatory molecular identification using the Wilkins vector sibling species differentiation PCR protocol. All vector mosquitoes were subjected to PCR for detection of the Vgsc-L1014F and Vgsc-L1014S knock down resistance alleles. P. falciparum sporozoite infection among the An. gambiae sl was detected using circumsporozoite ELISA and confirmed by PCR. Of the 1472 mosquitoes analysed 85.3% were An. quadriannulatus, 1.02% An. gambie ss, 6.3% An. arabiensis and 7.4% An .merus. Resistant kdr alleles occurred in 22.6% of the mosquitoes, the remaining 77.4% bore the susceptible allele. Plasmodium falciparum sporozoite carriage was 1.63% and 0.13% by ELISA and 1.5% by PCR, respectively. None of the vector mosquitoes that bore the kdr-resistant alleles was found with sporozoites. There was a highly significant association between kdr resistance Vgsc-L 1014F and Vgsc-L 1014 S alleles and absence *Plasmodium falciparum* sporozoite infection among the An. gambiae vector species (Chi-square = 7.13, p < 0.01, N = 1472). It was concluded that IRS may exert additional impact on transmission through selection for resistant Kdr mosquito variants that are refractory to malaria infection.

Key words: relationship, vector, Kdr resistance, plasmodium falciparum infection

Title: Biodiversity and dynamics of *Simulium* vector populations along the Menchum river in North-West Cameroon

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Background: In Africa, black flies are represented by 124 species of which 55 species are known to occur in Cameroon. Cytotaxonomic data and molecular information is only available for some subspecies of *S. damnosum sensu lato*. The Menchum valley in North West Cameroon is endowed with fast flowing tributaries and falls, which provide excellent breeding conditions for *Simulium* larvae. The main species found is *Simulium damnosum s.l.* which transmits, besides *O. volvulus*, a number of animal filariae from cattle, game animals and birds. Little is known about the prevalence of such filariae, but the occurrence of *O. ochengi* nodules in cattle has been confirmed at the Wum slaughterhouse.

Methods: *Simulium* adult flies were caught on man and larvae/pupae collected from 2015 to 2018 along the river Menchum (6°18'25''N10°01'01''E) and the tributary Mawong (6°19'26''N10°00'13''E). Female flies were dissected for *Onchocerca* larvae. The COI and the ITS2 regions were aligned using Geneious®. Phylogenetic analyses were performed using MEGAX64®.

Results: A total of 1.783 pupae were collected and 935 analyzed, showing 12 species (*S. cervicornutum 63.6%; S. katangae 13.3%; S.damnosum 8.2%; S. unicornutum 5.8%; S. pseudequinum 3.0%; S. dentulosum 2.4%; S. alcocki 1.9%; S. hargreavesi 1.0%; S. adersi 0.3%; S. hirsutum 0.2%; S. ruficorne 0.2%; S. schoutedeni 0.1%. The alignment of sequences gives us a maximum similarity of 75% of 11 species and 100% for <i>S. damnosum*. Only *S. damnosum s.l.* female flies (S. *squamosum*) were coming to bite on man and Annual Biting Rates were 86.000, *Onchocerca* L3 were seen (ATPs 98).

Conclusion: A possibility of continuous transmission is possible following the poor distribution coverage of Mectizan since 2016. The presence of Migratory *Simulium* that breeds in tributaries which access is difficult increases the ATPs. The molecular inventory and classification of Cameroonian *Simulium* species which is being prepared is likely to help control and eliminate onchocerciasis.

Keys words: black flies; Simulium damnosum; Onchocerca

Title: Assessment of social representations of malaria in the Pheuls ethnic population in nothern Benin.

Authors: Gado Dramane^{1*,} Jean Robert Klotoé¹, Elie Dannon¹. ¹Ecole Normale Supérieure de Natintingou, UNSTIM *gdramane@gmail.com

Background: The purpose of the study was to recognize the social representation in the Pheuls population by identifying cultural elements related to malaria, in order to create an intercultural approach to any health intervention to control and prevent the disease.

Methods: This qualitative study has an anthropological focus that used participant observation, informal conversations, focus groups, interviews, in-depth interviews with key actors. Analyses included review, classification and categorization of interviews.

Results: Malaria within the Pheuls culture is in harmony with several cultural factors, such as magic, religion, beliefs, myths, and nature. The health system must include these factors in its intercultural approach to ensure the sustainability of anti-malarial intervention measures. Until this is not properly addressed, the Pheuls population of northen Benin will remain a permanent source of risk for malaria transmission in this region and for the rest of the country.

Conclusion: The findings of this study contribute new variables that can facilitate an intercultural approach to improve the perception of malaria in the Pheul populations in Benin.

Keywords : Social representations, malaria, Pheul populations, Benin.

Title: Investigation of the influence of a glutathione S-transferase metabolic resistance to pyrethroids/DDT on mating competitiveness in males of the African malaria vector, *Anopheles funestus*

Authors: Magellan Tchouakui, Billy Tene Fossog, Brigitte Vanessa Ngannang, Doumani Djonabaye, Williams Tchapga, Flobert Njiokou, Charles S. Wondji

Background: Metabolic resistance is a serious challenge to current insecticide-based interventions. The extent to which it affects natural populations of mosquitoes including their reproduction ability remains uncharacterised. Here, we investigated the potential impact of the glutathione S-transferase L119F-GSTe2 resistance on the mating competitiveness of male *Anopheles funestus*, in Cameroon.

Methods: Swarms and indoor resting collections took place in March, 2018 in Tibati, Cameroon. WHO tube and cone assays were performed on F₁ mosquitoes from indoor collected females to assess the susceptibility profile of malaria vectors. Mosquitoes mated and unmated males collected in the swarms were genotyped for the L119F metabolic marker to assess its association with mating male competitiveness.

Results: Susceptibility and synergist assays, showed that this population was multiple resistant to pyrethroids, DDT and carbamates, likely driven by metabolic resistance mechanisms. Cone assays revealed a reduced efficacy of standard pyrethroid-nets (Olyset and PermaNet 2.0) with low mortality (<25%) whereas synergist PBO-Nets (Olyset Plus and PermaNet 3.0) retained greater efficacy with higher mortality (>80%). The L119F-GSTe2 mutation, conferring pyrethroid/DDT resistance, was detected in this *An.funestus* population at a frequency of 28.8%. In addition, a total of 15 mating swarms were identified and 21 *An. funestus* couples were isolated from those swarms. A comparative genotyping of the L119F-GSTe2 mutation between mated and unmated males revealed that heterozygote males 119L/F-RS were less able to mate than homozygote susceptible (OR=7.2, P<0.0001). Surprisingly, heterozygote mosquitoes were also less able to mate than homozygote resistant (OR=4.2, P=0.010) suggesting the presence of a heterozygote disadvantage effect. Overall, mosquitoes bearing the L119F-S susceptible allele were significantly more able to mate than those with 119F-R resistant allele (OR=2.1, P=0.03).

Conclusion: This study revealed that *An. funestus* swarms can be detected and characterized in the field allowing mating swarms to be targeted to implement alternative vector control strategies. Furthermore this study provides preliminary evidences that metabolic resistance potentially exerts a fitness cost on mating competiveness in resistant mosquitoes.

Title: Residual malaria transmission assessment: Quantification of the relative risk of malaria transmission at different night-time and location in Atacora region in Benin, West Africa

Authors: Rock Aïkpon*, Razaki Ossè and Martin Akogbéto

Background : A significant reduction in malaria incidence has been observed in Africa, due to the extensive use of indoor residual spraying and insecticide-treated net. Even so, residual malaria transmission continues to occur. This study aims to quantify the potentiel risk of the different night-time and location in the context of high coverage of ITNs and IRS in Benin.

Methods : Adult mosquito collections were carried out through Human Landing Catch. Vector species were identified using PCR. ELISA was used to determine sporozoite infections.

Results : The majority of biting occurred late at night with more infective bites observed outdoor. Considerable biting occurred early in the evening. The proportion of malaria transmission occurring before sleeping hours and outdoors is compromising the efforts towards malara control.

Conclusion : There is still significant residual malaria transmission across Benin, particularly in Atacora region despite the high coverage of ITNs and IRS in this area. To curtail residual malaria transmission, additional interventions able to target vectors escaping conventionnal vector control interventions should be prioritized.

Keywords: Residual malaria, quantification, Benin.

Title: Characterization of malaria vectors in prelude to genetic control strategy in four villages in Mali

Author: Niare D, Guindo A, Doumbia S, Diallo B, Yagoure B, Maiga Am, Sylla L, Camara H, Camara CO, Traore SF, Coulibaly MB

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Background: In Mali, malaria vector control is mainly based on the use of long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). These methods have proven efficient but do also have some limits. Due to they partial effectiveness, the search for alternative tools is necessary. A new approach based on the use of genetically modified mosquitoes to control malaria is being developed. However, in order to implement these new tools, it is imperative to understand the bionomics of local mosquito populations. Reason for that this study was initiated to characterize the local malaria vector populations in four villages in Mali (Tiénéguebougou, Kababougou, Sogolombougou and Ouassorola).

Method: Pyrethroid Spray Catch (PSC) and Human Landing Catch (HLC) were conducted monthly from April 2013 to March 2016.

Results: Anopheles gambiae s.l. was the predominant species in Sogolombougou (54, 7%, N=2381) and in Ouassorola (63, 1%, N=3130) respectively while its frequencies were lower in Tiénéguebougou (15,2%, N= 5049) and in Kababougou (28,4%, N= 2294). The peak of the density has been observed in August in the four villages. In terms of biting behavior, there was no significant differences between the number of mosquitoes collected indoors and that collected outdoor. The number of *An. gambiae s.l.* collected was 285 indoor vs 183 outdoor in Tiénéguebougou; 257 indoor vs 218 outdoor in Kababougou; 548 indoor vs 517 outdoor in Sogolombougou; 740 indoor vs 501 outdoor in Ouassorola. The peak of the human biting was observed in both indoor and outdoor of between 2:00 am and 3:00 am at the four sites.

Conclusion: The specific component of the specimen of *Anopheles An. gambiae s.l* is the following in the four villages: *An. coluzzii*, *An. gambiae* and *An. arabiensis. Anopheles coluzzii* was predominant in three villages: Tiénéguebougou, Kababougou and Ouassorola but *Anopheles gambiae* was predominant at Sogolombougou.

Title: Factors influencing Human African Trypanosomiasis (HAT) transmission in three endemic foci in the Republic of Congo

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Background: Human African Trypanosomiasis (HAT) remains a major public health problem in the Republic of Congo, where dozens of cases are recorded annually. The situation remains worrying in most parts of the country. The purpose of the study is to identify the factors favoring the occurrence of HAT cases and the maintenance of the disease in different epidemiological settings.

Description: The study will be conducted in three endemic foci in Congo, Yamba, Ngabé and Loukoléla. The search for parasites will be carried out during active screening campaigns organized jointly with the Congo National HAT control program. During these campaigns, humans and animals will be screened using TDRs and CATT tests followed by case confirmation by PCR analyses. The collected flies will be identified and dissected for the search for trypanosomes and molecular identifications. People living in the area will be submitted to a questionnaire.

Lessons learned: The study discusses the analysis plan put in place in order to collect and analyze field data in order to assess HAT risk in Congo. Through the use of questionnaires on field site, the study also provide useful data on how this information could be crosschecked with other data to have extremely refined information on HAT risk and contributing factors in different epidemiological settings. The awaited result in regard to the general evolution of the disease in Congo and neighboring countries is also discussed.

Conclusion/Next steps: Data recorded from the study will enable the government of Congo to prioritize interventions towards the elimination of HAT in the Republic of Congo.

Keywords: HAT, transmission, Republic of Congo

Title: Impact of refrigerated blood at $+ 4^{\circ}$ C and frozen blood at -20° C on the quality of an *Anopheles coluzzii* colony in the insectary in Mali

Author: Sylla L, Camara H, Yagoure B, Niare D, Doumbia S, Diallo B, Maiga AM, Camara CO, Guindo A, Coulibaly MB

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Background: The quality of blood can have a significant impact on the feeding and corresponding maintenance of wild mosquito colonies. Fresh blood is widely regarded as best but is not always readily available. Here we explore two methods of preservation (4°C and -20°C) over a period of 8 months by assessing impact on feeding, egg yield and subsequent fitness of *Anopheles coluzzii* in Mali.

Method: Human blood was obtained from blood bank under ethical approval and after required testing according to the blood bank's standards. Same age *Anopheles coluzzii* females were fed with blood stored at 4° C or -20° C for up to 8 months. Blood feeding was carried out every month and the quality was assessed by measuring the feeding rate, fertility, fecundity and the emergence rates were measured.

Results: Feeding rates were 97.5% [96,5; 98,2] and 88.3% [86,5; 89,8] for females fed on frozen and refrigerated blood respectively. Over the same period the average number of eggs laid per female in a single oviposition (fecundity) was 38.1 and 18.3 for frozen and refrigerated blood respectively. Fecundity was comparable for the first month and thereafter numbers drop considerably for 4° C vs -20°C stored blood.

There was no significant difference in any of the other parameters assessed; fertility (mean = 96.5% for both), Pupal emergence (mean = 98% for each), size as measured by wing length (mean = 3.1 mm for both) and longevity as measured by the maximum number of days lived by progeny (mean = 41.2 and 41.1 for 4° C and -20° C stored blood respectively),

Conclusion: Frozen blood can be used to blood feed mosquitoes for up to eight months while refrigerated blood may not guarantee good fecundity after one month of conservation at 4° C.

Key word: colony, mosquito, frozen blood, conservation.

Title: Comparative evaluation of resistance development and associated mechanisms between two insecticides and a novel combination in *Anopheles gambiae*

Author: Gonse

Background. Malaria control mainly relies on pyrethroids insecticides through the use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS). However, control efficacy is now threatened by PYRs resistance across Africa. To overcome this, Bayer recently developed a new formulation for IRS (brand name Fludora Fusion) consisting of a combination of deltamethrin (PYR) and clothianidin (neonicotinoid). In this context, this study aims at evaluating the long-term efficacy of this novel formulation through laboratory selection across multiple generations.

Methods. A field-derivated *Anopheles gambiae* strain showing low resistance to insecticides (Tiassalé-S, Côte d'Ivoire) was used as a parental strain and selected at the adult stage for 15 generations (selection pressure 60%-70% mortality) with deltamethrin, clothianidin and Fludora Fusion leading to 4 different lines (Tiassalé-S control, Delta-R, Clothia-R and Fludo-R). The resistances level of each line to each insecticide was followed by bioassays across the selection process and the underlying mechanisms were investigated.

Results. Adult bioassays confirmed the low resistance level of the Tiassalé-S strain to insecticides. Molecular analyses confirmed the presence at low frequency of common target site mutations (Ace1 and Kdr). Deltamethrin resistance remained low for 6 generations and then increased to reach < 20% mortality after 11 generations of selection while only a slight increased tolerance was observed with Fludora-Fusion after 13 generations of selection. Assessing the cross resistance of each line to all insecticides indicated that the Delta-R line showed the highest survival to pyrethroids although a slight increased pyrethroid tolerance was also observed in the Clothia-R and Fludo-R lines. Individual genotyping confirmed the association between target-site mutations and deltamethrin resistance.

Conclusions and perspectives. Although pyrethroid resistance was initially low, resistance to deltamethrin resistance was rapidly selected in link with known mechanisms while resistance to Fludora Fusion remained low. On-going RNA-seq will allow comparing mechanisms selected by each insecticide.

Keyword. Anopheles, insecticide resistance, Fludora fusion, IRS, pyrethroids,

Title: Genetic differentiation of Glossina pallidipes tsetse flies in Kenya

Author: Okeyo

Animal African Trypanosomiasis (AAT) remains a major impediment to agricultural economic growth in Kenya, where the major vector of the parasite that causes it is the tsetse fly *Glossina pallidipes*. The vector has been subject to intense but unsuccessful control measures in some parts of Kenya, creating a need to better understand how populations of this species are genetically connected to improve its control and monitoring. We analyzed 11 microsatellite loci and screened 250 G. pallidipes samples from eight locations in Kenya where tsetse densities are high, and thus risks to animal health. Bayesian and multivariate analyses grouped the samples into two genetically distinct eastern and western clusters (mean $F_{sr}=0.202$) separated by the Great Rift Valley, a well known biogeographic barrier. Locations within the western cluster had lower mean allelic richness (7.48 vs. 10.99) and higher within-cluster genetic differentiation (mean F_{sT} ; 0.183 vs. 0.018) than the eastern cluster. We also found higher mean percentages of related individuals in the western cluster (21.4% vs. 9.1%), and first generation migrants both within and between clusters. Isolation by distance was evident across the study area. We also found higher levels of genetic differentiation among populations from the western cluster relative to the eastern cluster that are consistent with changes in dispersal patterns caused by relatively recent anthropogenic influence such as land use changes and vector control programs. This data can inform both species- and region-specific best strategies for control and monitoring of this vector in Kenva.

Title: Fitness cost of deltamethrin resistance on life-traits of *Anopheles gambiae*, a major malaria vector in Africa

Title: Nkahe DL^{1,2}, Kopya E^{1,2}, Awono-Ambene P¹, Kekeunou Sévilor², Wondji C. S^{3,4}, Antonio-Nkondjio C^{1,4*}.

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Background: Pyrethroid resistance is rapidly expanding across Sub-Saharan Africa. Yet there is still not enough information of the influence of resistance on some life-traits parameters of the vector. This study aims to assess the fitness cost of deltamethrin resistance by comparing a susceptible laboratory strain to a resistant selected colony of *An. gambiae* by studying several life-traits parameters.

Methods: The resistant *An. gambiae* colony was established from mosquitoes collected on the field and selected for12 generations with deltamethrin 0.05%. The laboratory susceptible strain used as control was the Ngousso strain. A total of 100 females of each strains were blood fed and allowed for individual eggs laying in individual ponds, then different life traits parameters such as fecundity and fertility, larval development time and longevity were measured.

Results: The same ability to lay eggs was recorded between both groups (p = 0.1037) whereas, hatching was high in susceptible (p = 0.03488). Deltamethrin resistant larvae were found to have significantly long larval development time (11Days) compare to susceptible (8 Days) (p = 2.951e-15). In the progeny, the number of females was significantly high in susceptible group compare to the resistant colony (p = 0.03195). The longevity was also significantly high for the progeny of susceptible (22Days) compared to resistant strain (15Days) (p = 1.192e-13).

Conclusion: These results suggests that pyrethroid resistance is likely associated to some negative effects on mosquito life-traits. The addition of new tools targeting specifically larval stages could improve malaria vectors control in settings experiencing high pyrethroid resistance.

Key words: malaria vector, An. gambiae, life-traits, deltamethrin resistance, fitness cost.

Title: Susceptibility profile of *An. gambiae* populations to insecticides in the city of Yaoundé Cameroon

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Background: Insecticide resistance has emerged as one of the major challenges affecting malaria vector control in Africa. The present study presents data on the susceptibility status of *An. gambiae* population to insecticide in the city of Yaoundé.

Methods: Batches of 3-5 days old adult female mosquitoes reared from larval collections between 2017 and 2019 in 32 districts of the city of Yaoundé were exposed to all insecticides classes according to WHO guidelines. To also assess the level of resistance, field *An. gambiae* population and a susceptible reference laboratory strain of *An. gambiae* (Kisumu) mosquitoes were exposed to insecticides for a range of exposure times and assessed deaths 24 hours later. Control, dead and survivors mosquitoes from WHO susceptibility tests and only survivors and total population from CDC bottle bioassay were characterized for the target-site mutations (kdr) using Taqman method after DNA extraction from individual mosquitoes according to LIVAK method. Synergist PBO assays were conducted to assess the metabolic resistance. Molecular identification of mosquitoes was also conducted.

Results: High insecticide resistance levels were recorded with WHO susceptibility tests. We found an unexpectedly strong resistance phenotype to insecticides with CDC bottle bioassay in *An. gambiae* populations. Metabolic resistance was also present. Almost all mosquitoes tested had the West Kdr allele L1014F and this resistance allele was found at high frequency. Molecular analysis suggested the presence of both *An. gambiae* and *An. coluzzii* in different districts.

Conclusions: The study shows rapid evolution of insecticide resistance in vector populations of the city of Yaoundé and suggests the need for an integrated vector control approach to improve control mosquitoes in this city.

Keywords: Vector control, Mosquitoes, *Anopheles gambiae*, Insecticide resistance, resistance mechanism, Cameroon

Title: Physical integrity and survivorship of Long-Lasting Insecticidal Nets (LLINs) distributed to households of the same socio-cultural community in Benin, West Africa.

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Abstract Background: Long-Lasting Insecticidal Nets (LLINs) are designed to survive and sustain their physical barrier for 3 years in household conditions. However, studies have shown that most of these nets are usually torn or no longer present in the households before 3 years. This study was initiated in Benin to compare the survivorship and physical integrity of seven types of LLINs in a same socio-geographic area.

Methods: In August 2017, 1,890 households were selected in 9 villages in the municipality of Zagnanado in central Benin. Each one of the selected households received one of the seven LLIN products: Aspirational®, DawaPlus® 2.0, OlysetNet®, PermaNet® 2.0, PermaNet® 3.0, Royal Sentry® and Yorkool®. Overall, 270 LLINs of each type were freely distributed in Zagnanado. These bednets have been monitored and evaluated every 6 months to identify the most resilient and most preferred LLINs in the community. Net survivorship was assessed using the rate of net loss and physical condition.

Results: The survivorship of all types of LLIN was estimated at 92% (95% CI: 90.33-92.96) after 6 months and 70% (95% CI: 67.25-71.81) after a year of use. Only 1.73% of all types of LLIN had a visible loss of integrity after six months with a median pHI estimated at zero. The percentage significantly increased after 12 months with 10.41% of damaged nets (all types of LLINs). The median pHI for each brand of net was 23, 196, 141, 23, 23, 23, 121 and 72 respectively for Aspirational®, DawaPlus® 2.0, OlysetNet®, PermaNet® 2.0, PermaNet® 3.0, Royal Sentry® and Yorkool®. A significant difference was noted between the pHI at 6 and 12 months (p<0.0001). In 12 months, the OlysetNet® significantly suffered more damage compared to the others (P<0.001).

Conclusion: The results of this study revealed that after a year of use, the survivorship of the 7 LLIN products in households was lower than expected. However, all the LLIN products successfully met WHO standards for physical integrity after 12 months of use. The monitoring continues. The next steps will allow us to identify the most sustainable LLINs.

Keywords: LLINs, survivorship, physical integrity, malaria, Benin.

Title: Comparative Characterization of Transmission Profiles and Molecular Mechanisms Driving Insecticide Resistance in the Major Malaria Vector Anopheles Coluzzii From Sahel and Guinea Savannah of Central Africa

Author: Amen Nakebang Fadel

Background & Objective: Insecticide resistance is reversing the effectiveness of core malaria control tools: long-lasting insecticidal nets and indoor residual spraying; partly due to diversity in composition of the malaria vectors in regions with different ecology and heterogeneity of the resistance across sub-Saharan Africa. To facilitate malaria control in Central Africa populations of *Anopheles gambiae* s.l. from Sahel of Chad Republic and Guinea Savannah of Cameroon Republic were characterized.

Materials & Method: Blood-fed *Anopheles* were collected in the rainy season at Gounougou (northern Cameroon), N'Djamena and Massakory (central Chad) and forced to lay eggs. *Plasmodium* infection in heads/thoraces of the F_0 parents was determined using TaqMan assays. Resistance profiles were established using the WHO bioassays, cone bioassays, and synergist bioassays with piperonyl butoxide. Molecular mechanisms driving resistance was investigated by TaqMan genotyping of target site mutations and transcriptional analysis of metabolic resistance genes using RNA-sequencing.

Results & Discussion: Anopheles coluzzii was the major vector in all sites, with a low *Plasmodium falciparum* parasite rate of 4.7% in Gounougou; no infection was detected in Chad populations. Extremely high pyrethroid/DDT resistance was observed in all populations and uncommonly high bendiocarb resistance observed in Chad populations. Synergist bioassays recovered pyrethroid susceptibility implicating CYP450s. Cone bioassays indicated considerable loss of efficacy of bednets, e.g. PermaNet2.0 and PermaNet 3.0 (side panel). High frequency of the 1014F *kdr* mutation was observed in all sites. No *ace*-1 mutation was detected in the bendiocarb-resistant populations from Chad, suggesting metabolic resistance mechanism in play. RNA-seq detected *GSTe2* (e.g. Fold Change: 5x in Gounougou and 4.5x in N'Djamena) and *CYP6Z2* (FC: 3x in Gounougou and 4.1x in N'Djamena) as the most commonly, over-expressed metabolic resistance genes in pyrethroid-resistant mosquitoes compared with susceptible Ngoussou colony.

Conclusion & Recommendation: Multiple resistance in *An. coluzzii* from Central African Savannah is fueled primarily by metabolic mechanisms and poses a significant threat to malaria control/elimination.

Influence of color and presence of organic pollutants on the oviposition behavior and susceptibility status to deltamethrin of *Culex quinquefasciatus* and *Anopheles gambiae*.

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Background: Mosquitoes of the *Culex* and *Anopheles* genus are responsible for a high nuisance and disease transmission in most part of sub-Saharan Africa. However there is still not enough information on their bionomic and oviposition behaviour. The present study assesses the influence of color and organic pollutants presence on *Culex* and *Anopheles* females oviposition behaviour and susceptibility to deltamethrin.

Methods: Laboratory experiments using *Culex quinquefasciatus* and a pyrethroid resistant *Anopheles gambiae* strains were conducted. Gravid females were offered cups of different colors (red, green, yellow, black, purple and white) with water for oviposition their choices were recorded. Experiments were also conducted with cups containing different concentration of NPK fertilizers (1%, 0.75%, 0.5% and 0%) to assess the influence of exposition to organic pollutants on adult mosquitoes susceptibility status to deltamethrin.

Results: *Cx quinquefasciatus* gravids females were founded to be mostly attracted by black color cups with 35.88% of total eggs rafts laid in these cups. This was followed by green color cups 18.93%. Similar findings were recorded with *Anopheles gambiae* gravid females with 43.68% of eggs laid in black cups followed by red cups 28.54%. The F1 and F2 progeny were also found to display a similar oviposition behavior. Rearing larvae in water containing NPK fertilizers was found to accelerate the larval development time of *Culex quinquefasciaus* but not of *Anopheles gambiae*. Insecticide susceptibility tests done with *Culex* and Anopheline exposed during the larval stage to the fertilizer indicated *Culex* to be more susceptible to deltamethrin whereas *Anopheles gambiae* became more resistant to the same insecticide.

Conclusion: The study indicated several factors influencing vector bionomic on the field and the need to consider these variations in order to improve the efficiency of vector control tools implemented on the field.

Keys words: *Culex quinquefasciatus*, pyrethroid resistance, *Anopheles gambiae*, experiments, Color, NPK.

Title: Influence of Physico-chemical and biological parameters on the distribution of anopheline larvae in the city of Yaoundé, Cameroon

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Background: Malaria remains a major public health problem in Cameroon and the city of Yaoundé. Yet factors affecting malaria vectors distribution are not well understood. The present study was undertaken to assess the influence of physico-chemical and biological parameters affecting the distribution of anopheline larvae in the city.

Methods: Mosquito breeding site samplings were conducted monthly in eight districts of the city of Yaoundé from May to December 2017. All the breeding sites were geo-referenced using a GPS (Garmin) and were physically characterised. Physico-chemical measurements were conducted using a Wagtech (9100) portable kit. The following parameters were tested turbidity, pH, TDS, Conductivity, Temperature, Calcium, Hydroxide peroxide, Iron, Aluminium. Bivariate and multivariate regression analyses were conducted to assess any correlation between physico-chemical parameters the presence and the density of anopheline larvae (P < 5%).

Results: Four districts of Yaoundé Nkolbisson, Ekounou-palais and Tsinga were found with high anopheline larvae densities. The presence of anopheline larvae in a breeding site was positively and significantly correlated with temperature, turbidity, pH and distance to the nearest house while, it was negatively correlated with the presence of predators, the size and the depth of the breeding site. However, pH, conductivity, temperature, turbidity and Calcium were significantly correlated with larval density contrarily to the presence of predators, the depth, the surface area and presence of vegetation around the breeding sites. Two sibling species of Anopheles gambiae s.l were identified Anopheles gambiae ss and Anopheles coluzzii. These species were found in sympatry in 22 breeding sites (17, 47%).

Conclusion: The study suggests adaptation of An. gambiae to the urban domain and the need for further action to control this vector in the city of Yaoundé.

Key words: influence, Physico-chemicals, biological, distribution, anopheline larvae, Yaoundé.

Title: Population dynamics and larval habitats characterisation of *Culex* mosquitoes in Yaoundé : a study in prelude to both Bti and Bs-based larviciding control intervention

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Background: A larviciding control intervention was planned in the city of Yaoundé. Prior to this control trial, the present study was designed to collect information on distribution, density, seasonal variation and breeding sites characterization of *Culex* species, responsible in high nuisance and potential vector of Lymphatic Filariasis in the city of Yaoundé.

Methods: Both Human Landing Catches (HLC) and Center for Diseases Control Light Trap (CDC-LT) were used to monitor adult mosquito population in 26 districts of Yaoundé. Following the morphological identification of mosquitoes collected, density and seasonal variation of *Culex* spp were studied. PCR were performed to identify species of *Culex* pipiens complex. All water bodies in each district were assessed for the presence of mosquito larvae. Mosquito larvae were then identified to the genus level and water bodies positive for presence of *Culex* spp larvae were characterized.

Result: A total of 145,076 mosquitoes belonging to 5 genera were collected using both CDC-LTs and HLC. These included *Anopheles*, *Culex*, *Aedes*, *Mansonia* and *Coquillettidia*. With 94.98% of total mosquitoes collected, *Culex* genus was by far the most prevalent. *Culex* spp nuisance consisted of 71.71 bites per man per night and 12.91 mosquitoes per trap per night. *Culex* mosquitoes were present permanently all year long with mean densities varying according to rainfall. *Culex* spp larval habitats were categorized into seven types ; puddles (44.15%), gutters (23.07%) and agricutural trench (16.23%) being the most abundant. The values of some physicochemical parameters varied according to presence or absence of Culex spp larvae.

Conclusion : The permanent presence of *Culex* mosquitoes all year long in Yaoundé indicates that treatment with larvicide must be done regularly irrespective of seasons. During treatment, puddles, gutters and agricultural trench are types of larval habitats to be primarily targeted.

Keywords : Lymphatic filariasis, Culex, seasonal variation, larviciding, Yaoundé

Title: Assessment of the impact of Insecticide Resistance in *Anopheles gambiae* sl on Malaria Prevalence and Incidence in the presence of LLIN intervention in the North of Cameroon

Author: Njeambosay Boris

Introduction: Malaria control relies heaviest on vector control with LLINs being a major control tool. The spread of IR has been reported to compromise control efforts, but studies that provide direct evidence remain scanty. In this study, we measure the impact of IR on parasite prevalence and incidence in areas of high LLIN coverage in the north of Cameroon.

Method: A cross-sectional and a cohort study were respectively conducted during the malaria transmission season to determine the prevalence and the incidence of malaria in children recruited from 33 clusters selected from three health districts in the north of Cameroon. Data on the susceptibility status of the main malaria vector species, *An gambiae* sl, to deltamethrine 0.75%, were obtained following the WHO susceptibility tube test procedure with larvae collected from each cluster. Since resistance was wide-spread, the median 24h mortality was used as cut-off and clusters were classified as high resistant (HR) or low resistant (HR) and malaria parameter were compared.

Results: In the prevalence survey, 2744 children aged 2month- 10 years were recruited. The prevalence in the LR- and HR clusters were 40.9% and 19.4% respectively, and there was a positive relationship between 24 hr mortality and infection prevalence. In the cohort survey, 1980 children aged 2month-5 years were enrolled. The infection incidence in the LR- and HR clusters were 2.4 infection/year and 1.6 infection per year respectively, and there was no significant relationship between infection incidence and 24h mortality.

Conclusion: Even in areas of insecticide resistance LLINs provides protection aginst malaria. This is because they provide a physical barrier between man and the vector.

Keywords: Insecticide resistance, prevalence, incidence, Anopheles gambiae, malaria

Title: High Degrees of Spatial Heterogeneities in Malaria Prevalence Between Tanzanian Villages Formerly Experiencing High Transmission

Authors: Elihaika G. Minja, Johnson K Swai, Emmanuel Mrimi, Rukiyah Njalambaha, Halfan Ngowo And Fredros Okumu

Introduction: Malaria prevalence has significantly declined over the past decade in tanzania, due to scale-up of key interventions such as the long-lasting insecticidal nets (llins), indoor residual sprays (irs) and improved diagnosis and treatment. In the south-eastern districts of ulanga and kilombero, detailed entomological surveys have been done to track the declining malaria transmission and its drivers, but there have been no survey of actual malaria prevalence in recent times. We therefore conducted a cross-sectional active-case detection to assess prevalence *plasmodium* infections and associated factors in 12 wards of ulanga and kilombero districts, south-eastern tanzania.

Methods: Malaria infection was tested using rapid malaria diagnostic test (mrdt), and positive cases confirmed using microscopy and pcr. Structured interviews were done to identify households at risk.

Results: A Total Of 2,912 Individuals Were Tested Across All Villages. Multivariate Analyses Showed No Difference In Malaria Infections Between Males And Females (Or: 1.25 [0.99-1.57], P=0.064), But Living In Urban Or Peri-Urban Areas Was Associated With 99.7% Less Malaria (Or: 0.02 [0.01-0.07], P<0.001). Village By Village Prevalence Varied From As High As 52% (Tulizamoyo) And 43% (Igota) In The North, To 2% (Minepa) Or 1% (Lipangalala) In The Southernmost Part Of The Study Area. Three Wards Including Ifakara Town And Two Of Its Surrounding Wards Had No Positive Malaria Cases Detected. Malaria Prevalence Was Higher Among Under-Fives Than Other Age Groups (Or: 1.25 [0.99-1.57], P<0.001).

Conclusion: These findings highlight high levels of spatial variability in malaria transmission, and major decline in prevalence in some of the villages previously experiencing hyper to holoendemic transmission. Specific underlying drivers are not yet known until final analysis is completed.
Title: Exposure of Anopheles funestus and Anopheles gambiae s.l. to long-lasting insecticidal nets reduces longevity

Authors: Jorelle Ange, Tchakounte Betmbe

Background & Objective: Despite the increased report of insecticide resistance in malaria vectors, its impact on mosquito's life-traits after exposure to insecticide-treated nets remains less characterised. Here, we assessed the effects of exposure to PermaNet 2.0 on several life traits of malaria vectors in Cameroon.

Materials & Methods: Indoor resting mosquitoes were collected using electric aspirators in Centre Cameroon (Obout) in 2016. After assessing the resistance status of F_1 from the field collected-mosquitoes, progeny of the first generation (*An. funestus* s.l.) and seventh generation (*An. gambiae* s.l.) were used to assess the long-term effect of exposure to PermaNet 2.0 on several life-traits of these vectors in comparison to untreated net. In addition, the L119F-GSTe2 mutation associated with DDT/pyrethroids resistance in *An. funestus* was genotyped to assess its association with increased life-span post-exposure.

Results & Discussion: Both *An. funestus* and *An. gambiae* were resistant to pyrethroids and DDT with a greater level in the latter. Pyrethroid-only nets PermaNet 2.0 and Olyset exhibited a significantly reduced efficacy against *An. funestus* (mortality< 20%) in contrast to a greater efficacy for PBO-based Nets Olyset Plus (65% mortality), PermaNet 3.0 top (100% mortality). In both species, mosquitoes that survived exposure to PermaNet 2.0 exhibited a significantly reduced longevity than those non-exposed (6.95 days vs 12.46 for *An. funestus P*<0.001; 8.87 vs 11.25 days for *An. gambiae; P*<0.001). However, no significant difference was observed for blood feeding and fecundity in both species. In addition, molecular analysis of the L119F-GSTe2 mutation revealed that this mutation is associated with an increase in the chance of surviving after exposure to this net in *An. funestus*.

Conclusion & Recommendation: These results show that although the PermaNet 2.0 presents a reduced efficacy against resistant populations, it remains efficient after exposure by reducing life expectancy of the vectors which could contribute in the reduction of malaria incidence. Hence the need to sleep under bednets.

Title: Evaluating the feasibility and impact of house screening as an additional vector control intervention on malaria transmission in 3 southern African countries committed to malaria elimination.

Authors: Sangoro Peter, Fillinger Ulrike, Nkya Theresia, Kochelani Saili, Emmanuel Chanda, Clifford Mutero

Background: It is becoming increasingly clear that in order to further reduce vector densities from the low levels currently achieved with long lasting insecticidal nets (LLINs) or indoor residual spraying (IRS) to lower levels that may lead to faster and sustainable elimination of parasite transmission, these two primary indoor interventions will need to be augmented with additional interventions, for example, house screening to prevent mosquito entry, thus reducing human-vector contact. WHO have in recent years increasingly promoted integrated vector management (IVM), a rational decision-making process for the optimal use of resources for vector control.

Description: The main objective of the study is to implement an IVM demonstration project to evaluate the potential added benefit of integrating house-screening as a readily available but not-widely used vector control tool, in malaria control programmes of countries that are striving for malaria elimination. The project will be executed in three southern African countries namely Zambia, Zimbabwe and Mozambique. The integrated intervention comprising of house screening, community mobilization and LLINs will be compared to the use of LLINs alone in the project countries (Zambia, Zimbabwe and Mozambique). The primary outcomes will be clinical malaria morbidity and indoor mosquito densities, and the costs and benefits of these interventions. The study design will in each country involve a two-arm RCT trial with 400 houses in the intervention and 400 houses in the control arm. Entomological assessments will be made using CDC light traps while RDT will be used to test for P. falciparum infections in order to determine malaria incidence and prevalence.

Lessons learned: House screening is not a common intervention for self-protection against malaria vectors in the study area. There is need to advocate and promote house screening to increase community knowledge on this as an additional integrated vector management strategy for malaria control.

Next steps: Finalise evaluation of the epidemiological and socio-economic impact of house screening intervention among households in the project countries. Depending on the results, advocate for implementation and scaling up of house screening and community mobilization in addition to existing control tools to improve the efficacy, cost-effectiveness and sustainability of vector control, where applicable.

Title: Variation of Phenotypic Resistance and Increasing Kdr Allele Frequency in Western Kenya

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Anopheles mosquitoes are vectors of malaria, a tropical disease in Africa. Rampant use of insecticides to reduce human-vector contact has resulted in development of resistance. The objective of the present study was to determine resistance to multiple classes of insecticide in Homa Bay, a county with mixed crop irrigation. This study determined the frequency of knock down resistance (kdr) and insecticide resistance mechanisms in An. arabiensis malaria vector. Field collected Anopheline larvae were collected within the irrigated area and the non-irrigated area (over 10km from the irrigated area) reared in the insectary and subjected to standard WHO resistance bioassay tests for deltamethrin, melathion and DDT. Species identification was done on the An. gambiae s.l. mosquitoes using conventional PCR and the presence of knock down resistance (kdr) gene was determined using RT-PCR. Morphological identification revealed An. gambiae s.l. as the predominant Anopheline mosquitoes in Homabay. Molecular discrimination by PCR (n=181) reveled 2.21% An. gambiae s.s. and 97.79% An. arabiensis. Mortality against deltamethrin in An. arabiensis was observed at 97.9% (n=324) in irrigated area and 83.9% (n=114) in non- irrigated area while both the irrigated (n=104) and non- irrigated (n=109) areas recorded 100% mortalities against melathion. The frequency of kdr-east was observed to be 1% in the irrigated area (n=51) and 16% in the non-irrigated area (n=70) while the frequency of kdrwest is 9% and 4% in the irrigated (n=51) and non-irrigated (n=58) areas respectively. The frequency of ACE gene was observed to be 0 in both irrigated (n=73) and non-irrigated areas (n=60). From this study, it was established that the level of phenotypic resistance to deltamethrin in non- irrigated area is increasing. However, both zones were observed to be susceptible to melathion. Higher frequency of Kdr-east was observed in non-irrigated areas while kdr-west was observed in both zones with a higher frequency in the irrigated area. These preliminary findings will allow for further studies to investigate key contributors of insecticide resistance and thus provide timely and rational management of the insecticide resistance mechanism in question.

Title: The Forgotten Vectors in Insecticide Resistance Monitoring of Malaria Vectors

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Background: The reduction in global malaria cases since 2000 has been largely attributed to vector control with long lasting insecticidal nets and indoor residue spraying. This steady decline has stagnated, and the World Malaria Report 2018 highlighted an increase in malaria cases between 2016 and 2017. Insecticide resistance in malaria vector is one of the challenges identified. Monitoring of insecticide resistance in malaria vectors is essential for successful deployment of insecticidal vector control tools. Visualizing such data in time and space provides an indication where insecticide resistance may play a role in persisting malaria burden.

Methods: IR Mapper (<u>www.irmapper.com</u>) was launched in 2012 and continues to be updated monthly to geospatially display data from published literature on insecticide resistance in *Anopheles species*. IR Mapper was used for this analysis.

Results: As of April 2019, IR Mapper presented data from 21,123 tests from 436 published articles and reports of both phenotypic resistance and insecticide resistance mechanisms globally. 85.3% of this data is from Africa. *Anopheles gambiae s.l.* and *An. funestus s.l.* accounts for 90.1% and 9.5% of the tests, respectively. *An. coustani, An. pharoensis* and *An. labranchiae* together account for 0.4% of the tests. Among the phenotypic resistance tests between 2010 and 2018 using pyrethroids, 61.5% of the tests on *An. gambiae s.l.* reported confirmed resistance while 76.1% of tests on *An. funestus s.l.* reported confirmed resistance. *An. coustani* was tested only in Angola with no reports of confirmed resistance. There were no tests on *An. labranchiae* and *An. pharoensis* in this period.

Conclusions: All relevant species need to be part of insecticide resistance monitoring programmes, particularly where they are known to transmit malaria and occur in sympatry. Data gaps can present challenges for malaria control and elimination. IR Mapper is useful for identifying such data gaps.

Title: Influence of Water pH conditions on Teneral Reserve Accumulation in *Culex quinquefasciatus* (Diptera: Culicidae): Implication for Cost-effective Vector Control

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Background and Objectives: Water pH conditions of mosquito larval breeding habitats play critical roles in the availability and utilization of food materials essential for biological fitness. This study aims at determining, quantitatively, the roles of water pH on accumulation rates, life stages' mobilization, and utilization of teneral reserves for the processes of metamorphosis (pupation and eclosion) in *Cx quinquefasciatus*.

Materials and Methods: To this end, freshly laid egg rafts were incubated for 24 hours and the newly-hatched first instar larvae (LI) reared in ranges of pH conditions following standard procedures. The pH conditions were broadly categorized into acidic (pH 4.0 - 6.0), neutral (pH 7.0) and alkaline (pH 8.0 - 10.0). At each larval instar (LI – LIV) and life stage (pupa and adult), mosquitoes were randomly analysed for teneral reserve components (lipid, glucose, glycogen and protein) composition following established protocols.

Results and Discussion: Analyses revealed significant influence of pH on the entomological variables measured. Extremes of pH level significantly reduced the accumulation rates of the teneral components in the mosquito species. Mosquito cohorts reared at pH 7.0 had the highest larval daily accumulation rates (lipid, 3.70 ± 0.44 ; glucose, 2.74 ± 0.51 ; glycogen, 7.81 ± 1.01 and protein $6.52\pm0.81 \mu$ g/mosquito/day). At all pH levels, teneral components accumulation climaxed at the fourth larval instar. Pupation and eclosion resulted in

significant reduction in teneral components in the species. With mosquito cohorts at pH 4.0 and 9.0 requiring, respectively, the highest quantity for pupation ($20.25\pm1.98 \mu g/nutrient/mosquito$) and eclosion ($14.01\pm4.38 \mu g/nutrient/mosquito$), respectively.

Conclusion and Recommendation: The study revealed that pH 7.0 is the optimum for development of the species and extreme pH conditions significantly reduce teneral reserves for vectorial proficiency. The information generated could be integrated in the deployment of scarce resources for cost-effective mosquito vector control.

Keywords: Teneral Reserve, Mobilization, Pupation, Eclosion, Accumulation

Title: An Assessment of Ownership and Utilization of Long Lasting Insecticidal Nets in Makarfi Local Government Area, Kaduna State

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Background: Long lasting insecticidal nets (llins) use is an important cornerstone in malaria vector control. The level of ownership and utilization of long lasting insecticidal nets in makarfi local government area of kaduna state was assessed. The null hypotheses posed were thus: residents of makarfi local government area do not own long lasting insecticidal nets; and residents of makarfi local government area do not use long lasting insecticidal nets. The study area is mainly rural in nature and the main economic activity of the people is agriculture. **Methods:** multi-stage random sampling was used in selecting the wards and villages of the study area. Households were selected using systematic sampling. A total of 425 respondents were interviewed using a structured questionnaire. Ibm spss statistics version 20 was used to analyze data. An adult respondent represented each household.

Results: More than half of the sample studied (67.5%) had at least one bed net; and 95% of those who had bed nets reported that the nets were treated. Approximately half (56.1%) of respondents deployed bed nets the night before the survey. The most common reasons for non-use of bed nets were: heat (9.4%), not mosquito season (2.8%) and net size too small for bed (1.2%). Most respondents agreed that use of treated bed nets reduce mosquito bites (95.5%) and malaria (89.9%). The most likely channels of bed net acquisition in the study area were ante-natal clinics (30.6%) and mass distribution campaigns (22.4%).

Conclusion: This study rates ownership of LLINs to be fair and utilization of LLINs to be good, though universal coverage has not been attained. Improvement can be achieved through strengthening of all the channels of LLINs acquisition. gender or 1.07 95% (ci, 0.7 - 1.7)

Keywords: Longlasting insecticidal nets (LLINs), vector control, ownership and utilization.

Title: Assessment of Piperonyl Butoxide (Pbo) Synergist Efficacy on Pyrethroid and Dichlorodiphenyltrichloroethane (Ddt) Resistant Mosquitoes in Lekki, Lagos, Nigeria.

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Background & objective: Vector control forms an integral part of the global strategy for management of mosquito-borne diseases with insecticide application being the most crucial part. Development and spread of insecticides resistance is a major concern in mosquito control. We evaluated the effect of piperonyl butoxide (pbo) synergist on dichlorodiphenyl trichloroethane (ddt) and pyrethriods resistant *anopheles gambiae* s.L., *Culex quinquefasciatus* and *aedes aegypti* in lekki peninsula area of lagos, nigeria.

Materials & method: Larval of *anopheles, culex* and *aedes* mosquitoes collected from different breeding sites in lekki were allowed to emerge in the insectary and 2-3 days old female adults were subjected to susceptibility assays using who kits, test papers and standard procedure. Twenty female adult mosquitoes of each genus were exposed simultaneously to ddt (4%) and permethrin (0.75%) Alone. Subsequently, another similar set were pre-exposed to pbo (4%) for 1 hour before exposure to permethrin and ddt, all assays were carried out in four biological replicates and identification was done using morphological differentiation only. The knockdown time was recorded as the time interval for 60 minutes without any observable recovery and mortality at 24hr were also recorded.

Results & discussion: Resistance to ddt was detected with percentage mortalities of 55%, 60% and 87.5% For *a. Gambiae* s.L., *C. Quinquefasciatus* and *a. Aegypti* species respectively. High level of pyrethriods resistance was also recorded in the three mosquito species in the study location. Pre-exposure of mosquitoes to pbo significantly (p<0.05) Suppressed resistance to both insecticides in all the mosquito species.

Conclusion & recommendation: The result is indicative of the activities of p450 monooxygenase detoxifying enzymes in areas with high levels of ddt and pyrethriods resistance. Therefore, pbo should be incorporated in insecticide resistance management strategies in this area and others with similar mosquitoes' resistance profile for effective control of mosquito populations.

Title: Life Performance of host bloodmeal and its effect on feeding, fecundity and survivorship of Anopheles gambiae mosquitoes

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Background: Host blood feeding, reproduction and adult survivorship of *Anopheles gambiae* mosquitoes are fundamental aspects of malaria vector control efforts. Feeding, a twofold tool, can divert anthropophagic mosquitoes to feed on animals and kill them through treated livestock in areas with LLIN and IRS. Host blood-meal significantly influences the reproduction of *An. gambiae* while its survivorship determines the transmission rate sporozoites. The objective of this study was to determine life performance of host bloodmeal and its effect on feeding, fecundity and survivorship of *Anopheles gambiae*.

Methods: Human, chicken, pig, goat, sheep, and cow blood was presented to three days old *Anopheles gambiae* to feed on. Fully fed were allowed to lay eggs which hatched into larvae. Collected pupae were put in cages and monitored for adult survivorship until the last mosquito died. Statistical analysis was done in SAS 9.1 at P = 0.05.

Results: Human blood had the highest mean (66.3) in feeding while goat blood had the least mean (31.9) of feeding success. The F1 of mosquitoes fed on human (67.50) had the highest mean of eggs while the goat (20.62) gave the least mean of eggs laid. Human blood had the longest (36 days) while cow (23 days) had the shortest survivorship. Day 13 survivorship: chicken 71%, pig 54%, goat 43%, sheep 39% and cow 30%.

Conclusions: Domestic animals can divert *Anopheles gambiae* mosquitoes to feed on them and reduce malaria parasite transmission since they are dead end hosts. Vectorial feat of *An. gambiae* is depended on its reproductive power which has a great influence to mosquito densities. Cow blood which had the shortest survivorship can have few mosquitoes surviving past the extrinsic incubation period of malaria parasites. *Anopheles gambiae* feeding, reproduction and survivorship is vital in the vector control and management.

high in Dakar suburb, Diourbel and in Kedougou. Noteworthily, the Ace 1^{R} (G119S) was frequently found in association with the both kdr mutations.

Conclusion: This study provides a nationwide update and mapping of the insecticide resistance in Senegal, to support targeted and evidence-based vector control intervention to drive toward the malaria control and elimination as aimed by the Senegalese authorities. This work is also a solid support for insecticide resistance management to preserve the current core vector control tools as longer as possible.

Title: Malaria control situation in Cape Coast, Ghana

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Background and Objective: Despite the effort in malaria control, Ghana still contribute 4% to the global malaria cases. In order to make an informed decision on the global malaria agenda, we assessed both human and vector behavior that could affect malaria control in Cape Coast, Ghana.

Materials and Method: Anopheles distribution, feeding behavior, insecticide susceptibility against Deltamethrin 0.05%, Permethrin 0.75%, Cyfluthrin 0.15%, Etofenprox 0.5% and the bio-efficacy of insecticide treated nets (ITNs) used in households were assessed, using larval survey, human-landing-catches, WHO susceptibility and cone bio-assays. Structured questionnaire was also used to gather malaria control behaviors among pregnant women in Cape Coast.

Results and Discussion: An. coluzzii and An. gambiae were the major malaria vectors but their distribution varied in the city. None of the four insecticides caused more than 20% mortality. From human landing catches in 80 man nights, the probability of being bitten outdoors by Anopheles was 0.7%. ITNs and mosquito coil were popular control tools with similar usage on the night before the survey (Chi square=1.84, p = 0.17). The mean mortality (\pm SD) of local An. coluzzii tested against 17 LLINs was 25.4% (\pm 13.1). The frequency of L1014F kdr mutation in the local population was 87%.

Conclusions and Recommendation: Base on the feeding behavior of the local Anopheles in Cape Coast, ITNs could remain an effective malaria control tool. However, the level of efficacy of the ITNs, the level of insecticide resistance and adherence to its use were woefully below expectation. Thus, complete effectiveness of ITNs and its role in halting malaria in Ghana by 2030 remains doubtful, if other strategies are not scale-up to complement it.

Title: Insecticide resistance status and mechanism in *Anopheles gambiae* populations in Senegal in 2018

Authors: Dia A.K., Niang E.a., Samb B., Diedhiou S. Diallo M., Diallo M., Diop A., Faye O., Konate L.

Background & Objective: Like several other sub-Saharan African countries, Senegal has reduced the burden of malaria through the implementation of effective prevention and control strategies, including core vector control tools such LLINs and IRS. Despite the significant progresses, the sustainability of the current insecticide-based vector control interventions is threatened by the spread of vectors resistance to main insecticide used to control them. This study was carried out in 2018 to assess the current insecticide susceptibility/resistance status then characterize the main target site underlying mechanism (kdr and Ace 1^R mutations) among natural populations of *Anopheles gambiae* s.l. across the four eco-epidemiological areas of Senegal.

Materials and Method: Bioassays were performed on 3-5 days-old wild adult females of *An. gambiae* s.l. using WHO test kits for adult mosquitoes. The molecular identification of the *An. gambiae* complex species, and the characterisation of the kdr and Ace 1^{R} mutations were carried out by PCR methods described by Wilkins *et al.* (2006), Huynh *et al.* (2007) and Weil *et al.* (2003) respectively.

Results and Discussion: Molecular identification of *An. gambiae* s.l. species revealed the predominance or exclusive presence of *An. arabiensis* in almost all the study sites, except in Kedougou (sudano-guinean area) where *An. gambiae* and *An. coluzzii* were the most frequent species of the complex.

Results of the susceptibility tests showed that the surveyed population of *Anopheles gambiae* s.l. were resistant to pyrethroids in most of the surveyed health districts, excepted in Dioffior and Ndoffane (in the Sudanese area), where populations were susceptible to deltamethrin. Except a probable resistance recorded in Niayes district and the suburb of Dakar, all the populations were fully susceptible to pirimiphos-methyl. While the resistance to bendiocarb was recorded in most sites of the Sahel-Sudanese area (Dakar suburbs and Diourbel), and probable resistance in the cotton culture area in the south-eastern Senegal (Tambacounda and Kedougou).

Moreover, the assessment of the resistance intensity for pyrethroids (deltamethrin, permethrin and alpha-cypermethrin) has revealed high level resistance only among populations from Dakar suburb as well as from Kedougou. While the intensity was low to moderate in the other district.

Pre-exposure to synergist showed a potential contribution of metabolic mechanism to the observed resistance mostly to Deltamethrin and lesser to Permethrin.

The L1014F (Kdr-w), L1014S (Kdr-e) and G119S (Ace 1^R) mutations were found in all the surveyed health districts. Overall, the allelic frequencies of the three mutations varied spatially, being relatively lower to moderate in the Sahelian and Sudan-Sahelian areas, but



Title: Bionomics and Insecticide Resistance Profiles of Malaria Vectors in A Forested Location Near the Airport of Yaounde, the Capital City of Cameroon

Authors: Nkemingo Francis

Background: Malaria control remains challenging in equatorial regions of africa notably in cameroon. Reducing the burden of the disease requires a better characterization of vector populations particularly in forested regions where the incidence remains elevated. Here, we report an extensive characterization of malaria vectors in a location close to the international airport of yaoundé, the capital city of cameroon, including species diversity, *plasmodium* infection rate, resistance profiles and mechanisms.

Methods: Indoor resting blood-fed adult anopheles mosquitoes were collected from houses in march 2019 in elende, 2km from the airport, and forced to lay eggs to generate f1 adult progeny. Who standard bioassay tests were performed to assess resistance profile to insecticides. The molecular basis of resistance and *plasmodium* infection rate were investigated.

Results: Anopheles funestus s.S was predominant in elende (70%) followed by anopheles gambiae (20%). Its-2 pcr was used to identify the undetermined species. An. Gambiae exhibited high levels of resistance to pyrethroids and organochlorides (<12%% mortality) and to carbamates [bendiocarb (54.3% Mortality)] and organophosphates [(malathion (72.7% Mortality)]. However, an. Funestus showed moderate resistance to pyrethroids [permethrin (86.7% Mortality) and deltamethrin (77.8%)] And ddt (89.1% Mortality) and susceptibility to carbamates [bendiocarb (98.7% Mortality)] and organophosphates (malathion; 100% mortality). The ddt/pyrethroid 119f-gste2 resistant allele (12.5%) Was detected in an. Funestus s.S. The high pyrethroid/ddt resistance in an. Gambiae correlated with the high frequency of 1014f knock down resistance allele. A similar plasmodium sporozoite infection rate was detected in an. Funestus (6%) and an. Gambiae (5%).

Conclusion: These results highlight the challenges control programmes encounter to maintain the continuous effectiveness of insecticide-based interventions in forested regions.

Title: Characterization of the Swarming Behavior of the Natural Population of Anopheles Coluzzii and Anopheles Gambiae in the South and Center of Senegal

Author: Oumou Kalsom Gueye

Background : *Anopheles gambiae* s.S., The major malaria vector in africa, has long been known to exist in nature as two distinct and sympatric populations. Initially described as m and s molecular forms, now they split in two species and named as *anopheles coluzzii* and *anopheles gambiae* respectively. In most of their sympatric areas, the reproductive isolation between the incipient species is thought to be the main barrier to hybridization. However, in senegal, the barrier to the gene flow seems to be porous in some areas with relatively high hybridization rates. Here, we studied the swarming behavior of these two species to investigate its impact on the hybridization.

Methods: The study was carried out in the south and center of senegal during the 2018 raining season. Swarms were surveyed at sunset towards the lightest part of the sky, about 0.5 - 4M above the ground. Once located, swarm were collected using a net. Indoor resting populations was also collected during the same period by pyrethrum spray catch method to estimate the frequency of the two species and hybrids. All specimens collected were identified morphologically then by pcr.

Results: They show that *Anopheles gambiae* swarms mainly over bare ground whereas *anopheles coluzzii* swarms over various objects forming a dark-light contrast with the ground. The height of swarms varies between 0.5 To 2.5 Meters and the swarming duration was about 10 minutes. Start warming was correlated with sunset and no mixed swarm were found in the sympatric area despite the high level of hybridization rate.

Conclusion: Swarming behavior shows a pre-mating reproductive barrier between *Anopheles coluzzii* and *Anopheles gambiae* in senegal. No link was found between swarming behavior and hybridization, but the lack of mixed swarm may be the result of low number of samples obtained in the sympatric area.

Key words: An. Coluzzii, An. Gambiae, hybrids, swarming behavior.

Title: Effectiveness of complementary strategies to long-lasting insecticidal nets on malaria burden and transmission: a four-armed randomized controlled trial in the Korhogo area, Northern Côte d'Ivoire

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Background: Communication for human behavioural changes, indoor residual spraying and larviciding are proved effective against malaria in many settings. However, there are not generalizable evidences that their use in combination with the core vector control tool, long-lasting insecticidal nets (LLINs) provide additional benefit. In an effort to help decision makers in policy making, we conducted a four-armed randomized controlled trial to assess the effectiveness of each of these tools in combination with LLINs on malaria burden and transmission in an area with high pyrethroid-resistance.

Materials and Method: The trial was conducted in 28 villages in Korhogo area, Northern Côte d'Ivoire, from September 2017 to July 2018, after one year of baseline data collection. We selected the villages based on the population size and a minimum distance between villages of 2 km. We randomly allocated eight (8) villages to larviciding with *Bacillus thuringiensis israeliensis*, six (6) villages to IRS with pyrimiphos-methyl and six (6) other villages to intensive communication for human behavioural changes. All these villages as well as the remaining 8 villages (the control group) were covered with LLINs before the implementation of the complementary strategies. We then carried out four (4) entomological cross sectional surveys and five (5) epidemiological cross sectional surveys to measure entomological and epidemiological outcomes.

Results: Data analysis will be completed on June 7, 2019. The results will be presented at the conference.

Title: Impact of P450 metabolic based resistance on the effectiveness of Olyset and Olyset Plus against *Anopheles fune*stus, a major malaria vector in Africa

Authors: Benjamin D. Menze, Murielle. Wondji, Micareme. Tchoupo, Charles S. Wondji

Insecticide-based interventions are reducing malaria burden. Unfortunately, growing insecticide resistance in malaria vectors is threatening these successes. However, the extent of the impact of this resistance notably, metabolic resistance on the effectiveness of LLINs remains unclear. Taking advantage of the recent detection of the first DNA-based molecular marker for metabolic resistance in the Cytochrome P450 genes (CYP6P9a and CYP6P9b), we used an experimental hut study in Cameroon to assess the impact of P450 based metabolic resistance on the performance of LLINs. After experimental hut trial, mosquitoes were genotyped for the CYP6P9a and CYP6P9b to assess its impact.

Findings revealed higher mortality rate with Olyset Plus (99.1%) compared to Olyset (56.4.3%) (P < 0.001). Low mortality was observed with untreated bednets (9.9%). The blood feeding rate was reduced in Olyset Plus (5.6%) vs control (8.5%) but not significantly (P < 0.2). Correlation analysis revealed that homozygous resistant (RR) were better able to survive exposure to Olyset bednets than homozygous susceptible (SS) : (OR, 7.03; CI, 2.5 to 19.4; P < 0.009) for *CYP6P9a* and (OR, 5.1; CI, 1.8 to 14.6; P < 0.01) for *CYP6P9b*. A strong association was also observed between the genes and the ability to blood feed in the presence of Olyset bed nets: (OR= 3.1; p<0.0005) for CYP6P9a and (OR= 8.01; p<0.0001) for CYP6P9b. Further analysis demonstrates that mosquitoes double homozygous resistant (RR/RR) at both genes had by far a significant ability to reduce LLINs efficacy (Blood feeding: RR/RR v SS/SS; OR= 9.3; p<0.0001) compared to single homozygous (Blood feeding RR v SS; OR= 4.5; p<0.001).

Reduced efficacy of LLINs and the increased ability to survive and blood feed of CYP6P9a/b resistant mosquitoes highlight the impact of metabolic resistance. However, the high mortality observed with Olyset Plus suggest that PBO nets are a better solution in area where resistance is mainly mediated by P450.

Title: Mutiple insecticides resistance in dengue vectors, *Aedes aegypti* in southern Benin

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Insecticidal treatments for crop protection have often been cited as the main factor in resistance selection in *Aedes aegypti*, the main vector of yellow fever and dengue fever. To verify this hypothesis, firstly, a protocol was based on the collection of sociological data by interviewing women in households and restaurants in the district of the Dandji regarding the various means available to them to fight against mosquitoes daily.

Moreover, bioassay tests were performed to assess the susceptibility of dengue vectors to various agricultural insecticides where females mosquitoes aged 2-5 days old were subjected to susceptibility tests using four impregnated papers (permethrin at 0.75%; deltamethrin at 0.05% and DDT at 4%, lambdacyhalothrin (0.05%) and bendiocarb at 0.1%).

Finally, considering the massive use of pyrethroids and carbamates in these localities, we have investigated the enzymatic mechanisms (Glutathione-S-Transferase, esterase, monooxygenase P450 and total protein) from the F1 populations (from the parental population After breeding).

Results from this study showed that : **a**)-Sociological data showed that 95% of women use bombs and coils (mostly pyrethroids) to fight against the mosquitoes every time; **b**)- the massive use of bombs and coils have contributed in the resistance of *Ae. Aegypti* populations to organochlorines (10 % as a means of mortality) and pyrethroids (56 % As a means of mortality) but still susceptible to carbamates (100% as a means of mortality); **c**)- the presence of enzymatic activity (Esterase, Glutathione-s-transferase (GST) and P450 monooxygenase) in the wild population of *Ae. aegypti* was significantly higher than the control strain (P < 0.05).

Our research shows for the first time in Benin a mutiple insecticides resistance in dengue vectors, *Aedes aegypti* in Benin

However, base on the massive use of bombs and coils against *Ae. Aegypti* populations, it will be useful to search the knockdown resistance (KDR) as mechanisms of resistance in these populations of *Ae. aegypti*.

Keywords: Aedes aegypti, Insecticides, Résistance, Dandji, Benin

Title: Insecticide resistance status, frequency of L1014F Kdr and G119S Ace-1 mutations, and expression of detoxification enzymes in Anopheles gambiae (s.l.) in two regions of northern Benin in preparation for indoor residual spraying

Authors: Albert Sourou Salako*, Idelphonse Ahogni, Rock Aïkpon, Aboubakar Sidick, Fortune Dagnon, Arthur Sovi, André Aimé Sominahouin, Fiacre Agossa, Laurent Iyikirenga and Martin C. Akogbeto

Background: This study aims to provide baseline data on the resistance status to insecticides, the frequency of mechanisms involved and the impact of the association with the synergist piperonyl butoxide (PBO) on resistant *Anopheles gambiae* (*s.l.*) populations in two regions of northern Benin, prior to an indoor residual spraying campaign and introduction of next generation long-lasting insecticidal nets (LLINs) incorporating PBO.

Methods: Adult *Anopheles gambiae* (*s.l.*) originating from larvae collected in two study regions (Alibori within the Kandi-Gogounou-Segbana districts and Donga within the Djougou-Copargo-Ouake districts) were tested with impregnated papers (bendiocarb 0.1%, pirimiphos-methyl 0.25%, permethrin 0.75% and deltamethrin 0.05%). The synergist PBO was used to check for the involvement of detoxification enzymes in pyrethroid resistant populations.

Molecular analyses were performed for the identification of species within the *Anopheles* gambiae (s.l.) complex and kdr L1014F and G119S Ace-1 mutations. Biochemical assays assessed the activity of detoxification enzymes.

Results: Anopheles gambiae (s.l.) was resistant to pyrethroids, with a mortality range of 25–83% with deltamethrin and 6–55% with permethrin. A significant increase in mortality was observed after pre-exposure to PBO for both deltaméthrine (63–99%) and permethrin (56–99%). With bendiocarb, *An. gambiae* (s.l.) were susceptible in Kandi (99% mortality), with possible resistance (92–95%) recorded in Djougou, Copargo, Gogounou, Ouake and Segbana. All study populations were fully susceptible to pirimiphos-methyl. The frequencies of resistant mutations varied according to species and sites: 0.67–0.88 for L1014F *kdr* and 0–0.06 for G119S *Ace-1*. Three study locations (Djougou, Gogounou and Kandi) showed high oxidase activity and four sites (Djougou, Ouake, Copargo and Kandi) showed elevated esterase activity.

Conclusions: This study confirms resistance to pyrethroids and suggests emerging bendiocarb resistance in *An. gambiae* (*s.l.*) populations in northern Benin. However, recovery of susceptibility to pyrethroids after PBO exposure, and susceptibility to organophosphates in the *An. gambiae* (*s.l.*) populations indicate that next generation LLINs incorporating PBO synergist combined with an indoor residual spraying (IRS) campaign with organophosphate insecticides may be regarded as alternative control tools.

Keywords: Resistance, Anopheles gambiae (s.l.), IRS, Benin

Day 2: Tuesday 24th September 2019

Vector biology and control

Title: Challenges in Maintaining Anopheles Gambiae S.s. Tiassalé Colony Under Laboratory Conditions Over a Period of 9 Years.

Authors: Nukunu E. Akyea-Bobi, Ibrahim K. Gyimah, Charlotte Addae, Godwin K. Amlalo, Joannita Joannides, Dominic Acquah-Baidoo, Samuel Akpor, Alidu Iddrisu, Sampson Gbagba, Rebecca Pwalia, Melinda P. Hadi.

Background and objective: The spread of insecticide resistance worldwide has culminated in the need for new vector control tools. Development of new tools requires susceptible and resistant strains of mosquitoes. *Anopheles gambiae s.s. Tiassal*é strain was successfully colonised in Cote d'Ivoire in 2010 and has been maintained at Vestergaard-NMIMR Vector labs and other laboratories. The study aims to show the challenges with maintaining resistance within the colony over the period of time.

Materials and methods: *An. gambiae s.s. Tiassalé* larvae were collected from farms in Tiassalé, Ivory Coast, raised to adults and colonized. Resistance status of mosquitoes was determined routinely from 2012 to date by conducting WHO susceptibility tests to the four main classes of insecticides, pyrethroids, organochlorines, organophosphates and carbamates. *Vgsc-1014F and ace-1* mutations within the population were characterised using PCR techniques. Previously selection with deltamethrin at larval stages was adopted to maintain resistance. From January 2019 to date, adult mosquitoes were subjected to selection using the WHO tube assay with permethrin 0.75%.

Results and discussion: Over the years resistance to pyrethroids was lost despite selection activities. *An. gambiae s.s. Tiassalé* recorded mortality of 46.7%, 74.5%, 88.6%, 74.15%, 66.65%, 91.75% in 2014, 2015, 2016, 2017, 2018 and 2019 to pyrethroids respectively. *Vgsc-1014F* and *ace-1* mutations recorded from 2014 to 2019 remained fixed between 0.9 to 1.0 and 0.6 to 0.9 respectively. After adult selection with permethrin 0.75%, mortality recorded for pyrethroids reduced to 67.3%. *An. gambiae s.s. Tiassalé* lost their resistance to pyrethroids due to lack of selection pressure, though retained high frequencies to genotypic markers. Reduced genetic diversity in the colony may also be an issue. However, selection from January 2019 increased resistance status.

Conclusion and recommendations: The study is ongoing, and we hope to identify the ideal methods for maintenance of resistant colonies in the lab.

Title: Anopheles leesoni, a member of the Anopheles funestus (Diptera: Culicidae) group: first evidence of the implication in *Plasmodium falciparum* transmission in Cameroon

Authors: Edmond KOPYA^{1,2*}, Cyrille NDO^{1,3,4}, Flobert NJIOKOU², Parfait AWONO-AMBENE¹, Charles WONDJI³ and Christophe ANTONIO-NKONDJIO^{1,3}

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Background: Understanding the biology Anopheles malaria vector species is essential to plan more effective and sustainable malaria control strategies in endemic countries. This study reported the implication of *Anopheles leesoni* in malaria transmission in Cameroon, Central Africa.

Methods: Indoor resting mosquitoes were collected in three localities from May 2015 to March 2018 using electric aspirators and CDC light traps. *Anopheles funestus* s.l. mosquitoes were identified to species using polymerase chain reaction assay (PCR). Furthermore, *Plasmodium falciparum* infection status was analysed using the enzyme-linked-immunosorbent assay (ELISA).

Results: A total of 12744 *Anopheles* mosquitoes were collected by electric aspirator (N=4844) and CDC light traps (N=7900). *An. funestus* s.l. (86.95%) was the major species and the main malaria vector in rural savannah and rural forest sites followed by *An. gambiae* s.l. (13.05%) whereas in urban areas, *An. gambiae* s.l. was by far the most abundant representing 91.45% of *Anopheles* mosquitoes. Two members of the *An. funestus* group were identified among the 1396 analysed by PCR: 1307 *An. funestus* s.s (93.62%) and 89 *An. leesoni* (6.38%). *Plasmodium falciparum* infection rate determined by ELISA was 21.04% in *An. funestus* s.s. For the first time, *An. leesoni* was found positive for *P. falciparum* (infection rate: 13.48%) in Cameroon.

Conclusion: Very high *P. falciparum* infection rate observed in this study in *An. funestus* s.s highlighting the important role in malaria transmission in Cameroon. Furthermore detection of *P. falciparum* infection in *An. leesoni* calls for more attention towards this neglected vector species.

Keywords: Anopheles funestus group, An. leesoni, Malaria, Transmission, Cameroon

Title: Risk management in the vicinity of an arthropod containment level 2 (ACL2) in Burkina Faso

Authors: Roger Sanou¹, Robert K. Ouedraogo¹, Moussa Namountougou¹, M. Megan Quinlan², Andrew R. McKemey², Lucy C. Oliff², Haïda Wandaogo¹, Charles Guissou¹ and Abdoulaye Diabaté¹

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Background: Target Malaria in Burkina Faso has established an ACL 2 Insectary to evaluate novel genetically modified *Anopheles* mosquitoes, under research permits given by National Biosafety Authorities. ACL2 consists of a range of measures to prevent the escape of mosquitoes including; restricted access, training, biosafety procedures and infrastructure. The environment around a facility should also be considered in the risk assessment and management of potential unintended escape of mosquitoes. One aspect of this is the local mosquito population and species composition. The presence and relative abundance of conspecific species will inform the risk of escaped mosquitoes finding an environment where they could survive and potentially propagate. The purpose of outside surveillance trapping is to provide evidence of general trends in mosquito abundance and species composition around the Insectary.

Methods: Monitoring of mosquito fauna around the facility is performed weekly with four resting bucket traps located $\pm 5m$ from the facility to the North, West, South and East. Traps are lined with black cloth and include a wet cloth providing a humid microclimate attractive to resting mosquitoes. Trap openings are orientated towards the West. Traps are placed between 17:00 and 22:00 and collected the following day around 07:00 to 07:30. On collection the bucket opening is covered with a net, preventing escape, before mosquitoes are collected with a Prockopack electric aspirator. Captured mosquitoes are morphologically identified, the different species and number are recorded in a collection datasheet.

Results: Since the collection started in 2016, the most abundant species collected have been *Culex* species with no *Anopheles* species captured.

Conclusion: Although located in a region endemic for *Anopheles* mosquitoes, the urban location and habitat within the vicinity of the insectary are not conducive to *Anopheles*, suggesting a reduced risk that an unintended escape of *Anopheles* mosquitoes from the insectary would survive or propagate.

Key-words: Risk, Monitoring, Resting Bucket Trap, Insectary, Target Malaria.

Title: Characterisation of parasitological, epidemiological and entomological factors which could improve the surveillance of dengue and chikungunya in Cameroon.

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Background: Dengue and chikungunya are arboviral diseases transmitted by *Aedes* mosquitoes, and they are widely distributed across Africa and worldwide. In Cameroon, few cases have being reported in both urban and rural areas despite the wide distribution of their vectors. These diseases are largely overlooked because of the similarity of symptoms with those of malaria. The present project aim to generate information on dengue and chikungunya prevalence and identify factors which could improve their surveillance in Cameroon. More specifically, we will determine the prevalence of dengue and chikungunya among patients consulting for fever in 4 health districts; determine the exposure level of people living around the infection cases and characterise vectors populations involve in disease transmission close to infection cases.

Description: RDT_s for dengue and chikungunya will be performed on wiling patients attending 4 health districts centres in the city of Yaoundé. On positive patients, 100µL of blood will be sampled to confirm the presence of virus according to Trioplex PCR protocol, also to determine the viral strain of dengue involved using RT-PCR. An epidemiological survey will be conducted in the community alongside RDT_s testing and screening of biomarkers in order identify asymptomatic cases and exposure level to *Aedes* mosquito bites. Finally, we will collect *Aedes* mosquitoes to perform adult bioassays according to WHO protocol on reared larval stages and qPCR on collected adult stages to potentially isolate viruses.

Lessons learned: By the end of this study we intend to identify parasitological, epidemiological and entomological factors which could improve the surveillance of dengue and chikungunya in Cameroon such as the usage of RDT_s for clinical diagnosis.

Conclusions/Next steps: This study will enable the national health program to prevent potential dengue and chikungunya outbreaks in Cameroon.

Keywords: RDT_s ; viral strain ; prevalence ; surveillance.

Title: Assessment of urban malaria transmission in the city of Ouagadougou, Burkina Faso

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Background: Urban populations are growing rapidly, particularly in West Africa and this has a major implication for the risk and control of malaria. In the city of Ouagadougou, knowledges about malaria transmission and its vector ecology is not sufficient for appropriate vector control measures.

Methods: Three districts of the city of Ouagadougou were selected using a geographical approach that took into account the city heterogeneity. This approach was based on the analysis of the level of urbanization leading to urban and periurban sites. Timely adult mosquitoes and larva collection were done. Collected mosquitoes were identified and sorted by physiological status and genus. Blood meals sources and Plasmodium circumsprozoite Protein were assessed for anopheline mosquitoes.

Results: In total, 10158 mosquitoes including 7555 adults and 2603 larvae were caught. The population was composed of 71.9% of Culex spp., 22.2% of Aedes spp., 5.7% of Anopheles spp. and 0.2% of Mansonia spp. The majority of Anopheles specimens were caught in peri-urban area (84.2% while only few anophelines were found in central part of the city (15.8%). Puddles and metal dishes were found to be the productive breeding sites for anopheline mosquitoes. The most important part of blood meal source were human (66.7%) with some being mixed blood meal. A large amount of undetermined sources (21.1%) were found in peri-urban area leading to some animals sources not recognized in our antigen set. Plasmodium infection rate was 08.05% with the infectious mosquitoes found to be more prevalent in peri-urban setting (91.7%) compared to urban area (8.3%).

Conclusion: These results provided some useful information on anopheline distribution, breeding site, blood meal sources and sporozoite rate in the city of Ouagadougou. Such data will provide with a better management strategic for urban malaria control.

Key-words: Urbanization, Anopheline, Malaria, Blood meal, Plasmodium, Burkina Faso

Title: Assessing of risk of arboviral disease outbreaks in Brazzaville, major city of the Republic of the Congo based on Stegomyia indices

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Background: Dengue, chikungunya, yellow fever and Zika, are emerging and re-emerging in several countries, including the Republic of the Congo, where a chikungunya outbreak was reported in 2011 in Brazzaville. These diseases are mainly transmitted through infected bite of *Aedes* mosquitoes which major vectors are *Ae. aegypti* (African native species) and *Aedes albopictus* (Asian invasive species). Since 2011 there is no detailed information on larval ecology and infestation level of both species, that why this study aimed to perform a comparative analysis on larval ecology and assess the risk of arbovirus related diseases outbreak based on *Stegomyia* index in Brazzaville.

Methods: Surveys took place in December 2017 in Brazzaville. For this purpose, 27 neighborhoods were selected randomly in 9 districts. In each neighborhood, 10 dwellings were prospected, all the potential and positive containers for *Aedes* were recorded and referenced. Immature stages of *Aedes* found were collected and transported to the insectary, pupae numbers were counted and maintained until adulthood before morphological identification. *Stegomyia* indices: House Index (HI), Container Index (CI) and Breteau Index (BI), Pupae Index (PI) and Pupae per Person Index (PPI), were computed for entomological risk assessment.

Results: In total 269 dwellings were prospected and 3753 *Aedes* collected. 1993 *Aedes aegypti*, and 1760 *Aedes albopictus*, which were mostly found sharing the same breeding sites. Several containers type (455) were recorded and discarded tank was the most prevalent and productive container for both species. *Stegomyia* indices were found varying among the boroughs, but were overall high for both vectors; like BI, which was ranged between 6 to 83% and 20 to 78% respectively for *Ae. aegypti* and *Ae. albopictus*.

Conclusion: This study suggest that Brazzaville is under risk of transmission of arboviruses and gives more details about vectors ecology, which could help to support implementation of vector control strategies.

Title: Characterisation of physico-chemical factors influencing *Anopheles gambiae* larval distribution in districts of the city of Yaoundé (Cameroon)

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Background: For efficient larval source management, understanding vector's bionomic is key for the success of such a programme. The present report presents data of a longitudinal study assessing the influence of some bioecological factors on the distribution of *Anopheles* mosquito larvae in the city of Yaoundé.

Methods: Larval inspections were conducted in 8 districts of Yaoundé. Each water body inspected was mapped using Garmin GPS and recorded as positive or negative after checking the presence or absence of *Anopheles* larvae. Physico-chemical characteristics of breeding sites were recorded using a JENWAY multiparametric probe and Wagtech photometer. *Anopheles* larvae were collected by dipping. Mosquitoes were identified morphologically using identifications keys. DNA extractions and SINE-PCR tests were performed for molecular identification of sibling species of the *Anopheles gambiae* complex.

Results: Anopheles larvae were found in both common and uncommon habitats. Suitable breeding sites for anopheline larvae were small ($<3m^2$), shallow (<1m), without vegetation (0% of coverage) habitats. These habitats were characterized by the absence of Culicine larvae, larvivorous fishes and algae; but also close to households. Temperature, pH, turbidity and the concentrations of calcium, organophosphates and hydroxide peroxide means were high in positives breeding sites compared to negatives (p<0.05). No significant association was found between physico-chemical parameters and larval abundance. Anopheles coluzzii was the most prevalent species (91.49%) followed by Anopheles gambiae (9.51%). The two species were found to breed in the same habitat, temperature and pH were found to affect their distribution.

Conclusion: The study confirms the high prevalence of *An. gambiae* in the urban environment and also provided useful information to improve vector control strategies in the city of Yaoundé.

Keywords: Anopheles, distribution, breeding sites, physico-chemical.

Title: Status of insecticide resistance and sporozoite infection rates in *Anopheles* gambiae s.l in Teso Sub-County, Western Kenya

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Background: Control of malaria transmission heavily relies on the use of insecticide treated nets (ITNs) and indoor residual spray (IRS). However, evolvement of insecticide resistance is a threat to the gains already made. Malaria is endemic in Western Kenya including Teso sub-County. This study sought to investigate the levels of insecticide resistance and sporozoite infection rates in *Anopheles gambiae* s.l in Teso, Western Kenya.

Methods: Adult mosquitoes were collected using CDC light traps and hand catches, while larvae were collected using plastic dippers. Collection was done from randomly selected houses in six villages in Teso, Busia County during short and long rain seasons. Morphological and molecular species identification was done using Gilles de Melion 1968 taxonomic keys and Scott *et al* 1993 respectively. WHO standard bioassays and kdr allele genotyping were used for phenotypic and genotypic resistance analyses. Knock Down resistance genotyping was done by RT-PCR as described by Bass *et al* 2007. Female *An. gambie* s.1 mosquitoes were further screened for *Plasmodium falciparum* sporozoites and those blood fed assessed for host feeding preference by antigen specific Enzyme-Linked Immunosorbent Assays.

Results: A total of 728 mosquitoes were collected:45.47% (n=331) were *Anopheles gambiae s.s* and 54.53% (n=397) *Anopheles arabiensis*. During short rains 218 *Anopheles* were collected (*An. gambiae s.s* 13.6% (n=98), *An. arabiensis* 16.48% (n=120), while long rains collection was 510 (*An. gambiae s.s* 34.75% (n=253), *An. arabiensis* 35.16% (n=256). For indoor and outdoor densities; 75% were trapped indoor (*An. gambiae s.s* 30%, *An. arabiensis* 45%) while 25% (*An. gambiae s.s* 10%, *An. arabiensis* 15%) outdoors. Overall susceptibility to deltamethrin was 69.99% in both *An. gambiae s.s* and *An. arabiensis* and to permethrin was 66.54%. Of the samples collected (n=42), were sporozoite positive. (*An. gambiae s.s* 2.8%, *An. arabiensis* 8.96%. A total of 258 were subjected to kdr analysis; The East allele mutation (L1014S) was detected in (n=208) 80.62% both in *An. gambiae s.s* (n=114) and *An. arabiensis* (n=94). Sporozoite infection and knock down resistance in *Anopheles gambiae* complex species was found to be significantly correlated (R=0.87, P=0.001). A total of 229

were blood fed. Blood meal analysis showed a higher preference in human and cat feeding with 26.05% (*An. gambiae s.s* 8.12%, *An arabiensis* 17.93%) and 21.01% (*An. gambiae s.s* 7.84%, *An arabiensis* 13.17%) respectively, while the rest of the hosts tested recorded a preference of less than 10%.

Conclusions/Recommendations: This study reports presence of insecticide resistance to deltamethrin and permethrin in *An*opheles *gambiae s.s* and *Anopheles arabiensis* in Teso. Also, indicates a significant correlation of insecticide resistance with parasite infection rate in *An. gambiae*. More investigation on this in the entire Western Kenya and mesures put in place to counter insecticide resistance and upsurge of malaria cases.

Title: Comparison of Malaria transmission and vector dynamics during years when IRS was/was not conducted in Senegal

Authors: Abdoulaye Diop¹, Lassana Konate², Seynabou Diedhiou², Abdoulaye K. Dia², Mamadou Demba Sy ², Massila Senghor², Moussa Diagne², Badara Samb², Tiffany Clark ³, Joseph Chabi³, Richard Oxborough³ Libasse Gadiaga⁴, Mame Birame Diouf⁵ Ousmane Faye² Ellen Dotson⁶, Jenny Carlson⁷

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Introduction: In 2018, the National Malaria Control Programme of Senegal discontinued the President's Malaria Initiative (PMI)-funded indoor residual spraying (IRS) programme which had been implemented in the country over the past 10 years. Efforts to ensure access and use of ITNs continued at national level including former IRS districts. To determine the effects of the discontinuation on parameters related to malaria transmission, we compared data from 2017, the last year of IRS, and 2018. The scope of our study focused on entomological parameters, in particular malaria vector densities, behavior, sporozoite infection rates and transmission in Koumpentoum, Koungheul, Malem Hodar and Nioro, districts where IRS was withdrawn.

Method: Malaria vector density, behavior and *Plasmodium. falciparum* infection rates were monitored from July to December in both 2017 and 2018 in the four former IRS districts. Entomological data parameters were collected per site monthly using early morning pyrethrum spray catches (PSCs) and human landing catches (HLCs) during two successive nights both indoors and outdoors of three selected houses

Results: *Anopheles gambiae* s. l. remained the predominant vector in 3 of the districts but the density of *An. funestus* was still higher compared to *An. gambiae s.l.* in Nioro and Ndoffane. Overall biting rates of *An. gambiae* s.l. in IRS sites were very low for both 2017 (0.49 bites/person/night) and 2018 (0.47) after withdrawal. The sporozoite rates recorded during both years of collection were similar (2.69% in 2017 vs 1.54% in 2018) therefore induced approximately the same entomological inoculation rate (EIR) of 0.01 infected bite/ person/night, all sites included. Indoor resting density (1.88 females/house in 2017 vs 2.15 in 2018) and parity rate (54% in 2017 vs 62% in 2018) were slightly higher during the unsprayed year. The same trends were observed with *An. funestus* except the parity rate which decreased in 2018 (0.51 in 2017 vs 0.38 in 2018).

Conclusion: We observed no evidence of increased malaria transmission during the year after IRS withdrawal in Senegal. Though IRS had been halted, a reversal situation of high vector densities and malaria transmission did not occur.

Title: Challenges and ethical implications in vector control trials in south- eastern, Tanzania

Author: Rukia Njalambaha

Background: Entomological assessment of vector control tools which involves human volunteers faced a lot of challenges, includes ethical issues, low-compensations and sometimes lack of information regarding the study. Thus, this study will focus on the challenges and ethical implications occurred to human-volunteers during vector control trials.

Methods: Mosquitoes were collected in each study, to assess their ecological behaviours and response regarding the intervention tested. The collection of mosquitoes, which is often undertaken in such studies, can be dangerous to mosquito collectors, who are sometimes referred to as mosquito bait, and some entomologists still prefer human-landing catches. We will look at the entire entomological process involving human-volunteers, from the laboratory rearing mosquitoes to the test products which are applied in controlled semifield systems and in the field settings. The evaluation will be divided into three phases; Phase 1 will be on the ethical issue facing research participants in vector control research like feeding mosquitoes during the laboratory rearing. Phase 2 will mainly evaluate health risks which human volunteers encounters when exposed to the tested intervention. Phase 3 will be mainly focused on the assessment effect of the entomological products to the community. Also while doing intervention trials individual consent form is not adequate, the research team has a duty to obtain household, community, ward, or district-wide consent, depending on the extent of the spread of the intended trial.

Conclusion: Although the development of mosquito control tools must be tested on humans, fabrics, in their environment, and may cause a lot of harm. In order to avoid facing many ethical and practical challenges inherent in the research team developing new malaria mosquito control tools should provide ethical approvals for Internal Review Board (IRB) and National Institute for Medical Research (NIMR) before studies start to provide comprehensive institutional capacity strengthening based on entomological holistic needs assessments. While undertaking vector trials ensure involvement of majority of stakeholders, promote participatory research and community engagement, and minimize rumour mongering. Since mosquito control trials are community-wide, aim at obtaining and sustaining community consent even though it is much more demanding than in typical cases of individual consent. As there are many justice issues during trials and many more especially when trials are over, they must be adequately addressed.

Title: Measure the dynamics of malaria transmission using entomological approaches at Natitingou, Northern-East of Benin

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To evaluate the level of malaria transmission at Natitingou Northern-East of Benin, a cross sectional entomological study was carried out from April 2016 to March 2017 in urban and rural areas of this city. Adult mosquitoes were collected in both areas by Human Landing Catch and Indoor Pyrethrum Spray Catches (PSC). Entomological parameters like the human biting rate, the *Circunsporizoite protein* (CSP) rate and the Entomological Inoculation Rate (EIR) were evaluated. A total of 21,018 mosquitoes were collected where Anopheles spp which contributed to malaria transmission represented 17.09%. The highest bites of An. gambiae s.l. during the rainy season was found in August at urban (33.75bites/p/n) and rural (25.83 bites/p/n) but, there is no significant different between the average HBR of An. gambiae s.l. in urban area (11.41 bites/ p/n) and in the rural area (8.21 bites/p/n) (P>0.05). Transmission was high during the rainy season (June to November) and low during the dry season (December to May) and was vehicle by An. gambiae Colluzzi (65%). and An. arabiensis (35%). The EIR was significantly higher in the dry season in urban area (0.125bi/p/n) than in rural area (0.021 bi/p/n) (P < 0.05). However, during the rainy season, there was no significant difference between the EIR from urban and rural areas (P > 0.05).

These findings showed that malaria is permanent at Natitingou both in urban and in rural area and was vehicle by *An. Coluzzi* and *An. Arabiensis*. These results will be useful to implement a strategy against malaria in this city.

Key words: Anopheles gambiae, Malaria, Transmission, Natitingou, Benin.

Title: Key role for water storage containers with no specific purpose in *Aedes aegypti* production

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Introduction: There is evidence of continuous low transmission of dengue fever in Kenya throughout the year. In the absence of effective antiviral drugs or vaccines, control of immature mosquitoes remains a key intervention component. In most areas where dengue and other infections transmitted by *Ae. aegypti* are endemic, water is stored due to complete or erratic lack of water supply. Depending on the community, container-stored water may be used for drinking, cooking, bathing, laundry and sanitation, or for no particular purpose. The current study focused on the relationship between the purpose for which water was stored in different types of containers found in houses in Msambweni sub-county, Kwale County, Kenya and *Ae. aegypti* pupal production.

Methods: Three larval surveys were conducted in different study locations within Msambweni Sub-county. In all the 3 surveys participating households were assessed for immature mosquito infestation. All water-holding containers in the peridomestic area were inspected for *Ae. aegypti* larvae and pupae.

Results: The no-purpose containers were the least common containers (9%), yet they contributed 68% of Ae. *aegypti* pupae. Among the different no-purpose container types, container index ranged 9% in drums to 41% in tires. Container index in all other containers ranged between 1% and 41%. Container uses that require hygienic handling of the containers and frequent refilling, as is the case for water used for drinking and cooking, are intuitively protective against *Ae. aegypti*.

Discussion: Containers without any designated use found within peridomestic areas were shown to contribute the majority of *Ae. aegypti* pupae. Water was allowed to remain in these no-purpose containers for more than a week (irrespective of container type), making them important sources of *Ae. aegypti*. Stored water use dictates how often the container is refilled, whether it will be covered or not, and where it will be placed within the household (indoor or outdoor). Source reduction interventions targeting no-purpose containers have the potential to reduce Ae. *aegypti* production significantly.

Title: Blood feeding pattern and seasonal distribution of dengue mosquito, Aedes aegypti (Diptera: Culicidae) in Southern Benin

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To assess the seasonal distribution of *Ae. aegypti* and its blood feeding pattern in Cotonou, southern Benin, a cross seasonal entomological study on adults of *Ae. aegypti* was carried out from May 2016 to April 2017 in southern Benin. *Ae. aegypti* were collected by Human Landing Catches (HLC), Indoor Pyrethrum Spray Catches (PSC), Biogents (BG) sentinel trap and Gravid traps (GT) in order to evaluate the seasonal distribution of *Ae. Aegypti* in southern Benin. Moreover, blood-meal sources were searched from mosquitoes collected using Polymerase Chain Reaction (PCR). During the year of study, a total number of 18,658 mosquitoes were collected. 15,204 were sampled by HLC; 303 with BG trap, 3,038 with PSC, 48 with the GT. From 13,834 females, 1,380 were blood-fed. Adult's collection was high during the two rainy seasons (June to July and October to November) but declined in the two dry seasons (December to March and August to September). The average of Human Biting Rates (HBR) obtained during the rainy seasons (58.62 bites/prn) (p < 0.05). Identification of blood-meal sources indicated humans as the primary host.

These findings showed the presence of *Ae. aegypti* year round at Cotonou in southern Benin. Data on blood feeding patterns of *Ae. aegypti* collected during this study showed that the most important host are humans. These results provide valuable information about potential dengue virus transmission cycles and will help to get a greater understanding about DENV ecology in Benin

Key words: Aedes aegypti, Season, Traps, Blood, Benin

Title: High Plasmodium infection and insecticide resistance in *Anopheles gambiae* from Bankeng, a recent irrigated rice growing village in a forest area in central Cameroon

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Background: The impact on malaria transmission of recent government-led development of large scale irrigated rice farming projects across Cameroon remain poorly characterised. Here, we assessed the impact of a recent rice field farm on malaria transmission risks in a forested area in central Cameroon.

Methods: Mosquitoes were collected in the locality of Bankeng in July 2018 and morphologically identified whereas members of the *Anopheles gambiae* complex were identified to species by PCR. The *Plasmodium* infection rate was assessed using TaqMan genotyping. Susceptibility of *An. gambiae* s.l to different insecticides was assessed using adult mosquitoes obtained from collected larvae. The frequency of knockdown resistance (kdr) mutation in *An. gambiae* population was estimated by PCR whereas the expression of two major P450 genes in *An. gambiae*, CYP6P3 and CYP6M2, was assessed by qRT-PCR.

Results: *An. gambiae s.l* represented 98% of the 1087 mosquitoes collected with *Anopheles gambiae* as the predominant species. The total human biting rate was 44.5 bites/person/ night although higher outdoor (50.2 b/p/n) than indoor (38.9 b/p/n). A sporozoite infection rate of 8.5% (56 infected out of 654) was observed, mainly from *Plasmoduim falciparum* (82.1%). Entomological inoculation rate was 3.8 ib/p/n although r higher indoor (4.9 ib/p/n) than outdoor (1.8 ib/p/n). The Bankeng *An. gambiae* population exhibited high resistance level to almost all insecticides except to organophosphates with mortality rates ranging between 2.5% and 21% for pyrethroids and DDT. A partial recovery of the susceptibility was observed for deltamethrin after pre-exposure to PBO and DEM synergists with a high frequency of *kdr* mutation (93.9%) and a 6-fold over-expression of CYP6P3 P450 gene.

Conclusion: This study revealed an elevated risk of malaria transmission in Bankeng caused by a highly insecticide resistant *An. gambiae* population. These results will guide the implementation of suitable control strategies to protect populations in new rice growing areas.

Key words: Malaria, risk of transmission, irrigated rice, Bankeng, *An. gambiae*, insecticide resistance.

Feeding Behaviour in Anopheline Mosquitoes and Implications for Malaria Transmission in Shendam LGA, Plateau State, Nigeria

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Evidence of malaria vectors changing their behaviors in response to control measures has been documented. These changes include a shift in the biting time and place of biting behavior, where mosquitoes have been observed to bite in the earlier hours of the night before people are under bed nets, or have developed a preference towards outdoor biting to avoid insecticide treated surfaces. These changes could possibly render the current intervention tools such as Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) inefficient. This study investigated the feeding behaviour of anopheline mosquitoes and implications for malaria transmission in Shendam LGA, Plateau State, Nigeria between March and June 2014 using a safe sampling tool known as the CDC light trap. The CDC light traps was set up monthly in randomly selected two houses from 1800 to 0600 hours for 3 nights in both indoor and outdoor points. The trap was placed close to the leg of a person sleeping under an untreated bed net both indoors and outdoors with cups changed hourly. Temperature and relative humidity were recorded at the beginning of each hour. The mosquitoes were then placed in labeled paper cups and morphologically identified, and later preserved in Eppendorf tubes for further processing. A total of 735 (76.40%) anophelines were caught in which the indoor point was higher 568 (59.04%) individuals than outdoors 394 (40.96%). There was a high significant variation ($\gamma^2 = 31.472$, df = 1, P < 0.001) in anopheline abundance between feeding points. Seven female Anopheles species were morphologically identified. An. gambiae had the highest abundance of 718 (97.69%), followed by An. squamosus (7; 0.95%), An. funestus (5; 0.68%), An. maculipalpis (2; 0.27%) and the least were An. pharoensis, An. rufipes and An. coustani, each with only 1 female (0.14%). Abundance between anopheline species varied significantly ($\chi^2 = 4175.5$, df = 6, P < 0.001). Anopheles species feeding peaked in the indoor and outdoor points between the hours of 12-1am and 1-2am respectively as temperature decreases with an increase in relative humidity. The man-biting rate per night from Anopheles species at both points generally increased as the rainy season got under way from March to June (Indoors: 0.5, 3.3, 12.5, 64; Outdoors: 1, 1.3, 2.8, 37). In Conclusion, Anopheles prefers to feed indoors demonstrated by an increase in man-biting rate across the months which possibly suggests a high man-vector contact and a rise in the transmission of malaria and implications for malaria control in this high-risk area.

Title: The Importance of Copy Number Variation in Metabolic Insecticide Resistance in *Anopheles Gambiae*

Authors: Lizzie Tchongwe

Background and objectives - Insecticide resistance is a threat to the success in malaria control due to intensified use of the few available insecticides. Genetic variations in the *Anopheles gambiae* genome are important in increased resistance as evidenced in target site mutations. However, genetic variations such as copy number variations (CNVs) have been poorly studied and their role poorly understood in metabolic resistance. The aim of this study was to identify metabolic resistance genes that exhibit copy number variation and their significance in metabolic resistance.

Materials and methods - Pyrethroid phenotyped samples from Malawi (59) and Kenya (354) were screened for the presence of a specific *Cyp6aa1* duplication known to exist in East Africa (*Cyp6aa1*-Dup1) with PCR. CNV discovery was done using the *Anopheles gambiae* 1000 genomes phase 3 data from Uganda and Tanzania, focusing on known metabolic resistance genes; *Cyp6aa1*, *Gstes* and *Cyp9k1*.

Results and Discussion - A high frequency of *Cyp6aa1*-Dup1 was found in both dead and alive phenotypes. A total of 177 (43 %) individuals had the duplication, 137 (33 %) did not have a duplication and 99 (24 %) were dropouts. The *Cyp6aa1*-Dup1 duplication was significantly associated with the resistance phenotypes. The CNV discovery revealed high CNV frequencies in the candidate genes screened. *Cyp6aa1*-Dup1, so far only known to exist as a duplication, was found to exist as a triplication in the Tanzania data, and a new duplication was found on *Gste2* in 2 individuals from Uganda.

Conclusion and recommendations - These results have shown an importance of gene duplication in pyrethroid resistance, as well as high frequency of CNVs in the three metabolic resistance genes. More work should be done to functionally validate *Cyp6aa1* in *Anopheles gambiae* and understand its role in insecticide resistance and as a diagnostic marker in metabolic resistance.
Title: Contribution of F1534C and V1016I *kdr* mutations to pyrethroid resistance of *Aedes aegypti* mosquito from Somgandé, a district of Ouagadougou, Burkina Faso

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Background & Objective: Pyrethroid-resistance involving *kdr* mutations is widespread in *Aedes aegypti* and may impact control efforts in endemic countries. Since 2006, dengue cases have been sporadic in Burkina Faso until outbreaks of 2016 that occurred mainly in Ouagadougou and of 2017 resulting in 15,074 suspected cases and 36 deaths. These outbreaks highlighted a lack of information on local dengue vectors that is required for their control. In this study, we determined the resistance profile to insecticides and the *kdr* mutations involved in pyrethroid-insecticide resistance of *Ae. aegypti* from Somgandé, a district of Ouagadougou.

Materials & Method: Unfed 3-5-day old females from collected larvae were exposed to insecticide-impregnated papers using WHO bioassay protocol. DNA was extracted from bioassayed mosquitoes and amplified by allele specific-PCR to genotype the V1016I and F1534C *kdr* mutations. Sequencing of the region of the voltage-gated sodium channel gene encompassing these mutations was performed to confirm allele specific-PCR genotypes.

Results & Discussion: The local *Ae. aegypti* population was resistant to pyrethroids with mortalities of 15% for permethrin and 37% for deltamethrin. Mortality was 55% for propoxur and 90% for bendiocarb indicating resistance to carbamates. Susceptibility to organophosphates (malathion and fenitrothion) was observed (mortality>97%). Very high frequency of the F1534C *kdr* allele was found with 93% while the V1016I *kdr* mutation frequency was 46%. The F1534C and V1016I mutations were in Hardy-Weinberg equilibrium and associated to permethrinresistance (OR=6.80, P=0.012 for V1016I and OR=29.50, P<0.001 for F1534C) with a synergistic effect (OR=6.8, P=0.029 for double mutants). Neither F1534C nor V1016I mutations showed any association with deltamethrin resistance in *Ae. aegypti*.

Conclusion & Recommendation: *Ae. aegypti* population in this locality of Burkina Faso is widely resistant to pyrethroids and carbamates insecticides but still susceptible to organophosphates providing useful information for possible future control.

Title: Detecting changes in deltamethrin resistance intensity in sub western Burkina Faso

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Background: Pyrethroid resistance is increasing in strength and this could seriously affect pyrethroid-based control strategies. The class II pyrethroid insecticide deltamethrin is commonly used in long lasting insecticidal treated nets (LLINs) and resistance to this chemical has spread in Burkina Faso. This study evaluates spatial and temporal variation in the intensity of deltamethrin resistance within malaria vector population to inform resistance monitoring.

Methods: Quantitative WHO tube bioassays were performed in the Cascades region of Burkina Faso to evaluate pyrethroid resistance level during the rainy (September-October) and dry (February-March) seasons of 2017 and 2018 in the villages of Tengrela and Tiefora. The lethal concentration resulting in 50% mortality (LC₅₀) within 24 hours of exposure was determined.

Results: Anopheles coluzzii was the predominant species within the An. gambiae complex at Tengrela (> 87%). In Tiefora, the An. gambiae complex fluctuated somewhat between seasons (An. coluzzii: 83% dry and 62% wet season in 2017; 68% dry and 40% wet season in 2018). Overall, the mortality rate in vectors exposed to diagnostic dose for determining deltamethrin resistance was low in the dry season (5.6% (CI: 2.08 - 11.80) vs. 35.4% (CI: 28.19 - 43.70) and 6.9% (CI: 3.02 - 13.14) vs. 15.3% (CI: 10.05 - 21.87) respectively recorded in Tengrela and Tiefora in 2017). The LC₅₀ in vector populations also varied between seasons; being higher in the dry vs. wet season in both localities in 2017 (Tengrela: 0.15 (CI: 0.13 - 0.17) vs. 0.10 (CI: 0.09 - 0.11); Tiefora: 0.19 (CI: 0.16 - 0.22) vs. 0.22 (CI= 0.19 - 0.25). In Tengrela, the estimated LC₅₀ increased substantially between 2017 and 2018 (e.g from 0.10 (CI: 0.09 - 0.11) in wet season 2017 to 0.23 (CI= 0.17 - 0.31) in dry season 2018).

Conclusions: These preliminary results suggest that levels of insecticide resistance in malaria vector populations may vary seasonally, as well as between years. Investigation of potential mechanisms underlying these apparent season fluctuations should be undertaken. If confirmed, such seasonal changes in resistance should be considered when deploying insecticide based control strategies.

Title: Plasmodium falciparum gametocyte carriage and transmission in Anopheles gambiae : Cross-sectional study among children in some public primary schools of Ouidah (Benin)

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Background & Objective: Recently, the progress of malaria control has declined after an unprecedented session of successfulness in worldwide malaria control. Asymptomatic reservoirs of malaria parasites are common yet are difficult to detect, posing a problem for malaria control. However, gametocyte stage of Plasmodium falciparum is considered an important target for disrupting malaria transmission. Identifying natural parameters that influence gametocyte carriage will contribute to a better understanding of the dynamics of the sexual stage parasites for transmission reducing strategies. This study attempted to identify the impact of asymptomatic Plasmodium gametocytes carriage on the transmission in Anopheles gambiae.

Materials & Method: Asexual parasites and gametocytes were detected from 1006 fingerprick blood specimens throughout a cross-sectional study in nine public primary schools of Ouidah using light microscopy. Gametocyte prevalence and density were analyzed according to some natural parameters. Human to mosquito transmission was assessed using standard membrane feeding assays.

Results & Discussion: Plasmodium sp. prevalence was 30% (299/1006). Prevalence of P.falciparum gametocytes was 3.77% (38/1006). Children 8-11 years old had higher gametocyte and asexual parasites densities (1200/ μ l and 97304/ μ l respectively) compared to others age groups. Anopheles gambiae mosquitoes were infected at low gametocytes densities (3.03% mosquitoes at 10/ μ l).

Conclusion & Recommendation: Our findings indicate that asymptomatic children harbor infectious Plasmodium falciparum gametocytes. Interventions to reduce gametocyte density in these localities need to be highly targeted. Therefore, in order to achieve the goal of malaria elimination, there is an urgent need to halt human–mosquito transmission by targeting both symptomatics and asymptomatics Plasmodium carriers.

Key words: P. falciparum, gametocytes, asymptomatic, malaria, Ouidah, Benin.

Title: Measuring the level of malaria risk in rural areas of western Burkina Faso

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Malaria is the main cause of consultation in western Burkina Faso. The natural environment is favourable to the development of mosquitoes, vector of the disease. Despite the number of malaria control programmes, the risk remains high, hence the need to investigate among environmental factors the most influential ones in the development of the disease. The objective of this study is to measure malaria risk in the Houet province (Burkina Faso) through environmental factors and the distribution of the disease prevalence. Malaria cases recorded in 2017 by the Houet province's health centres has been spatialised at the level of health areas. An exploratory regression on 10 variables identified the variables statistically correlated with malaria. The ordinary least squares regression method was used to model the relationships between these variables and malaria. Then, malaria prevalence was interpolated by empirical Bayesian kriging. The result of this modelling was discredited by natural thresholds to determine risk areas and the proportion of the population living there. Exploratory regression showed that precipitation, temperature, proportion of clay in the soil and distance to surface water are the variables statistically correlated with malaria. Ordinary least square regression indicated that these variables explained for 63% of malaria cases. The discretized results of the empirical Bayesian kriging revealed that 23.33% of the province's surface area was at low risk, 52.6% at moderate risk, 22.71% at high risk and 1.36% at very high risk. These areas are respectively inhabited by 14.58%, 21.11%, 13.29% and 51.03 of the province's population. This study assessed the contribution of environmental factors to malaria transmission and to measure the risk in the Houet province. These results could help to improve the effectiveness of malaria control programmes.

Keywords: malaria, risk, prevalence, modelling

Title: The Laboratory Assessment of Behavioural and Physiological Responses of *Anopheles Gambiae* to Piperonyl Butoxide

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Introduction: Malaria occurrence has decreased remarkably across sub-Saharan Africa and this is largely due to the mass distribution of insecticide treated bed nets. However, over the years, mosquitoes have acquired resistance to pyrethroids and this threatens the future effectiveness of bed nets, therefore alternative solutions and compounds are urgently needed. Piperonyl Butoxide (PBO) incorporation in nets treated with LLINs is one potential solution as it is a synergist that enhances the pyrethroid activity by inhibiting the P450 enzymes. However, little is currently known regarding whether exposure to this compound in an LLIN affects the way in which mosquitoes interact with the net.

Aim: This project assessed the impacts of exposure to PBO-LLINs on *Anopheles gambiae* with focus on longevity, behaviour, host-seeking and reproductive capacity.

Methods: The thumb test assay (developed by the McCall group at LSTM) was used to assess mosquito behaviour at the net interface, these were coded on BORIS and exported to SPSS. The experiment compared two strains of *Anopheles gambiae*, Kisumu and Banfora.

Results: The study observed three study arms; untreated net, deltamethrin only and PBOdeltamethrin combination net (Permanet3.0 roof). The results showed a reduction in net contact time and blood feeding duration on the treated nets in comparison to the untreated, additionally, Banfora spent more time on the treated net than the untreated, while Kisumu spent more time on the untreated net. The study also showed that blood meal concentration and oviposition rates are affected by net type and Deltamethrin only net showed higher hatch rates than other treated net types in the Banfora. It was observed that PBO LLINs exhibit repellency properties in Kisumu strain. The experiment revealed mortalities of 100% with the PBO net on both the resistant and susceptible strain.

Conclusion: The PBO LLIN (PN3) is very effective and has significant effects on the longevity, fecundity and blood feeding duration of susceptible and resistant vectors.

Title: Unexpected Anopheles Species Diversity in Western Kenya

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Background: Historically, most vector sampling in malaria endemic areas was done indoors owing to the fact that primary malaria vectors are highly endophilic. With the increased coverage of indoor vector control measures, outdoor vector populations are recently given more consideration. This study aimed to investigate the species composition and malaria transmission potential of *Anopheles* in two locations in Western Kenya.

Methods: Female *Anopheles* mosquitoes were collected using mouth aspiration indoors and cattle baited traps outdoors. Morphological and molecular species identification was done. The sporozoite infection rate was determined for all species.

Results: A total of 2464 female *Anopheles* mosquitoes were morphologically identified. Whilst indoors catches were dominated as expected by primary vectors, *An. funestus s.s* and *An. arabiensis*, an unexpected diversity of *Anopheles* species was collected outdoors. Thirteen distinct *Anopheles* species were found in the study areas; including *An. gambiae s.s, An. arabiensis, An. funestus, An. rivulorum, An. maculipalpis, An. squamous, An. pharaoensis, An. symesi, An. ziemanni, An. caliginosus, <i>An. paludis, An. tenebrosus, and An. coustani coustani.* Secondary vectors such as *An. symesi* and *An. ziemanni* among others were more abundant outdoors than *An. funestus* and *An. gambiae s.s* two of the most important primary vectors in the regions during the season. All sporozoite positive mosquitoes were found indoors in Ahero and outdoors in Mbita. The positive species were *An. funestus* s.s and *An. arabiensis* in both sites. In addition two (2) *An. rivulorum* an outdoor-biting species, tested positive for *Plasmodium* sporozoites in Mbita giving an infection rate of 10% (2/20).

Conclusions and recommendations: Despite the intense indoor vector control interventions during sampling in Mbita, outdoor-biting mosquitoes were associated with residual malaria transmission therefore outdoor control tools need to be developed and applied for effective malaria control and eventual elimination.

Title: Effect of Irrigation on Malaria Vector Population in Western Kenya

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Background and Objective: Environmental degradation exacerbated by climate change has necessitated adaptation programs such as irrigation, in order to increase food security. However, irrigation may increase the risk of malaria through increasing the number of suitable breeding habitats, enhancing habitat stability and productivity and providing an excess of vectors to bridge transmission seasons. The aim of the current study was to evaluate the effect of irrigation on malaria vector populations and malaria transmission risk in western Kenya.

Materials and Method: The study was conducted in a semi-arid irrigated area in Homa bay county, western Kenya from January to December 2018. Indoor resting anopheline mosquitoes were collected using pyrethrum spray catches in eight clusters from irrigated and non-irrigated areas. Mosquitoes were morphotyped and analyzed for distribution, abundance and spatiotemporal density variation. *Anopheles gambiae* sibling species were identified by polymerase chain reaction (PCR).

Results and Discussion: Of the 1,050 vector *Anopheles* mosquitoes collected 97.1% were from the irrigated area while 2.9% were from the non-irrigated area; they comprised of *An. gambiae* s.l. (93.4%) and *An. funestus* s.l. (6.6%). In the irrigated (n=1020) area *An. gambiae* s.l. comprised 93.7% and *An. funestus* s.l. 6.3%. In the non-irrigated area *Anopheles gambiae* s.l. was also dominant 83.3% compared to *An. funestus* s.l. 16.7 %. PCR analysis (n=241) indicated that 99.6% were *An. arabiensis* while 0.4% were *An. gambiae* s.s. There was no significant variation in *An. arabiensis* densities between wet and dry season in either irrigated areas ($t_7 = 0.1$, p>0.05) or in non-irrigated areas ($t_7 = 0.4$, p>0.05). There was a significant variation in *An. arabiensis* densities between irrigated and non-irrigated areas (p<0.05).

Conclusion and Recommendations: The irrigation increased vector population densities. There is potential increase in malaria transmission risk in association to irrigation existing in western Kenya county of Homa bay.

Title: Non-Inferiority testing of Insecticide Treated Nets (ITNs): comparison of results from experimental huts and Ifakara Ambient Chamber tests

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Candidate Insecticide Treated Nets (ITNs) with a claim of improved control of vector borne disease must demonstrate both non-inferiority to the first-in-class product and superiority to the current standard of care on both mortality and feeding inhibition endpoints. Experimental hut trials with free-flying mosquitoes are currently the gold-standard method for evaluating ITNs. However, due to variability in mosquito densities at field sites it may be necessary to carry out large studies to get enough power to measure the non-inferiority of ITNs. Therefore, the WHO has encouraged exploration of other potential alternative test methods including the I-ACT (Ifakara ambient chamber test). In this study, I-ACT and experimental huts were compared for non-inferiority studies.

Three net products were evaluated in fully randomized, double blinded equivalence field studies to compare the performance of the investigational interventions to active comparators following WHO guidelines. Study 1: an alpha-cypermethrin + PBO LN in comparison to alpha-cypermethrin only ITN. Study 2: alpha-cypermethrin and pyriproxyfen ITN against alpha-cypermethrin only ITN. Study 3: deltamethrin incorporated polyethylene ITN compared deltamethrin-coated ITN. The nets were first evaluated in experimental huts and then the same nets were subsequently evaluated in the I-ACT using both pyrethroid susceptible and pyrethroid resistant mosquitoes.

In each experimental hut study, products performed very similarly to active comparators with wide 95% confidence intervals. However, in the I-ACT where 30 mosquitoes of each strain are used each night, confidence intervals were smaller and the studies were powered to detect non-inferiority at the 10% effect difference. Using the I-ACT, after 20 nights of data collection superiority of the PBO product was seen against the CYP450 resistant mosquito strain. A difference in mosquito fertility between the susceptible and resistant strains with the pyriproxyfen net was also observed and for the pyrethroid only net the incorporated product showed superiority over the coated product after 20 washes against the pyrethroid resistant strain. The I-ACT assay is useful for measuring small effect differences between new products needed for non-inferiority studies using both insecticide susceptible and resistant strains.

Title: Geographical distribution and prevalence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), two major arbovirus vectors in Central Africa

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Background: Arboviral diseases including dengue are increasingly spreading in the tropical/subtropical world including Africa. Both epidemic vectors are *Aedes aegypti* and *Ae. albopictus*. *Aedes albopictus* originated from Asia was first reported in Central Africa in 2000, in Cameroon, with the indigenous mosquito species *Ae. aegypti*. Updated knowledge on the distribution and abundance of the major vectors *Ae. aegypti* and *Ae. albopictus* constitutes crucial surveillance action to prepare African countries such as Cameroon, Central African Republic and the Republic of Congo for potential arbovirus outbreaks. Here, we present a survey carried out in three Central African countries to assess the current geographical distribution and prevalence of both vectors.

Methods: Immature stages of *Aedes* were collected in several locations in the Republic of the Congo (nine), Cameroon (28) and CAR (seven) between 2012- 2017. In some important cities of the region like Yaoundé and Bangui surveys were performed in early and late wet season. Immature stage of *Aedes* were transported in to the insectary and reared to adult stage. Adults were morphologically identified, counted and grouped according to species and location.

Results and Discussion: Analysis revealed that *Ae. aegypti* is widely distributed across the Central Africa region whereas *Ae. albopictus* is limited to the southern part, around 6°4'N. However, *Ae. albopictus* is the most prevalent species in all southern locations where both species are sympatric except in some locations as Douala (Cameroon) and Brazzaville (Congo) where *Ae. aegypti* is predominant. This suggests that factors such as climate, vegetation, and building density impact the distribution of both species in central Africa. *Ae. aegypti* was found more abundant earlier in the wet season and *Ae. albopictus* in the late wet season in Bangui (CAR), while in Yaoundé (Cameroon) no major difference was found between both species in both seasons.

Conclusion and Recommendation: This information of arbovirus vectors across central Africa could help in planning vector control programmes against possible outbreak of arbovirus related diseases in the region.

Title: Distribution of *Wolbachia* infection prevalence in natural populations of *Anopheles gambiae* in three different Ecological settings in Burkina Faso.

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Background: Traditional malaria control strategies have reduced the burden of malaria in many endemic areas. However, due to the emergence and rapid spread of insecticide and drug resistance, new control tools are required to sustain the gains. Vector endosymbionts, mainly *Wolbachia*, are known for their ability to protect insects against viruses and are of great interest for their potential using for transmission blocking in Africa, where many diseases of humans and livestock are caused by insect-vectored parasites and viruses. The presence of *Wolbachia* in *Aedes aegypti* prevents dengue and other flavivirus infections in the laboratory. Based on these results, a programme for the dissemination of *Wolbachia*-infected mosquitoes at several test sites around the world was implemented. The prevalence of *Wolbachia* in *Anopheles*, the malaria vector, has not been extensively studied. In order to understand the feasibility of using *Wolbachia* or other bacterial symbionts to control malaria transmission, more information is required on the prevalence of these bacteria in natural populations of *Anopheles* and the relationship between bacteria and malaria infections. In this study, we propose to assess the spatial and temporal distribution of natural *Wolbachia* in field population of *Anopheles gambiae* s.l.

Methods: Mosquitoes will be collected in 3 villages from different climatic zones of Burkina Faso. Two collection methods will be used: mechanical aspiration of mosquitoes resting inside houses, and larvae collection from natural breeding sites. PCR will be conducted on the mosquitoes to determine the species and assess the prevalence of *Wolbachia* and *Plasmodium falciparum*. Sequencing and PCR will be used to determine the presence of other symbiotic bacteria.

Results: Final results from the prevalence evaluation will be available in September-2019.

Conclusion: This study will allow us to better assess *Wolbachia* prevalence and then characterize their diversities in natural populations of *Anopheles gambiae* s.l and provide insight into the interaction between mosquitoes and *Wolbachia*.

A Novel Microsporidian Blocks *Plasmodium Falciparum* Transmission in *Anopheles Arabiensis* Mosquitoes

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Background and Objectives: Global malaria control approaches have been majorly compromised by the evolution of insecticide-resistant mosquitoes. Research is currently focusing on developing more sustainable control tools such as the use of mosquito symbionts. This study aimed at identifying and characterising a novel microsporidian isolated from *Anopheles* mosquitoes in Kenya. The study further demonstrated the correlation of the symbiont with *Plasmodium* within these major malaria vectors to determine its potential use as a biological vector-control.

Material and Methods: Wild *Anopheles arabiensis* were collected in regions around Western and Central Kenya using indoor aspiration technique. The molecular detection and isolation of the novel microsporidian symbiont was done by designing highly sensitive primers targeting the conserved 18S small-subunit RNA region. These were used for both the phylogenetic characterisation of the organism and quantitative PCR to monitor co-occurrence with *Plasmodium*. Fluorescence microscopy was used to show microbial tissue localization and transmission routes among successive generations.

Results and Discussion: Phylogenetic studies demonstrated that *Microsporidia MB* lies within the same clade as *Crispospora chironomi*- previously isolated from nonbiting midges in Siberia. Additionally, fluorescence microscopy showed different spore development stages of *Microsporidia MB* within infected larval midgut tissues. A negative correlation was observed between *Plasmodium* and the symbiont indicating a plausible transmission-blocking agent.

Conclusion and Recommendation: This is the first report of a novel symbiont with a strong refractory effect against *Plasmodium* transmission within *Anopheles arabiensis*. This study developed a highly-sensitive molecular-based assay for the isolation and phylogenetic classification of the novel microsporidia. Furthermore, fluorescence microscopy images developed in this work demonstrate its different spore development stages; key in comprehending the nature of infection of *Microsporidia MB*. Fundamentally, this research offers a baseline to further ongoing work in understanding the biology of transmission and the type of protection conferred by this interesting micro-organism.

Title: Small Scale Field Evaluation 0f Bio-Larvicides for Mosquito Control in Wakiso District in Uganda

Authors: Shady M. A., Kato A. B., Nambatya G., Okedi L. M. O., Okia M., Omujal F., Kigongo S., Mukwaya L., Tarek A-El-Tayeb

Background: A small scale trial of three candidate bio-larvicides: a microbial product Bactivec (*Bacillus thulingiensis* var *israeliensis* - *Bti* SH-14), a microbial product Griselesf (*Bacillus sphelicus* strain 2362) and a photo-larvicide SAFE (chlorophyll a and b) was conducted in Wakiso District, Central Uganda. The bio-larvicides were assessed for the potential of reducing larval populations of mosquitoes in different breeding sites and ecological settings in the Ugandan environment.

Methodology: A descriptive study to assess field bio-efficacy and residual effect of the three bio-larvicides was conducted in defined mosquito breeding sites, applying bio-larvicides at various dosages.

Results: The study indicated that larviciding with Bactivec, SAFE and Griselesf was able to significantly reduce the mean densities of *Anopheles* and *Culex* late stage larvae and pupae per habitat, by an order of magnitude, ranging from 50-100% per day for a period varying between more than one week and more than three weeks, with no significant toxic effect on non-target organisms. Bactivec inhibited adult malaria vector emergence for more than 2 weeks post-treatment, while SAFE and Griselesf inhibited adult malaria vector emergence for more than 3 weeks. None of the bio-larvicides inhibited vector oviposition.

Conclusions: Larviciding using Bactivec, SAFE and Griselesf, can be an effective tool in reducing malaria vector populations and malaria transmission in Uganda. Further, trials with additional larvicides will help the choice of the safest and cost-effective larvicides to be integrated in the Ugandan Integrated Vector Management policy.

Title: Climatic variation and abundance of the vectors of the malaria, in two villages of the district of Kati (Region of Koulikoro, Mali)

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Background: Meteorological and seasonal variations affect the abundance of the malaria vectors. The relationship between climatic parameters and vector populations is important to understand. This study estimates the effect of three climatic variables on the abundance of mosquitoes in two villages.

Methods: The result from longitudinal data а study villages. in two Tieneguebougou (TN) and Ouassarola (OS) over two years of collection: from January 2016 to December 2017. In each village, Pyrethroid Spray Catch was used monthly in twenty five rooms (15 fixed / 10 randomly selected). There are meteorological stations in these villages and the data are downloaded monthly. **Results**: In both villages, the highest mean temperature was in April of both years. The highest mean annual relative humidity in Ouassorola was in September 2016 and in August 2017. In Tieneguebougou, these were earlier in the year in August 2016 and in June 2017.

In three of four possibilities, the most rain was observed in August. The exception belongs to Tieneguebougou in 2017 when July was the wettest month. Anopheles *gambiae s.l.* was the only malaria vector identified in the mosquito catch. The two villages had a similar number of mosquitoes collected every month (t=0.51, d.f=23, p=0.61). In individual models to consider the size of the effect of the climatic parameters on the number of mosquitoes in the sample, temperature explained 4% of the monthly variation (F=1.22; d.f=46.47; p=0.27), rainfall accounted for 47 % (F=20.56; d.f=46.47; p0.001) and relative humidity had the largest effect at 56 % (F=38.91; d.f=46.47; p0.001).

Conclusions: It is possible that relative humidity may be a better proxy for available water, and thus breeding sites, in the environment than rainfall itself and it may also account for a lag between rain and mosquito numbers.

Title: Status of insecticide susceptibility of key malaria vectors in the Kenyan coast

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Background: Malaria vector susceptibility to insecticides is key in the disease transmission elimination campaigns. The current study evaluated the status of insecticide susceptibility in *Anopheles gambiae* and *Anopheles funestus* mosquitoes from selected loci along the Kenyan coast.

Methods: *Anopheles* mosquito larvae were collected from larval habitats around Kilifi using the standard dipping technique. The larvae were reared to resultant adults under insectary conditions and exposed to insecticides using the standard WHO test kit for phenotypic insecticide susceptibility testing. Permethrin, Deltamethrin, Bendiocarp, Fenitrothion and DDT were the insecticides employed.

Results: A total of 1,200 mosquitoes were successfully reared to adults, where 491 were identified as *An. funestus* s.l., 700 identified as *An. gambiae* s.l. and the remaining 9 were *An. pretoriensis*. A proportion of 2.04 % of *An. funestus* s.l. showed some resistance to Permethrin. On the other hand, 3.71 % of *An. gambiae* s.l. showed resistance to Deltamethrin. *An. pretoriensis* exposed were all susceptible to the insecticides tested. There was no significance difference in the observed resistance between Permethrin and DDT. The average insecticide susceptibility level to Permethrin was 96 % (0.92-0.99, 95 % C.I), Deltamethrin - 94 % (0.89-0.99, 95 % C.I), Bendiocarp - 100 % (0.97-1.01, 95 % C.I), Fenitrothion - 100 % (1.00-1.00, 95 % C.I) and DDT - 97 % (0.94-1.00, 95 % C.I)

Conclusion: Although a bigger proportion of the malaria vectors tested showed susceptibility, there was some sort of resistance to pyrethroids. In line with the reference susceptibility test results interpretation, there could be presence of resistant genes in the vector population. The possibility of resistant genes in this vector population cannot be ignored. Further assessment of resistant mechanisms need to be evaluated like *kdr* gene analysis and even metabolic-based resistant testing.

Title: Exposure to the insecticide-treated bednet PermaNet 2.0 reduces the longevity of wild *Anopheles funestus*, major African malaria vector, but *GSTe2*-resistant mosquitoes lives longer

Authors: Ange Tchakounte, Magellan Tchouakui, Mu-Chun Chiang, Williams Tchapga, Kopya Edmond, Patrice Takam Soh, Flobert Njiokou, Jacob M. Riveron and Charles S. Wondji

Background & Objective: Despite the increased report of insecticide resistance in malaria vectors, its impact on mosquito's life-traits after exposure to insecticide-treated nets remains less characterised. Here, we assessed the effects of exposure to PermaNet 2.0 on several life traits of malaria vectors in Cameroon.

Materials & Methods: Indoor resting mosquitoes were collected using electric aspirators in Centre Cameroon (Obout) in 2016. After assessing the resistance status of F_1 from the field collected-mosquitoes, progeny of the first generation (*An. funestus* s.l.) and seventh generation (*An. gambiae* s.l.) were used to assess the long-term effect of exposure to PermaNet 2.0 on several life-traits of these vectors in comparison to untreated net. In addition, the L119F-GSTe2 mutation associated with DDT/pyrethroids resistance in *An. funestus* was genotyped to assess its association with increased life-span post-exposure.

Results & Discussion: Both *An. funestus* and *An. gambiae* were resistant to pyrethroids and DDT with a greater level in the latter. Pyrethroid-only nets PermaNet 2.0 and Olyset exhibited a significantly reduced efficacy against *An. funestus* (mortality< 20%) in contrast to a greater efficacy for PBO-based Nets Olyset Plus (65% mortality), PermaNet 3.0 top (100% mortality). In both species, mosquitoes that survived exposure to PermaNet 2.0 exhibited a significantly reduced longevity than those non-exposed (6.95 days vs 12.46 for *An. funestus* P<0.001; 8.87 vs 11.25 days for *An. gambiae*; P<0.001). However, no significant difference was observed for blood feeding and fecundity in both species. In addition, molecular analysis of the L119F-GSTe2 mutation revealed that this mutation is associated with an increase in the chance of surviving after exposure to this net in *An. funestus*.

Conclusion & Recommendation: These results show that although the PermaNet 2.0 presents a reduced efficacy against resistant populations, it remains efficient after exposure by reducing life expectancy of the vectors which could contribute in the reduction of malaria incidence. Hence the need to sleep under bednets.

Title: What is the role of aestivating mosquitoes in maintaining Plasmodium between wet seasons and in transmission during the dry season?

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Mosquitoes are the engine of malaria transmission and have remained the most effective target for disease control for over a century. The dry season biology of the African malaria mosquito and its consequences for disease transmission represent a key gap in our understanding. Recent findings showed that aestivating mosquitoes survive during the 7 month-long dry season. Whether these mosquitoes maintain malaria during the dry season by low level transmission or whether they act as a reservoir of the parasite besides humans, remains unknown. If so, aestivating mosquitoes will initiate disease transmission after the first rains, even if all humans were successfully cleared of their infection, complicating elimination efforts. We measured mosquito infection rate and malaria case load during the dry season in the Sahel. If mosquitoes act as a plasmodium reservoir, we predict that i) mosquito infection rates during the dry season are moderate to high, and ii) 2-3 weeks after the late dry-season peak in mosquito density (mid-March-early April) malaria case load rise and so will happen after the first rain (June). On the other hand, if aestivating mosquitoes are not infectious, the malaria case load will not change after the late dry season peak or shortly after the first rain. Mosquito sporozoite infection, determined by CSP ELISA on ~10,000 Anopheles coluzzii females collected in the village Ballabougou between 2012 and 2016 showed that contrary to our prediction, infection rate during the dry season was very low (<1%), suggesting that these mosquitoes had minimal exposure to plasmodium, or have lost their infection. Likewise, malaria cases in the regional clinic 5 km from Ballabougou was low and stable throughout the dry season, with minimal case load after the high biting frenzy during the late dry-season peak. Our preliminary analysis provides evidence that aestivating mosquitoes do not act as a reservoir for *Plasmodium* during the dry season and that transmission rate by aestivating mosquitoes is minimal.

Title: Efficacy of a new mode of action compound ImergardTM Wettable Powder for Indoor Residual Spraying compared to Actellic TM measured in a community randomised entomological study

Aurhors: Rose Phillipo¹, Carly Marshall¹, Olukayode Odufuwa¹, Jason Moore^{1,2}, Adam Saddler^{1,2}, Amanda Ross¹, Sarah J Moore^{1,2}

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The emergence of pyrethroid resistance resulted in the gradual replacement of relatively inexpensive pyrethroids, firstly with bendiocarb (carbamate) and subsequently with pirimiphos methyl capsule suspension (CS) (organophosphate) both more expensive alternatives. This increase in cost of IRS contributed to the halving of structures sprayed by Presidents Malaria Initiative (PMI) funded IRS programmes. Therefore, if IRS is to remain an important element in an integrated vector control approach both in Tanzania and the rest of sub-Saharan Africa, alternative insecticides, particularly ones with novel modes of action (MOA) are essential.

A new MOA IRS, ImergardTM wettable powder (WP), was evaluated in a non-inferiority comparison to Actellic, the standard of care IRS in Tanzania. ImergardTM WP has a new physical mode of action (mechanical disruption of the cuticle) which deters the development of resistance and is not toxic to humans, non-target organisms and the environment.

A Phase 3 community level evaluation of ImergardTM WP was executed according to standard WHO approved methodology. The primary outcome is sporozoite rate. Secondary outcomes are Entomological Inoculation rate, residual efficacy of the insecticide up to 8 months (biological efficacy), insecticide susceptibility of target vector species, vector density and vector mortality of wild populations of strongly pyrethroid resistant *Anopheles* (*An.*) arabiensis and *An. funestus* sensu stricto (s.s.). Full data from the trial will be presented.

Title: Bednet migration and implications for bednet durability studies, national distribution campaigns, and vector control.

Authors: Guglielmo, F.¹; Toé, H.²; Sayers, E.¹; Ranson, H.¹; Sagnon, N.²

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Background: Between 2015 and 2017, a study was conducted in two villages in Burkina Faso to assess the durability of long-lasting insecticidal nets (LLINs) in field conditions. Net survivorship and fabric integrity were recorded through surveys at 6, 12, 18, 24, 30, and 36 months interval (AprilOctober) using methodologies proposed by the World Health Organisation. Data suggested high levels of attrition (>75%), largely due to bednet destruction.

Methods: Using bednet IDs, we followed the individual LLINs throughout the three years, and crossreferenced LLIN, household, and compound IDs to trace bednet movement. Patterns of migration were triangulated with ethnographic data collected between 2016 and 2018 in the same region to account for seasonal and intra-household dynamics that could shape bednet use.

Results: Throughout the surveys, 10% of the 545 bednets distributed moved at least once between compounds and 35% between households. The number of individuals and of sleeping spaces recorded throughout the surveys, as well as the number of bednets present in the participant households, varied routinely, following predictable religious and socio-economic dynamics of human migration.

Conclusions: In Burkina Faso, bednets are considered to be useful enough to be carried, moved, and exchanged. Tracing bednet migration is vital to plan population census, national procurement, replacement, and the timing of bednet distribution campaigns. While further research is necessary in order to make these data comparable with those from other countries, more precise sociodemographic information about bednet users and about interhousehold allocation should be collected in order to improve bednet coverage.

Title: Infection by Trypanosoma congolense influence field Glossina bacteriome

Author: Jean Marc Tsagmo Ngoune

Background: A number of reports have demonstrated the role of insect bacterial flora on their host's physiology and metabolism. The tsetse host and vector of trypanosomes responsible for human sleeping sickness (human African trypanosomiasis, HAT) and nagana in animals (African animal trypanosomiasis, AAT) carry bacteria that influence its diet and immune processes. However, the mechanisms involved in these processes remain poorly documented. This underscores the need for increased research into the bacterial flora composition and structure of tsetse flies. The aim of this study was to identify the diversity and relative abundance of bacterial genera in *Glossina palpalis palpalis* flies collected in two trypanosomiasis foci in Cameroon.

Methods: Samples of *G. p. palpalis* which were either negative or naturally trypanosomepositive were collected in two foci located in southern Cameroon (Campo and Bipindi). Using the V3V4 and V4 variable regions of the small subunit of the *16S* ribosomal RNA gene, we analyzed the respective bacteriome of the flies' midguts.

Results: We identified ten bacterial genera. In addition, we observed that the relative abundance of the obligate endosymbiont *Wigglesworthia* was highly prominent (around 99%), regardless of the analyzed region. The remaining genera represented approximately 1% of the bacterial flora, and were composed of *Salmonella*, *Spiroplasma*, *Sphingomonas*, *Methylobacterium*, *Acidibacter*, *Tsukamurella*, *Serratia*, *Kluyvera* and an unidentified bacterium. The genus *Sodalis* was present but with a very low abundance. Globally, no statistically significant difference was found between the bacterial compositions of flies from the two foci, and between positive and trypanosome-negative flies. However, *Salmonella* and *Serratia* were only described in trypanosome-negative flies, suggesting a potential role for these two bacteria in fly refractoriness to trypanosome infection. In addition, our study showed the V4 region of the small subunit of the *16S* ribosomal RNA gene was more efficient than the V3V4 region at describing the totality of the bacterial diversity.

Conclusions: A very large diversity of bacteria was identified with the discovering of species reported to secrete antiparasitic compounds or to modulate vector competence in other insects. For future studies, the analyses should be enlarged with larger sampling including foci from several countries.

Keywords: *Glossina*, Bacterial flora, Sleeping sickness, Nagana, Trypanosome, Metabarcoding.

Title: Habitat Characterization And Insecticide Susceptibility

Author: Najat Kahamba

For the past few decade malaria, a disease transmitted by *Anopheline* mosquitoes has been significantly reduced. Despite of such achievement, there is worldwide a growing concern about other mosquito-borne diseases for instance dengue, yellow fever, chikungunya and zika all transmitted by *Aedes* mosquitoes. Recently in sub Saharan Africa, outbreaks of these diseases have been reported in several countries and the viruses have been detected in places where there is no outbreak yet. Though it is widely distributed in urban areas, disease distribution, mosquito species dynamics and susceptibility of mosquito to public health pesticides remain unknown across small towns and rural areas in Tanzania. Therefore, the aim of this study was to characterize the aquatic habitats of *Aedes aegypti* in Ifakara town and its surrounding wards, and to assess their susceptibility status to common public health insecticides.

The study area was divided into grids $(200m \times 200m)$ using GIS technique. Grids with human habitation and buildings were randomly selected to search and identify aquatic habitats. Once the aquatic habitats were identified, they were characterized for their physical properties (such as size, location, water clarity, water movement, vegetation, presence of shades and surrounding environment). From these habitats larvae of *Aedes* were collected and reared in the insectary for insecticide susceptibility tests which was evaluated using standard WHO guideline.

A total of 41,465 larvae were collected, 63% (n=25,916) were *Aedes aegypti*, 33.8% (n=14,015) *Culex* and 3.6% (n=1,493) were other *Aedes*. The most common identified habitats used were tires, clay pot, flower pot, garage pits, coconut shells, pits, wells, tree holes and containers. Habitats with clear water and shades were the most commonly infested with *Aedes*. From the analysis it has been revealed that the larval density significantly differs across the habitats, wards and seasons. During dry season susceptibility status was relatively higher in most of the tested insecticides. A reduced susceptibility during wet season was observed to 0.1% bendiocarb for Mlabani, Viwanja sitini and Lipangalala stains.

These findings provides a baseline information on the infestation of *Aedes* species which is very useful to plan for vector control programs against arboviral infections in southeastern part of Tanzania. But also, the *Aedes* resistance status findings is very important for strengthening the current intervention against the vector.

Title: Perspectives of key stakeholders in Tanzania on alternative technologies for malaria elimination

Authors: Marceline Finda, Nicola Christofides, Javier Lezaun, Brian Tarimo, Prosper Chaki, Ann Kelly and Fredros Okumu

Background: Global burden of malaria has significantly reduced since 2000 due to both public health efforts and improvements in socioeconomic conditions. As we approach elimination however, it is crucial to understand and address socio-cultural factors associated with persisting transmission. Novel technologies such as mass releases of genetically-modified mosquitoes, mass drug administrations of Ivermectin and larviciding require strong collaborations between scientists and communities to ensure the technologies are effective and meet local needs. This study aims to use innovative anthropological techniques to bridge current gaps between researchers and key stakeholders on various issues relative to alternative malaria control technologies.

Methods: Focus group discussions (FGDs) were held with stakeholders including scientists, policy makers, officials from regulatory bodies and community members in Tanzania. The discussions focused on exploring opinions of the stakeholders on the need for alternative technologies for malaria elimination, and their views on novel technologies that are aiming towards elimination. This is an on-going study.

Preliminary findings: All participants of the FGDs agreed that while malaria prevalence and incidence have declined over the past decade, currently available strategies and their levels of utilization are not sufficient for malaria elimination in Tanzania by 2030. However there were varying opinions among the stakeholders on which alternative technologies should be put in place to achieve elimination by 2030. Popular technologies across the stakeholder groups included larviciding, genetically-modified mosquitoes, and spatial repellents. Less preferred options included space-spraying of mosquitoes and mass drug administration of Ivermectin. Community members and scientists also preferred improving housing for the lowest income people for malaria control, but policy makers challenged the sustainability of this strategy given its high costs. More FGDs are in progress and more comprehensive findings will be shared during the conference.

Title: Effects of nectar phytochemicals on life-history traits of the invasive Asian tiger mosquito, *Aedes albopictus*

Author: Teresia Njoroge

Plant nectar is an essential component of adult mosquito diets. During nectar-feeding, mosquitoes ingest various phytochemicals, including phenolics, terpenoids, and alkaloids. Compared to other nectar-feeders (e.g., bees), the ecological significance of nectar phytochemicals for mosquitoes has not been extensively explored. The aim of this study was to conduct laboratory-based assays to evaluate the effects of nectar phytochemicals on the longevity, fecundity and sugar-feeding behavior of adult female Aedes albopictus. Longevity was assessed by subjecting newly emerged females to 10% sucrose solution containing quercetin, p-coumaric acid and caffeine and recording daily mortality. For fecundity assays, the mosquitoes were subjected to the same dietary phytochemicals for seven days and eggs counted following a single blood meal. The cold-anthrone test was used to quantify the amount of sugar consumed by mosquitoes exposed to the dietary phytochemicals. Dietary quercetin and pcoumaric acid were associated with lifespan extension (mean longevity= 69 and 67 days, respectively, compared to control =54 days). Although caffeine ingestion resulted in low sugar consumption (33.84 µg/µl) compared to the control group (67.84 μ g/ μ l), none of the three phytochemicals affected the fecundity of gravid females. Our results demonstrate that dietary phytochemicals can affect mosquito longevity and sugar-feeding behavior and thereby may influence their fitness and vectorial capacity. We are currently conducting an experiment using a whole transcriptome-based approach to identify mosquito-nectar phytochemical interactions at the genomic level.

Title: Microbiomes Underlying Mosquitoes Along the Kenyan Coast

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Coastal climate, Urbanization and globalization have contributed greatly towards the spread of mosquitoes as disease vectors. Mosquitoes harbour microbiomes, which could be explored as vector bio-control agents along the Kenyan coast. However, the distribution of potential microbiomes in mosquitoes along the Kenyan coast has not been well understood.

We hypothesized that there could be differential distribution of target microbiomes for mosquito biocontrol. Key objective was to screen various microbiomes associated with mosquitoes along the Kenyan coast.

Mosquitoes were sampled from three sites at the Kenyan coast and reared to larvae, pupae and adult mosquitoes at Pwani University laboratory and were tested for various microbiomes at the International Centre of Insects Physiology and Ecology. Specific primers were used to tease out the microbiomes bionomics.

The results showed that various microbiomes including microsporidia, *Crispospora spp*, *Spiroplasma spp*, and *Wolbachia* spp were present in larvae, pupae and adult mosquitoes sampled along the Kenyan coast. It would be interesting to extend the screening to a larger part of the Kenyan coast and target *Aedes aegypti* possible exploratory control effort of these diseases.

Key words: Microbiomes, Mosquitoes, Kenyan Coast, Biocontrol

TITLE: ETUDE PRELIMINAIRE SUR LES CONNAISSANCES ATTITUDES ET METHODES DE LUTTE ANTIVECTIRIELLE DES POPULATIONS DE QUATRES LOCALITES URBAINES ET RURALES DE L'OUEST CAMEROUN

Résumé: O. E. Ngo Hondt · W. Ekoko · P. Ntonga Akono · P. Awono Ambene · R. Ngaha · L.G. Lehman

Introduction: Deux enquêtes relatives aux connaissances du paludisme et des arboviroses, ainsi qu'aux attitudes et pratiques des populations vis-à-vis des moustiques ont été réalisées dans les départements du Haut-Nkam et du Ndé dans la région de l'ouest Cameroun.

Méthodes: Les enquêtes se sont déroulées dans les localités urbaines de Bangangté (5°09'00''Nord, 10°31'00''Est) et Bafang (5°09'23''Nord, 10°10'44''Est) et les localités rurales de Bakassa (5°07'24''Nord, 10°14'52''Est) et Bangoua (5°13'13''Nord, 10°27'461'') en février 2019. Les informations ont été recueillies à l'aide d'un questionnaire CAP. Des équipes de deux se déplaçaient de maison en maison, et les questions étaient posées à des adultes responsables. Les questions portaient sur le paludisme et les arboviroses, les vecteurs et mode de transmission ainsi que les méthodes de lutte antivectorielle utilisées par les populations locales. Les données recueillies ont été ensuite analysées à l'aide du logiciel SPSS 20.

Résultats: Le taux de nuisance était de 7,05% en zone urbaine et 16,84% en zone rurale. En zone rurale, seul 1,12% des populations interrogées font le lien entre les moustiques et les arboviroses contre 0% en zone urbaine. 56,02% et 71,35% des populations font le lien entre les moustiques et le paludisme respectivement en zone urbaine et en zone rurale. Les méthodes de lutte antivectorielle les plus utilisées sont les fumigènes, les plantes à essences (frottis) et les décoctions, tant en milieu urbain qu'en milieu rural. En zone urbaine, 81,81% utilisent les fumigènes contre 38% en zone rurale et des décoctions à 13,64% en ville uniquement. Par ailleurs, des plantes locales à essence répulsive sont utilisées sous forme de frottis à 4,55% en ville et 62% en zone rurale. La moustiquaire est connue par la majorité de personnes interrogées et presque pas utilisée du fait du faible taux de nuisance. Le moyen de protection contre les piqures de moustiques en journée reste l'utilisation des vêtements longs.

Conclusion: La faible nuisance Culicidienne dans ces localités peut être due à l'altitude et aux températures qui peuvent descendre jusqu'à 16°c dans la nuit. A cela pourraient s'ajouter les vents et les plantes à essence très utilisées comme plantes ornementale et sous forme de fumigène.

Keywords : Enquête CAP ; Paludisme ; arboviroses ; plante à essence ; Ouest Cameroun.

Title: Distribution of *Anopheles gambiae*. sl larvae in the city of Yaoundé and spatial mapping of the landscape (Cameroon)

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Background: The rapid and unplanned urbanization of African cities is considered to increase the risk of urban malaria transmission. The present study objective was to assess factors influencing the spatio-temporal distribution of *Anopheles gambiae* sl larvae in the city of Yaoundé.

Methods: All water bodies were checked monthly for the presence of mosquito larvae from March 2017 to May 2018 in 32 districts of Yaoundé. Physico-chemical characteristics including the size, depth, turbidity, pH, temperature, conductivity, sulfates, orthophosphates, hydroxide peroxide, conductivity, iron and calcium were recorded and analyzed according to anopheline larvae presence or absence. High resolution satellite images from landsat sentinel ETM were used for spatial mapping of both field and environmental variables. Bivariate and multivariate logistic regression models were used to identify variables closely associated with anopheline larvae presence.

Results: A total of 18,696 aquatic habitats were sampled and only 2,942 sites (15.7% of sites) contained anopheline larvae. A high number of sites (\geq 69%) presented late instar larvae (L3, L4 and pupae). Larvae of anopheline mosquitoes were sampled in a high variety of breeding sites. Puddles were the most common (51.61%), followed by tire prints, wells and drains (12.85%, 11.72% and 11.34% respectively). Bivariate logistic regression analyses associated anopheline larvae presence with the absence of predators, absence of algae, absence of vegetation and depth of less than 1 m. Conductivity, turbidity, organophosphates, H₂O₂ and temperature were significantly high in breeding sites with anopheline larvae than in breeding sites without larvae (P<0.1). Both *Anopheles coluzzii* and *An. gambiae* were recorded. GIS mapping indicated a heterogeneous distribution of anopheline breeding habitats in the city of Yaoundé. Land cover analysis indicated high variability of the landscape of the city of Yaoundé.

Conclusion: The data confirms adaptation of *An. gambiae* to the urban domain in the city of Yaoundé and calls for urgent actions to improve malaria vector control.

Key words: Larval habitats, Anopheles dynamics, malaria, GIS, Yaoundé

Title: Preliminary post-distribution assessment of household usage of LLINs and their residual efficacy against Anopheles gambiae malaria vectors in sentinel sites in Cameroon.

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Background: Following the national distribution of Long Lasting Insecticidal Nets (LLINs) in 2015-2016, the Cameroon National Malaria Control Program has identified the constant monitoring of the effectiveness of this vector control strategy as relevant to assess his impact in the field. To provide a baseline set of data on community use of LLINs and on their residual lethal effect against natural vector populations, we conducted cross-sectional surveys one year following the second round of national distribution of LLINs in 2015-2016.

Methods: These surveys consisted of: i) household investigations on the use and washing conditions of LLIN and ii) assessment of residual efficacy of used LLINs exposed to a laboratory susceptible *An. gambiae* strain and to anopheline vector strains from six sentinel sites belonging to regions of North (Pitoa + Garoua), North-West (Bamenda), East (Bertoua), South (Kribi) and Littoral (Douala). The household surveys were based a questionnaire to collect the net use, coverage and washing pratices of LLINs, and the cone bioassays were performed to check for the residual efficacy and mortality rate due to LLINs against both laboratory and field *An. gambiae* mosquitoes.

Results: The overall number of households investigated was 1,135. In the selected regions, the net distribution coverage in 2016 reached 97%, and the effective use rate 88%. The percentage of households showing compliance with national/WHO recommendations for net washing practices was less than 41%. The 25 used nets checked for their bioefficacy were coated with α -cypermethrin (76%), 5 with permethrin (20%) or with deltamethrin (4%). All used nets revealed optimally effective against reference susceptible strain (100% mortality) and a remarkable reduction of induced mortalities against *Anopheles gambiae* s.l. field populations.

Conclusions: this finding suggests the need of supplementary and integrated actions that will promote community education and the development of alternative vector control tools. **Key words:** household usage, LLINs, residual efficacy, *Anopheles gambiae*, malaria, Cameron.

Title: Impact of copper on the mortalities of *Anopheles* populations which have different insecticides susceptibility profiles in laboratory breeding sites

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Background: The rapid rise of insecticide resistance is threatening the efficiency of malaria vector control interventions. However, current knowledge of factors inducing pyrethroid resistance, the main insecticide used in vector control remains incomplete. In the present study, the effect of copper on the life story of resistant wild anopheles was investigated.

Methods: Four strains of Anopheles were selected for this experiment: Kisumu (Susceptible strain), VKPER (Permethrin resistant strain), Ladji wild Anopheles (Resistant) and Houeyiho wild Anopheles (Resistant). These strains were reared in a simulated breeding site containing copper. Six replicates, 4 tests (exposed) and 2 controls (non-exposed) of 5 larvae each were made for each of the strains, Larvae were fed with Tretramin baby fish food. Mortality recorded after 24, 48, 72, 96, 120, 144 and 168, Hrs. Alive mosquitoes were reared in insectarium till emergence.

Results: Globally, we recorded higher tolerance levels to Cu for was higher in the insecticide resistant field strains of anopheles (Ladji and Houeyiho) followed by resistant laboratory strain (VKPer) and finally, the susceptible laboratory strain Anopheles Kisumu which was far less tolerant to Cu contaminations. Exposure to Cu had no impact the emergence rate

Conclusion: The present study shows that Copper can actually threaten the development of *An. gambiae* larvae even if it have no effect on their emergence. More works still on to assess the contribution of Cu in cross-resistance selection to lambdacyhalothrin

Key words: Anopheles populations, insecticides susceptibility, contaminated breeding, copper, and life story

Title: Seasonal Malaria Vector and Transmission Dynamics in Western Burkina Faso

Author: Patric Stephane Epopa

Background: In the context of widespread mosquito resistance to currently available pesticides, novel, precise genetic vector control methods aimed at population suppression or trait replacement are a potentially powerful approach that could complement existing malaria elimination interventions. Such methods require knowledge of vector population composition, dynamics, behaviour and role in transmission. Here we characterized these parameters in three villages, Bana, Pala and Souroukoudingan, of the Sudano-Sahelian belt of Burkina Faso, a region where bed net campaigns have recently intensified.

Methods: From July 2012 to November 2015, adult mosquitoes were collected monthly using pyrethroid spray catches (PSC) and human landing catches (HLC) in each village. Mosquitoes collected by PSC were identified up to species level, to reveal the seasonal dynamics of local vectors. Monthly entomological inoculation rates (EIR) that reflect malaria transmission dynamics were estimated. Finally, population and EIR fluctuations were fit to locally-collected rainfall data to highlight the strong seasonal determinants in this region.

Results: The principal malaria vectors found were in the *Anopheles gambiae* complex. Mosquito abundance peaked during the rainy season, but there was variation in vector species composition between villages. Mean survey HLC was similar across villages and ranged from 18 - 48 mosquitoes/person/night. The resulting monthly EIRs were extremely high during the rainy season (0.91 - 2.35 infectious bites/person/day) but decreased substantially in the dry season (0.03 - 0.22). Vector and malaria transmission dynamics generally tracked seasonal rainfall variations, and the highest mosquito abundances and EIRs occurred in the rainy season. However, despite low residual mosquito populations, malaria infected mosquitoes remained present in the dry season.

Conclusion: These results highlight the important vector control challenge facing countries with high EIR despite the recent campaigns of bed net distribution. As demonstrated in these villages, malaria transmission is sustained for large parts of the year by a very high vector abundance and high sporozoite prevalence, resulting in seasonal patterns of hyper and hypo-endemicity. There is, therefore, an urgent need for additional vector control tools.

Keywords: Vector control, Genetic control, Seasonal dynamic, Anopheles gambiae s.l., EIR

Title: Mosquito surveillance and malaria transmission dynamics in Taveta sub-county within Taita-Taveta County, Coastal Kenya

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Background: Mosquito surveillance is a prerequisite in evaluating mosquito densities to access disease transmission within the population. Proper estimation of mosquito densities is helpful in monitoring mosquito abundance, disease transmission and advise policy makers on impact of vector interventions.

Materials and Method: Six villages representing different ecological and hydrological (irrigated vs non-irrigated) set up in the area were used for vector-borne diseases surveillance including Kiwalwa, Mwarusa, Njoro, Kimundia, Kingwareni and Challa. Adult mosquito collections indoor and outdoor using backpack aspirators and Center for Disease Control (CDC) miniature light trap inside 10 selected houses. All *Anopheles* mosquitoes were identified morphologically and sibling species of *An. gambiae* and *An. funestus* complex further identified using rDNA Polymerase Chain Reaction (PCR) technique. All the *Anopheles* collected were tested for sporozoite infectivity using *P. falciparum* sporozoite enzyme linked immunosorbent assay (ELISA) technique.

Results: A total of 1,627 *Anopheles* mosquitoes were collected in 6 villages within Taveta Sub-county. The species composition for the *Anopheles* mosquitoes included *An. gambiae* (78.05%, n = 1,270), *An. funestus* (8.05%, n = 131), *An. coustani* (11.86%, n = 193), *An. pretoriensis* (1.54%, n = 25), *An. pharoensis* (0.37%, n = 6) and *An. squamosus* (0.12%, n = 2). Most of *Anopheles gambiae* and *An. funestus* mosquitoes were collected from Kiwalwa while the least were collected from Challa. For mosquitoes collected indoors, only *An. gambiae* was found to have *P. falciparum* infections. Mosquitoes collected resting in the houses through backpack aspiration had the highest sporozoite infection rates (3.06% for *An. gambiae* and 2.99% for both *An. gambiae* and *An. funestus*). For mosquitoes collected to have sporozoite infections. *An. coustani*, *An. gambiae* and *An. funestus*) were found to have sporozoite infections. *An. coustani* had the highest sporozoite infection rate (3.66%) followed by *An. funestus* (1.18%) and *An. gambiae* (1.13%).

Conclusion: Anopheles gambiae, An. funestus and An. ccoustani are responsible for indoor and outdoor transmission of malaria in Taveta Sub-county. Irrigated areas had more Anopheles mosquitoes compared to non-irrigated areas. For effective malaria control strategies in this area, outdoor biting/resting mosquitoes should be targeted using complementary vector control tools.

Title: Annual Estimates of Entomological Indicators in Zambia During Three Years of Indoor Residual Spraying with Pirimiphos Methyl

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Background: Indoor residual spraying (IRS) is an important vector control strategy for the National Malaria Elimination Program in Zambia. Due to pyrethroid resistance, Zambia has used an organophosphate—pirimiphos-methyl (Actellic 300CS)—for IRS since 2012.

Description: The U.S. President's Malaria Initiative (PMI) Africa Indoor Residual Spraying (AIRS) Project conducted IRS annually in 2015, 2016, and 2017 with pirimiphosmethyl in Luapula, Northern, Muchinga, and Eastern provinces in Zambia. Following each IRS campaign, monthly vector surveillance using three standard collection methods, monthly evaluation of residual efficacy of pirimiphos-methyl by cone bioassays, and yearly insecticide susceptibility testing by WHO tube assays were performed. Entomological monitoring was conducted in seven districts at two sentinel sites, one sprayed and one unsprayed per district.

Lessons learned/Findings: *Anopheles funestus* s.l. was the predominant malaria vector identified during the three years of surveillance. The average number of mosquitoes per collection effort in the sprayed sites was 4.35, 8.87, and 6.44 for *An. funestus* s.l. and 0.36, 0.42 and 0.44 for *An. gambiae* s.l. in 2015, 2016, and 2017, respectively. In the sprayed sites, the indoor density of *An. funestus* s.l. one month after IRS fell by 17% in 2015, 28% in 2016, and 56% in 2017, while concomitant increases of 73, 60, and 47% were observed at matched unsprayed sentinel sites. Vector densities returned to pre-spray levels at five to six months after IRS indicating a waning effect of the insecticide sprayed. The overall residual efficacy of pirimiphos-methyl was four to five months. Local vectors were susceptible to pirimiphos-methyl during all three years of surveillance.

Conclusions/Next steps: IRS with primiphos-methyl was effective at reducing mosquito populations in this area of Zambia, but the duration of the effect was short relative to the transmission season. Alternatives such as use of longer lasting insecticides, twice per year spray, or use of next generation LLINs may need to be considered.

Title: Patterns of Anopheline feeding/resting behaviour and *Plasmodium* infections in North Cameroon, 2011-2014: implications for malaria control

Authors: Wolfgang Eyisap Ekoko^{1,2}, Parfait Awono-Ambene¹, Jude Bigoga³, Stanislas Mandeng^{1,4}, Michael Piameu^{1,5}, Narcisse Nvondo⁴, Jean Claude Toto¹, Philippe Nwane¹, Salomon Patchoke⁶, Lili Ranaisse Mbackop^{1,4}, Jerome Achille Binyang^{1,4}, Martin Donelly⁷, Immo Kleinschmidt^{8,9}, Tessa Knox¹⁰, Arthur Mbida Mbida², Alain Dongmo², Etienne Fondjo⁶, Abraham Mnzava^{10,11}, Josiane Etang^{1,12,13}

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Background: Variations in malaria vector feeding and resting behaviours alongside infection rates influences the outcome of vector control strategies, thus effective malaria control relies on evidence-based interventions. Anopheline behaviour and *Plasmodium* infections were investigated in North Cameroon, following Long Lasting Insecticidal Net (LLIN) distribution in 2010.

Methods: During four consecutive years from 2011 to 2014, adult mosquitoes were collected indoors, outdoors, and in exit traps during the high malaria transmission seasons in 38 localities selected in the Garoua, Pitoa and Mayo-Oulo health districts. Anophelines were morphologically and molecularly identified, then analysed for blood meal origins and *Plasmodium falciparum* circumsporozoite protein (*Pf*-CSP) infectivity rates.

Results: Overall, 9,376 anophelines belonging to fourteen species/sibling species were recorded with *An. gambiae s.l.* (*An. arabiensis* (73.3%), *An. coluzzii* (17.6%) and *An. gambiae s.s* (9.1%)) the predominant (72%), followed by *An. funestus s.l.* (20.5%) and *An. rufipes* (6.5%). The 3 sibling species of the *An. gambiae* complex were found indoors (38-55%) as well as outdoors (18-39%) and in exit traps (16-35%) suggesting variable resting behaviour. A great variability was also observed in their trophic sources, among which 28% had human blood meals and 25% animal blood meals, mainly cattle (15.6%) and sheep (11.6%). Mixed blood meals where recorded as well. *An. funestus* and *An. rufipes* presented exophilic tendency (up to 33% outdoor biting rates), coupled with zoophagic tendency (27% animal blood meals) . *Pf*-CSP rates were higher indoors (3.2-5.4%) versus outdoors (0.8-2.0%), and increased yearly (p<0.03).

Conclusion: The current study reveals behavioural plasticity of the major malaria vectors and continuous *Plasmodium* infection in the presence of LLINs in households thus supplementary interventions to LLINs are needed in North Cameroon.

Keywords: Mosquitoes, Vector behaviour, Alternative hosts, Long Lasting Insecticidal Nets, Malaria infections, North Cameroon.

Title:ABS-19-0230

Title: Using a miniaturized double-net trap (DN-Mini) to assess relationships between indoor-outdoor biting preferences and physiological ages of two malaria vectors, *Anopheles arabiensis* and *Anopheles funestus*

Authors: Alex J. Limwagu, Emmanuel W. Kaindoa, Halfan S. Ngowo, Emmanuel Hape, Marceline Finda, Gustav Mkandawile, Japhet Kihonda, Khamis Kifungo, Rukiyah M. Njalambaha, Damaris Matoke-Muhia and Fredros O. Okumu.

Background:Effective malaria surveillance requires detailed assessments of mosquitoes biting indoors, where interventions such as insecticide-treated nets work best, and outdoors, where other interventions may be required. Such assessments often involve volunteers exposing their legs to attract mosquitoes (i.e. human landing catches (HLC)), a procedure with significant safety and ethical concerns. Here, an exposure-free miniaturized double-net trap (DN-Mini) is used to assess relationships between indoor-outdoor biting preferences of malaria vectors, *Anopheles arabiensis* and *Anopheles funestus*, and their physiological ages (approximated by parity and insemination states).

Methods: The DN-Mini is made of UV-resistant netting on a wooden frame and PVC base. At $100 \text{cm} \times 60 \text{cm} \times 180 \text{cm}$, it fits indoors and outdoors. It has a protective inner chamber where a volunteer sits and collects host-seeking mosquitoes entrapped in an outer chamber. Experiments were conducted in eight Tanzanian villages using DN-Mini to: **a**) estimate nightly-biting and hourly-biting proportions of mosquitoes indoors and outdoors, **b**) compare these proportions to previous estimates by HLC in same villages, and **c**) compare distribution of parous (proxy for potentially-infectious) and inseminated mosquitoes indoors and outdoors.

Results: More than twice as many *An. arabiensis* were caught outdoors as indoors (p<0.001), while *An. funestus* catches were marginally higher indoors than outdoors (p=0.201). *An. arabiensis* caught outdoors also had higher parity and insemination proportions than those indoors (p<0.001), while *An. funestus* indoors had higher parity and insemination than those outdoors (p=0.04).

Conclusions: Malaria vectors that are behaviorally-adapted to bite humans outdoors also have their older, potentially-infectious sub-populations concentrated outdoors, while those adapted to bite indoors have their older sub-populations concentrated indoors. Here, potentially-infectious *An. arabiensis* more likely bite outdoors than indoors, while potentially-infectious *An. funestus* more likely bite indoors. These observations validate previous evidence that even outdoor-biting mosquitoes regularly enter houses when young.

Title: Abundance and Diversity of *Culex* Mosquitoes in Pristine And Disturbed Habitats in a Tropical Rainforest of Southwest Region of Cameroon

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Background: anthropogenic changes have been linked to the recent emergence of certain infectious diseases. Little data exists on how deforestation affects the overall species diversity in mosquito communities such as *Culex* despite their known role in the transmission of diseases. We proposed to monitor the abundance and diversity of *Culex* mosquitoes in three habitats along a gradient of anthropogenic disturbance in a tropical rainforest of South west region of Cameroon.

Methods: the collection survey of mosquitoes was conducted in 2017 in three types of habitats (unlogged forest, selectively logged forest and young palm plantation) using net traps, sweep nets, resting traps and larval dipping from breeding sites. Immature stages were reared to adults and mosquitoes were identified to species with the aid of a stereomicroscope and morphological identification keys.

Results: a total of 2,081 *Culex* mosquitoes were collected (n=527-25.32% in the unlogged forest, n=1,077-51.75% in the selectively logged forest and n=477-22.92% in the young palm plantation with a significant difference (p<0.005) between the sites. The average diversity indices (Shannon and Simpson) were high in the selectively logged forest and low in the unlogged forest. According to Chao1 and ACE indices, the highest richness was found in the selectively logged forest and the lowest richness was found in the young palm plantation on clear-cut forest.

Conclusions: the results of this study show that habitat modification due to the conversion of rainforest into palm oil plantation affects the abundance and diversity of mosquitoes and favours the invasion of human bitters as well. With the highest mosquito abundance and diversity, the selectively logged forest appears to be a convenient habitat for the development of mosquitoes. Selective logging is therefore a dangerous activity to avoid during the deforestation process since it could favour mosquito-borne diseases emergence or outbreak.

Title: Multiple mechanisms insecticide resistance to deltamethrin in Anopheles gambiae sensu lato main malaria vector of Mali

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Background: Resistance to pyrethroids is a great challenge for vector control in Africa. The measurement of the level of susceptible of major vectors to these insecticides and the determination of the molecular and metabolic bases of this resistance are indispensable to the updating of the control strategies.

Methods: WHO standard bioassay test was used to assess 3-5 days old F_0 *An. gambiae s,l.*, adults susceptibility to deltamethrin. Synergists tests (PBO, DEM and DEF) were performed to screen metabolic resistance mechanisms. The TaqMan technique was used for genotyping of insecticide resistant alleles.

Results: A strong phenotypic resistance to deltamethrin was observed with mortality rates of 15% and 13% respectively at Karadié and Dangassa. Results of synergist tests showed an increase of 69.7% and 74.0% in mortality for PBO in Karadié & Dangassa, respectively. This increase was 26.8% in Karadié for DEF and 42.0% for DEM in Dangassa. There was no effect on mortality after pre-exposure to DEM at Karadié and to DEF at Dangassa. Hight KdrW resistance allele frequency was observed in all species at all sites. The KdrE resistance allele frequency was relatively high in *An. arabiensis* compared to the other species. The N1575Y resistance allele frequency was higher in both *An. gambiae* & *An. coluzzii* (major vector species) at all sites, but it was found in *An. arabiensis* in Karadié site only.

Conclusion: Our study showed the presence of multiple mechanisms of resistance to deltamethrin in the study localities including the cytochrome P450 resistance mechanism in Mali.

Keywords: Malaria, Mali, *Anopheles gambiae s.l.*, Insecticide resistance, Resistance mechanisms, Malaria vector control

Title: Resistance to insecticides and LLINs based on pyrethroids in *Anopheles gambiae* s.l. and *Culex* mosquitoes, a challenge for vector control in the city of Yaoundé, Cameroon

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Background: the effectiveness of long lasting insecticidal Nets and indoor residual spraying in mosquito control is threatened by vector resistance to insecticides. Our study sort to assess the susceptibility of mosquito populations to insecticides versus the biological efficacy of LLINs on the local mosquito populations.

Methods: Anopheles gambiae s.l. and Culex sp. larvae and pupae were collected from a variety of breeding sites from July-august and October-Oovember 2017 in the city of Yaoundé and reared until adult emergence. Adult females were used for the WHO susceptible tests with deltamethrin and permethrin impregnated papers at 1x, 5x and 10x concentrations and bio efficacy test with three net brands; permanet 2.0®, Olyset® and Interceptor® distributed during the 2016 mass campaign. Mosquitoes were morphologically identified and the Anophelines further identified to species level by the PCR technique.

Results: Overall, 8 259 mosquitoes were used for both assays. *Anopheles gambiae* s.l. represented 43.8% and *Culex* sp. 56.2%. The *Culex* genus, was constituted of *Culex quinquefasciatus* (36.8%), *Culex antennatus* (36.4%), *Culex duttoni* (14.8%) and *Culex perfuscus* (9%). For the *Anopheles gambiae* complex, *Anopheles coluzzii* was the only species in all the sites, except in Mvan and Emana where 12% of *Anopheles gambiae* was recorded. The two genus of mosquito displayed high levels of resistance to 1x, 5x and 10x deltamethrin and permethrin, with a variability in mortality rates from 1% to 86.5%. The mortality rates recorded with all the different LLIN were very low ranging between 1.3-74.5%.

Conclusion: Our study confirms high levels of insecticide resistance among urban mosquito populations with a decrease in the effectiveness of LLINs. This information should contribute to a better management of insecticide resistance among malaria vectors and mosquitoes involved in annoyance in the cities of Cameroon.

Keywords: Resistance; Insecticide; Mosquito nets; Anopheles gambiae s.l. Culex sp.
Title: Status of insecticide resistance in Anopheles gambiae (s.l) of The Gambia

Authors: Saihou Cham

Background: Long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS), have contributed significantly to the decreasing malaria burden observed in The Gambia since 2008. Nevertheless, insecticide resistance may threaten such success; it is important to regularly assess the susceptibility of local malaria vectors to available

Methods: In the transmission seasons of 2016 and 2017, *Anopheles gambiae* (*s.l.*) larvae were sampled in or around the nine vector surveillance sentinel sites of the Gambia National Malaria Control Programme (GNMCP) and in a few additional sampling points. Using WHO susceptibility bioassays, female adult mosquitoes were exposed to insecticide-impregnated papers. Molecular identification of sibling species and insecticide resistance molecular markers was done on a subset of 2000 female mosquitoes. All statistical analyses were performed in R statistical package.

Results: A total of 4666 wild-caught female adult mosquitoes were exposed to either permethrin (n=665), deltamethrin (n=744), DDT (n=1021), bendiocarb (n=990) or pirimiphos-methyl (n=630) insecticide-impregnated papers and control papers (n=616). Among the 2000 anophelines, 1511 (80.7%) were *Anopheles arabiensis*, 204 (10.9%) *Anopheles coluzzii*, 75 (4%) *Anopheles gambiae* (*s.s.*), and 83 (4.4%) *An. gambiae* (*s.s.*) and *An. coluzzii* hybrids. There was a significant variation in the composition and species distribution by regions and year, P=0.009. Deltamethrin, permethrin and DDT resistance was found in *An. arabiensis*, especially in the coastal region (odds ratio=34, P=0.014). There was suspected resistance to pirimiphos-methyl (actellic 300CS) in the North Bank Region in 2017.

Conclusion: As no confirmed resistance to bendiocarb and actellic 300CS was detected, the national malaria control programme can continue using these insecticides for IRS. Nevertheless, the detection of *Ace-1 119S* mutation warrants extensive monitoring. The source of insecticide pressure driving insecticide resistance to pyrethroids and DDT detected at the coastal region should be further investigated in order to properly manage the spread of resistance in The Gambia.

Title: Spatio-temporal distribution and insecticide resistance status of Aedes mosquitoes in Ghana

Authors: Christopher Mfum, Owusu-Asenso

Background: The *Aedes* has been transmitting Yellow fever and dengue fever viruses and other unknown arboviruses in Ghana. This study investigated the spatio-temporal distribution and insecticide resistance status of *Aedes* mosquitoes in Ghana.

Method: This study was carried out in three ecological landscapes of Ghana. Indoor and outdoor sampling was done with BG traps, human landing catch (HLC) and prokopack aspirator (PPK) during the dry and rainy seasons of 2017/2018 to determine spatio-temporary distribution. Phenotypic insecticide resistance status of *Aedes* was determined using the WHO susceptibility bioassay. Host blood meal sources was determined by PCR.

Results: A total of 2193 adult *Aedes* mosquitos were collected comprising; *Aedes aegypti* (97.3%), *Aedes africanus* (2.2%) and *Aedes luteocephalus* (0.05%). The dry and rainy season had 73.5 (42.1%) and 103.95 (57.9%) *Aedes* respectively. HLC had the highest densities of 210.9 (77%) followed by PPK 74 (17.8%) and BG trap 15.5 (5.2%). The test results showed that *Aedes* mosquito populations from all study sites were resistant to DDT (0 - 84%). Vectors showed resistance to deltamethrin in Tema (68%) and patchy resistance in the other sites. Vectors showed resistance to permethrin in Accra (40.0%) and Larabanga (88.8%), suspected resistance in Konongo (90%), Navrongo (90%) and Paga (96%). *Aedes* mosquitoes showed resistance to bendiocarb in Larabanga. *Aedes* mosquitoes were susceptible to organophosphates at all sites. Blood meal analysis showed that the *Aedes* mosquitoes were mostly anthropophilic with HBI of 0.9.

Conclusion: The development of resistance by *Aedes* mosquitoes to DDT, pyrethroids and carbamates may have an operational impact on the efficacy of insecticides on vector control interventions

Title: Host preference and feeding patterns of primary malaria vectors, *Anopheles arabiensis* and *Anopheles gambiae* s.s. in sites with or without Indoor Residual Spraying in Rwanda.

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Background: Blood-meal source analysis provides insight into host preference of disease transmitting insects and their efficiency in pathogen transmission. In this study, blood meal source of *Anopheles gambiae* s.l. mosquitoes known to be the dominant malaria vectors in Rwanda was investigated, the composition of sibling species identified and the potential impact of indoor residual spraying (IRS) on species composition described.

Method: Pyrethrum spraying catch method was used to collect mosquitoes resting inside houses from IRS and non-IRS sites. Blood fed *An. gambiae* s.l. collected from July to December 2018 were analyzed using direct enzyme linked- immunosorbent assay and sibling species identified by polymerase chain reaction (PCR).

Results: Overall 225 blood fed *An. gambiae* s.l. were identified by PCR; 55.1 % were *An. gambiae* s.s., 39.1% *An. arabiensis* and 5.8% of samples failed to amplify. Species composition in IRS sites was 16.1% *An. gambiae* s.s. and 83.9% *An. arabiensis*. In the non-IRS sites, the proportion was 93.2% *An. gambiae* s.s. and 6.8% *An. Arabiensis*. Single-source blood meal in IRS sites was 75% human and 10% bovine for *An. gambiae* s.s; whereas 26.8% and 65.9% of blood meals taken by *An. arabiensis* were from humans and bovines, respectively. Other identified sources of blood meals were either goat, mixtures from hosts, or unidentified animals.

Conclusion: The dominant malaria vector in IRS sites is *An. arabiensis* with greater preference for feeding on cattle than on humans. *An. gambiae* s.s. was found to be the primary vector in non-IRS sites and fed mainly on humans. The dominance of *An. arabiensis* in IRS sites is likely related to selection with *An. arabiensis* less affected by IRS due to its zoophagic and exophagic behaviors. Regular treatment of cattle with effective insecticide could provide additional protection against human malaria in areas where *An. arabiensis* is becoming the dominant malaria vector.

Title: Complications and Risk Factor Associated with Jigger Infestations In Kilifi County, Coastal Kenya

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Background: Tungiasis has reemerged in epidemic dimensions in Africa ravaging resource poor communities despite being regarded a neglected tropical disease. The causative parasitic jigger flea, *Tunga penetrans* thrives in sandy dry and dusty environments where domesticated animals serve as reservoir hosts. Infestations results in various health complications and impede implementation of control measures. The current study evaluated the risk factors contributing to jigger infections and the resultant complications.

Methodology: A cross-sectional study was carried out in three foci in Kilifi County in 2018. Semi-structured questionnaires was administered to individuals from selected households to evaluate risk factors for infections. Infected individuals were examined for jigger flea infection and any complications arising evaluated by collection swabs from cavities left after surgical extraction of the jiggers. Anaerobic and aerobic cultures were performed on the swabs for complications arising from bacterial infections.

Results: Majority of the jigger infestations were associated with mud walled houses with earthen floors (OR 3.52; 95% CI 0.20 -0.89, P <0.02). Presence of domesticated animals inside houses were show to significantly increase the odds of being infected (OR 4.37; 95% CI 2.05 - 9.69; P = <0.05). Gender and distance from watering points had significantly influence on infestation. Six pathogenic aerobic bacteria species were isolated from jigger infested individuals dominated by *Staphyloccous aureus* (32.5%). Others were *Staphylococcus* (16.9%), *Escherichia coli* (14.5%), *Enterococcus feacalis* (8.4%), *Klebsiella* species (1.2%) and nonpathogenic *Streptococcus pyogens* (3.6%).

Conclusions: Bacterial pathogens are key contributors to the complications associated with jigger infestation in coastal environments. The type and features of the inhabitant house and presence of domesticated reservoir animals are significant contributors to jigger infestations and should be considered in designing and implementation of intervention strategies against jigger infestation.

Title: Assessment of the sensitivity of Anopheles gambie s.i. to insecticides in Congo

Authors: Nianga Bikouta Grâce

Background and Objective: In Congo, malaria vectors are mainly controlled through the use of Long-Lasting Insecticidal Impregnated Mosquito Nets (LLINs).

However, the emergence of insecticide resistance in Anopheles remains a threat. In particular, resistance to pyrethroids (insecticide recommended in the impregnation of mosquito nets) could constitute an obstacle to the use of LLINs, the efficacy of which is well established.

The objective of this study was to evaluate the sensitivity level of *Anopheles gambiae s.l.* to insecticides.

Materials and Method: This study was conducted between 2016-2018 in three localities in Congo (Brazzaville, Kintelé and Djoumouna). Anopheles larvae and nymphs were collected from the breeding sites and reared. The adults obtained were used for sensitivity testing.

The tests were carried out on females 2- 5 days old. Seven insecticides were used: 3 pyrethroids, 2 Organophosphates, 1 carbamate and 1 Organochlorine. Pre-exposure to the synergist piperonyl butoxide (PBO) 4% was added to Deltamethrin 0.05% to determine the metabolic resistance related to P450 monoxygenesis. The efficacy of these insecticides was determined by their knockdown effect at 60 minutes and the mortality observed after 24 hours after exposure.

Results and Discussion: Sensitivity test results showed that all tested Anopheles gambiae populations are resistant to DDT and pyrethroids and have become resistant to Bendiocarb and pirimiphos-methyl. On the other hand, these populations have all been sensitive to malathion. For this purpose, malathion may be proposed for the control of Anopheles gambiae s.l. After pre-exposure to PBO, the mortality was 98.6%. Resistance mechanisms have not yet been identified. But the PBO test revealed that monooxygenases are involved in pyrethroid resistance.

Conclusion and Recommendation: *Anopheles gambiae s.l.* is resistant to many insecticides. Malathion can be used as an indoor residual spray. The use of MIILDAs with PBOs could be an effective alternative to control the emergence of resistance.

Title: Awareness and Adherence of Proprietary Patent Medicine Vendors (Ppmvs) in Ondo State to Malaria Rapid Diagnostic Test(RDT).

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Background: Nigeria policymakers, researchers, and practitioners are debating methods of curbing self-treatment which is increasing to about 80%. The use of RDTs has proven to be a cost effective way of addressing this concern and the involvement of PPMVs is key to it's actualization. This study assessed the awareness and adherence of proprietary patent medicine vendors in Ondo state to malaria rapid diagnostic test(RDT).

Materials and Methods: A descriptive cross-sectional study was carried out on a total of 196 Proprietary Patent Medicine Vendors(PPMVs) registered under National Association of Patent Medicine Dealers in Akure South, Ondo State, Nigeria from August to November 2018 using simple random and purposive sampling. A self-designed questionnaire was used in data collection and data analyzed using SPPS version 12 software.

Results: Awareness of the RDT was high as 85.9% of the respondents have heard of RDT, while 75.0% know about the malaria treatment policy. The study also reveals that arthemeter was the most familiar drug among respondents with 58% of the respondents stating their familiarity with the drug. 83.3% of respondents believed the use of RDT has improved the treatment of malaria. Only 58.9% of the PPMVs conducted RDT before prescribing anti-malaria drugs and just 32.3% of the respondents had enough supply of RDT. Furthermore, only 44.3% had RDT in their stores as at the time of conducting this study while 55.7% did not have RDT. The cost and unavailability of malaria RDT were the major factors influencing the use of RDT by PPMVs.

Conclusion and recommendation: Awareness of the ACT malaria policy was high, but adherence was unsatisfactory. Vendors should be trained and provided with health education information on the policy to improve awareness and adherence.

Title: Population genetic structure of *Rhipicephalus microplus* in Cameroon: implications for ticks control.

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Background: *Rhipicephalus microplus* is the most important arthropod vector of livestock diseases globally. Its notorious character is attributed to its competency to transmit pathogens, to develop resistance against acaricides and to displace endogenous ticks. Although we have reported for the first time the presence of the tick in Cameroon as a result of uncontrolled transboundary movement of these vectors, few studies have been undertaken on the genetic characterization of tick population in central Africa. This study investigated the population genetic structure of *R. microplus* collected in cattle from Cameroon. The generated data will be important in underpinning future vector control programs.

Methods: 7091 ticks were collected in 54 sites during a countrywide cross-sectional survey. Partial COI and 16sRNA sequences were generated and analyzed from 76 specimens. Population genetic structure was evaluated using Haplotype diversity (Hd), Nucleotide diversity (pi), and number of haplotypes (h) parameters. Phylogenetic tree was constructed.

Results: Three tick genera were identified: *Amblyomma* spp, *Hyalomma* spp, and *Rhipicephalus* spp. Intriguingly, *R. microplus* (1112/7091) was more abundant and widely spread than *R. decoloratus* (708/7091), depicting an apparent displacement of the endogenous tick by *R. microplus*. Sequences analysis reveals low Hd and pi values for both COI (0.052 and 0.00009) and 16sRNA (0.026 and 0.00006) genes. Only two haplotypes were identified, indicating a very low genetic polymorphism among isolates. Phylogenetic trees revealed that *R. microplus* were grouped into Africa/Americas clade. Interestingly, a new haplotype that seem specific to Cameroon was identified using 16sRNA. Our data do not show any subdivision in *R. microplus* populations from Cameroon.

Conclusion: The study highlighted a very low genetic differentiation of *R. microplus* isolates from Cameroon, suggesting that a generalized tick control strategy could be effective on the field.

Title: EVIDENCE OF ELEVATED CYTOCHROME P450 AND GLUTATHIONE S TRANSFERASE IN ANOPHELES RESISTANT TO DELTAMETHRIN AND DDT INSECTICIDES IN SOUTH WEST NIGERIA

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Background: Insecticide resistance in the major malaria vector, *Anopheles gambiae*, is a major concern to malaria vector control program in Nigeria. Target-site insensitivity arising from a single point mutation called *kdr* have been implicated in the resistance process with little information available on metabolic based resistance mechanisms. We therefore investigate the involvement of metabolic enzymes in *Anopheles gambiae* populations that showed cross resistance to Deltamethrin and DDT from South West Nigeria.

Method: Larval samples of *Anopheles* were collected from New Garage in Ibadan and the adult emergence exposed to 0.05% Deltamethrin and 4% DDT according to WHO criteria. All the samples were morphologically identified and with PCR. Cohorts of the populations were further exposed to Pyperonil Butoxide and the insecticides (PBO+Deltamethrin; PBO+DDT) to implicate metabolic resistance. Survivors exposed and unexposed were analyzed for Monooxigenases, Esterases and Glutathione S Tranferases enzyme levels according to WHO criteria. The data was analyzed, and the results compared between the exposed and the unexposed samples using paired two tailed t-test (P=0.05).

Results: Susceptibility testing showed high levels of resistance to both Deltamethrin and DDT with 26% and 2% mortalities respectively. All the mosquitoes were morphologically identified as *Anopheles gambiae s.l.* and subsequently identified as *Anopheles gambiae s.s.* and *Anopheles arabiensis*. Result of the synergist assay implicate the involvement of metabolic proteins in the process. The mortality values of the samples exposed to PBO+Deltamethrin increased from 26% to 64% while that of DDT also increased from 2% to 10%. Biochemical analyses revealed significantly high levels of both Cytochome P450 monooxygenases and Glutathione S Transferases in the exposed samples as compared with the unexposed (p<0.05) in both the samples exposed to Permethrin and DDT. However, esterase level between the exposed and the unexposed samples was not statistically significant (p>0.05).

Conclusion: Cytochrome P450 monooxygenases and Glutathione S Transferases are involved in the resistance of *Anopheles gambiae s.s.* and *Anopheles arabiensis* to Deltamethrin and DDT insecticides in South West Nigeria.

Keywords: Insecticide resistance, *Anopheles gambiae s.s.*, *Anopheles arabiensis*, Cytochrome P450, Gluthathione S transerases.

Title: Evaluation of Long-Lasting Microbial Larvicides for Malaria Vector Control in a Rice Irrigation Ecosystem at Ahero, Western Kenya.

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Introduction: Malaria remains a major health challenge in Kenya with chemical-based vector control interventions threatened by development of insecticide resistance and changes in vectors' behavior. This study was designed to evaluate the efficacy and persistence of two briquet formulations namely LL3 and FourStar[®] consisting of *Bacillus thuringiensis* variety *israelensis* (Bti) and *Bacillus sphaericus* (Bs) in a rice irrigation ecosystem.

Methods: Testing for efficacy in irrigated rice fileds was conducted at Ahero irrigation scheme, Western Kenya whereby an acre of rice paddy was subdivided into 15 equal plots and randomized into three arms, namely (i) LL3; (ii) FourStar®; and (iii) untreated control plots. The larvicides were applied at manufacturers recommended dosage of 1 briquet per 100 square feet. The plots were sampled for emergent *Anopheles* adults in emergent traps by using prokopak aspirators for a period of 12 weeks.

Results: The LL3 and Fourstar briquettes resulted in 78% and 70% overall reduction of emerging anophelines from the irrigated rice ecosystems. After two weeks of application, reduction was remarkably high at over 60% for the rest of the evaluation period. Both larvicides were very effective in reducing populations of *An.arabiensis* the major malaria vector in this region.

Conclusions: The LL3 and FourStar® briquettes larvicides proved effective in reducing adult emergence in *Anopheles* mosquitoes for a prolonged duration (over three months). However, there is need to integrate these long-lasting microbial larvicides with the existing tools for malaria vector control for significant reduction in malaria transmiss

Key words: Bacillus thuringiensis var. israelensis, Bacillus sphaericus, Anopheles gambiae complex

Title: Seasonality and Diversity of Malaria Vectors Caught in a Malaria Pre-Elimination Area in Southern Zambia

Authors: Twig Mudenda¹, Limonty Simubali¹, Harry Hamapumbu¹, Ben Katowa¹, Philip E. Thuma¹, ², Douglas E. Norris² and Jennifer Stevenson^{1,2} for the International Centres for Excellence in Malaria Research

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Introduction: In Zambia, few mosquito species are thought to play a role in malaria transmission. Macha, a malaria pre-elimination area in Southern Province, has a community malaria prevalence of about 1%. Here, the primary vector is *Anopheles arabiensis* which is mainly found in November to March during rainy season and disappears in the dry season. However, malaria cases with no travel history are seen at facilities within the hospital catchment area, during this time. This study aimed to assess vector species composition, feeding behaviour and spatio-temporal trends over a 2 year period to assess whether there are species that could be maintaining transmission during the dry season in Macha.

Methods: Mosquitoes were sampled indoors and outdoors monthly from 2016 to 2017 using CDC light traps set next to occupied bed nets and next to animal enclosures. *Anopheles* species were identified morphologically and molecularly. Infections with *Plasmodium falciparum sporozoites* were detected by circumsporozoite protein ELISA. Multiplexed blood meal PCR was used to determine host feeding preferences

Results: Multiple *Anopheles* species were found throughout the year except for the commonly known vector *An. arabiensis*, whose population dwindles in the dry season. Eight distinctspecies were identified. Four of these species groups, *An.squamosus*, *An.coustani*, *An.rufipes* and *An. pretoriensis* were found by CSP ELISA to carry *Plasmodium* sporozoites, all being caught outdoors. Sequencing of mitochondrial DNA to confirm identities and confirmation of *Plasmodium* infection by qPCR continues.

Conclusion: *An. squamosus, An. coustani, An. rufipes* and *An. pretoriensis* are present all year round and have potential to extend the malaria transmission period through the year. They potentially sustain transmission after the main indoor resting and indoor biting vectors have been reduced by indoor-targeted control. Additional control tools that complement the existing interventions are required. Further studies to understand the behaviour of these secondary vectors are required.

Title: Where do the malaria vectors actually rest inside houses?

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While countries have set ambitious goals towards malaria control, more efforts are directed towards scaling up interventions such as long-lasting insecticide-treated nets (LLINs), indoor residual spraying (IRS) and effective case management. LLINs and IRS particularly target indoor-biting and indoor-resting malaria transmitting mosquitoes. It is therefore important to understand resting behaviours of the major malaria vectors inside houses and how much they can be affected by indoor interventions. Thus, we investigated the preferred resting surfaces for *Anopheles arabiensis* and *Anopheles funestus* inside house in rural south-eastern Tanzania.

Study houses were selected based on the following inclusion criteria: i) thatched roofs and un-plastered mud walls, ii) thatched roofs and un-plastered brick walls, iii) corrugated iron roofs and un-plastered brick walls, and iv) corrugated iron roofs and plastered brick walls. In each of the selected house, mosquitoes were collected from multiple surfaces including floors, walls, roofs, bed-nets and household goods (furniture, utensils and clothes) using Prokopack aspirators.

We have detected differences between preferred resting sites by the two malaria vector species. Particularly, *An. funestus* are more of generalists in terms of their resting surfaces compared to *An. arabiensis*, although both species preferred thatched roofs over iron roofs. While in houses with thatched roofs *An. arabiensis* rest mostly on roofs (>55%), *An. funestus* readily also rests on other surfaces as well, such as walls and household goods (>40%). In houses with iron roofs *An. arabiensis* were found distributed mostly on floors, bed-nets and household goods (>80%), while *An. funestus* preferred walls and household goods (>55%). Our findings suggest that vectors do not only rest on walls, where they could be targeted with IRS, but also on roofs and other surfaces, such as bed-nets, floors, furniture, clothes and utensils. We, therefore, recommend that house designs should be considered when planning for interventions such as IRS.

Title: Effects of Repeated Exposure to a Pyrethroid Llin in Anopheles Gambiae s.s. Opeibea.

Authors: Joannitta Joannides, Samuel Akpor, Alidu Iddrisu, Sampson Gbagba, Joseph Chabi, Rebecca Pwalia, Melinda P. Hadi.

Background and objective: Insecticide resistance poses a threat to the control of malaria. There is need to understand the relationship between resistance and mosquito survival after exposure to LLINs. Previous studies have demonstrated that while resistant mosquitoes are no longer killed within 24 hours of contact with a pyrethroid LLIN, they may suffer from delayed mortality. However, as resistance increases, delayed mortality effects may decrease. The study aims to show the outcomes of repeated exposure to a pyrethroid LLIN in a highly resistant strain of *Anopheles gambiae s.s.*

Materials and methods: *An. gambiae s.s.* Opeibea mosquitoes, previously characterised to be highly resistant, were collected from a vegetable farm in Accra, Ghana. Female mosquitoes were exposed to a pyrethroid LLIN, PermaNet®2.0, according to a previously published experimental protocol: regime A, mosquitoes were exposed to the LLIN daily for 5 consecutive days; regime B, exposure to LLIN every 4 days (maximum 4 exposures); and regime C, exposure to LLIN and human bloodmeal every 4 days (maximum 4 exposures). mortality was recorded daily for 21 days.

Results and discussion: Mortality rates 24 hours following exposure for all regimes ranged from 0% to 5%. Even with daily exposure to the LLIN (regime A), 72-hour mortality did not exceed 5% and did not differ from the control untreated net. Initial results suggest that delayed mortality effects following repeated exposures to LLINs (all regimes) are substantially reduced in a highly resistant strain of *An. gambiae s.s.* Analysis of mosquito survival curves are ongoing.

Conclusion: The study explored how even with repeated exposure of a highly insecticide resistant strain of *An. gambiae s.s.* in Ghana to a pyrethroid LLIN, delayed mortality effects may not be observed. These early results signal that at high level of resistance, the protective efficacy of pyrethroid LLINs may be lost.

Tittle: Detection of *Wolbachia* and different trypanosome species in *Glossina palpalis palpalis* populations from three sleeping sickness foci of southern Cameroon

Authors: Sartrien Tagueu Kanté, Trésor Melachio, Elvis Ofon, Flobert Njiokou and Gustave Simo

Background: Tsetse flies are the cyclical vector of human and animal African trypanosomiasis. To improve vector control in order to achieve the elimination of human African trypanosomiasis (HAT) and boost the control of animal diseases, investigations have been undertaken on the tripartite association between tsetse, trypanosome, and symbionts. Understanding the vectorial competence of tsetse requires decrypting these tripartite associations. In this study, we identified *Wolbachia* and trypanosomes in *Glossina palpalis* from three HAT foci in southern Cameroon.

Methods: Tsetse flies were captured with pyramidal traps in the Bipindi, Campo and Fontem HAT foci. After morphological identification, DNA was extracted from whole flies. *Wolbachia* and trypanosomes were identified by PCR using different trypanosome-specific primers and two *Wolbachia*-specific primers (wsp and16S rRNA). Statistical analyses were performed to compare the trypanosome and *Wolbachia* infection rates and to look for an association between these microorganisms.

Results: From a total of 2122 tsetse flies, 790 *G. p. palpalis* were analyzed. About 25.32% of flies harbored *Wolbachia* and 31.84% of non-teneral flies were infected by at least one trypanosome species. The global *Wolbachia* prevalence revealed by the two markers was no significantly difference while some differences were observed between HAT foci. From 248 *G. p. palpalis* with trypanosome infections, 62.90% were with *T. vivax*, 34.68% with *T. congolense* forest, 16.13% with *T. brucei* (s.l.) and 2.42% with *T. congolense* savannah. Of all trypanosome-infected flies, 29.84% harbored *Wolbachia* and no association was observed between *Wolbachia* and trypanosome co-infections.

Conclusions: This study revealed differences in the prevalence of *Wolbachia* and trypanosomes in *G. p. palpalis* according to HAT foci. The use of only one marker has underestimated the prevalence of *Wolbachia*. The presence of *Wolbachia* seems to have no impact on the establishment of trypanosomes in *G. p. palpalis*.

Keywords: Glossina palpalis palpalis, Symbiont, Wolbachia, Trypanosoma sp

Title: Baseline malaria transmission indicators prior to a Randomized Controlled Trial of Eave Tubes in Bouaké, central Côte d'Ivoire.

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The study is part of a cluster randomised controlled trial (CRCT) of the eave tube strategy in central Côte d'Ivoire and aimed to provide baseline information on transmission of malaria from 40 village clusters involved in the study. In December 2016, Human Landing Catches (HLC) data were collected over 6 months between 18.00 and 08.00 in all village clusters. Mosquitoes collected were identified to species and Anopheles dissected for parity rates. The thorax of the parous females were then processed by Polymerase Chain Reaction (PCR) for detection of sporozoites. The density, Entomological Inoculation Rates (EIR), Sporozoite Index (SI) were estimated. Data on the molecular forms of *Anopheles gambiae* s.1, the allelic frequencies of the *Kdr-w*, and *Ace-1* mutations were included.

Overall, 15,632 mosquitoes were collected. *Anopheles gambiae* s.l was the most abundant species (66%) followed by *Anopheles funestus* (10%). The other Culicidae represented less than 25% of the total mosquito population. On average, 79% of the mosquitoes were captured between 22.00 and 05.00. Of the 5,174 *An. gambiae* s.l females dissected, 92% were parous. Sporozoite prevalence was 4.6% in those processed by PCR, with *P. falciparum* accounting for 94% of the infected *An. gambiae* s.l and very few (6%) of *P. malariae* found in this species. *An. coluzzi* represented 84% of the *An. gambiae* s;l. population. The resistant allele frequency was 0.94 for the *Kdr-w* and 0.19 for the *Ace-1* genes. On average, sleepers from the 40 village clusters experienced 568.7 infective bites per year, with great variation in EIR between villages.

These baseline data gathered appeared very useful for the randomization process of the interventions before the CRCT per se.

Day 3: Wednesday 25th September 2019

Vector biology and control

Title: Vectorial Role Of Ticks In The Transmission Of Hemoparasites In Cattles In The Menoua Subdivision (West, Cameroon)

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Backgroud: Ticks are obligate and hematophagous ectoparasites that affect most cattle farms in Cameroon and hinder their development. Unfortunately, very few studies have focused their attention on these ectoparasites in the Menoua Subdivision. Thus, the aim of this study was to do an inventory on the differents species of ticks in the Menoua and to bring out their involvement in the transmission of hemoparasites in cattle.

Methodology: A total of 939 cattle were examined for the collection of ticks and blood. In addition, questionnaires were administered to the owners and sheperds in order to collect the informations concerning the herd. These ticks were identified in the laboratory using a binocular steroscope. Blood samples helped in preparing the thick blood smear and observation was done at the oil immersion objective.

Results: The boidiversity of ticks in Menoua constitutes 15 species which are Boophilus decoloratus (30.44%), Amblyomma variegatum (26.46%), B. microplus (9.12%), B. geigyi (8.1%), B. annulata (2.15%), Rhipicephalus mushamae (6.35%), Rhi. lunutatus (2.73%), Rhi. Guilhoni (2,53%), Rhi. Sanguineus (1,58%), Hyalomma rufipes (3,87%), Hya. truncatum (2.4%), Hya. anatolicum (1.05%), Hya. excavatum (1.05%), Hya. impeltatum (1.05%) et Haemaphysalis leachi (1.02%). Amongst the 939 animals examined, 782 hosted at least one tick with a general prevalence of 83.28%. *Anaplasma marginale* (31.68%), Theileria sp (25.20%), *Babesia bovis* (22.92%), *Babesia bigemina* (11.95%) et *Anaplasama. Centrale* (8.25%) were the principal hemoparasites transmitted to cattle by these ectoparasites with the global prevalence of 75% (270/360). Inaccessibility and inefficiency of acaricides, absence of pasture, sensibilisation by veterinary services constituting the principal risk factors predisposing the cattle to parasitism in our study area.

Conclusion: Several tick species hinder the development of cattle production and more complimentary studies are needed in order to set up effective control strategies

Keys words: Ticks, hemoparasites, cattle, infection, infestation, Menoua.

Title: Results of Larval Control in Some African Countries Using Biolarvicides.

Author: Dr. Alfredo Vera Estrada, (PhD), Mrs. Mavy Hernandez Rodriguez (MSc), Labiofam, SA Technical Team, Cuba.

Labiofam SA is a Cuban company specialized in biological products to prevent human vector-borne diseases. Among other products Labiofam has developed two Biolarvicides for mosquito control (Bactivec and Griselesf) based on Bacillus thuringiensis, var. israelensis, H-14, strain 266/2 and Bac. sphaericus, strain 2362 respectively, which have been used in an integrated strategy with other methods of Vector Control in the Malaria Control Programs of the Ministries of Health in some countries, along with the use of Impregnated bednets and Indoors Residual Spraying and the consultancy of Labiofam experts, obtaining very good results.

It targets not only the diseases transmission, but also the reduction of all the species of mosquitoes present in the breeding sites to attain the well-being of the people in the communities involved, therefore can be applied also in touristic areas to diminish the mosquito's bites in outdoors locations.

Taking into account the results of the applied programs it is presented, as has been recorded by the local health authorities, the reduction of the Relative Larval Densities in the breeding sites and the reduction in malaria cases in Ghana, Angola, Equatorial Guinee, Burkina Faso, Gabon and Zambia.

As a conclusion of the results obtained we recommend the inclusion of Larval Source Management strategies and especially biolarviciding in the National Malaria Control Programs of the countries, mainly in those trying to achieve the elimination of this disease in the coming 2020 year.

Title: Identification of Two Intermediate Snails Hosts of *Schistosoma mansoni* in the Southern Cameroon Using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism of the Ribosomal DNA Gene Internal Transcript Spacer 2.

Authors: Mureille Carole, Tchami Mbagnia

Background: Two *Biomphalaria* species (*B. pfeifferi* and *B. camerunensis*) are involved in intestinal schistosomiasis transmission in Cameroon. Few studies are related to vector control; the two vector species are highly closely related morphologically. A clear identification of these *Biomphalaria* snails is key to sharpen the disease cartography and to evaluate the transmission risk in localities where they are found.

Methods: The study was conducted from July to August 2017 in the Centre, South, West, East and Far North administrative Regions of Cameroon. Snails were collected using a dip net, fixed in tubes containing 95% ethanol and transported to the Parasitology and Ecology Laboratory of the University of Yaoundé I. Total DNA was extracted from the foot of 400 *Biomphalaria* snails using Cethyl Trimethyl Ammonium Bromide. We used Polymerase Chain Reaction and Restriction Fragment Length Polymorphism technique for the internal transcribed spacer 2 region of ribosomal DNA analysis with Hpa II and TaqαI enzymes; samples were sequenced using ABI Prism 3730 automated DNA sequencer.

Results: The amplification of the ITS2 region of *Biomphalaria* snails resulted in a total of 450bp and produced two profiles with two distinct bands after digestion with Hpa II for each species between 100 and 300 bp: *B. camerunensis* profile 1 and 2 respectively (220 bp and 145 bp; 220 bp and 200 bp), *B. pfeifferi* profile 1 and 2 respectively (220 bp and 105 bp; 280 bp and 105 bp) and 03 profiles with 03 bands with TaqaI: 02 for *B. camerunensis* and 1 for *B. pfeifferi*. A total of nine haplotypes were obtained, gene flow in populations under study was very low.

Conclusions: The results show that the addition of PCR-RFLP using Hpa II enzyme to morphological method could improve the identification of *B. pfeifferi* and *B. camerunensis* in Cameroon.

Key words: Molecular taxinomy, *Biomphalaria pfeifferi*, *Biomphalaria camerunensis*, *Schistosoma mansoni*, vector control, South Cameroon.

Title: Effects of the synergist piperonyl butoxide on *Anopheles gambiae* **s.l. resistance to deltamethrin in North Cameroon**

Authors: Stanislas Elysée Mandeng^{1,2}, Herman Parfait Awono-Ambene¹, Jude D. Bigoga³, Wolfgang Eyisap Ekoko^{1,4}, Jérome Binyang², Michael Piameu^{1,5}, Lili Ranaise Mbakop^{1,2}, Betrand Nono Fesuh⁶, Narcisse Nvondo², Raymond Tabue^{3,12}, Philippe Nwane¹, Rémy Mimpfoundi², Jean Claude Toto¹, Immo Kleinschmidt^{7,8}, Tessa Bellamy Knox⁹, Abraham Peter Mnzava¹⁰, Martin James Donnelly¹¹, Etienne Fondjo¹², Josiane Etang^{1,13,14*}

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Background: Insecticide resistance is considered as a serious obstacle to malaria prevention. New tools are therefore needed to manage insecticide resistance and sustain vector control interventions. The present study aimed at evaluating the effect of synergists on deltamethrin resistance in Anopheles. gambiae s.l. populations in North Cameroon.

Methods: Mosquito larvae were collected from five settings in Garoua, Pitoa and Mayo Oulo Health Districts (HDs) in 2011, 2012 and 2015. Susceptibility of mosquito to deltamethrin was assessed using WHO susceptibility tests protocol for adult mosquitoes. Sub samples of test mosquitoes were identified down to species using PCR-RFLP. The Kdr alleles were genotyped using the hot ligation oligonucleotide assay (HOLA).

Results: The An. gambiae species complex included Anopheles arabiensis (68.5%), Anopheles coluzzii (25.5%) and Anopheles gambiae (6%). Mortality rates of An. gambiae s.l. to 0.05% deltamethrin varied from 35% to 47%, 63% to 90% and 81% to 93% in Garoua,

Pitoa and Mayo Oulo HDs respectively. Mosquito contact with the synergist 4% piperonyl butoxide prior to exposure to deltamethrin induced a significant increase (P<0.0001) of mortality to 0.05% deltamethrin to up to 100%, with reduction of the knockdown times. Besides, the Kdr 1014F allele was variable in the three species ie An. arabiensis, An. coluzzii and An. gambiae (s.s.) with frequencies varying from 3% to 79%; globaly the highest frequencies were observed in mosquito populations of Garoua and Pitoa HDs (47.85% to 33.5%) compared to the mosquito population in the Mayo Oulo HD (6%) (P<0.0001). No Kdr L1014S allele was recorded.

Conclusion: The effect observed of PBO synergist suggests the involvement of detoxification enzymes in addition to the Kdr gene observed in the tested populations. This study confirm the implication of several mechanisms confering resistance in An. gambiae s.l. populations and highlight the new control tools for fighting against malaria vector in Northern Cameroon.

Keywords: *Anopheles gambiae* s.l.; deltamethrin; Kdr alleles; synergist PBO; insecticide resistance; malaria vector control; resistance management; Northern Cameroon.

Title: Investigating the influence of larval nutrition on the life traits and vector competence of *Anopheles coluzzii* major vector of malaria in Cameroon

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Background: In some mosquito species, the conditions experienced by larvae during development have been shown to influence the life traits and the development of the pathogens which they transmit. If mosquitoes are not reared under ideal conditions, vector competence experiments in the laboratory may not accurately reflect vector-pathogens relationships in nature. In this work, the influence of larval nutrition on the life traits and vector competence of *Anopheles coluzzii* (Ngousso strain), has been studied.

Methods: Larvae were reared to a fixed densities but the daily amount of food available for each larva varied across experiments. Thus, five food amount (diets) were used: 0.025mg/larva/day (d1), 0.05mg/larva/day (d2), 0.1mg/larva/day (d3), 0.2mg/larva/day (d4) and 0.3mg/larva/day (d5). For each diet, three replicates were made and the following parameters were assessed: larval mortality rate, larval development time, larval pupation rate, emergence rate, adult longevity, and adult body size. Moreover, the influence of larval nutrition on *Plasmodium falciparum* development was assessed by comparing oocyst load in midgut after experimental infection of females with *P. falciparum* gametocytes taken from the blood of children aged between 5 and 11 years.

Results: Larval nutrition deprivation resulted in increased development time, decreased pupation and emergence rates and smaller adult female body size. Moreover, larval nutrition has strongly influenced blood intake in adults and reproductive success. The fecundity, the prevalence of the infection and the oocyst load in midguts were higher in mosquitoes that were abundantly fed at the larval stage.

Conclusion: These results suggest that larval nutritional rearing condition is a major factor in laboratory estimates *An. coluzzii* vector competence. This observation emphasizes the importance of considering larval development factors in all studies aiming to understand the interactions between this vectors and *Plasmodium* parasites.

Key words: Anopheles coluzzii, Plasmodium falciparum, diet, life trait, vector competence.

Title: Evidence of high intensity resistance in Anopheles gambiae mosquitoes from South-Togo

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Background: Insecticide resistance in malaria vectors threatens malaria prevention and control efforts. WHO has recently suggested that, tube susceptibility test for adult mosquitoes should be further expanded to assess the resistance intensity using a variety of quantitative bioassays. Effective control of malaria vector populations requires more broadly information on resistance intensity displayed against different classes of insecticides. We describe the resistance levels and intensity in Anopheles gambiae from natural population in Togo.

Methodology: Bioassays were used to quantify the level of resistance to deltamethrin and pirimiphos-methyl in well know laboratory colonies and one field population of Anopheles gambiae sensu lato. WHO susceptibility tube assays were used to produce data on mortality, and in order to quantify the intensity the specimens were exposed to various concentrations of deltamethrin: 0.05%; 0.1%; 0.25%; 0.375% and that of pirimiphos-methyl: 0.125%; 0.25%. The presence of kdr^R (L1014F/L1014S) and ace-1^R (G119S) point mutations was investigated from the field population using PCR-RFLP analysis and Taqman assay respectively.

Results: The results diplayed that Baguida (field population used) showed evidence of high resistance intensity to deltamethrin when compared to the well know laboratory colonies. However relatively low resistance intensity were recorded when used pirimiphos-methyl. Individuals with both kdr^R (L1014F) and ace-1^R (G119S) point mutations were recorded.

Conclusion: The high resistance levels displayed by the natural population compared to the laboratory strains of Anopheles gambiae, reveal a new challenge for malaria vector control strategies. Further investigation will be needed to more understand the mechanisms underlying the real phenotypic resistance observed in wild vector populations.

Key words: Anopheles gambiae, resistance intensity, Baguida, Insecticides.

Title: Characterizing the molecular and metabolic mechanisms of insecticide resistance in *Anopheles gambiae* s.l. and its association with malaria transmission dynamics in Guinea

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Background: Insecticide resistance across sub-Saharan Africa is a pervasive problem among *Anopheles*, with implications for the effectiveness of malaria vector control interventions. We investigated the association between insecticide resistance and malaria prevalence and characterized molecular and metabolic mechanisms in field populations of *Anopheles gambiae* s.l. from Guinea.

Methods: During the long rainy seasons in 2017-2018, we sampled mosquitoes using human landing collections in Maferinyah and Faranah, Guinea, two areas of high malaria transmission. Levels of susceptibility to seven public health insecticides (alpha-cypermethrin, bendiocarb, chlorfenapyr, clothianidin, deltamethrin, permethrin and pirimiphos-methyl, were assayed using CDC resistance intensity bioassays. PBO synergist assays were performed to assess the role of elevated cytochrome P450s in resistance. Vectors were screened by PCR for the presence of *Plasmodium falciparum*, target site mutations (L1014F, N1575Y, I1527T and G119S), and relative expression of three metabolic genes (*CYP6M2, CYP6P3* and *GSTD3*).

Results: Deltamethrin and permethrin resistance was intense, with a third of mosquitoes surviving ten times the diagnostic dose of insecticides. Vector populations were completely susceptible to alpha-cypermethrin, chlorfenapyr, clothianidin and pirimiphos-methyl. The L1014F*kdr*-N1575Y haplotype and I1527T mutation were associated with mosquito survival following permethrin exposure. Partial restoration of pyrethroid susceptibility following PBO pre-exposure suggests a role for mixed-function oxidases. Carbamate resistance was lower and significantly associated with the G119S mutation. *P. falciparum* infection rates were 6.8% and 4.2% among resistant and susceptible mosquitoes, respectively; survivors of bendiocarb exposure were more likely to be infected. All metabolic genes were found to

be overexpressed in field *An. gambiae* s.s., compared to a susceptible G3 colony. *CYP6P3* was significantly overexpressed in bendiocarb survivors.

Conclusions: Full vector susceptibility to alpha-cypermethrin, clothianidin, chlorfenapyr and pirimiphos-methyl, and potential synergism with PBO, is encouraging, and new LLIN and IRS interventions incorporating these chemicals should be considered by the National Malaria Control Program as part of management strategies to mitigate future selection for resistance.

Day 3: Wednesday 25th September 2019

Mosquito genomics: progress and challenges

Title: Insecticide resistance profiles of *Anopheles gambiae* s.l. in Togo and genetic mechanisms involved, during 3-year survey: is there any need for resistance management?

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Introduction: A study was conducted to provide a temporal data on insecticide resistance status in the major malaria vector *Anopheles gambiae s.l.* from Togo.

Material and Methods: Two to five days old females of *An. gambiae s.l.*, originating from three localities (Baguida, Kovié, and Kolokopé) were subjected during three years (2012, 2013, and 2016) to: organochlorides (4% DDT), pyrethroids (0.05% deltamethrin, 0.75% permethrin, and 0.05% lambdacyhalothrin), carbamates (0.4% bendiocarb and 0.1% propoxur), and organophosphates (5% malathion, 0.4% chlorpyrifos methyl, and 1% fenitrothion) following the WHO standard protocol. Dead and surviving mosquitoes were stored separately for DNA extraction, species identification, and *kdr* and *ace-1* genotyping. Knockdown times (KDT₅₀ and KDT₉₅) were high in *An. gambiae s.l.*

Results: The lowest KDTs were recorded at Baguida in 2013 for deltamethrin (KDT₅₀ = 24.7, CI = [22.4-27.12] and KDT₉₅ = 90.78, CI = [76.35-113.49]). No KDTs were recorded for DDT and in some instances for permethrin. Mosquitoes were fully susceptible to fenitrothion (Kolokopé: 100% and Kovié: 98.05%, CI = [95.82-100.26]) and malathion (100% at both Kolokopé and Kovié) in 2013 and only malathion (Kolokopé; 100%) in 2016. Three species, *An. coluzzii, An. gambiae*, and *An. arabiensis*, were identified at the three localities with some hybrids at Baguida (2013), and Kovié (2012 and 2016), respectively. *Anopheles gambiae* was relatively dominant (61.6%). The *kdr* 1014F allele frequency was >0.9 in most of the cases, except at Kolokopé (f(1014F) = 0.63, CI = [0.55-0.71]) in 2013. The *kdr* 1014S allele frequency was below 0.02. The highest *ace-1* frequencies were

identified in An. gambiae at Baguida (2012: 0.52, CI = [0.34-0.69] and 2013: 0.66, CI = [0.46-0.86]).

Conclusion: The resistance status is worrying in Togo and should be considered in the future malaria vector resistance management program by decision-makers.

Keywords: Malaria, Anopheles gambiae s.l., Resistance, kdr, ace-1, Vector control, Togo.

Title: Impact of Sunlight Activated Formulation Extract (SAFE) larvicide against mosquito population in Uganda

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Introduction: Malaria is endemic and of health public concern in Uganda. Vectors that are known to transmit malaria in the country include: Anopheles gambiae ss, Anopheles funestus and Anopheles arabiensis. Larval source management (LSM), a strategy that includes Larviciding and source reduction (environmental manipulation, modification and elimination of aquatic habitats) for mosquito larval control, has long been used as a measure for malaria control in many parts of the tropics. In this study we conducted a large scale on all the existing breeding sites and all stages of all mosquitoes were targeted in Nakasongola District before country wide implementation of larviciding as an additional tool in malaria control and elimination in the Country.

Methods: Entomological and Epidemiological baseline data was collected from both the households and health facilities in Nakasongola District. Few, Fixed and Findable (3Fs), accessibility of the breeding sites and acceptance by the community members and its ruralurban nature was used as a criteria during the selection of breeding sites to be treated. Local Village Health Team members were trained on larvicide application according to the WHO protocol, 2005. using dosages and specifications provided by the manufacturer. The application dose of larvicide was depending on the habitat and was calculated from: Dose (g) = application rate (g/m^2) x surface area (m^2) . Adult mosquitoes were collected from the nearby houses 10M form the breeding sites before the application of safe. Vector densities were monitored on a monthly basis and a significant reduction was identified. Samples were identified using a mosquito identification key. Larvae collection was done as baseline, during and after application. There was a significant reduction of larvae calculated from Mulla's Formula for calculating the percentage reduction of L1+L2, L3+L4 instar and pupae in the potential breeding places and identification was done using standard morphological characteristics (Russell, 1996, Gillies and De Meillon1968). Monitoring and evaluation of the efficacy of the larvae was done on interval of two days. Live larvae were counted and entered in the data collection forms.

Sunlight Activated Formulation Extract (SAFE) is a photo biological larvicide powder, a natural product that contains chlorophyll a and b. SAFE is acronym for a natural "sunlight activated formulation extract" made of chlorophyll in silicate base. SAFE is a novel formula derived using a nano technique and all active ingredients are of plant origin. Vector densities and species: A. gambiae, A. funestus and Culicines were identified. 4mg/m² per habitat was used, vector identification (pre, during and after spray period), was monitored and 100% reduction was registered on all species. Malaria cases: incidence, prevalence reduced by more than 50% and very few, cases were reported during this period.



Results: A 100% reduction in mosquito densities was record in the treatment area after the application of SAFE. This was recorded from the data collection forms collected for the period of 21 days before the reapplication.

Conclusion: Malaria cases reduced tremendously in Nakasongola where larviciding took place and the drugs from the health center was given to the next sub county. After the withdraw of larviciding activities in Nakasongola, malaria cases increased.

Recommendation: Larviciding as an additional intervention should not just be withdrawn without community participation on both environmental manipulation and modification which are more sustainable.

Title: A 6.5kb structural variation is driving the up-regulation of major P450 genes conferring pyrethroid resistance in the major malaria vector Anopheles funestus

Authors: Leon M. J. Mugenzi, Benjamin D. Menze, Gareth D. Weedall, Gareth J. Lycett, Jacob M. Riveron, Fidelis Cho-Ngwa, Charles S. Wondji

Introduction:_Malaria control relies heavily on insecticide-based control interventions notably with pyrethroids to which *An funestus* is increasing being reported resistant to. Elucidation of the molecular factors driving resistance is a prerequisite to the design of suitable resistance management strategies. Here, we established the contribution of a structural variation (SV) in conferring P450-based metabolic resistance and designed a simple PCR assay allowing to detect and track the spread of this resistance in the field.

Methodology: Whole genome sequencing (WGS) of Pooled mosquitoes from different African regions was carried out. Luciferase reporter assay and Quantitative Real time PCR (qRT- PCR) were used to investigate the potential role of the SV on the increased expression of *CYP6P9a* and *CYP6P9b* in resistant mosquitoes.

Results: WGS of resistant and susceptible mosquitoes detected a 6.5kb insertion in resistant mosquitoes in the intergenic region of two major cytochrome P450 genes (*CYP6P9a* and *CYP6P9b*) conferring pyrethroid resistance. Luciferase assay with the 8.2kb intergenic region from resistant mosquitoes containing the 6.5kb SV revealed a significantly elevated promoter activity compared to the 0.8 kb core promoter suggesting a central role played by this SV in the over-expression of resistance genes. A simple PCR was designed to genotype this 6.5kb SV and used to show that it is nearly fixed in southern Africa and present in part of East Africa but absent elsewhere. A qRT-PCR revealed that mosquitoes with two copies of SV had the highest expression of both P450 genes compared to those without SV. Using experimental hut trials, we showed a strong association between the presence of the insertion and the ability of mosquitoes to survive pyrethroid exposure and also to increased blood feeding success.

Conclusion: Our novel PCR diagnostic assay is a reliable tool for insecticide resistance monitoring as well as assessing its impact on malaria control.

Abstract: Abs-19-0074

Title: Anopheles funestus mosquitoes in contemporary malaria transmission, Kenya

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Anopheles funestus is among malaria vector species complexes whose biology, genetic diversity, and ecology remain understudied. Here, I will present data on the population structure of *Anopheles funestus* mosquitoes in relation to possible ongoing variations in vector-parasite associations among sibling species in the group in Kenya. Findings from our recent work show *Anopheles funestus* s.s. as the main vector species in the Funestus group; however, with occurrence of *An. longipalpis* C and for the first time, naturally infected with *Plasmodium falciparum*. Also, our data reveal a previously unreported potential malaria vector in the Funestus group whose *COI* or ITS2 sequences did not match reference sequences yet i) found to be infected with the *Plasmodium* parasite, ii) displayed high human feeding abilities. *Anopheles funestus* was found to be subdivided into 3 unique genetic clusters in Kenya, based on microsatellite analysis, with genotypes mirroring the degree of malaria prevalence in the different risk zones of Kenya. The implication of the findings in the context of active surveillance for effective malaria control and elimination will be discussed.

Abstract: Abs-19-0075

Title: Microsatellite Data Reveals Low Level of Barrier to Gene Flow Between Populations of *Anopheles Coluzzii* From South West Nigeria

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Background: Malaria vector control in Nigeria is currently facing a lot of challenges. Prevalence data over the years have indicated that much needs to be done with less emphasis laid on the importance of genetic control. These genetic approaches require proper understanding of the population structure of the local mosquito vectors. To this end, we evaluate the population genetic structure of the major malaria vector, *Anopheles coluzzii*, from South West, Nigeria.

Method: Anopheles mosquitoes were collected from eleven localities which span the Mangrove/Forest and Forest/Savannah zones in South West Nigeria. The mosquitoes were identified morphologically and with PCR. For comparison, only the *Anopheles species* found across all the sites was genotyped for microsatellite sequencing. Microsatellite genotyping was conducted at 10 microsatellite loci chromosome 2. The result was analyzed using Arlequin software.

Result: Three species of the Anopheles family (*Anopheles coluzzii, Anopheles gambiae s.s.* and *Anopheles arabiensis*) were detected in the study. The most abundant and widespread was the *Anopheles coluzzii*. Microsatellite analysis revealed equivocal results. Number of alleles across all loci decreased from the populations found in the mangrove/forest to the forest/savannah. Noticeable among these were that of the AG2H26 (30 to 4), AG2H637 (16 to 8) and AG2H79 (23 to 14). Observed heterozygosity was high across all loci irrespective of climatic zone (0.52-0.89). Gene flow was more extensive in the mangrove/forest populations across all loci (F_{sT} 0.02- 0.09, P>0.05), however, genetic differentiation across loci increased as we moved to the forest/savannah (F_{sT} 0.03-0.27, P>0.05). Notable among these are alleles AG2H772, AG2H637 and AG2H79, two of which has been previously found to be responsible for nearly all the genetic differentiations across cline in Anopheles mosquitoes in Nigeria. However, locus AG2H637 is located within inversion 2La, an inversion that has also been linked with resistance to insecticide in field populations of *Anopheles coluzzii* in Nigeria.

Conclusion: This study hence shows that four loci, AG2H26, AG2H772, AG2H637 and AG2H79 are responsible for nearly all the genetic differentiation in *Anopheles coluzzii* from South West, Nigeria.

Title: Seasonality and impact of vector control interventions on population genetics of *Anopheles funestus* and *Anopheles gambiae* malaria vectors in Zambia

Author: Mbanga, Muleba

Background: The varied biology and advent of insecticide resistance of mosquitoes that transmit malaria pathogens complicate vector control for most national programmes. It remains challenging to tailor the optimum suite of control activities for reduction of the entomological inoculation rates. Although Infrastructure exists for gathering data, there are important gaps in the information necessary to inform optimized vector control programs in Africa. Vector genomics may be key to designing and implementing effective vector control strategies. The Anopheles 10000 genome project at the Sanger Institute presents an opportunity for Zambia to collect mosquito specimens for whole genome sequencing (WGS). Specimens from Zambia will represent genetic samples currently missing to describe the range of genetic variation and the rate of gene flow through *An. funestus* and *An. gambiae* species complexes across Africa. We designed a study to facilitate elimination of malaria in sub-Saharan Africa by providing essential data from Zambia for vector mosquito risk assessment, surveillance, and control.

Methods: In consultation with the National Malaria Elimination Program, we established three entomological collection sites representing three different epidemiological zones in areas where data on vector populations including WGS in the context of insecticidal control would help guide control activities.

Results: Information will be used to make decisions about where to target vector control, what methods are most likely to succeed, and coverage required to achieve health results. This will also inform other efforts, like modeling to translate evidence into policy, efficient regulation of new products, and risk evaluation of either performing or not performing vector control strategies.

Conclusion: The WGS will be used to investigate mosquito population structure as they represent the regional geographic and environmental vector species variation. The study will help track occurrence and movement of resistance genes, fundamentally guiding vector control and insecticide resistance management strategies in Zambia and the African region.

Title: Prevalence of genetic markers associated with insecticide resistance and resting behavior in *Anopheles* mosquitoes in Kenya

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Background: Effective control of malaria vectors is one of the world's top public health priorities. With challenges such as resistance to insecticides by vectors arising, WHO recommends that endemic countries conduct surveillance on malaria vectors and monitor and evaluate their control interventions and use the information obtained to identify challenges that pose a threat to effective vector control.

Methods: 731 archived *Anopheles* specimens from Migori, Baringo, Mwea and Tana River collected within the context of malaria vector surveillance in Kenya were analysed. Mosquitoes were collected indoors using light traps and aspirators between August 2016 and November 2017. Specimens were identified using morphology and further by PCR method following DNA extraction by the alcohol precipitation method. *Anopheles gambiae* s.l. were screened for the presence of *kdr* mutation associated with resistance to pyrethroids and DDT, *ace-1*^{*R*} mutation associated with resistance to organophosphates and carbamates and 2La inversion associated with indoor resting behaviour using PCR assays.

Results: Of the 731 *Anopheles gambiae* s.l. tested, 90.5% (n=603) were identified as *An. arabiensis* and 9.5% (n=63) as *An. gambiae* s.s. The frequency of *kdr* alleles was 16.9% (n=124) in Migori, 3.1% (n=192) in Baringo and 0.5% (n=196) in Mwea. No kdr mutants were found in Tana River. The 2La inversion in *An. gambiae* s.s. occurred at frequencies of 87% (n=30), 80% (n=10) and 52% (n=50) in Baringo, Tana River and Migori respectively.

Conclusion: These result show that *An. arabiensis* plays a significant role in malaria transmission in Kenya and that the *kdr* mutation is spreading especially in western Kenya where Migori is located, calling for further studies to understand resistance patterns in the area. The lower tendency of *An. gambiae* s.s. to rest indoors in Migori compared to the other areas suggest a need to consider outdoor control intervention in addition to indoor ones in this area.

Title: Patterns of gene flow correlate with the expression profile of detoxification genes associated with pyrethroid resistance in the malaria vector *Anopheles funestus* in East Africa

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Background & Objective: Vector control remains the cornerstone for the control and elimination of malaria. However, understanding the molecular basis of insecticides resistance and population genetic structure in *Anopheles funestus* is an important step for the design of suitable resistance management strategies to control this vector. In this study, we investigated how patterns of the genetic structure of populations of *An. funestus* impact the molecular basis of pyrethroid resistance in this vector.

Materials & Method: Blood-fed mosquitoes *An. funestus* were collected across the four localities in Uganda (Arua, Bulambuli, Lira, and Tororo) and neighboring Kenya (Kisumu). The presence of resistance patterns to 0.75% permethrin insecticide was determined using WHO bioassay. A Microarraybased genome-wide transcription analysis was performed to identify the set of genes associated with permethrin resistance. 17 microsatellites markers were genotyped and used to establish patterns of genetic differentiation.

Results & Discussion: The five populations exhibited a high over-expression of several cytochrome P450 genes suggesting that this enzyme family plays a major role in the pyrethroid resistance in this region. The most commonly up-regulated P450s include *CYP6M7*, *CYP9K1*, *CYP4C36*, and *CYP6Z1*. However, the level of expression of some of these genes varies between locations. Genetic analysis indicated significant genetic differentiation (F_{sT}) between Arua and other localities and not caused by an isolation by distance. Population structure analysis revealed a barrier to gene flow between Arua and other areas suggesting that ecological and geographical barriers were responsible for the genetic differentiation, which may prevent or slow the spread of resistance alleles to other localities.

Conclusions & Recommendation: This study provides evidence that a geographical shift in the resistance mechanisms corresponds to patterns of population structure in Uganda and neighboring Kenya. The genetic structure and variation in gene expression could be used to inform future interventions especially as new insecticides used to malaria control interventions across these countries.

Title: Genetic determinants of the extrinsic incubation period (EIP) of *Plasmodium falciparum* in anopheles vectors

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Background: The extrinsic incubation period (EIP) of plasmodium is a determining factor in the intensity of malaria transmission. Many studies have shown that EIP is highly temperature dependent. However, the extent to whicg genetic variation of the parasite and/or mosquito can influence EIP remains unkown. The objective of our study was to use a natural *Anopheles/Plasmodium* combination to study the genetic determinants of *Plasmodium falciparum (P.f)* development time in anopheles.

Methods: *An. gambiae*, *An. arabiensis* and *An. coluzzii* were experimentally infected with blood from four natural *P.f* isolates. The EIP was estimated using a novel non-destructive approach based on the detection of parasites in cotton balls soaked in a 10% glucose solution.

Results: Our results indicate that the overall median EIP of *P.f* at 27°C was 12 days. Although this period was similar in all three mosquito species, it significantly varied between parasite isolates. There also was a mosquito species-*P.f* isolate interaction on the EIP. Finally, we found that the fastest growing parasites were also those that induced the highest mortality among mosquitoes, suggesting the existence of a trade-off between two key transmission traits.

Conclusions: More work will be needed to confirm that some parasite clones develop faster in some mosquito species than others. The results of this work will provide important information on malaria control.
Day 3: Wednesday 25th September 2019

Harnessing research capacity in Africa to empower programmes

Title: Pregnancy and CYP3A5 genotype affect day 7 plasma Lumefantrine concentrations

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Background: Pregnancy and pharmacogenetic variation alters drug disposition and treatment outcome. The objective of this study was to investigate the effect of pregnancy and pharmacogenetic variation on day 7 lumefantrine plasma concentration and therapeutic responses in malaria-infected women treated with artemether-lumefantrine (ALu) in Tanzania.

Methodology: Atotal of 277 (205 pregnant and 72 non-pregnant) women with uncomplicated *Plasmodium falciparum* malaria were enrolled at Mkuranga, Kisarawe, Utete hospitals; and Mohoro and Kibiti health centers in the Coast Region of Tanzania between May 2014 and December 2017. Patients were treated ALu, and followed up to day 28 to monitor clinical and parasitological response using parasite microscopy, screening and genotyping by qPCR and nested PCR. *CYP3A4*, *CYP3A5* and *ABCB1* genotyping were done. Day 7 plasma LF concentration and the PCR-corrected adequate clinical and parasitological response (ACPR) at day 28 as the primary outcome were determined.

Results: The mean log day 7 plasma LF concentrations were significantly lower in pregnant women than non-pregnant women (geometric mean ratio = 1.40, 95% CI =1.120 to 1.745, p = 0.003). A univariate followed by multivariate analysis showed that being pregnant, low body weight and *CYP3A5*1/*1* genotype were significantly associated with low day 7 LF concentration (p < 0.01). PCR-corrected ACPR was 93 % (95 % CI = 89.4 - 96.6) in pregnant women and 95.7 % (95 % CI = 90.7–100) in non-pregnant women. Day 7 LF concentration was significantly associated with the treatment outcome, whereby patients with lower concentration had a high risk of treatment failure (median 667 vs. 178.1 ng/mL, P < 0.001).

Conclusion: We report significant effect of pregnancy, low body weight and *CYP3A5*1* allele as a significant predictor of low day 7 plasma exposure. In turn lower day 7 LF concentration is associated with a higher risk of recrudescence (treatment failure).

ABS-19-0094

Title: Molecular Markers of Plasmodium Falciparum

Author: Njobe

Resistance to Sulfadoxine/Pyrimethamine (SP) is growing in many malaria endemic areas with risk of jeopardizing its use as intermittent preventive treatment during pregnancy. Resistance is associated with SP uptake which causes emergence and accumulation of mutations on the *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthetase (*Pfdhps*) genes. Monitoring resistance markers frequently can provide information on temporal trends of SP efficacy in combatting malaria in pregnancy and guide our preventive treatment policies. The aim of this study was to assess the association between recent SP uptake and frequency of mutations and how these mutations affect maternal outcomes.

Blood samples were collected from malaria positive pregnant women who had either taken at least a dose of SP or were SP naive. Parasite density and haematocrit were determined and PCR-RFLP was used to determine the prevalence of *Pfdhfr* N51I, C59R, S108N and *Pfdhps* A437G, K540E mutations.

The two genes were successfully genotyped in 63 patients. Thirty-six of them (57%) had taken at least a dose of SP. The most common mutations were dhfr N51I and S108N (100%). The K540E mutation was not detected. The prevalence of the quadruple mutation, a combination of *dhfr* triple mutation and *dhps* A437G was significantly higher in those who had taken just a dose of SP (P=0.02). The presence of the quadruple mutant did not adversely affect maternal outcome as the parasite density, haematocrit and clinical presentation was comparable to those of patients without this mutant combination.

In this study, SP uptake was associated with a higher frequency of the quadruple mutation and taking just one dose of SP was more likely to increase frequency of mutations than 2 or more doses. This means accumulation of mutations can possibly be slowed if pregnant women receive at least 2 doses of SP.

Title: Population Dynamics and *P. Falciparum* Sporozoite Rates of *An. Gambiae S.l.* and *An. Funestus* in Low, Moderate and High Malaria Prevalence Regions, Tanzania

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Introduction: Despite of the several efforts in implementing Integrated Malaria Vector Control initiatives in Tanzania, the knowledge on malaria vectors dynamics and and sporozoite rates is limited. Therefore, National Malaria Control Programme (NMCP) has been implementing the malaria vectors entomological surveillance countrywide since 2016, aiming to obtain information on malaria vectors population dynamics and their infectivity rate for evidence-based decision making.

Methodology: The malaria vectors entomological surveillance was carried out in 62 selected district councils countrywide. Monthly mosquito collection was performed using CDC-Light traps indoors for over two years. *An. gambiae s. l.* and *An. funestus* mosquitoes were identified morphologically using identification key and by polymerase chain reaction (PCR) technique to reveal their sibling species. *Plasmodium falciparum* sporozoites rates in these malaria vectors were detected by using ELISA technique.

Results: A total of 74,611 mosquitoes were collected. Among the mosquito collected, *An. gambiae s.l.* and *An. funestus* accounted for 21.4% (N=15,993) and 7.0% (N=5,218), respectively. The trends of malaria vectors in low (below 1% to 2%), moderate (3% to 7%) and high (8% to 24%) malaria prevalence regions were revealed. Generally, in high malaria prevalence regions, the proportions of both *An. gambiae s.l.* and *An. funestus* were higher than in moderate and low malaria prevalence regions. PCR results have shown that, majority (77.8%) of the *An. gambiae s.l.* were *An. arabiensis*, while in *An. funestus* group they were all *An. funestus* s.s. In majority of the district councils, *An. arabiensis* was a dominant malaria vectors and the *P. falciparum* sporozoite rate of this sibling species was highest (2.7%) compared *An. gambiae* s.s. and *An. funestus*. The sporozoite rates among malaria vectors differ from one district council to another; however, the overall sporozoite rate was 2.13%.

Conclusion: The present findings have revealed that, malaria vectors population dynamics and sporozoite rates varied from one district councils to another. In some district councils, *An. arabiensis* dominated for 100% and having highest sporozoite rates, despite of its behaviours of bite and rest outdoors where the current indoor interventions seems to be ineffective for this species. More efforts to strengthen outdoors interventions such as biolarvicides are inevitable. The proportions of both malaria vectors were highest in high malaria prevalence regions. This situations call for a closer monitoring on the ongoing vector control interventions countrywide.

Key words: Population dynamics, Sporozoite rates, *An. gambiae s.l., An. funestus*, malaria prevalence, district councils, Tanzania



Title: Evaluation of Malaria Surveillance System in Kano State, Nigeria, 2014-2018

Authors: Visa I. Tyakaray, O. Ajumobi, M. Dalhat, C. Michael, P. Nguku

Background: An estimated 3.2 billion people worldwide are at risk of having malaria and Africa accounts for 90% of malaria cases. Nigeria has the highest global burden with 51 million cases and 207,000 deaths annually. Kano State has a prevalence of 27.7%. We evaluated the malaria surveillance system to determine whether it is meeting set objectives and assessed key system attributes.

Methods: We evaluated the system using the "Centre for Disease Control's Updated Guidelines for Evaluation of Public Health Surveillance Systems, 2001". We did a retrospective review of malaria web-based data of the National Health Management Information System case monthly summary forms, 2014-2018 and conducted descriptive analysis of cases using Microsoft Excel. We interviewed five key stakeholders and administered semi-structured questionnaires to 35 Malaria Focal Persons (MFPs) in the state.

Results: Thirty-two (91%) of the MFPs described the system as simple, 27 (77%) reported that changes in the data tool have been accommodated and all MFPs understood malaria case definitions. All stakeholders interviewed and 34 (97%) of MFPs described the system as acceptable and 33 (94%) recounted that data collected are analyzed and used for decision-making. Data was essentially from public health facilities and therefore not representative. Individual health data collected mostly within ten minutes with 29 (82%) reporting data monthly within first week of new month. Data generated by the system are incomplete data and stability not guaranteed as it is donor driven since 83% funding is from partner agencies.

Conclusion: Malaria surveillance system in Kano State, Nigeria is simple, flexible, useful, acceptable and timely. We recommended involvement of private health facilities, strengthening and increasing human capacity as well as funding to enhance its representativeness and improve data quality.

Keywords: Evaluation, Malaria, Surveillance, Kano State, Nigeria.

Title: Decision Support to Integration of Larval Source Management in the National Ivm Malaria Control Strategy: Piloting A Community-Based Larviciding Model in Nakasongola District, Uganda.

Authors: Kato A. B., Shady M. A., Nambatya G., Okedi L. M. O., Okia M., Omujal F., Egitat G., Kigongo S., Onek D., Mukwaya L., Tarek A-El-Tayeb

Background: Large populations could be protected from malaria by controlling aquatic stages of mosquitoes if cost-effective and scalable implementation systems can be designed.

Methodology: A large-scale larviciding trial was conducted in Lwabyata Village, Nakasongola District in Uganda, employing modestly paid community members, known as Village Health Technicians (VHTs) and Community-Owned Resource Persons (CORPs), fielding a Bio-larvicide called Sunlight Active Formulation Extract (SAFE).

Results: We were able to demonstrate the applicability of mosquito larviciding for malaria control to village level. The population of both aquatic and adult stages of Anopheline and Culicine mosquitoes reduced. Analysis of variance showed significant difference (P \ge 0.05) between the treatment and control sites. Likewise, malaria cases showed reducing trends by the end of the study period (y = -149.9x + 659.5; R² = 0.67502), suggesting that larviciding could substantially contribute to malaria reduction. Much as the community exhibited moderate to low levels of awareness about larviciding, their attitudes and opinions were significantly positive, indicating a strong willingness to accept larviciding as a new tool.

Conclusions & recommendations: Larviciding can be an effective tool in reducing malaria transmission, especially when integrated with other methods. Further, longer-term trials with additional larvicides will however help make the choice of the most cost-effective larvicides to be integrated in the Ugandan IVM policy.

Day 3: Wednesday 25th September 2019

Adopting the one health approach - breaking down silos

Title: Impact of Landscape Anthropisation And Land-Use Change on the Ecology Of *Aedes* Arbovirus Vectors In Large Industrial Palm Areas, Côte D'ivoire

Authors: Zahouli JBZ^{1,2*}, Koudou BG^{1,3}, Müller P^{4,5}, Malone D⁶, Tano Y³, Utzinger J^{4,5}

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Background: In Côte d'Ivoire, the transformation of large rainforests into industrial palm plantations has coincided with a tangential transmission of zoonotic *Aedes* -borne arboviruses to humans. We assessed the impact of landscape anthropisation and land-use change on the distribution and host-seeking behaviours of *Aedes* mosquitoes among land-covers in dengue and yellow fever foci.

Methods: From January to December 2014, *Aedes* immatures and adults were collected across gradients of landscape anthropisation (sylvatic, peridomestic and domestic ecozones), and land-use change (forest, polyculture, palm monoculture and housing area) using ovitraps, larval surveys and human-baited double-net traps.

Results: Totals of 28,276 *Aedes* specimens belonging to 11 species (*Ae. aegypti*, *Ae. africanus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. lilii*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. palpalis* and *Ae. vittatus*) were found. *Aedes* species richness (7 species) and abundance (49.7%) were higher in peridomestic ecozones. *Ae. aegypti* proportion was higher in domestic (81.0%), followed by peridomestic (62.6%) and sylvatic (37.3%) ecozones. Only four *Ae. aegypti* specimens were found in palm monocultures. *Aedes* showed higher species richness in forest (11 species), and higher abundance in polyculture (n = 28,276; 60.9%). *Ae. aegypti*, *Ae. dendrophilus* and *Ae. vittatus* biting rate was 34.6 and 7.2-fold higher in polycultures (21.5 bites/person/day (b/pd)) and housing areas (4.5 b/p/d), respectively, compared to forests (0.6 b/p/d). *Ae. aegypti* displayed strong anthropophagy inflicting 93.0% of bites to humans. *Aedes* biting activity showed bimodal daily feeding cycles, with stronger magnitude in polycultures.

Conclusion: Landscape anthropisation and land-use change alter the ecology of *Aedes* resulting in the spray of forest-dwelling zoophagic and anthropophagic vectors primarily biting humans around their homes and farms. This may link arbovirus enzootic to epizootic cycles thus raising the risk of transmission of dengue and yellow fever viruses from wild animals to humans. An integrated vector management should be recommended.

Title: Plasmodium falciparum histidine- rich protein (PfHRP2 and 3) diversity in Western and Coastal Kenya

Author: Thiong'o, Kelvin Kariuki

Plasmodium falciparum histidine-rich proteins 2 (PfHRP2) based RDTs are advocated in falciparum malaria-endemic regions, particularly when quality microscopy is not available. However, diversity and any deletion in the *pfhrp2* and *pfhrp3* genes can affect the performance of PfHRP2-based RDTs. A total of 400 samples collected from uncomplicated malaria cases from Kenya were investigated for the amino acid repeat profiles in exon 2 of *pfhrp2* and *pfhrp3* genes. In addition, PfHRP2 levels were measured in 96 individuals with uncomplicated malaria. We observed a unique distribution pattern of amino acid repeats both in the PfHRP2 and PfHRP3. 228 PfHRP2 and 124 PfHRP3 different amino acid sequences were identified. Of this, 214 (94%) PfHRP2 and 81 (65%) PfHRP3 amino acid repeat types were identified. PfHRP2 levels were not correlated with parasitemia or the number of PfHRP2 repeat types. This study shows the variability of PfHRP2, PfHRP3 and PfHRP2 concentration among uncomplicated malaria cases. These findings will be useful to understand the performance of PfHRP2-based RDTs in Kenya.

Title: Biophysical Basis For The Proliferation Of Malaria Vectors In Northern Benin (West Africa)

Authors: Sominahouin, Aimé André

Background: The environment is a major determinant of malaria biodiversity because of the vectorial nature of plasmodium transmission and the bioecological preferences of vectors. Seasonality, distribution and quantity of rainfall, temperature, humidity, altitude, presence of surface water as well as anthropogenic factors.

The identification of risky malaria transmission sites using remote sensing, combined with other known factors influencing malaria transmission, have resulted in maps of priority risk areas for control intervention using the GIS approach. This system has been used to study the impact of environmental factors on the proliferation of malaria vectors.

Methods: The study was conducted in the northern Benin. Landsat OLI TIRS ortho-rectified satellite : images, SRTM DEM satellite image, population density in each municipality (2017-2018) were used.

Results: The results of our research show slope instability. The areas of steep slopes are identified .However, the higher the altitude, the more the terrain slopes and the kinetics of the water is high, demonstrating a strong runoff of water. Similarly, the lower the altitude; the lower the kinetics of water.

Our results suggest that water stagnation or infiltration will support runoff; hence if water stagnates; this is favorable for the development of mosquito breeding sites, given therugged terrainassociated with the predominance of a plateau. The spatial distribution of human population density is characterized by a denser population in the districts of Kandi I, Kassakou and Gogounou in the health zone of Kandi-Gogounou-Ségbana. A similar observation is also madein the districts.

Conclusion: Malaria transmission by anopheles is conditioned by environmental factors.Benin will make significant progress in eliminating malaria by using geospatial technologies at different levels for risk mapping and targeted interventions in endemic areas. Therefore, this strategy should be set as one of the prioritiesofthe Benin NMCP in its malaria elimination goal.

Title: Composition of intestinal human parasites from market gardening environments of city of Yaoundé, Cameron

Authors: Djieukap N. Laurelle^{1,2}, Awono-Ambene H. Parfait², Zebaze T. Serge.^{3*}

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Background: Intestinal parasites remain a neglected public health problem in poorest communities and are found throughout the world. In Cameroon, children are the most vulnerable group and the environmental causes of such health issue remain questionable.

Methods: A survey was conducted from October to December 2016 to assess the environmental risks of intestinal parasite infections in lowland areas of Yaounde city, also known as main breeding places for the local malaria vector species, *Anopheles gambiae*. Samples (water and soil) were collected in wells, rivers, and furrows associated with agricultural practices in Nkolondom, Nkolbisson, Tsinga. Physico-chemical parameters such as electrical conductivity and turbidity were measured and soil samples were analyzed for the detection of intestinal parasites using Kato Katz and Formaline-Ether methods.

Results: Of the 82 samples analyzed, physicochemical parameters of water and the composition of soil samples showed significant variations per location and environments. In total 14 parasite species were identified from the selected studied locations, including 3 protozoans and 11 helminths. The most frequent parasite species was *Strongyloides stercoralis* (2,000 larvae/L) for helminths and *Entamoeba histolytica* (>4,000 cysts/L) for protozoans. Species diversity and richness also varied from 8 to 14 according to locations and environments.

Conclusion: The presence of viable stages of some helminths (*Ascaris lumbricoides*, *Strongyloides stercoralis*, *Diphyllobothrium latum*, *Hymenolepis nana*, *Trichostrongylus sp.*, *Shistosoma mansoni*) confirm the potential risks for market gardeners and consumers of vegetable products from urban agriculture. This finding demonstrates the importance of the environmental management of the potential health issues in order to prevent some of these diseases.

Key words: Composition, intestinal parasites, market gardening environments, urban areas.

Title: Zoonotic circulation of canine leishmaniasis in Bobo-Dioulasso, an urban areas of Burkina Faso : need of One Health approach to control the disease ?

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Background: Even considered as neglected tropical disease, leishmaniasis is still being transmitted in Africa particularly in Burkina Faso occurring actively in regional hotpots in the Western area of the country. Indeed, visceral species of *Leishmania* was found in 2015 circulating within domestic dog populations in Bobo-Dioulasso whereas cutaneous form was identified within human population from Larama in rural setting. Unfortunately, any molecular and genetic analysis were performed to identify properly species phylogeny and circulating story. The present study aimed to update the prevalence of *Leishmania* spp in domestic dogs from Bobo-Dioulasso with the ultimate objective to refine the genetic profiles of *Leishmania*.

Methods: A cross sectional survey was carried out in six quarters of Bobo-Dioulasso differing in terms of human density, urbanisation level and central or peripheral location in the town. Blood samples were collected from domestic dogs into EDTA tubes (4-5mL) by veinipuncture. Serodiagnostic, an immunochromatographic test (rK39-ICT/DiaMed-IT LEISH[®]) was performed together with the STAT-NAT[®] *Leishmania*, a multiplex qualitative Real-Time PCR, to detect infectivity status of dogs.

Results : A total of 147 dogs have been sampled. The mean age was $3,32 \pm 2.52$ years. 53.74% were females, with a ratio sex M/F (68/79) of 0.86. Serological and Real-Time PCR assays reported respectively prevalence of canine leishmaniasis at 4.76%, and 10.88% indicating that the RT-PCR was more sensitive. The disease was not significantly associated to age, gender and clinical signs. The species identification of *Leishmania* parasites detected in positive dogs was still being processed.

Conclusion: Our study confirmed that zoonotic *Leishamia* is still circulating in Bobo-Dioulasso with a big risk for humans. Whereas we do not know yet the phylogeny of such parasites there is a need to implement One Health Approach to overcome this disease in Bobo-Dioulasso.

Key words : Parasite reservoir, dogs, *Leishmania*, One health, Bobo-Dioulasso, Burkina Faso

Title: Effect of Land-use change on mosquito abundance and the prevalence of avian malaria parasite in a tropical rainforest of Cameroon

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Anthropogenic land-use change is considered a key driver of disease emergence; it usually modifies the interactions of many biotic and abiotic factors and can alter the pattern of diseases in wildlife birds. Whether land-use changes increase directly or indirectly the prevalence of avian malaria parasites through the effect on hosts and/or vectors is still not well understood. We sampled understory bird communities and mosquitoes in three habitat types (Unlogged forest, selectively logged forest, and young palm plantation) to assess the effects of habitat type on avian *Plasmodium* prevalence and its relationship with mosquito abundance. Blood samples of 845 bird individuals belonging to 86 species and 26 families were collected and screened using microscopy and PCR. Plasmodium infections were detected in 136 individuals (16.09%) and varied significantly between forest types. The intensity of *Plasmodium* infection was low in general and did not differ significantly according forest types. We also observed that, the prevalence of *Plasmodium* varied significantly according to bird feeding groups. Nectarivorous birds had the highest *Plasmodium* prevalence while the lowest prevalence was recorded in granivorous birds. The abundance of mosquito varied significantly according to forest types and the highest mosquito abundance was recorded in the selectively logged forest while young palm plantation had the lowest abundance. Also, the prevalence of *Plasmodium* significantly and positively correlated with mosquito abundance and this correlation was associated to selectively logged forest. This study highlighs the importance of biotic and abiotic factors in the transmission dynamic of avian malaria. Habitat type affects the abundance of mosquito and play determinant role in the prevalence of avian Plasmodium parasites. Species feeding behavior is also important for a better understanding and prediction of patterns of parasite infections in a changing environment.

Keywords: Selective logging, palm plantation, mosquito abundance, bird feeding groups, avian malaria.

Title: Establishment of Technical Vector Control Advisory Groups (Tvcags): Experiences from Three Countries (Cameroon, Burkina Faso, And Malawi) Within the Partnership for Increasing the Impact of Vector Control (Piivec) Programme

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Background: Vector control is fundamental in reducing the burden of vector borne diseases (VBDs) however, implementation in many settings is challenging. This can partly be attributed to vertical implementation of specific VBD control programmes. Against this background the PIIVeC programme has facilitated the establishment TVCAGs in three countries to enhance integration of vector control planning and implementation.

Description: Through in-country stakeholder analyses TVCAGs comprise a wide range of stakeholders including from government, implementing partners, university and research institutions. The in-depth interview with TVCAG members (Cameroon (12), Malawi (12), and Burkina Faso (7)) highlighted opportunities and challenges in setting up TVCAGs, areas of integration, and sustainability.

Lessons learned: In Cameroon, the TVCAG is managed by a private research institution which has a memorandum of understanding with the Ministry of Health (MoH). In Malawi and Burkina Faso, the TVCAGs are managed within the MoH. Additionally, in Burkina Faso an independent TVCAG board was put in place for overall steering. In Malawi the existence of other functional technical working groups within MoH provided key lessons, while in Burkina Faso the efforts of the PIIVeC country coordinator, who is within the MoH, were advantageous. Introduction of TVCAGs was viewed as positive in pooling and managing resources for vector control efforts and putting into practice the World Health Organization 'One Health Approach'. The major challenge recognized by all countries is maintaining continuous funding from donors and/or governments after the PIIVeC programme conclusion.

Conclusion: The three countries offer three unique models from which subsequent evaluations in the short- and long-term will reveal their resilience and sustainability. The ultimate impact of the TVCAGs is likely to be linked to ongoing support from various stakeholders including the donor community. Lessons from these processes can be used to inform setting up similar groups within countries of similar settings.

Title: Low Transmission of *Wuchereria Bancrofti* Within Cross-Border Districts of Côte D'ivoire: An Important Step Towards Elimination of Lymphatic Filariasis in West Africa

Authors: Firmain N Yokoly^{1,2*}, Julien Z. B. Zahouli^{2,3}, Aboulaye Méite⁴, Millicent Opoku^{5,6}, Bernard L Kouassi², Dziedzom K. de Souza⁵, Benjamin G. Koudou^{1,2}

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Background: The Global Program to Eliminate Lymphatic Filariasis (GPELF) was launched in 2000 with the goal of interrupting transmission of lymphatic filariasis (LF) through multiple rounds of mass drug administration (MDA). In Côte d'Ivoire, MDA is ongoing since 2015. In order to guide the LF control and elimination efforts, entomological surveys were carried out to determine the abundance and distribution of mosquitoes and the transmission of *Wuchereria bancrofti* in four health districts located at the borders of the country and implementing MDA.

Methods: Adult mosquitoes were collected in four health districts (Aboisso, Blolequin, Odienne and Ouangolodougou) using window exit traps (ETC) and pyrethrum spray catches (PSC) during two consecutive years from July to December 2016 and July to December 2017. In each district, 420 households were selected. Mosquitoes collected were morphologically identified and ovaries of females dissected for parity. Furthermore, 184 pools of *An. gambiae* and 217 pools of *Culex* spp. were randomly formed and analyzed for *W. bancrofti*, using conventional polymerase chain reaction (PCR).

Results: A total of 15,562 mosquitoes were collected including *Culex* (62%), *Anopheles* (31%), *Mansonia* (4%), *Aedes* (2%) and *Coquillettidia* (0.4%). Fifty six percent of mosquitoes collected were unfed and 54% parous. Overall, *W. bancrofti* infection rates were very low in all districts. None of the mosquitoes was found infected with *W. bancrofti* in Blolequin and Ouangolodougou. However, 0.1 % of *Culex* spp analyzed were positive in both Aboisso (n = 57) and Odienné (n =68). *An. gambiae* showed *W. bancrofti* infection rates of 1% (n = 11) in Aboisso and 0.08 % (n = 60) in Odienné.

Conclusion: Our study revealed high diversity and abundance of mosquito species, but very low transmission of *W. bancrofti* in cross-border districts of Côte d'Ivoire showing that the MDA-based interventions could contribute to the elimination of the disease in the country.

Key words: Lymphatic filariasis, *Wuchereria bancrofti*, mass drug administration, crossborder districts, Côte d'Ivoire

Day 3: Wednesday 25th September 2019

Innovation in vector surveillance and control



Title: The interplay between malaria vector population, seasons, and malaria prevalence.

Authors: Aju-Ameh, Celina Onyawoibi

Background: Globally malaria is the most advanced and prevalent vector-borne disease. Human activities, migration, land use, control interventions and climate are some of the factors that contribute to fluctuations in malaria transmission. Malaria epidemiology is fundamentally influenced by short-term seasonal cycles, though they are irregularities with climatic factors and malaria transmission. This semi-longitudinal investigation reveals a dynamic interaction between seasons, vector population density and malaria prevalence. Thus highlighting the importance of innovative vector surveillance as a core control tool.

Methods: Indoor resting mosquitoes collected between the hours of 06:00 and 09:00 using Pyrethrum Spray Catch (PSC), were preserved dry over silica gel and used for morphological and molecular identification. Asymptomatic malaria prevalence was determined through Rapid Diagnostic Test (RDT), Microscopy test and PCR. A line list hospital attendance record was collated to explore the symptomatic malaria situation. Metrological data was collected from a weather station in the study community to see impact of some weather elements on the malaria situation.

Results: The four seasons under study shows a progressive increase in vector population density with increase in rainfall. A strong positive correlation exists between vector population and asymptomatic malaria prevalence [PCRtest- $R^2=0.439(43.9\%)$; RDT+MicroT - $R^2=0.425(42.5\%)$; RDT- $R^2=0.342(34.2\%)$]. Symptomatic malaria peaked during the dry season (12.54%) with lower vector density while onset of rains with higher vector density recorded the least (4.7%). In terms of mean monthly temperature and minimum and maximum relative humidity no marked difference was observed. However, a marked difference was observed in number of rain days.

Conclusion: Established here is a dynamic interaction between malaria vector population, prevalence and the environmental landscape; hence the imperative for an ecological input for strategic malaria control. Control efforts should be solution tailored to locale specifics in the context of essential data generated.

Keywords: Malaria, Prevalence, Vector population, Seasons, Essential data, Nigeria

Title: Insecticide susceptibility of *An. gambiae* s.l to chlorfenapyr and piperonyl butoxide (PBO) effect in areas of high pyrethroid resistance from Cote d'Ivoire: Implications for malaria vector control

Author: Bernard L. Kouassi¹, Joseph Chabi¹, Ndombour G. Cissé¹, Cecilia Flatley², Dereje Dengela², Constant Edi³, Antoine M. Tano⁴, Pascal Zinzindohoue⁵, Blaise Kouadio⁵, Andre McKenzie⁵, Seth Irish⁶, Jennifer Armistead⁷.

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Background: Pyrethroid-treated mosquito nets are currently the main stay of vector control in Côte d'Ivoire. However, resistance to pyrethroids has been reported across the country, limiting options for insecticide resistance management. Interceptor®G2, a long-lasting net with a combination of chlorfenapyr and alpha-cypermethrin, believed to help in the control of pyrethroid-resistant mosquitoes, recently received World Health Organization prequalification listed (WHO PQ). We investigated the susceptibility of *An. gambiae* s.l to chlorfenapyr and furthermore the resistance status to pyrethroid insecticides with and without pre-exposure to piperonyl butoxide (PBO) in ten sites across the country.

Methods: Susceptibility tests were conducted on 2-4 day old adult females *An. gambiae* s.l. emerged from larval collections. Two chlorfenapyr doses (100 and 200 μ g/bottle) were used to test the susceptibility of the mosquitoes using CDC bottle assay methods. The resistance status, intensity and synergist (PBO) assays of deltamethrin, permethrin and alpha-cypermethrin were tested using WHO susceptibility test kits.

Results: The results indicated that *An. gambiae* s.l. was susceptible to chlorfenapyr in five of the ten sites (98–100% mortality) at the dose of 100 μ g/bottle; and in seven sites (99–100% mortality) at the dose of 200 μ g/bottle. Furthermore, *An. gambiae* s.l. from 9 sites were highly resistant to the three pyrethroids tested (mortality between 0 and 20%), the only exception being Bouna. Pre-exposure to PBO did not yield full susceptibility but induced significant increment of mortality in all sites for deltamethrin than alpha-cypermethrin and permethrin respectively.

Conclusion: The susceptibility of *An. gambiae* s.l. to chlorfenapyr in most sites indicates that the Interceptor[®]G2 should be considered as an alternative to pyrethroid only nets for vector control in these locations in Côte d'Ivoire. Results from the synergist assays could also support the NMCP in terms of selection of areas that could be considered for PBO nets.

Key words: Malaria, *Anopheles gambiae*, Resistance monitoring, Long lasting nest, Interceptor[®]G2, PBO nets.

Title: Vector control against malaria: Contribution of natural plant extracts as an alternative to synthetic insecticides.

Authurs: Foko D.G.A*, Tamesse J.L.

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Background: Insecticide resistance to malaria vectors was first reported in the 1950s and resistance to the main chemical insecticide classes used in Public health is now widespread. Resistance is predicted to impair malaria control efforts. Although public health use of insecticide has an impact on the development of resistance in target vectors, it may also pollute the environment and affect the health of humans and animals. The World Health Organization recommended the use of integrated control approaches to improve the control and elimination of malaria. New insecticide candidates deriving from plants extracts or essential oils are one of these approaches which, if well implemented could help control malaria with no impact on the environment and humans.

Material and Methods: We investigated the insecticidal properties of 15 natural plants belonging to nine families in Cameroon. These plants were characterized and active biocide compounds determined and used to produce phytoinsecticides against the main malaria vector *Anopheles. gambiae s.l.*

Results: Overall, 8 plants out of 15 revealed an important insecticidal activities including ovicidal activity [5 plants, yield (0.24%-1.67%), active compounds (26-52) and LC50 after 24hrs (0.14%-17.8ppm)], larvicidal activity [7 plants, yield (0.08%-1.67%), active compounds (26-81) and LC50 after 24hrs (0.44%-20ppm)] and adulticidal activity [3 plants, yield (0.044%-0.24%), active compounds (52-81) and LC50 after 24hrs (0.10%-0.44%)]. Monoterpenes (60%-80%) constituted the main active ingredients of the essential oil of these plants. Although these biocides have not yet undergone field evaluations, their activity was confirmed with field mosquito's populations and different phytoinsecticides were prepared and evaluated.

Conclusion: Essential oils from selective plant families in Cameroon stand as promising tools to overcome the phenomenon of insecticide resistant vectors in malaria endemic regions.

Keywords : Phytoinsecticide, Essential oils, Vector control, malaria.

Title: Indoor residual spray bio-efficacy and residual activity of a clothianidin-based formulation (SumiShield® 50WG) provides long persistence on various wall surfaces for malaria control in the Democratic Republic of the Congo

Authors: Ngwej Leonard M.

Background: Bio-efficacy and residual activity of SumiShield[®] 50WG (50%, w/w) with active ingredient clothianidin, a neonicotinoid compound, was assessed using an insecticide-susceptible laboratory strain of *Anopheles arabiensis*. Implications of the findings are examined in the context of potential alternative insecticides for indoor residual spraying in Lualaba Province, Democratic Republic of the Congo.

Methods: Contact surface bioassays were conducted for 48 weeks on four types of walls (unbaked clay, baked clay, cement, painted cement) in simulated semi-field experimental conditions using two different doses of clothianidin active ingredient (200 mg ai/sq m and 300 mg ai/sq m). Additionally, two types of walls (painted cement and baked clay) were examined in occupied houses using the 300-mg dosage. Laboratory-reared *An. arabiensis* were exposed to treated surfaces or untreated (controls) for 30 min. Mortality was recorded at 24-hr intervals for 120 hrs.

Results: Under semi-field experimental conditions, there was no significant difference in mortality over time between the two doses of clothianidin. The mortality rates remained above 60% up to 48 weeks on all four wall surface types. The formulation performed better on cement and unbaked clay with a mean final mortality rate above 90%. Under natural conditions, there was no significant difference in response between baked clay and painted cement walls with a mean final mortality rate above 90%. The insecticide also performed significantly better in natural settings compared to semi-field experimental conditions.

Conclusion: Depending on the type of experimental surface, the residual activity of the two doses of clothianidin was between 28 and 48 weeks based on a 60% mortality endpoint. Clothianidin at 300 mg ai/sq m applied on two house walls (baked clay or painted cement) performed equally well (>80% mortality) on both surfaces up to week 41 (approximately 9.5 months).

Title: Reduction of malaria vector transmission in Malian endemic villages.

Authors: Alpha Seydou YARO^{1, 2}*, Boubacar COULIBALY¹, Adama DAO¹, Bernard SODIO², Moussa DIALLO¹ and Sekou F TRAORE¹.

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Malaria in the Sahel occurs typically over a short period of three months, targeting aestivating mosquitoes could reduce the early "rounds" of vector-human amplifications and potentially reduce malaria transmission exponentially. An integrated fight against malaria since *Plasmodium falciparum* resistance to chloroquine has been undertaken in Mali, to slow down malaria burden. In the current study we compared malaria vector transmission data collected in two endemic areas: Bandiagara and Toukoro. In the first site data were collected prior to the intensive dissemination of ACTs and the policy of large-scale free distribution of long-lasting mosquito treated nets. In the second site, data were collected 15 years after, when ACTs and free MTNs distribution take place. Results comparison show a significant reduction of Entomological Inoculation Rate (EIR) from 15 infective bites per man per month in Bandiagara in 2001 to 0.27 infective bites per man per month in 2016 in Toukoro. The reasons for this significant reduction of vector transmission level could be due not only to the political authorities' involvement, but especially because of population's massive adhesion to the global integrative strategy of the fight against this disease. That show positive impact of collaboration between scientists, heath centers, communities and decision makers.

Key words: Malaria, vector transmission, EIR reduction, Mali

Title: Sporozoite infection rates and insecticide resistance and its association with 2La chromosomal inversion in *Anopheles gambiae* complex species (Diptera: Culicidae) in Teso Sub-County, Western Kenya

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Background: The control of malaria vectors is heavily reliant on the use of insecticides treated nets (ITNs) and indoor residual spray (IRS). Pyrethroids are the main insecticides recommended for use, however, evolvement of insecticide resistance is threat to the gains made. Chromosomal inversions have been reported to play a role in mosquito adaptive behavior. Among them is the 2La chromosomal inversion (2La), a trait that is believed to influence the resting behavior of *Anopheles* mosquitoes. It is also thought to cause *Anopheles* mosquitoes resting indoors to withstand prolonged exposure to the pesticide without being "knocked-down". The ability of *Anopheles* mosquitoes to withstand exposure to insecticide can contribute to their efficiency in malaria transmission. This study sought to understand the correlation of 2La chromosomal inversion with sporozoite infection rates and insecticide resistance in *An. gambiae* in Teso, Western Kenya, an area known to be malaria endemic.

Methods: Adult mosquitoes were collected from both indoors and outdoors using CDC light traps and hand catches, while larvae were collected using plastic dippers. Sampling was done from six randomly selected villages representing Teso North and Teso South Sub-counties of Busia County. Collection was done during the short rains (September -November 2015) and long rains (April-June 2016) season. Mosquitoes were identified morphologically using Gillies and Coetzee 1987 keys. Sub-species identification of the *An. gambiae* and *An. funestus* complexes was done as described by Scott *et al.*, (1993) and Koekemoer *et al.* (2002) respectively. Knock Down Resistance (kdr) allele genotyping was done using real time PCR. Female adult mosquitoes were analyzed for *Plasmodium falciparum* sporozoite infection, and host blood meal source through Enzyme-Linked Immunosorbent Assays (ELISA). Mosquito insecticide susceptibility test was done on a 3-5 day old emerged adult *Anopheles* mosquitoes following WHO 2013 guidelines.

Results: On densities, *An. gambiae s.s* constituted 45.47% and *An. arabiensis* 54.53%. Phenotypic resistance to deltamethrin and permethrin was observed at 24.64% and 30.37% respectively in *An. arabiensis* while in *An. gambiae s.s* resistance > 18% to both deltamethrin and permethrin was recorded. The overall sporozoite infection rate was at 14.34%, (5.18% in *An. gambiae s.s* and 9.16% in *An. arabiensis*). The L1014S mutation was observed in



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both *An. arabiensis* and *An. gambiae s.s.* There was a significant correlation in sporozoite infection with knock down resistance in *An. gambiae s.l* mosquitoes by Pearson's correlation coefficient of R=0.87, P=0.001. Homozygous 2La chromosomal inversion in *An. gambiae s.s* was recorded at 32.92% and was significantly correlated with sporozoite infection (χ^2 = 32.7925, df=4, p=<0.01). A higher preference of feeding on human than other hosts was observed in *An. arabiensis* (human, 25.39%, cat 16.23%, other hosts dog, goat, chicken and bovine were less than 10%.) while 17.0% *An. gambiae s.s* had fed on human and 6.59% on cat.

Conclusions: Here we report a significant correlation of sporozoite infections rates in *An. gambiae* s.s with 2La/a chromosomal inversion, however, no association with insecticide resistance. Further, we confirm that there's a correlation between insecticide resistance and sporozoite infection in *An. gambiae* s.s and *An. arabiensis* indicating the fitness of insecticide resistance mosquitoes to transmit malaria. Deltamethrin and permethrin resistant mosquitoes are more likely to withstand prolonged exposure to insecticide thus influence continuous malaria transmission in Teso. Alternative mosquito control measures should be considered to counter insecticide resistance to commonly used pyrethroids.

Title: Contribution of GIS for mapping malaria vectors distribution in the city of Yaoundé.

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Background: The GIS technology (Geographic Information System) is now widely used in public health to drive control efforts and identify hotspot areas. This report present data of the mapping of *An. gambiae* distribution in the city of Yaoundé

Methods: The study was conducted monthly in 32 districts of the city of Yaoundé from April 2017 to August 2018. All breeding sites were characterised and georeferenced using Garmin GPS. High resolution satellite images ETM2 from Sentinel-2 were used for spatial mapping of both field and environment variables. The index (NDVI, NDWI and BI) were calculated with Sentinel-2 satellite images. All the data were registered in a GIS database and analysed using Arc catalog, from ArcGis software. Seasonal and spatial variability of mosquito breeding sites and the landscape were analysed.

Results: The Building Index (BI) range from 10.68 to 73.27 in 2017 to 1 to 99 in 2018. NDWI, and NDVI estimates also showed important variations strongly correlated with the rapid modification of the landscape of the city of Yaoundé. Decrease in the vegetation cover associated to increased human settlement was recorded. Seasonal variation of breeding sites and *An. gambiae* larval distribution were all in conformity with the changing landscape of the city of Yaoundé. The use of the "IDW" interpolation technique helped identify hotspots areas across the city of Yaoundé. Hotspot areas were mainly distributed along river or agriculture cultivated sites and could be associated to high malaria transmission risk in the city of Yaoundé.

Conclusion: The use of GIS prove to be a useful tool for mapping and controlling malaria in high urbanized city as Yaoundé. The use of this technique could be determinant to drive efforts towards malaria elimination in the city of Yaoundé.

Key words: Anopheles, breeding sites, malaria, GIS, satellite images, mapping, Yaoundé.

Title: Determining species of field-collected mosquitoes using near infra-red spectroscopy

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Tracking changes in vector populations such as species abundance, age distribution and infection constitutes a direct measure of the efficacy of vector control interventions. Near infra-red spectroscopy (NIRS) is a rapid and non-destructive scanning technique that has been shown to be able to determine the species of morphologically indistinguishable mosquitoes. In this study, we evaluate the potential of NIRS to determine the species of laboratory-reared mosquitoes using machine learning (ML) models calibrated using colony mosquitoes. Mosquitoes of different species (Anopheles arabiensis, Anopheles coluzzii, Anopheles gambiae) were collected in three different field locations in Burkina Faso and reared to adult under laboratory conditions alongside colony mosquitoes. Following emergence colony mosquitoes were killed and scanned daily using NIRS. Maternal generation (F0) and offspring generation (F1) reared from field collected mosquitoes were killed and scanned 4 days post-emergence and the species determined by PCR. ML models were constructed to predict mosquito species from their spectra. NIRS was able to differentiate the three species of colony mosquitoes of constant age, including the previously untested An. coluzzii, with misclassification rates below 7% in all cases. Species could also be determined in colony mosquitoes where the age of the mosquito varied and was unknown though the accuracy was lower. Colony mosquitoes could predict the species of laboratory reared F0 and F1 mosquitoes with some loss of accuracy depending in species, location and generation. The work indicates that NIRS is able to identify the three most epidemiologically important species in the Anopheles gambiae complex with relatively high accuracy. The precision of the method diminishes with the increased realism investigated here (location of the population, age of mosquito) though NIRS still appears to a promising tool for mosquito surveillance that needs to be further validated in the field.

Title: Entomological evaluation of indoor residual spraying (pyrimiphos-methyl CS) on malaria transmission in Diébougou district, South-West Burkina Faso

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The rapid spread of insecticide resistance in malaria vectors and the possible recent increase in malaria cases in Africa require to develop and evaluate new vector strategies able to manage resistance. The combination of two insecticides targeting different time of the life cycle of the Anopheles vector is one possibility. A randomized controlled trial was performed with the objective to evaluate in communities, the effect of indoor residual spraying (IRS) with pyrimiphos-methyl (PM) in combination with Long-Lasting Insecticidal Nets (LLINs) on malaria transmission and the vectors' diversity, resistance and behaviour. The study took place in 13 villages (5 sprayed and 8 control) between November 2017 to June 2018. We performed 4 surveys of hourly mosquito collection following the implementation of IRS using the human-landing collection technique from 17:00 to 09:00 in each village. Malaria vectors species, *Plasmodium* infection, blood-meal source, *kdr-west*, *kdr-east* and *ace1* target-site mutations were searched by molecular technique. Residual activity of PM was monitored with susceptible "Kisumu" and wild strains using the WHO cone wall bioassay technique. The residual efficacy of the PM ranged 88-100% for 7 months on mud and cement walls against both susceptible and wild strains of Anopheles gambiae s.l. Average densities of Anopheles sp were 0.69 bites per human per night in the sprayed villages, significantly lower than in the control villages (3.2 b.h⁻¹.n⁻¹; RR=0.38; 95%CI[0.15-0.94]). The majority of biting activities of Anopheles occurred between 02:00 to 3:00h and 06:00 to 7:00h in the control villages and sprayed villages respectively. Overall, entomological inoculation rate was lower in the sprayed villages (0.14 infective bites per human per night) compared to the control villages (0.84 ib.h⁻¹.n⁻¹). PM insecticide IRS allowed to drastically reduce malaria transmission in our trial. PM should be considered as a potential good complementary tool to LLINs in high transmission areas of Burkina Faso but in association with surveillance of malaria vector behaviour.

Keywords: Vectors diversity, resistance, Long-Lasting Insecticidal Nets and behaviour

Prospects for developing efficient traps and targets for the xenomonitoring of onchocerciasis vectors in the Cascades region of Burkina Faso.

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Background: Following the recrudescence of onchocerciasis in the Cascades region of Burkina Faso, the national NTDs programme adopted mass drug administration of Ivermectin as the strategy of disease elimination. The impact of this strategy is monitored by quantifying *Onchocerca volvulus* infection prevalence in the human and blackfly vector (*Simulium damnosum*) populations. The gold standard blackfly collection method, the human landing collection (HLC), is not ideal due to ethical concerns of exposing volunteers to bites. Therefore, the absence of a standard trap for blackfly collection limits entomological surveillance activities. An efficient trap must be able to attract and catch blackflies in quantities close to the HLC. The attractiveness of blackflies toward their host is complex and is a result of a combination of the different visual and olfactory cues. Therefore, the search for an efficient trap requires investigating different cues which stimulate the host-seeking behaviour of blackflies.

Objective: In the present study, we are trying to develop traps and targets by assessing artificial visual and olfactory cues that will increase the number of blackflies lured and caught by the e-target traps along the Comoé river of Burkina Faso.

Method: For this purpose, we will use the e-target traps which are experimental tools used to develop traps and targets for hematophagous insect control. In a series of experiments, we will assess (i) the visual attractiveness of different e-target traps (colour, size, and shape) and (ii) the efficiency of synthetic odours to increase the number of blackflies collected. The most efficient trap will be compared to the HLC in subsequent experimental series. The activities are in progress and the first series of experiments will end in July 2019. The development of a standard trap will support the strategy of elimination of onchocerciasis in Burkina Faso.

Title: Transgenic fungus: A new era in biological control of malaria vectors

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Background: Malaria control efforts require innovations in vector surveillance and control. Here, we have tested the efficacy of a mosquitocidal *Metarhizium* strain (Met-Hybrid) engineered to express an insect-specific, spider neurotoxin (Hybrid) and GFP under the control of a hemolymph-specific promoter

Methods: The experiments were conducted in a multi-chambered MosquitoSphere (a contained, near-natural environment) constructed for this purpose in Soumousso, a region of Burkina Faso where malaria is endemic. We used the sphere to test a variety of low technology treatment protocols that could be used routinely by householders and found that suspending Metarhizium in locally produced sesame oil and spreading that on black sheets achieves a long term effect in the sphere.

Results: Compared to a strain of wild-type virulence expressing RFP (Met-RFP), Met-Hybrid killed anopheline mosquitoes in half the time and at much lower spore doses, which increased the percent of lethally infected mosquitoes and the effective persistence of the fungus. We also demonstrated that Met-Hybrid had important pre-lethal effects that include reduced blood feeding and flight capacity by infected mosquitoes. In addition, Mp-Hybrid infected mosquitoes laid eggs earlier (~5 days) compared with Mp-RFP and untreated mosquitoes (~7 and ~8 days respectively). This is the first evidence that transgene expression can induce an increased terminal investment response, and it could potentially undermine transgenic control approaches by affecting the evolution of infected mosquitoes. Finally, based on WHOPES susceptibility bioassays, Met-Hybrid provided effective biological control for mosquitoes that may be used to synergistically manage insecticide resistance with current methods.

Conclusions: This study represents an important step in the progression of transgenic mosquito control technologies into field application. We are currently working on community engagement and policy for an eventual open field release to test epidemiological and clinical impacts of transgenic Metarhizium.

Title: High throughput approach for entomological surveillance

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Background: The emergence of Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) as a robust, rapid, high throughput, cheaper and accurate tool for vector identification is revolutionizing the field of medical entomology. This study aimed to determine if MALDI-TOF MS could distinguish mosquitoes of *Anopheles gambiae* and *Anopheles funestus* complexes.

Materials and methods: Two legs from each mosquito used in database set up (31) and blind tests (99) were homogenized manually in 20 μ l of 70% formic acid in 1.5 ml microtubes using pellet pestles, followed by the addition of 20 μ l of 50% acetonitrile. The supernatant was then deposited on a steel target plate then overlaid with an equal volume of CHCA matrix. The mixture was allowed to dry for several minutes at room temperature before being introduced into the MALDI-TOF-MS instrument for analysis.

Results: A total of 31 specimens belonging to 4 mosquito species (*An. gambiae ss, An. arabiensis, An. funestus ss, An. rivulorum*) were subjected to MALDI-TOF-MS analysis to create reference databases. Querying the spectra using the databases yielded satisfactory results, with identification log score values of above 1.8. A total 99 mosquito specimens (*An. gambiae ss (40), An. arabiensis (29), An. funestus ss (25), An. rivulorum (5)*) were queried against the newly created database. Out of these, 92 were correctly identified into their respective species, giving a general sensitivity of ~98%.

Conclusion: MALDI-TOF MS can replace PCR for mosquito species identification of the *Anopheles gambiae* and *Anopheles funestus* complexes, dramatically reducing costs and sample processing.

Title: Efficacy of pirimiphos methyl (Actellic[®] 300CS) and clothianidin (SumiShield[®] 50WG) for indoor residual spraying in 2018 against pyrethroid resistant *Anopheles gambiae* s.l. in Burkina Faso

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Background: The Burkina Faso National Malaria Strategic Plan (2016-2020) recommends that non-pyrethroid indoor residual spraying (IRS) be used as a complementary vector control tool together with long-lasting insecticidal nets (LLINs) in locations where pyrethroid resistance occurs. This study aimed to evaluate the residual efficacy of pirimiphos methyl (Actellic[®] 300CS) and clothianidin (SumiShield[®] 50WG) in three districts in Burkina Faso.

Methods: Insecticides were applied at target dosages of 1000mg active ingredient (a.i)/m² for Actellic[®] 300CS and 300 mg a.i/m² for SumiShield[®] 50WG in houses with concrete and mud walls. Pirimiphos-methyl was sprayed in Kongoussi district while clothianidin was sprayed in Kampti and the central part of Solenzo districts. Residual efficacy of insecticides was monitored monthly from June 2018 to May 2019 with susceptible *An. gambiae* Kisumu strain and wild *An. gambiae* s.l. using the WHO cone wall bioassay technique

Results: In Kongoussi district, the 24h mortality rate of *An. gambiae* Kisumu strain was 98 to 100% during the first 4 months after which it declined to 90-95% up to six months after spraying and remained above 80% for 9 months. Wild *An. gambiae* s.l. presented high mortality rates during the first four months, but the rates declined to 50 to 56% on concrete walls in five and six months after spraying. In Kampti and Solenzo districts, clothianidin achieved full efficacy (mortality rates of 100 %) for 6 months and the mortality remained above 90% for at least ten months on cement and mud walls with both Kisumu and wild *An. gambiae* s.l. within 72 hours of exposure.

Conclusion: Clothianidin WG provided efficacy of at least ten months on cement and mud walls with both insectary and wild *An. gambiae* s.l. while pirimiphos-methyl CS had a residual efficacy of at least nine months with the susceptible strain but only four months in Kongoussi with wild *An. gambiae* s.l. This result could be an early indication of reduced pirimiphos-methyl susceptibility in *An. gambiae* s.l. from Kongoussi, although this was not detected in WHO resistance tests.

Keywords: IRS, Anopheles gambiae s.l, residual efficacy, Burkina Faso.

Title: Larvicide-based vector control to accelerate the elimination of onchocerciasis in a hyperendemic focus of the West Region, Cameroon

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Key Words : Onchocerciasis - Vector control - Larviciding - Temephos - Cameroon

Ivermectin (IVM) mass distribution through community-directed approach has been so far the main option to control onchocerciasis in most endemic areas accross the World. Despite efforts deployed to regarding this affection, it remains a public health concern in several countries in Sub-saharan region. While proofs of elimination are reported in few West African countries, in Cameroon the goal in achieving elimination by 2025 as stated the World Health Organization is still uncertain. Indeed high prevelances of microfilaria (Mf) are still reported even in foci receiving an annual dose of IMV for more than 20 consecutive years. This situation prompts the need to explore complementary intervention strategies to achieve the ultimate goal in the near future. A trial in the Massangam hotspot combining vector control based on hand application of temephos (Abate[®]) in Simulium breeding sites and mass treatments of populations with IVM and Doxvcvcline is launched since 6 months. Entomological activities include susceptibility test of Simulium blackfly larvae to temephos and evaluation of population densities through collection of larvae and adult blackflies. Complete susceptibility of larvae collected in rivers Mbam and Nja was noted at concentrations 0.3 mg/L and 0.5 mg/L of temephos. Prospection of breeding sites prior larviciding revealed presence of several larvae in natural and artificial supports in Nia and Mbam. A day after larviciding, only cocoons were observed on these supports. During the 10 consecutive weeks of larviciding, the daily biting rate decreased from 352 to 194 bites/collector/day in Nja, and from 956 to 371 bites/collector/day in Mbam. Applying temephos in Simulium breeding sites at year period of low blackflies population densities may have a significant impact in controlling onchocerciasis in this focus. The upcoming epidemiological evaluation may clearly demonstrate the decrease in Mf prevalences in humans and adult blackflies.

Authors: Rosine Danale, Metitsi Tesongang

Background: The development of insecticide resistance in Anopheles vector populations represents a major threat for effective malaria control. Factors influencing expression of their mechanisms are poorly understood. The purpose of this study was to investigate the influence of blood digestion on the susceptibility of *Anopheles coluzzii* to DDT 4 %.

Methods: Two days old female mosquitoes were divided into two groups. One was given blood meal using artificial parafilm-glass feeder system, and the other one was feed with 10% sucrose solution. Test to assess susceptibility to DDT 4 % were performed 24 hours and 72 hours after feeding on the two groups using WHO protocol. Besides, other tests were performed in groups of mosquitoes that received blood meal after pre-exposition to PBO.

Results: Tests conducted 24 hours after feeding showed lower mortality rates (p < 0.5) in the mosquitoes that ingested blood (26.25%) compared to those who did not ingest blood (91.2%). However, susceptibility to the insecticide was restored 72 hours later, with high and comparable (p > 0.5) mortality rates (91.25 and 93.75 %) observed in the two groups of mosquitoes. Pre-exposition of blood fed mosquitoes to PBO allowed restoration of the susceptibility to DDT from 26.25 % to 95 % suggesting induction of cytochrome P450 oxydases during blood digestion.

Conclusion: The study showed that tolerance of *An. coluzzii* to DDT increase during blood digestion through induction of cytochrome P450 oxydases. These observations should be considered in the implementation of malaria control strategies such as indoor residual spraying, which targets the fraction of mosquitoes resting indoors to digest the blood ingested the day before. Nevertheless, our results emphasize the advantage of combining insecticides with synergist in to enhance the efficacy of malaria vector control in the current context of spreading insecticide resistance.

Key words: malaria, Anopheles coluzzii, DDT, PBO, resistance

Title: A vector control decision tool to help strategise options for indoor interventions

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Background: Anti-malarial efficacy of key vector control interventions – long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) - primarily protects against indoor biting mosquitoes. Pyrethroid resistant mosquito populations are causing concern as the efficacy of pyrethroid-based interventions is reduced. Novel synergist or non-pyrethroid LLINs developed to mitigate this challenge are now available. LLIN and IRS performance will differ in different settings because of location-specific seasonality, mosquito habitat, pyrethroid resistance profiles, malaria endemicity and historic control.

Methods: To help decision-makers determine which cost-effective mosquito control to employ, we investigated how product efficacy varies given pyrethroid resistant status and behaviour of local mosquitoes using systematic reviews and transmission modelling. A webtool is introduced that allows users to explore the cost-effectiveness plane to guide decisions on intervention implementation. We demonstrate the utility of this tool by: i) predicting epidemiological results of randomised control trials using the transmission model that underpins the webtool, and; ii) comparing cost-effectiveness predictions based on data from specific settings to those made using the webtool options.

Results: Both standard and synergist piperonyl-butoxide (PBO) LLINs have reduced efficacy where mosquitoes are pyrethroid resistant. Longer-lasting, non-pyrethroid IRS formulations can mitigate against this reduced protectivity, but impacts vary given setting-specific spray quality, wall-surface, coverage and house size. Substantially more protection is afforded by LLINs and IRS where mosquitoes are preferentially indoor biting.

Discussion: Decision makers must evaluate the cost-effectiveness of different strategies when novel products enter the market. The webtool, which considers products endorsed by the WHO (PBO-LLIN and long-lasting IRS), provides some guidance to help make challenging decisions on intervention strategy given the non-linear relationships driving product efficacy and the impacts of pyrethroid resistance.

Title: Identification of *Plasmodium falciparum*-infectious *Anopheles coluzzii* mosquitoes using near-infrared spectroscopy

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The prevalence of infectious mosquitoes is used to assess the effectiveness of vector control against malaria. Mosquitoes with salivary gland sporozoites can be identified by microscopy following manual dissection, ELISA technique or using expensive molecular methods. These low throughput techniques are impeding routine entomological surveillance especially in areas with high mosquito densities. This study evaluates the potential of nearinfrared spectroscopy (NIRS) technique to differentiate between Plasmodium infected and uninfected mosquitoes. Colony of Anopheles coluzzii mosquitoes were fed on blood collected from gametocyte positive volunteers naturally infected with *Plasmodium falciparum* using direct membrane feeding assay in Burkina Faso. Two hundred and ten mosquitoes (111 in exposed group and 99 in control group) were killed 14-18 days-post blood-feeding and scanned using NIRS. Infection status was confirmed in individual mosquitoes using PCR as the reference method. The overall predictive accuracy for differentiating infectious from uninfectious mosquitoes was 72%, and the false negative and false positive rates were 37% and 20% respectively. The area under the receiver operating characteristic curve was 0.80. The moderate diagnostic accuracy observed here might be improved by modifying the machine learning methods used to convert spectral data into estimates of infectious status or by improvements in the lights source, fibre optics, or NIR sensors. NIRS is a rapid and non-destructive scanning technique that has the potential to be used to measure sporozoite prevalence though further work is needed to increase its precision and validate the technique in wild caught mosquitoes.

Key words: Plasmodium, Anopheles, near-infrared spectroscopy

Title: Insects to feed insects: Larval diets based on insects for mass-rearing a major vector of malaria, *Anopheles arabiensis*, Patton (*Diptera: Culicidae*).

Authors: Nanwintoum Séverin Bimbilé Somda, Hamidou Maiga, Wadaka Mamai, Hanano Yamada, Antoine Sanon, Abdoulaye Diabaté, Kounbobr Roch Dabiré, Jérémie Gilles, Jeremy Bouyer,

Background: Efficiency of novel genetic control strategies requires cost-effective production of mosquitoes. To provide more affordable and suitable mass production of *Anopheles arabiensis*, we investigated the use of edible insects as alternative larval diet ingredients. Ten different insect meal (IM) of black soldier fly (BSF), yellow mealworm (YM) or house fly (HF) were assessed on mosquito life history traits.

Methods: The IM were evaluated either alone and/or in replacement of the bovine liver powder in the reference IAEA diet. Furthermore, optimal combinations between tuna meal (TM), brewer's yeast (BY) and IM were figured out through an augmented simplex design. Finally, five more promising mixtures were assessed on both larva and adult mosquito life history traits.

Results: *An. arabiensis* larvae developed successfully from L1 to L4 but did not pupate, when reared in pure IM diets. Interestingly, when used individually as BLP replacement in the IAEA diet, eight IM led to enhanced or similar effects on the larval development parameters. Three-component mixture design experiment, considering TM, BY and BSF, revealed promising mixtures, most of which included only two ingredients such as $\frac{1}{2}TM+\frac{1}{2}BSF$, $\frac{1}{3}TM + \frac{2}{3}BSF$. Further assessment of the five most promising mixtures on mosquito development parameters including fecundity, longevity showed enhanced or similar results compared to the control. Based on all parameters, including diet cost reduction, the mixtures 50%TM+35%BSF+15%BY and $\frac{1}{2}TM+\frac{1}{2}BSF$ can be recommended for *An. Arabiensis* mass rearing.

Conclusions: Our study provided effective *An arabiensis* larval diets, more than 10-fold cheaper than the reference IAEA diet, highlighting the possibility to use insects to feed insects. More broadly, these results could be applicable to the species of the *An gmabiae* complex knowing their similarity in larval nutrition.

Title: Susceptibility testing of *Anopheles* malaria vectors with the neonicotinoid insecticide clothianidin; results from 16 African countries, in preparation for indoor residual spraying with new insecticide formulations.

Authors: Richard M Oxborough^{1*}, Aklilu Seyoum^{1,}, Yemane Yihdego¹, Roch Dabire², Virgile Gnanguenon³, Francis Wat'senga⁴, Fiacre R Agossa ⁵, Gedeon Yohannes⁶, Sylvester Coleman⁷, Samdi Lazarus Musa⁸, Abdoulaye Diop⁹, Ousmane Faye¹⁰, Stephen Magesa¹¹, <u>Alphaxard</u> Manjurano¹², Michael Okia¹³, Evelyne Alyko¹⁴, Hieronymo Masendu¹⁵, Ibrahima Baber¹⁶, Arthur Sovi¹⁷, Jean-Desire Rakotoson¹⁸, Kenyssony Varela¹⁹, Bernard Abong'o²⁰, Bradford Lucas¹, Christen Fornadel²¹, Dereje Dengela¹

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Washington, DC, USA.

Background: In 2017 more than 5 million house structures were sprayed through the U.S. President's Malaria Initiative, protecting more than 21 million people in sub-Saharan Africa. New IRS formulations, SumiShieldTM 50WG and Fludora FusionTM WP-SB, became World Health Organization (WHO) prequalified vector control products in 2017 and 2018 respectively. Both formulations contain the neonicotinoid active ingredient, clothianidin. In preparation for rollout of clothianidin formulations as part of national IRS rotation strategies, baseline susceptibility testing was conducted in 16 countries in sub-Saharan Africa.

Methods: There is currently no WHO guidance regarding clothianidin susceptibility procedures or diagnostic concentrations. Therefore, a protocol was developed for impregnating filter papers with 2% SumiShieldTM 50WG dissolved in distilled water. Susceptibility tests were conducted using insectary-reared reference *Anopheles* and wild collected malaria vector species. Mortality was recorded daily for 7 days, due to the slow-acting nature of clothianidin against mosquitoes. *An. gambiae* s.l. adults from wild collected larvae were tested in 14 countries, with wild collected *An. funestus* s.l. tested in Mozambique and Zambia.

Results: One-hundred percent mortality was reached with all susceptible insectary strains and with wild *An. gambiae* s.l. from all sites in 11 countries. However, tests in at least one location from 5 countries produced mortality below 98%. While this could potentially be a sign of clothianidin resistance, it is more likely that the diagnostic dose or protocol requires further optimization. Repeat testing in 3 sites in Ghana and Zambia, where possible resistance was detected, subsequently produced 100% mortality. Results showed susceptibility to clothianidin in 38 of the 43 sites in sub-Saharan Africa, including malaria vectors with multiple resistance mechanisms to other insecticides.

Conclusions: This study provides an interim diagnostic dose of 2% clothianidin on filter papers which can be utilized by national malaria control programs and research organizations until WHO concludes multi-center studies and provides further guidance.

Title: Evaluation of attractive insecticidal treated bed nets against resistant anopheles mosquitoes and malaria parasites transmission: a modelling study

Author: Nicolas Moiroux^{1,2,§}

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There is recent evidences from the field and the lab that Long Lasting Insecticidal nets (LLIN) may be attractive to *An. gambiae* populations and may increase bite exposure of LLIN users. However it was not clear how these attractive LLINs may impact malaria transmission at a community level.

We developed a model to answer this question under various scenario of LLIN coverage and vector's physiological (mortality), quantitative (indoor escaping) and qualitative (spatial-temporal avoidance of LLINs) behavioral resistances. We compared the effect of attractive, inert and deterrent LLINs on the spread of the common Kdr mutation in a malaria vector population using a genetic model.

According to our model, attractive LLINs are expected to reduce malaria transmission in comparison to inert or deterrent LLINs whatever the coverage percentage. Maximum reduction was reached at 63-65% coverage for which a highly attractive LLIN is expected to reduce malaria transmission by 69% and 85% compared to an inert and a deterrent LLIN, respectively. Our simulations show that whatever the level of physiological or behavioral resistance in the vector population, attractive LLINs are always most efficient than inert or deterrent LLINs but the reduction in transmission lowered as resistance increased. According to our genetic model, attractive LLIN allowed a slightly faster spreading of the Kdr mutation in the vector population than inert and deterrent LLINs.

Our model clearly showed that attractive LLIN are expected to reduce malaria transmission in all scenarios even in the presence of resistant phenotypes of vectors, when compared to inert or deterrent LLINs. The possibility to increase attraction of LLINs might be considered as a new opportunity. Combining new knowledge in chemical ecology and techniques to bi-impregnate LLINs may lead to provide to the current malaria control toolbox a most efficient version of a still well-accepted and utilized tool.

Title: A Single Mutation in a Cytochrome P450 Cyp6p9a is Associated with a High Blood Meal Volume in Anopheles Funestus Resistant Mosquito

Author: Lynda Nouage Djounkwa

Background & Objective: More recently, the *L119F-Gste2* and *AA-CYP6P9a* mutations were reported to be useful molecular markers to track metabolic resistance in *An. funestus*, a major African malaria vector. The markers offer the opportunity to assess the impact of insecticide resistance on different life trait of *An. funestus*. In this light, the present study aimed to investigate the impact of resistance on the blood feeding process by assessing the association between blood meal size and the *L119F-GSTe2* and *AA-CYP6P9a* mutations in *An. funestus*.

Materials & Methods: Mosquitos were collected from January to June 2018in the Adamaoua region. F1 progenies from field-collected mosquitoes were used to assess the impact of *L119F-GSTe2* mutation, whereas for *CYP6P9a*, we used females from crossing between insecticides susceptible and resistant *An. funestus* lab strains. Then, mosquitos were allowed to feed on human volunteers. Human haemoglobin was quantified from the abdomen of fed mosquitoes whereas, head and thorax were used for *L119F-GSTe2* and *AA-CYP6P9a* mutations genotyping. The influence of markers on the blood meal size was assessed by comparing the median volumes between the genotypes of each marker.

Results & Discussion: Overall, 360/1200 and 134/273 field and laboratory mosquito strains respectively, successfully fed. The median volume of blood was 3.4 µl/mg (field mosquito) and 4.9µl/mg (lab mosquito). Among those mosquitoes, 24/360 were homozygotes, 103/360 heterozygotes and 233/360 homozygotes susceptible for the *L119F-Gste2* mutation, whereas for *Cyp6p9a*, 31/134 were homozygotes resistant 67/134 heterozygotes and 36/134 homozygotes susceptible. The blood meal size was observed significantly higher in resistant compared to susceptible genotype for *CYP6P9a* (*P*=0.0056), but not for *L119F-GSTe2* mutation.

Conclusion & Recommendations: The present study shows that the *CYP6P9a* mutation, was associated with high blood meal size in resistant compared to susceptible *An. funestus*. However, further studies are needed to better investigate the effect of metabolic resistance on the life traits of *An. funestus*.

Title: Overview of Inesfly Insecticide Paints Scientific Results in Vector Control

Authors: Eustace Anye, Ignacio Gil, Rainer Sonnek and Pilar Mateo

Background: Insecticide paints are receiving interest as a promising new tool for the control of disease vectors. Several scientific reports have been published over the years about the performance of the technology, its long lasting activity, resident's acceptance and its alignment with corrent trends in modern housing in tropical countries.

Methods:, The scientific reports evaluating Inesfly insecticide paints have been reviewed and summarized with the focus on efficacy data and resident's acceptability.

Results: Several paint compositions were tested (containing pyrethroids, organophosphates, IGR and carbamate) against vectors of infectious diseases (Chagas disease, malaria, dengue fever, leishmaniasis and African trypanosomyasis) under laboratory and field conditions.

Principal findings were related to the long lasting effect exerted by the paints and measured in housing infestation index for *T. infestans* (reduction infestation index from 68.9%-96.7% to 0%-6.6% 6 years after application), *Gl. papalis* densities (90% reduction after 15 months of paint application), mortality of *Ae. albopictus* (100% in cone bioassays, 2.5 years after treatment), of *An. Coluzzi* (79%-82% in Early Morning Collections 1 year after paint application), of *An. gambiae* (100% in cone bioassays at month 19 after application), and of *Ph. argentipes* (>80% for 2 years , as well as blood feeding inhibition for *An. gambiae* (blood fed rates 68.5%-76.1% in control houses and 2%-13% in painted ones). A decrease in fertility and fecundity (*Cx. Quinquefasciatus*) and vapour phase effects were reported. Acceptance rates by residents for painting were high (>94%) and no significant side effects observed.

Conclusions: The review showed evidence of efficacy of paints against a varierty of diesease transmitting vectors and the accaptability of painting as intervention tool. The results showed potential of paint application in vector borne diseases prevention either in government driven programmes or comunity based interventions with self-application by homeowners.

Title: Phase II experimental hut evaluation of the efficacy and wash resistance of Royal Guard[®] LN against wild pyrethroid resistant *Anopheles gambiae* sl in Cove, Benin

Authors: Abel AGBEVO¹*, Dr Corine NGUFOR^{1, 2}

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Background: Pyrethroid resistance in malaria vectors has spread across sub-Saharan Africa. One of the most promising strategies to prevent or delay the development of resistance is to use at least two molecules having unrelated modes of action in combination in the same bed net. LNs containing a mixture of pyriproxyfen (an insect growth regulator) and pyrethroid could provide personal protection through the pyrethroid component and reduce vector abundance in the next generation through the sterilizing effect of pyriproxyfen.

Method: The efficacy of Royal Guard[®], a newly developed mixture LN containing pyriproxyfen and alpha-cypermethrin, was evaluated in experimental huts in Cove, southern Benin against pyrethroid resistant *Anopheles gambiae* sl. Comparison was made with Duranet[®] (a WHOPES recommended pyrethroid-only treated net) and with pyriproxyfen alone (PPF LN).

Results: In experimental huts, with unwashed nets, Royal Guard[®] LN induced significantly higher mortality rates than Duranet[®] LN (22.5% vs. 19.4%, P<0.05); When washed 20 times, mortality rates did not differ significantly between both LN types (13.2% with Royal Guard[®] and 11.2% with Duranet[®], P>0.05). Royal Guard[®] LN induced significantly higher blood-feeding inhibition than Duranet[®] when unwashed (53% with Royal Guard[®] and 34% with Duranet[®], P<0.05) and similar levels of blood feeding inhibition when washed 20 times (10% with Royal Guard[®] and 7% with Duranet[®], P>0.05) whereas PPF LN did not induce any blood-feeding inhibition both when unwashed and after 20 washes. However, the overall reduction in offspring with PPF LN and Royal Guard[®] relative to the control was 94% and 95% respectively when unwashed and 49.8% and 50.4% after 20 washes. Duranet[®] did not induce any reduction in offspring when unwashed and after 20 washes.

Conclusions: The efficacy of Royal Guard[®] is encouraging and clearly illustrates that this new net might be a promising tool for malaria transmission control and resistance management.

Key words: Malaria, *Anopheles gambiae* sl, Royal Guard LN, Pyrethroid resistance, Experimental huts.

Title: Trends in Insecticide Resistance in African Malaria Vectors

Authors: Hancock PA, Hendricks CMJ, Tangena J-A, Bhatt S, Moyes C, Coleman M.

Background: Vector control has had a significant impact on reducing the malaria burden in Africa, however, insecticide resistance has the potential to derail these gains. To fully understand the impact of insecticide resistance we need to quantify the trends through space and time. This project used disaggregated susceptibility test results and spatial data to quantify the potential drivers of resistance, and modelled trends from 2005 to 2017 across east and west Africa.

Methods: Variation in the prevalence of resistance to pyrethroids and DDT over the period 2005-2017 was quantified using results from a collated data set of WHO susceptibility tests performed on 6,423 *Anopheles gambiae* s.l. samples. The east and west models were informed by a suite of potential explanatory variables including the coverage of insecticide-based vector control interventions, agriculture and the relative abundance of the sibling species. Models were generated using a Gaussian process regression of the spatial-temporal correlations in the susceptibility data. Data sets were collated from published and unpublished data.

Results: There has been a dramatic increase in the prevalence of insecticide resistance during this 13-year period. The proportion of land area with less than 90% resistance has increased form 10% in east and west Africa to approximate 40% in east Africa and 90% in west Africa.

ITN and IRS were key explanatory variables in the increase of insecticide resistance.

Discussion: The predictions generated by the spatial-temporal model can be used to generate tools that are potentially useful in vector control decision-making. An example of this is the calculated probability of mean percentage mortality to an insecticide being below a threshold value, or, a sliding scale (10 to 90%). These data sets and models can be utilised to develop insecticide resistance management plans by malaria control programmes.

Title: Community and Stakeholder Engagement for Scaling up Acceptance / Consents of Innovative Genetic Technologies for Vector Control to reduce malaria transmission.

Elinor Wanyama Chemonges, Emmanuel Magala, Annet Namukwaya, Richard Linga, Jonathan Kayondo

Stakeholder engagement is as important as the scientific development of new malaria control technologies in the laboratories. Community and stakeholder engagement is one of the three pillars in Target Malaria research whose aim is to develop and share a novel accessible, sustainable and cost effective gene drive technology for malaria control. Target Malaria has dedicated stakeholder engagement teams working in communities to share information, consult, obtain acceptance/consent and get feedback on the research. A stepwise approach for both the research and engagements takes into consideration the need to provide evidence based information of one phase before proceeding to the next. Engagement aims at getting stakeholders to understand, appreciate and give acceptance/ consent for development of additional malaria control tools.

Informed consents/acceptance require innovative knowledge sharing methods appropriate and effective for different stakeholders. Target Malaria is exploring use of age appropriate communication and engagement tools for children and youths in school using unconventional teaching aids e.g. comic and work books - with a potential ripple effect that cascades to their families and peers and use of short video clips to convey messages in grassroots communities. It is anticipated that these will be effective methods for scaling up acceptance /consents for Target Malaria research.

Target Malaria research involves testing an area-wide vector control method that does not conform to the familiar clinical trial model employing individual informed consent, a different set of research ethics considerations is required. Trial authorization by communities is therefore necessary. Early and ongoing community engagement is essential for sharing of knowledge, experience and perceptions between the project and its stakeholders to help ensure that pathways for development respond to expectations.

Effective community and stakeholder engagement using innovative awareness methods is closely linked to scaling up acceptance/consent of innovative genetic technologies for vector control to reduce malaria transmission.

Title: Investigating chemical cues associated with natural mosquito larval habitat grasses in western Kenya

Getachew E. Bokore^{1,2}, Tullu Bukhari², Patrick Onyango², Mike N. Okal¹, David Tchouassi¹, Ulrike Fillinger¹

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Complimentary control strategies are required to target malaria vectors with pre-dominant outdoor feeding behaviour and those resistant to insecticides. The development of attract and kill strategies based on effective attractants for vectors in search of egg-laying sites could be highly beneficial since all gravid females would be targeted irrespective of their level of insecticide resistance and feeding behaviour. Previous research has been shown that gravid females respond to cues released from plants, soil and microbes associated with aquatic habitats.

Oviposition preference responses of gravid *Anopheles gambiae* to grasses were measured in olfactometer and large semi-field systems at icipe, Mbita, western Kenya. Three different grasses namely *Cyperus rotundus*, *C. papyrus* and *Panicum repens* were evaluated for their attractiveness to gravid mosquitoes.

Gravid mosquitoes were 5 times more likely to respond to *C. rotundus* than the reference experiment (water vs water) in the olfactometer. In the semi-field gravid mosquitoes were 2.2 times more likely to be trapped in modified BG-sentinel oviposition traps containing *C. rotundus*. *Cyperus papyrus* was 3.5 and 1.9 times more attractive than water in olfactometer and semi-field system respectively. In the olfactometer, *Panicum repens* was 2.1 times more attractive to the gravid mosquitoes than water alone.

All three grass species studied were attractive to gravid *Anopheles* mosquitoes with *C. rotundus* performing the best. Volatile chemicals released by the intact plants increase the odour-orientation of gravid malaria females towards the source of the volatiles. It suggests that volatiles from grasses strongly associated with water are not very specific but are general volatiles released by many grasses.

Keywords: Attractant, gravid, attract and kill, malaria vectors, grasses, oviposition

Title: A Case for Change in Vector Control Registration

Presenter: Angus Spiers

Background & Objective: Innovation 2 Impact was established to streamline the path to market for innovative vector control tools. Since its inception, much of the focus has been on supporting the development of the new WHO Prequalification process for vector control tools. That process is now functional and with a number of products already prequalified meaning attention has turned to addressing country access to innovative vector control tools. To this end, i2i has undertaken a study to characterise the registration process for vector control tools and look into potential efficiencies that can be gained through the use of the prequalification process and product dossier.

Materials & Method: A 12 country study was undertaken comprising the review of available documentation, over 100 interviews with numerous stakeholders and country visits to discuss the registration process with the regulators. Results have been collated and discussed with key partners to provide recommendations on a way forward.

Results & Discussion: The results clearly demonstrate the challenges of registering products in African countries from the perspective of the countries and manufacturers. Six main challenges will be discussed 1) Registration authority fragmentation 2) Available expertise to evaluate products 3) Need for specific requirements for VC tools 4) Lack of financial resources 5) communication between authorities 6) Transparency of the registration process.

Conclusions/Next Steps: The diversity of regulatory authorities, processes and requirements make streamlining country registration difficult. However, it is imperative that with the raft of new tools on the horizon that countries are prepared to review and register these products in an informed and effective manner. The new prequalification dossier, combined with renewed political commitment and regional leadership provides a unique opportunity for progress.

Title: The protective gap of indoor residual spraying (IRS): wall modifications after spraying affects actual coverage and hampers malaria elimination efforts

Authors: Mercy Opiyo

Whilst the residual effect of the insecticides and changes in mosquito behaviors are monitored after IRS implementation, human behaviors that may affect IRS efficacy such as replastering, painting or washing of treated wall surfaces are never. The question remains to what extent the number of people truly protected by IRS are overestimated, and how this affects the ongoing large-scale efficacy studies on the additional effect of IRS on top of nets. The purpose of the study **is to quantify the frequency of those human behaviors over time.** Together with data on the residual effect of insecticides and information on mosquito bionomics, the protective gap of IRS can be properly quantified, which will allow designing appropriate mitigation strategies.

The study is conducted in Matutuine and Boane districts in Maputo Province, southern Mozambique from November 2018-June 2019. Different IRS products were implemented; Matutuine was sprayed with Actellic® and Boane with SumiShield®. The data is collected monthly using structured questionnaires in each of the 700 households selected in all the districts.

Our preliminary data demonstrates that in Matutuine, during month one, about 15 % of all the people interviewed had their walls modified by either washing, replastering or painting, and by month six, over 40 % had modified their walls either once or twice. In Boane, the modification is about 4 % and 7 % in the first month and the sixth month respectively. Bedrooms and living rooms are the most targeted for modification in each district. Frequency of adding a new room/house on the compound after IRS application is 8 % and 24 % in months one and six respectively in Matutuine. In Boane, this is about 2 % and 7 % by months one and six respectively. The analysis is still ongoing, and complete results will be presented during the meeting.

Title: Exploring the Spatio-Temporal Distribution of Malaria Cases in the Presence of Indoor Residual Spraying (IRS) Within a Sugar Estate in Southern Malawi

Authors: Hoek Spaans R.¹, Mkumbwa, A.², Nasoni, P.², Stanton M.C.^{1,3}, **Jones C.M**.^{1,4} ¹Liverpool School of Tropical Medicine, Liverpool, UK; ²Illovo Sugar Malawi, Nchalo, Malawi; ³ Lancaster Medical School, Lancaster University, UK; ⁴Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

Background: Whilst indoor residual spraying (IRS) is a highly effective malaria control measure, it is not widely implemented by Malawi's National Malaria Control Programme (NMCP) due to challenges related to costs and emerging resistance to the most widely used insecticides. Privately-run IRS campaigns are however occurring across the country such at that being conducted by Illovo Sugar Africa. Illovo Sugar Malawi consists of two sites, one of which is in Nchalo, Chikwawa district, southern Malawi and covers an area of 150km² with a resident population of 13,500. The aim of this study is to use routinely collected malaria case data from the seven health clinics within the estate, in addition to remotely sensed environmental data and local weather station data, to identify spatial and temporal patterns in malaria risk plus measure the impact of IRS on malaria incidence.

Methods: The spatial and temporal dynamics of monthly malaria cases were investigated for 2015-2018 using time series plots and prevalence maps. The relationship between monthly malaria incidence and environment and weather (temperature, rainfall) was explored, and the temporal lag between these variables and the subsequent impact on malaria incidence was identified. Generalised linear mixed models were then used to quantify the impact of environment, weather and IRS on malaria incidence.

Results: A clear seasonal trend in malaria cases was observed, with inter-annual variability in incidence potentially being driven by fluctuations in rainfall. Spatial patterns in incidence at the clinic-level were observed which we hypothesise is related to socio-economic and environmental factors. Data analysis relating to the impact of IRS on malaria incidence is ongoing and is expected to be completed by September 2019.

Conclusion: Spatial and temporal heterogeneities in malaria cases identified in this analysis can be used to guide future control activities such as more targeted IRS or larval source management.

Title: Predicting *Anopheles gambiae* s.l. mosquito age and species in Burkina Faso by using mid Infra-red spectroscopy

Authors: Roger Sanou¹, Abdoulaye Niang¹, Doreen Siria², San Coulibaly¹, François Wiminga¹, Adrien Marie Gaston Belem³, Fredros O. Okumu², Mario Gonzalez⁴, Simon Babayan⁵, Heather Ferguson⁵, Francesco Baldini⁵, and Abdoulaye Diabaté¹

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Background: Mid infra-red spectroscopy (MIRS) is a measurement of molecular vibrational bands in the mosquito cuticle, a new approach that could be used for mosquito age and species identification as part of malaria vector surveillance. Here, we report the results of the analysis of the MIRS and machine learning on major malaria vectors to identify the age and species of *Anopheles gambiae* s.l in Bobo-Dioulasso, Burkina Faso.

Methods: The study was carried out in Bobo-Dioulasso on females collected from the field and the lab to set up respectively the training and the validation sets. The field samples were randomly collected among indoor resting mosquitoes in two villages, Soumousso and Vallee du Kou. Mosquitoes were brought to the semi-field vector sphere in Vallee du Kou, for oviposition under natural conditions. Individual egging was performed, and maternal females were identified later using PCR to isolate *An. gambiae* from *An. coluzzii*. The offspring from both species were pulled into trays and fed until adulthood and F1 adult mosquitoes collected from Day1 to Day17 as well as lab strains and at 3 different physiological states (unfed, blood fed, and gravid). In addition, samples from both lab strains were transferred as pupae to the semi-field vector sphere and emerging adults were collected at different dates under field conditions. And then all the samples collected were kept for at least 3 days before scanning.

Results: Preliminary results from the scanned samples showed that MIRS could predict and identify mosquito age up to 90% of accuracy with lab mosquitoes whereas age prediction was above 80% of accuracy for the field and lab mosquitoes.

Conclusion: This new approach combining MIRS and machine learning enables to develop new easy-to-use method requiring no reagent and less time consuming for the analysis and prediction of age and species of malaria vectors.

Key words: machine learning, age, species, prediction, accuracy, *Anopheles gambiae* s.l., vector sphere.

Title: Vectorbase.Org, Popbiomap Tool For Surveillance Data Visualization And Sharing

Authors: Samuel Rund

VectorBase is a Bioinformatics Resource Center for Invertebrate Vectors of Human Pathogens that hosts data and builds tools to facilitate research, monitor and control of vector-borne diseases. The population biology (PopBio) data includes insecticide resistance phenotypes and genotypes, population abundance, pathogen infection status, genomic variation and blood-meal identification, which can be freely accessed here www.vectorbase.org/popbio/ map. PopBio data is geotagged and can be queried and browsed with a map-based interface that has autocomplete, summary statistics and graphs, share screen, spreadsheet-ready data download for any user query, among other features. PopBio data comes from scientific papers, vector control districts, ministries of health, public and private health agencies and international initiatives like the Worldwide Insecticide Resistance Network (WIN). PopBio allows the interrogation and visualization of worldwide populations for more than 300 species. We operate a continuous process of literature curation to add new data every two months. Since 2017, our collections have grown dramatically. As of April 2019 we have population count records from 233 thousand trap collections from 4491 trapping locations, representing over 39 million mosquitoes, 15 thousand insecticide resistance genotypes, 20 thousand insecticide resistance phenotypes, 58 thousand pathogen assays, and 10 thousand blood meal analyses. Records we are currently processing will eclipse these numbers. VectorBase has been funded since year 2004 by the National Institutes of Health (NIH) -National Institute of Allergy and Infectious Diseases (NIAID).

Title: Assessment of five *Anopheles* collection methods for xenomonitoring of lymphatic filariasis and malaria transmission in two regions of Burkina Faso

Authors; Coulibaly S.^{1,2}, Bamogo R.^{1,2}, Hien A.^{1,2}, Sangaré I.^{1,2}, Soma D.D.^{1,2}, Ouari A.¹, Koalas L.^{1,2}, Sawadogo P.S.¹, Nikièma A.^{1,2}, Bationo R.¹, Tapsoba J.¹, Ouedraogo A.¹, Diabaté A.¹, Dabiré K.R.¹

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Background: entomological surveillance of lymphatic filariasis and malaria infection can play an important role in the decision-making of national programs to eliminate these diseases. As potential vector infection rates are often low for *Wuchereria bancrofti*, a method for collecting large numbers of mosquitoes is necessary. To overcome this challenge, this study assessed the efficiency of five vector collection methods for xenomonitoring of lymphatic filariasis in malaria co-infection context.

Methods: Vector collections were performed during the rainy season with different collection methods: Human landing catches (HLC) indoor and outdoor houses, Window exit-trap, Human-baited double net trap (HDN) and Pyrethrum spray catches (PSC). Molecular analyses were performed to identify *Anopheles gambiae s.l.* sibling species and to determine vector infection rates both for *Wucheria bancrofti* and *Plasmodium falciparum*.

Results: A total of 3,322 mosquitoes were collected during eight collection days and *Anopheles gambiae s.l.* was the vector caught in largest proportion (63.82%) in all sites. The HLC (indoor and outdoor) showed higher efficiency by collecting the highest mean number of anophelines per night in the Eastern (33.69 and 31.69) and Southwestern (53.81 and 30.06) health region. Screening of vectors infection index for *W. bancrofti* and *P. falciparum* showed a 0.004 and 0.13 respectively. PSC, HLC and Window Exit-trap were been the most effective collection methods for sampling vectors infected to *W. bancrofti* and *P. falciparum*.

Conclusion: This study revealed the probability of using others collection methods that HLC and PSC for surveying Anopheles populations and can be useful for xenomonitoring for both LF and malaria.

Keywords: Xenomonitoring, Anopheles gambiae s.l., lymphatic filariais, malaria, Burkina Faso

Title: Secondary vectors, resistance to insecticide and human behavioural pattern as drivers of residual malaria transmission in southern forested area of Cameroon

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Despite the use of indoor vector tools, people still complain about malaria in some setting. To better understand the fundamental limits of core interventions and to guide the prioritization of interventions targeting transmission that persists in the context of high vector control coverage, drivers of this persistence were investigate. Increase usage of treated nets in communities has reduced density of primary vectors and has opened way for competition between primary and secondary vectors which are becoming as abundant as primary vectors. Secondary vectors were found to display high biting rate during the first part of the night while primary vectors showed high densities mostly in the second part of the night. High human biting rate was observed in the morning in both sites. Mosquitoes were found to bite frequently outdoors. Local primary vectors were less frequently found resting indoors whereas An. gambiae was recorded resting indoors due to it high resistance level to pyrethroids. Several mosquitos' species were detected infected sustaining continuous malaria transmission in the locality. Mosquito infection rate was not significantly different from indoor to outdoor collection. Outdoor transmission contribute to 61.33% while secondary vector contribute to 36.45% of the total transmission. An. gambiae was found to display high resistance to permethrin and deltamethrin, insecticide used for bed net impregnation in Olama and Nyabessan. A variable level of susceptibility was recorded for the other species screened (An. moucheti, An. nili, An. marshallii, An. *paludis*). Despite a high LLINs ownership rate, usage was affected by the factors such as the use of damage nets, the non-frequent use of nets and the habit of going to sleep late. The following stress the necessity to develop vector control targeting different behaviour of mosquito in the locality for further actions in order to improve malaria control in the equatorial forest region.

Keywords: *Anopheles* sp, residual malaria transmission, insecticide resistance, forest, Cameroon

Title: Larval species diversity, seasonal occurrence and breeding habitat preference of mosquitoes transmitting Rift Valley fever and malaria in Baringo County, Kenya

Author: Isabella Ondiba

Background: Baseline information that is essential for determining the areas to target with larval control includes estimates of vector diversity and larval habitat preferences. Due to a lack of such information in Baringo County, Kenya, this study assessed species diversity and larval habitat preference of potential mosquito vectors of Rift Valley fever (RVF) and malaria.

Methods: Mosquito larvae were sampled from nine types of larval habitats and were identified morphologically. Species diversity was estimated by the Shannon's diversity index while larval habitat preference by RVF and malaria vectors was determined by ANOVA.

Results: A total of 7724 immature mosquitoes comprising 17 species belonging to four genera, namely *Anopheles*, *Culex*, *Aedes* and *Mansonia*, were identified. Among the 17 species, three *Anopheles* species are responsible for malaria transmission: *An. gambiae* (*s.l.*), *An. funestus* (*s.l.*) and *An. pharoensis*. Rift valley fever vectors included *Mansonia* spp. and *Culex* spp. The highest Shannon's diversity index was observed during the cold dry season (H = 2.487) and in the highland zone (H = 2.539) while the lowest diversity was recorded during the long rain season (H = 2.354) and in the riverine zone (H = 2.085). Ditches had the highest mean number of *Anopheles* larvae (16.6 larvae per sample) followed by swamp (12.4) and seasonal riverbed (10.7). Water pit and water pan had low mean numbers of *Anopheles* larvae (14 and 1.8, respectively) but relatively high mean numbers of culicines (16.9 and 13.7, respectively). Concrete tank was the least sampled type of habitat but had highest mean number of culicine larvae (333.7 1) followed distantly by water spring (38.9) and swamp (23.5). Overall, larval habitats were significantly different in terms of larval density (F(8,334) = 2.090, P = 0.036).

Conclusions: To our knowledge, the present study reports culicine larval species diversity in Baringo for the first time and the most preferred habitats were concrete tanks, water springs and swamps. Habitats preferred by *Anopheles* were mainly riverbed pools, ditches and swamps. Environmental management targeting the habitats most preferred by potential vectors can be part of integrated vector control in Baringo, especially during dry seasons.

Key words: Baringo, Species diversity, Larval habitats, Malaria, RVF, Season

Title: Importance of Semi Field Systems in Low Malaria Prevalence Settings with Multiple Species of Mosquitoes in Macha Zambia.

Authors: Limonty Simubali¹, Twig Mudenda¹, Gift Mwaanga¹, Alpha Simudoombe¹, Charlton Munsanje¹, Buster Munsanje¹, Chris Book^{1,2}, Philip E. Thuma^{1,2}, Douglas E. Norris² and Jennifer Stevenson^{1,2} for the International Centres for Excellence in Malaria Research

- 1. Macha Research Trust
- 2. Johns Hopkins Bloomberg School of Public Health

Introduction: Macha Research Trust is a research institute set in rural Southern Province, Zambia. Here, malaria prevalence as well as densities of the major vector, *An. arabiensis*, have declined greatly over the past 15 years. Studies of this and other vectors' behaviors and responses to control have become increasingly challenging due to low catch sizes. To be able to determine what factors may be driving this residual transmission and to study novel control, a system that allows for detailed studies is needed. Based on valuable input from Ifakara Heath Institute (IHI), Macha Research Trust (MRT) decided to construct a semi-field system to better understand these vector factors.

Description: With the help of IHI, MRT established its first semi-field system termed "N'ganda ya mansenya" the "Mosquito House". Measuring 29.2 x 21.4 x 5.3M, its domed roof and netted walls allows for equilibrium with the external environment whilst containing released mosquitoes. Three chambers are seeded with vegetation, water bodies allow for larval development, and huts similar to those in Macha have been constructed. Indoor sprinklers simulate rainfall, and goats provide blood meals for a self-replicating colony of *Anopheles gambiae*. A further three chambers with concrete floors allow for easy recapture of mosquitoes, and optimization of control tools. Weather loggers monitor temperature, humidity, wind direction and speed and illumination.

Lessons Learned: The Mosquito House has allowed for expansion of entomological studies and is used by multiple partners to study mosquito behavior and responses to novel control tools. Vigilance of staff and commitment of funds for regular maintenance of the facility integrity combined with regular surveillance for mosquito escape and establishment of mitigation measures are essential.

Conclusion/Next Steps: The Mosquito House continues to provide a unique facility for detailed entomological studies. Increased interest within Zambia, has led to its use by multiple partners. Funds have been identified for its continued maintenance.

Title: Assessment of *anopheles gambiae* and *anopheles arabiensis* oviposition site preference with aging of natural habitat materials.

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Background: Although there about eight species of mosquito under complex Anopheles gambiae sensu lato, An.arabiensis and an. gambiae remain most effective vectors of Malaria in sub-saharan Africa. These species tend to lay their eggs in small, temporary sunlit pools, however preference of these species on oviposition with the aging of natural habitat materials have not been studied. The study was experimental approach to investigate the oviposition preference of an. gambiae s.s. and an. arabiensis in Muheza Tanga Tanzania.

Methods: This study used water from natural habitat material and distilled water as control. Water was sampled from natural breeding habitats and test on day 0(fresh), 5, 10 20and 30 days old water to determine oviposition preference of gravid mosquitoes in different water aging. In each trial a total of forty replicates were used with five mosquitos each. The set up was done by using double paper cup technique with four cups per cage, two with distilled water(control) and other two with habitat water. Eggs were counted the next day and the numbers were recorded on the special form.

Also this was done by collection of water daily before experiment for the assessment of mosquitoes aging and response to oviposition site selection. Mosquitoes were of the same batch from same hatching day and were fed and given chance to oviposit till when they die.

Results: This study reveals the actual oviposition site selection for An. gambiae s.s. and an. arabiensis.

Conclusion: The information obtained from this study can be used to improve our understanding of oviposition preference of Afrian Malaria vectors especial an. gambiae s.s. and an. arabiensis so as to assist the improvement of the mosquito control techniques.

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Notes	

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Getting Back on Track to End Malaria

Since 2000, global efforts to fight malaria have greatly reduced the incidence of malaria by more than 500 million cases. The majority of the cases prevented can be attributed to vector control tools, with long lasting insecticide nets (LLINs) responsible for 68 percent of all malaria cases averted in Africa.

Set against the back drop of early success, however, the WHO World Malaria Report 2018 announced that after an unprecedented period of success in global malaria control, progress has stalled. Data from 2015– 2017 highlight that no significant progress in reducing global malaria cases was made in this period. There were an estimated 219 million cases and 435,000 related deaths in 2017.

The increase in malaria cases occurring is particularly worrying, given the significant increase in the distribution of LLINs. Between 2015 and 2017, a total of 624 million insecticide-treated mosquito nets (ITNs), mainly longlasting insecticidal nets (LLINs), were reported by manufacturers as having been delivered globally. This represents a substantial increase of nearly 200 million more nets over the previous period 2012–2014, when 465 million ITNs were delivered globally.

Although 56% percent of people at risk of malaria now have access to LLINs throughout the world, Africa carries the highest burden with 200 million cases reported in 2017. This may be further complicated by of the threat of insecticide resistance, which has been reported throughout Africa and threatens the efficacy of current tools.

As the malaria community explores what is required to get back on track, questions are emerging around the quality of LLINs, and the associated concerns around accountability and equitable access to the most effective tools.

Previous success supports optimism, but we must speak out on changes needed to get back on track. Let us work together to assure the right actions are taken.

On quality

There is a growing consensus for the need for consistent standards around the quality of LLINs with a greater adherence to WHO prequalification



An alarm bell is ringing around the world today: for the second straight year, there is a flatlining of what had been a steady decline in the global malaria epidemic.

Dr. Tedros Adhanom Ghebreyesus, Director-General, World Health Organization 2017 World Malaria Report





specification. Numerous reports of physical and chemical quality issues have been raised during the last two years. The issues appear systemic and in some instances involve large quantities of nets. Among the issues around physical dimensions: incorrect mesh size, knitting defects, imperfect seams and poor trimming have been reported. Among the issues around chemical content: LLINs with reduced pyrethroid content and poor wash resistance have been reported. With substandard chemical content, some WHO bioefficacy tests on new LLINs reported nearly 0% mortality after 20 standard WHO washes – far below the WHO cut-off of 80% mortality.

To ensure the consistent quality of LLINs, we call for a rigorous and systematic system of post shipment inspections and routine monitoring in country that looks at both the physical and chemical attributes of LLINs. This is crucial to ensure high quality LLINs are distributed to the community, providing effective protection.





On accountability

As a social good company, our mandate is to deliver innovative global health tools. We believe each LLIN manufacturer must be held accountable for the quality of each net. Accountability is about responsibility – holding each other responsible for the best way to achieve our common goal – eliminating malaria.

We welcome the establishment of a transparent WHO PQ complaints procedure so that instances of nonadherence to the product specifications are reported by National Malaria Control Programmes (NMCP) and donor agencies. While countries have reported on the poor quality of LLINs received, the information is not always shared with procurement agencies, donors and other vector control organisations.

We ask for an investigation of the issues reported on non-adherence, and we call on WHO PQ to take timely action resulting in the suspension and the delisting of substandard quality vector control products. Today, the actions of procurement agencies towards suppliers of substandard nets are not standardized. A transparent and active WHO PQ system with feedback to relevant stakeholders will guide informed procurement decisions. It will provide NMCP with a formal mechanism to ensure populations at the risk of malaria receive high quality LLIN and other vector control tools.

On equitable access to the right tools

In the drive for universal coverage, greater efforts should be directed to ensure NMCPs have equal access to data to inform decisions that best support their Vestergaard is a global company known for its innovative solutions to create a healthier, more sustainable planet. We operate under a humanitarian entrepreneurship model, whereby doing good is good business. More than 795 million PermaNet[®] LLINs have been distributed, protecting more than 1.6 billion people in more than 200 countries. In Africa, 550 million nets have been distributed protecting 1.1 billion.

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country's need. A country driven approach is more than simply the number of nets needed, but what product meets local needs. Countries must be confident that they have access to innovative tools to meet their elimination targets.

Pyrethroid resistance is threatening the efficacy of LLINs and jeopardizing the larger public health goal of eliminating malaria. While developing and testing the next generation of LLINs, in areas of pyrethroid resistant malaria mosquitoes, we call for shifting to the most efficacious LLINs available today as a key measure to put the fight against malaria back on track: PBO LLINs.

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"If you think you are too small to make a difference, you haven't spent the night with a mosquito."

An African Proverb