2nd WIN international
Conference on

"Integrated approaches and innovative tools for combating insecticide resistance in arbovirus vectors"

October 01-03th, 2018

Grand Copthorne Waterfront Hotel
Singapore

www.winsingapore2018.com

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October 1-3 2018, Grand Copthorne Waterfront hotel, Singapore

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2nd WIN International Conference on **"Integrated approaches and innovative tools for combating insecticide resistance in arbovirus vectors"**

October 1-3 2018, Grand Copthorne Waterfront hotel, Singapore

General information

Visit the conference website at https://WINSingapore2018.com

Venue

The 2nd WIN international Conference is held at the:
Grand Copthorne Waterfront Hotel
392 Havelock Road, Singapore 169 663
Tel +65 6733 0880
enquiry.gcw@millenniumhotels.com

https://www.millenniumhotels.com/en/singapore/grand-copthorne-waterfront/

From Singapore Airport to the Grand Copthrone Waterfront Hotel

The Singapore Changi Airport is on the eastern tip of Singapore and is located about 30 minutes from Grand Copthorne Waterfront Hotel by taxi. From the airport there are a number of ways to transfer to the hotel:

> **Taxi** is the easiest – simply follow the signs after clearing Customs. Meters are always used in Singapore and prices are reasonable. A trip to the city during the day will be between S$20 – S$30, including a-S$3 – S$5 airport surcharge. An additional 50% surcharge applies between midnight and 06.00am.

> **Mass Rapid Transit (MRT)** – the trains run from a station between Terminal 2 and Terminal 3, but you'll need to change trains at the Tanah Merah station to a city-bound train. The closest station to the Grand Copthorne Waterfront Singapore is Tiong Bahru MRT (EW17) station, which is on the East West Line (Green line).

> **Bus** – Bus terminals can be found in the basements of Terminal 1, Terminal 2 and Terminal 3. The bus service runs from 06.00am to midnight only.

The closest MRT station to Grand Copthorne Waterfront Hotel is the Tiong Bahru Station on the East West Line (Green line). Note this is the same MRT line as Changi Airport.

For more information on the public transport service, please refer to the website: http://journey.smrt.com.sg/journey/mrt_network_map/

Social media

Follow the WIN network on twitter @theWIN_network and tweet about the conference using #WINSingapore2018

In case of emergency

Ambulance: 995 / Police: 999 / ComfortDelgro (Taxi): +65 6552 1111

2nd WIN International Conference on **"Integrated approaches and innovative tools for combating insecticide resistance in arbovirus vectors"**

October 1-3 2018, Grand Copthorne Waterfront hotel, Singapore

Context of the conference

Arboviruses transmitted by mosquitoes such as Dengue, Zika, Chikungunya and Yellow fever, represent a major threat to public health worldwide. The Global Vector Control Response (GVCR) recently adopted at the WHO assembly aims to reduce the burden and threat of vector-borne diseases by 2030 through effective locally adapted sustainable vector control. The evidence base to support effective vector control is however limited for arboviral diseases due to a lack of vector surveillance and by the emergence and spread of insecticide resistance in mosquito vectors.

A recent systematic review highlighted that 40 countries already reported resistance to at least one chemical class in the mosquitoes *Aedes aegypti* or *Ae. albopictus*. Resistance is now recognized as a major threat for the control of arboviral diseases and has likely contributed to their re-emergence and spread in some parts of the world. Important knowledge gaps remains on mosquito resistance including its distribution, dynamics, mechanisms and its impact on vector control. Furthermore, there is an urgent need to review progress and achievements made in the deployment of integrated approaches and/or innovative technologies for the surveillance and control of arbovirus vectors and to discuss their potential for mitigating insecticide resistance.

Aim of the conference

Organized jointly by the French Institut de Recherche pour le Développement (IRD) and Duke-NUS Medical School of Singapore, the 2nd WIN conference will serve as a forum for addressing the progress, research gaps and priorities for the control of vectors of emerging arboviruses and the management of insecticide resistance. The conference has been labelled **"France-Singapore Year of Innovation 2018"**.

The conference will facilitate the involvement of scientists, stakeholders, members of the private and public sectors, and decision makers from around the world to share knowledge and ideas and to provide recommendations for the improvement of insecticide resistance surveillance and deployment of innovative vector control tools.

Objectives of the conference

- To update the **global burden of arbovirus diseases** and **spread of their vectors worldwide**
- To review the current knowledge on the **distribution, levels and mechanisms of insecticide resistance**
- To understand the **operational implications of resistance** and detail the options for management when strategies fail
- To review the performances of **new technologies and strategies for the control of invasive vectors** and their potential for **mitigating insecticide resistance**
- To foster **private-public partnership** for the development of new insecticide products for vector control

Expected outcomes

The conference is expected to raise awareness for strengthening the capacity of national authorities in resistance monitoring and deployment of alternative tools for arboviral diseases control. The conference will focus on basic and translational research with the scope of enhancing vector control and fostering

innovation. Round tables open to all participants will be set up to leverage the knowledge of the audience into strategies that may accelerate the translation of vector research into policies and programs.

A follow up of the 1st WIN conference, Rio de Janeiro, Brazil

The first international conference on Insecticide resistance in vectors of arboviruses, held in Rio de Janeiro, Brazil, from December 5 to 8 2016, aimed to identify strategies for the development and implementation of standardized insecticide resistance management and to define research priorities for control of vectors of arboviruses. Representatives from 94 institutions working on vector-borne diseases were present including research institutions and universities, international organizations (WHO), development agencies (USAID), ministries of health and members of the private sector.

Supported by the WHO Research & Training program on Tropical Diseases (TDR), the Departement of Neglected Tropical Diseases (NTDs) and the U.S. Centers for Disease Control and Prevention (CDC), the first WIN conference attracted more than 160 participants of around 30 different nationalities and, was followed by 70,000 people on the web.

The workshop proceedings of the 1st WIN conference were published in **Parasites and Vectors** (https://doi.org/10.1186/s13071-017-2224-3)

WIN: a multidisciplinary approach

The WIN (Worldwide Insecticide resistance Network) was founded in March 2016 by the French National Research Institute for Sustainable Development (IRD) and the French National Centre for Scientific Research (CNRS) in response to a call of the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Department of Neglected Tropical Diseases (NTD), which provided first financial support. It currently involves 19 universities and research organizations, all internationally recognized in the field of vector-borne diseases caused by mosquitoes. The network is represented on the six continents and benefits from international leadership in vector resistance.

Uniting the international scientific community. The Worldwide Insecticide resistance Network (WIN) aims to support international efforts to mitigate insecticide resistance in arbovirus vectors. The missions are to:

- Review evidence, fill knowledge gaps and identify research priorities for vector control;
- Collect comparable, representative and accurate Insecticide resistance data for policy decisions;
- Strengthen national capacities for early detection and surveillance of resistance;
- Advice to decision makers to establish IRM and deploy resistance breaking tools.

The WIN brings the scientific community together by organizing a biennial international conference on insecticide resistance and innovative control strategies for arbovirus vectors.

Promoting research. Since it was founded, the WIN has published several review articles identifying research priorities and current shortcomings in the field of vector control and resistance management. The network's goal is to support research by attracting funds specifically for basic and applied research projects led jointly by its institutional members. The network also aims to promote innovative, alternative control strategies to improve the control of resistant mosquitoes.

Guiding public health policies. The WIN aims to support the WHO and public health bodies to take decisions on resistance management and mosquito control and, thanks to its international experts, is able to offer consultancy services and produce technical reports in this area. The network has built a global reference database, enabling each country to identify the state of resistance locally and to use this information as a basis for guiding strategies to deal with resistant mosquitoes.

Training public health professionals. The network also plans to organize and deliver basic to expert training workshops for public health professionals and academics with a view to strengthening capacities in insecticide resistance monitoring in the countries affected by these diseases.

A public-private coalition for the general good. The WIN is based on a membership system, bringing together universities, research institutes, international organizations, not-for profit organizations and industries, to put insecticide resistance in the global vector control agenda. The WIN aims to foster innovation by strengthening "public-private" partnerships and promoting the latest technological advancements in the area of chemical, biological and genetic control.

Hosted at the IRD center in Montpellier, France, the WIN is welcoming new partnerships to support research, training, networking and expertise activities in vector control with a view to reduce the global burden of vector-borne diseases.

Programme at a glance

Monday October 1st, 2018

08:15-09:00	Registration
09:00-09:30	Welcome notes
09:30-12:30	Scientific Session 1: Challenges and prospects for the control of emerging arboviral diseases
10:45-11:15	*Coffee break & Poster session*
12:30-14:00	*Lunch (Grand Ballroom 2) - Group photo*
14:00-18:00	Scientific Session 2: Insecticide resistance, operational consequences and options for management
15:45-16:15	*Coffee break & Poster session*

Tuesday October 2nd, 2018

09:00-12:30	Scientific Session 3: New technologies and strategies for the control of invasive or insecticide-resistant vectors
10:30-11:00	*Coffee break & Poster session*
12:30-14:00	*Lunch (Grand Ballroom 2)*
12:30-14:00	Side-Event: Perspectives for the development of an insecticide testing laboratory (ITL) at Singapore *(by invitation only)*
14:00-18:00	Session 4: Public / private initiatives to foster innovation in public health
15:45-16:15	*Coffee break & Poster session*
19:00-22:30	*Cocktail & dinner (at Hotel Promenade)*

Wednesday October 3rd, 2018

09:00-12:30	Round tables	
09:00-10:00	Round table 1: Are SDGs for dengue and other arboviral diseases achievable with existing vector control tools?	All members
10:00-11:00	Round table 2: Insecticide resistance: a trick or a real threat for vector control? Where's the proof that it's having an operational impact?	All members
11:00-11:45	*Coffee break & Poster session*	
12:30-14:00	Lunch (*Grand Ballroom 2*)	
14:00-18:00	WIN internal meeting	

Scientific programme

Monday October 1st, 2018
- Grand Copthorne Waterfront hotel-

08:15-09:00 Registration

09:00-09:30 Welcome notes

Room: Grand Ballroom, level 4

09:00-09:05	Welcome address from the National Environmental Agency	**Mr. Derek Ho**, Director-General Public Health, NEA, Republic of Singapore
09:05-09:10	Welcome address from the French Ministry of Foreign Affairs in Singapore	**Mr. Marc Abensour,** Ambassador of France to the Republic of Singapore
09:10-09:15	Welcome address from the DUKE-NUS medical school	**Prof Patrick Casey**, Senior Vice Dean, Research, DUKE, NUS, Republic of Singapore
09:15-09:20	Welcome address from the Department of Control of Neglected Tropical Diseases (NTD), World Health Organization	**Dr. Raman Velayudhan,** Coordinator, WHO-NTD, Switzerland
09:20-09:25	Welcome address from the Special Programme for Research and Training in Tropical Diseases (TDR), World Health Organization	**Dr. Florence Fouque,** Team Leader on vectors, WHO-TDR Switzerland
09:25-09:30	Objectives of the conference	**Dr. Julien Pompon**, Duke-NUS Medical School, Republic of Singapore **Dr. Vincent Corbel**, Institut de Recherche pour le Développement, France

Monday October 1st, 2018 (continued)

09:30-12:30	**Scientific Session 1: Challenges and prospects for the control of emerging arboviral diseases**	
Room:	**Grand Ballroom, level 4**	
Chair persons:	**Dr. Raman Velayudhan (NTD) / Dr. Florence Fouque (TDR)**	
09:30-09:50	Public Health Priorities for Prevention and Control of Epidemic Arboviral Diseases (pdf)	**Dr. Duane Gubler**, Duke-NUS Medical School, Singapore
09:50-10:10	The WHO global vector control response to reduce the burden of vector borne diseases (pdf)	**Dr. Raman Velayudhan**, World health Organization, NTD, Switzerland
10:10-10:30	The use of Wolbachia to disrupt dengue, Zika and chikungunya transmission by *Aedes aegypti*	**Dr. Scott O'Neill**, World Mosquito Program, Vietnam
10:30-10:45	Discussions	
10:45-11:15	***Coffee break & Poster session***	
Chair persons:	**Assoc. Prof. Lee Ching Ng (NEA) / Dr. Greg Devine (QIMR)**	
11:15-11:35	Arbovirus vectors in SEA: a plea against ignorance (pdf)	**Dr. Didier Fontenille**, Institut Pasteur, Cambodia
11:35-11:55	Project Wolbachia Singapore – Exploring a novel tool for *Aedes* control	**Assoc. Prof. Lee Ching Ng**, National Environment Agency, Singapore
11:55-12:15	Detection of Resistance in Insects Vectors of Human Disease: The Road Forward (pdf)	**Prof. Jeffrey Scott**, Cornell University, USA
12:15-12:30	Discussions	
12:30-14:00	***Lunch (Grand Ballroom 2) - Group photo***	

Monday October 1st, 2018 (continued)

14:00-18:00	**Scientific Session 2: Insecticide resistance, operational consequences and options for management**	
Room:	**Grand Ballroom, level 4**	
Chair persons:	**Dr. Fabrice Chandre (IRD) / Dr. Shinji Kasai (NIID)**	
14:00-14:15	Insecticide susceptibility status and activity of detoxifying enzymes in larvae and adult *Aedes albopictus* from six regions of Madagascar (pdf)	**Dr. Fara N. Raharimalala,** Institut Pasteur, Madagascar
14:15-14:30	Insecticide resistance status of arbovirus vectors, *Aedes aegypti* and *Ae. albopictus* in Laos: distribution, levels and mechanisms (pdf)	**Dr. Sébastien Marcombe,** Institut Pasteur, Lao PDR
14:30-14:45	The last frontier: origin and insecticide susceptibility of a recently introduced *Aedes albopictus* population from Portugal (pdf)	**Dr. João Pinto,** Instituto de Higiene e Medicina Tropical, Portugal
14:45-15:00	Insecticide Resistance Monitoring in *Aedes aegypti* from Brazil after Zika outbreak (pdf)	**Dr. Ademir Martins,** FIOCRUZ, Brasil
15:00-15:15	Systematizing insecticide resistance testing for *Aedes* spp. in Latin America: What will it take? (pdf)	**Dr. Nelson Grisales,** Abt associates, USA
15:15-15:30	A geographical perspective on insecticide resistance and mechanisms in the major *Aedes* vectors of arboviruses infecting humans (pdf)	**Dr. David Weetman,** Liverpool School of Tropical Medicine, UK
15:30-15:45	Discussions	
15:45-16:15	***Coffee break & Poster session***	
Chair persons:	**Dr. Julien Pompon (DUKE-NUS) / Dr. Jean-Philippe David (CNRS)**	
16:15-16:30	Novel DNA markers of insecticide resistance in Arbovirus vectors for improving resistance monitoring and management	**Dr. Jean-Philippe David,** Centre National de la Recherche Scientifique, France
16:30-16:45	First detection of a Vssc allele V1016G in *Aedes albopictus* collected from Asia and Europe: A new emerging threat to controlling arboviral diseases	**Dr. Shinji Kasai,** National Institute of Infectious Diseases, Japan

Monday October 1st, 2018 (continued)

16:45-17:00	Insecticide resistance and its operational implications for dengue control on the Indonesian island of Bali (pdf)	**Mrs Erly Sintya Dewi,** Universitas Warmadewa, Indonesia
17:00-17:15	Experimental evaluation of the impact of household aerosolized insecticides on pyrethroid resistant *Aedes aegypti* (pdf)	**Dr. Gabriela Gonzalez-Olivera,** Universidad Autónoma de Yucatán, Mexico
17:15-17:30	Pyrethroid-resistant mosquitoes compromise Australia's aircraft disinsection procedures	**Dr. Gregor Devine,** QIMR Berghofer Medical Research Institute, Australia
17:30-17:45	Management of insecticide resistance in arbovirus mosquito vectors: advances and challenges (pdf)	**Dr. Fabrice Chandre,** Institut de Recherche pour le Développement, France
17:45-18:00	Discussions	

Tuesday October 2nd, 2018
- Grand Copthorne Waterfront hotel -

09:00-12:30 Scientific Session 3: New technologies and strategies for the control of invasive or insecticide-resistant vectors

Room: **Grand Ballroom, level 4**

Chair persons: **Prof. Hassan Vatandoost (TUMS) / Dr. João Pinto (IHTM)**

09:00-09:15	Neighbors help neighbors control urban mosquitoes (pdf)	**Prof. Dina Fonseca,** Rutgers University, USA
09:15-09:30	Gravitrap as a surveillance and control tool	**Dr. Chee-Seng Chong,** National Environment Agency, Singapore
09:30-09:45	Promising new tools to fight *Aedes* mosquitoes in Thailand	**Dr. Alongkot Ponlawat,** USAMD-AFRIMS, Thailand
09:45-10:00	Alarming *Aedes aegypti* resistance in Cambodia: Skirting resistance with an integrated vector management in an entomological / epidemiological approach	**Dr. Sébastien Boyer,** Institut Pasteur, Cambodia

Tuesday October 2nd, 2018 (continued)

10:00-10:15	Integrated *Aedes* management (IAM) for the control of *Aedes*-borne diseases (pdf)	**Dr. David Roiz,** Institut de Recherche pour le Développement, France
10:15-10:30	Discussion	
10:30-11:00	***Coffee break & Poster session***	
Chair persons:	**Dr. John Grieco (NDU) / Dr. Dina Fonseca (RU)**	
11:00-11:15	Alternative Strategies for Arbovirus Control	**Dr. Nicole Achee,** Notre Dame U., USA
11:15-11:30	Overview of Auto-dissemination technology for mosquito controls (pdf)	**Dr. Devi Shankar Suman,** Ministry of Environment, India
11:30-11:45	Targeted Indoor Residual Spraying (TIRS) for the control of *Aedes aegypti*	**Dr. Gregor Devine,** QIMR Berghofer Medical Research Institute, Australia
11:45-12:00	New developments in the use of the Sterile Insect Technique (SIT) (pdf)	**Dr. David Damiens,** IRD-CYROI, La Réunion, France
12:00-12:15	Gene editing population sex determination pathway for vector control	**Dr. Kyrou Kyros,** Imperial College, UK
12:15-12:30	Discussion	
12:30-14:00	***Lunch** (Grand Ballroom 2)*	
12:30-14:00	**Side-Event: Perspectives for the development of an insecticide testing laboratory (ITL) at Singapore**	Private session (by invitation only)
Room:	**Toucan, Level 4**	

Tuesday October 2nd, 2018 (continued)

14:00-18:00	**Session 4: Public / private initiatives to foster innovation in public health**	
Room:	**Grand Ballroom, level 4**	
Chair persons:	**Dr. K. Raghavendra (NIMR) / Dr. W. Juntarajumnong (KU)**	
14:00-14:15	Filling the resistance management gap: the power of intrinsic resistance management in Bacillus thuringiensis israelensis strain AM65-52 protects public health with effective control of organophosphate and pyrethroid resistant vectors	**Mr. Peter DeChant,** Valent Bioscience, USA
14:15-14:30	Fludora Co-Max®: A new space spray combination for mosquito resistance management programs (pdf)	**Mr. Jason Nash,** Bayer, Singapore
14:30-14:45	Effective *Aedes* Control based on resting behaviour & New IRS Tools (pdf)	**Mr. Iñigo Garmendia,** Goizper spraying, Spain
14:45-15:00	Innovative formulation for long lasting residual insecticides (pdf)	**Mr. Siao Jing Sam,** Syngenta, Singapore
15:00-15:15	Managing *Aedes* spp. with push-pull tactics: how non-repellent chemistries may mitigate behavior and vectorial capacities (pdf)	**Mr. James Austin,** BASF, USA
15:15-15:30	Friendly™ mosquitoes for efficient and safe vector control	**Dr. Kevin Gorman,** Oxitec, UK *(excused)*
15:30-15:45	Discussion	
15:45-16:15	***Coffee break & Poster session***	
Chair persons:	**Dr. Dominic Schuler (WHO-PQT) / Dr. Vincent Corbel (IRD)**	
16:15-16:30	Innovation to Impact: Update on progress and future priorities (pdf)	**Dr. Angus Spiers,** Innovation to Impact (I2I), UK
16:30-16:45	Overview of the prequalification of vector control products (pdf)	**Mr. Dominic Schuler,** WHO-PQT, Switzerland
16:45-17:00	Building a vector control toolbox for Integrated Vector Management (IVM) (pdf)	**Dr. Nick Hamon,** Innovative Vector Control Consortium, UK

Tuesday October 2nd, 2018 (continued)

17:00-17:15	Legislative framework for vector control: the situation for European countries, including overseas territories (pdf)	**Dr. Florence Fouque**, WHO-TDR, Switzerland
17:30-17:45	VectorBase.org's PopBioMap: An online resource for insecticide resistance data (pdf)	**Dr. Samuel Rund**, Notre-Dame University, USA
17:45-18:00	Toward a global partnership for the control of insecticide resistant Arbovirus vectors: The WIN initiative (pdf)	**Dr. Vincent Corbel**, IRD, France
17:45-18:00	Discussion	
19:00-22:30	***Cocktail & dinner** (at Hotel Promenade, Level 1)*	

Wednesday October 3rd, 2018
- Grand Copthorne Waterfront hotel -

09:00-12:30 Round tables

09:00-10:00	**Round table 1: Are SDGs for dengue and other arboviral diseases achievable with existing vector control tools?**	All members
Room:	**Grand Ballroom, level 4**	
Chairman:	Dr. Raman Velayudhan, WHO-NTD, Switzerland	
Rapporteur:	Dr. Nicole Achee, NDU, USA	
10:00-11:00	**Round table 2: Insecticide resistance: a trick or a real threat for vector control? Where's the proof that it's having an operational impact?**	All members
Room:	**Grand Ballroom, level 4**	
Chairman:	Dr. Greg Devine, QIMR, Australia	
Rapporteur:	Dr. Dave Weetman, LSTM, UK	
11:00-11:45	***Coffee break & Poster session***	
11:45-12:15	Conclusions / Recommendations	Rapporteurs of each round table

Wednesday October 3rd, 2018 (continued)

12:15-12:30	Official closure of the conference	**Dr. Florence Fouque**, WHO-TDR, Switzerland
12:30-14:00	***Lunch** (Grand Ballroom 2)*	
14:00-18:00	**WIN internal meeting**	
Room:	**Canary, Level 4**	
14:00-17:00	WIN General Assembly	WIN members
17:00-18:00	WIN steering committee meeting	WIN members

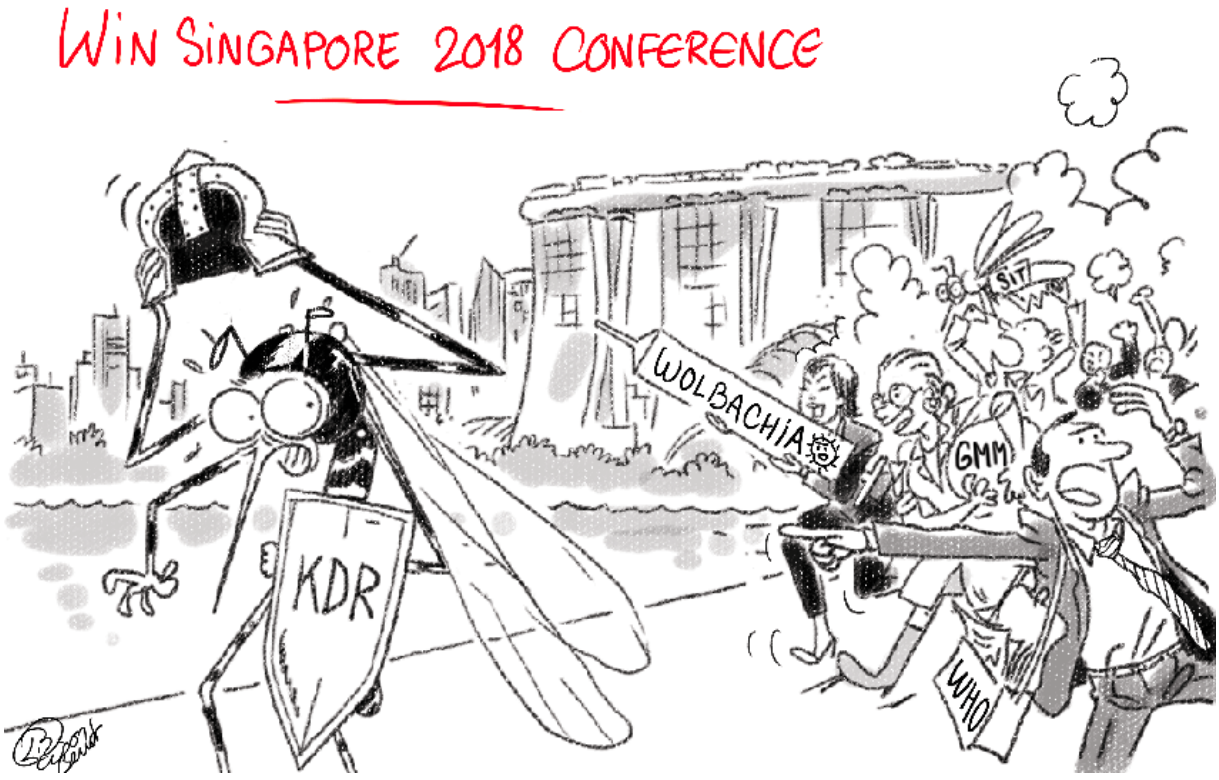

Table of abstracts

Keynote Speaker

Scientific session 1: Challenges and prospects for the control of emerging arboviral diseases

Public Health Priorities for Prevention and Control of Epidemic Arboviral Diseases

Duane J Gubler [1]

duane.gubler@duke-nus.edu.sg

[1] Duke-NUS Medical School, Singapore

The past 40 years has seen a dramatic emergence of epidemic arboviral diseases transmitted primarily by mosquitoes. The frequency and magnitude of the epidemics, especially those transmitted by urban *Aedes* species, have progressively increased over time, accelerating in the past 10 years. Dengue was the first of these viruses to emerge in the post-World War II period in Southeast Asia, spreading from less than 10 known endemic countries and only a few thousand cases reported each year in the 1960s, to 124 endemic countries worldwide, an estimated 400 million infections and 100 million symptomatic cases annually. Pandemic dengue has been followed by chikungunya and Zika fever, both of which have followed the same pattern of global geographic spread and increasing epidemic frequency and magnitude. There has also been an increase in local and regional epidemics of other arboviruses such as Japanese encephalitis, West Nile encephalitis, Venezuelan equine encephalitis, eastern equine encephalitis, St Louis encephalitis, Ross River, Rift Valley fever and others, as well as the emergence of newly recognized arboviruses. Recent high-profile epidemics of yellow fever in both Africa and the Americas has underscored that the risk for urban epidemic transmission of yellow fever is the highest in 60 years. If urban epidemics of yellow fever begin to spread geographically to the Asia Pacific region, it could threaten global economic security and create a global public health emergency. This talk will briefly review the changing epidemiology of these and other potential epidemic arboviral diseases and the prospects for prevention and control.

Global Vector Control Response 2017-2030

Raman Velayudhan [1]

VelayudhanR@who.int

[1] Department of Control of Neglected Tropical Diseases (CDS/NTD), World Health Organization

The Global Vector Control Response (GVCR) aims to reduce the burden and threat of vector-borne diseases through effective locally adapted sustainable vector control. Success will depend on the ability of countries to strengthen their vector surveillance and control programmes with capacity and financial resources. WHO will support the development of regional action plans and country activities based on GVCR strategies and priorities identified in the Country Cooperation Strategy (CCS) as part of country focus.

The key areas of activity that will radically change the control of vector-borne diseases:

• Aligning action across sectors, since vector control is more than the work done by ministry of health (MOH). MOH has to coordinate work with other relevant ministries and with city planners to eradicate breeding sites of urban vectors (*Aedes* mosquitoes, rodents etc.);

• Engaging and mobilizing communities to protect themselves and build resilience against future disease outbreaks (including impact of climate change);

• Enhancing surveillance to trigger early responses to increases in disease or vector populations, and to identify when and why interventions are not working as expected; and

• Scaling-up vector-control tools (including new tools) and using them in combination to maximize impact on disease while minimizing impact on the environment.

Specifically, the new integrated approach calls for national programmes to be realigned so that public health workers can focus on the complete spectrum of relevant vectors and thereby control all of the diseases they cause. Recognizing that efforts must be adapted to local needs and sustained, the success of the response will depend on the ability of countries to strengthen their vector-control programmes. National strategic plans need to be revised and country-specific targets defined till 2030.

The use of *Wolbachia* to disrupt dengue, Zika and chiikungunya transmission by *Aedes aegypti*

Scott O'Neill [1]

scott.oneill@worldmosquito.org

[1] World Mosquito Program, Institute Vector Borne Disease, Monash University, Clayton, VIC, Australia.

Novel methods for control of *Aedes* transmitted diseases are urgently needed. I will provide an update on the use of Wolbachia by the World Mosquito Program to reduce virus transmission by *Aedes aegypti* without the need to suppress the mosquito population. Recent results indicate that deployment can be done at scale and current indications are that this approach is having large positive impacts on disease in human populations. Moreover, the approach appears sustainable and in many settings, may be cost saving for governments to implement.

Arbovirus vectors in SEA: a plea against ignorance

Didier Fontenille*[1], Sebastien Boyer [1]

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[1] Institut Pasteur du Cambodge

In 1932, the eminent Nobel Prize winner Charles Nicolle prophetically wrote: "Nature's attempts to create new infectious diseases are constant. It is a fatal fact and we will never be able to detect them from their origin." In terms of vector borne diseases, both Chikungunya and Zika viruses have recently confirmed Nicolle's predictions, causing outbreaks despite being discovered decades earlier. In spite of the immutable certainty of emerging infectious diseases, we are not condemned to observe, suffer and react. Entomologists, modestly, can also help to know, predict and prevent. In Southeast Asia, particularly Cambodia, we know that numerous factors such as climate change, land use, deforestation/reforestation, urbanization, pesticides, and human behavior can contribute dramatically to vector risk. However, we are largely ignorant of the total distribution, abundance and biology of the mosquito species present. Previous studies have shown there is a wide diversity of arbovirus vectors in Asia. Greater than 430, 220 and 240 species of mosquitoes have been identified in Thailand, Vietnam, and Cambodia, respectively. Unfortunately, defensive strategies for vector control in most Asian countries remain a very classical fight with rare successes and numerous failures. There appears to be several reasons for this stalemate including paucity of field research as well as lack of interest and/or competency in research laboratories. Based on a few examples, we will see that there are more questions than answers: How can we effectively control vectors? How can we assess the risk of emergence and outbreak in the frame of environmental, social and climate change? What do and don't we know about the biology and biological evolution of mosquitoes (Ae. aegypti vs Ae. albopictus) and mosquito-virus coadaptation in Asia? What about sylvatic cycles? Overall, this talk makes a plea against ignorance, even by entomologists themselves!

Project *Wolbachia* Singapore – Exploring a novel tool for *Aedes* control

Lee Ching Ng [1]

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The National Environment Agency (NEA), Singapore is evaluating the use of male *Wolbachia*-carrying *Aedes aegypti* mosquitoes to further suppress the *Ae. aegypti* mosquitoes in the community. When these released male *Wolbachia-Aedes* mosquitoes mate with our urban *Ae. aegypti* females, the resultant eggs do not hatch. The continual release of male *Wolbachia-Aedes* mosquitoes over time is thus expected to bring about a reduction in the urban *Ae. aegypti* mosquito population. *Wolbachia* is a naturally occurring bacterium found in more than 60 per cent of insect species. The Phase 1 field study – conducted from October 2016 at three selected study sites at Braddell Heights, Tampines West and Nee Soon East – demonstrated that the released male *Wolbachia-Aedes* mosquitoes had successfully competed with the urban *Ae. aegypti* males and mated with some of the urban *Ae. aegypti* females. As a result, the releases led to a 50 per cent suppression of the urban *Ae. aegypti* mosquito population at the study sites. In the Phase 2 field study that began in April 2018, NEA will further improve the release methodologies and tactics to mitigate the challenges posed by Singapore's high-density and high-rise urban landscape. NEA hopes to use the *Wolbachia* technology to complement its current vector control programme.

Detection of Resistance in Insects Vectors of Human Disease: The Road Forward

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Insecticides have been a reliable method for the control of insect vectors of diseases for decades. The evolution of resistance has the potential to render these important tools ineffective and the costs, in terms of human health, would be catastrophic. In order to slow the evolution of insecticide resistance I propose that two pieces of information are critically important: accurate assessment of the phenotype, and methods for the rapid determination of the frequency of the mutations that confer resistance in field populations. First, the phenotype of resistance is not a binary trait and vector biologists should refrain from using methods that are inaccurate and/or do not capture the actual level of resistance within a population, such as single concentration assays. Second, if we are to make progress with understanding the factors that influence the evolution of resistance, the mutations responsible for the phenotype must be identified. This is somewhat simpler for mutations in target site genes (e.g. Vssc), but is much more challenging for other major resistance mechanisms, such as detoxification-mediated resistance. Some techniques, such as the use of model substrates to approximate a mechanism of resistance should be avoided. Even new high throughput sequencing technologies, as powerful and elegant as they are, have limitations that must be recognized. Examples will be provided, and a proposed blueprint for future studies of insecticide resistance in insect vectors of human disease will be presented.

Abstracts for oral presentation

Scientific session 2: Insecticide resistance, operational consequences and options for management

Insecticide susceptibility status and activity of detoxifying enzymes in larvae and adult *Aedes albopictus* from six regions of Madagascar

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Larvae or eggs were sampled in six localities: Antananarivo, Toamasina, Farafangana, Antsiranana, Mahajanga and Morondava and reared in the insectary. Bioassays in adults were assessed on F1 females using WHO diagnostic concentrations with propoxur, DDT, deltamethrin and fenitrothion and in larval stage, temephos and Bti were used with different concentrations. Three-day old non-blood-fed F1 females and 4th star larvae non-exposed to insecticides were stored at - 80°C for further biochemical analyzes.
In adult stage, bioassays revealed a possible resistance to DDT in all localities except Morondava. Mosquitoes from Mahajanga demonstrated a confirmed resistance to deltamethrin. Resistance to propoxur was observed in all localities. All mosquito populations were susceptible to fenitrothion except those of Antananarivo and Toamasina. For larval bioassay with Bti, all localities were susceptibles with the different concentrations tested except those Antsiranana and Toamasina. For the bioassay with different concentrations of temephos, all localities demonstrated a confirmed resistance.

Biochemical studies allowed highlighting an overproduction of alpha-esterases in adult mosquitoes from Morondava, of beta-esterases from Antsiranana, Mahajanga and Morondava and of CytP450 from Mahajanga and Farafangana. From larval detection, an overproduction of alpha-esterase was observed from Antsiranana, Mahajanga and Farafangana, of beta-esterases from Antsiranana and Farafangana, and of CytP450 from Antsiranana and Morondava.

The overexpression of these detoxifying enzymes appears well associated with the observed phenotypic resistance in *Ae. albopictus* from Antsiranana, Farafangana, Mahajanga and Morondava. The absence of overexpression in the two last sites (Antananarivo, Toamasina) suggests another type of insecticide resistance mechanism. These results provide the first baseline information on insecticide resistance and its mechanisms in populations of *Ae. albopictus* in Madagascar. Investigation of other types of mechanisms, such as alteration of insecticide target-sites due to single gene mutations is in progress to complete this study.

Insecticide resistance status of arbovirus vectors, *Aedes aegypti* and *Ae. albopictus* in Laos: distribution, levels and mechanisms

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Background. Knowledge on insecticide resistance in *Aedes* mosquitoes is a basic requirement to guide arbovirus vector control programs. In South-Est-Asia, resistance of *Ae. aegypti* and *Ae. albopictus* to chemical insecticides has been reported in several countries, but until now, there was no records in Lao PDR. To fill this gap, we investigated the frequency and distribution of insecticide resistance to several larvicides and adulticides in *Aedes* spp. collected in 12 provinces. We also investigated the underlying molecular mechanisms, including the identification of enzymes and knock-down mutations (kdr), involved in resistance. **Methods & Results**. Standard WHO larval bioassays revealed various levels of resistance to the commonly used larvicide temephos (Abate). Cylinder test on adults showed strong resistance to malathion (organophosphate), DDT (organochlorine) and the pyrethroids permethrin and deltamethrin insecticides. Synergist bioassays with the use of detoxification enzymes inhibitors showed a significant increased susceptibility of mosquitoes to insecticides, indicating that metabolic-based resistance mechanisms play a major role in resistance. Biochemical assays confirmed these results by showing significant elevated activities of cytochrome P450 monooxygenases (P450), glutathione S-transferases (GST) and carboxylesterases (CCE) in adults. The kdr mutations, V1016G and F1534C were detected by qPCR at low and high frequency, respectively, in all the *Ae. aegypti* populations tested. There was no significant difference between the frequency of kdr mutations (for both 1534C and 1016G) and the survival rate from DDT and permethrin ($p>0.05$) suggesting that kdr alleles may have only a minor role in resistance. The presence of Copy Number Variations (CNV) of insecticide resistance candidate genes previously associated with insecticide resistance was investigated by qPCR method and the results showed an important polymorphism of gene copy number for the gene encoding the carboxylesterase CCEAE3A. **Conclusions**. These results suggest that the high levels of insecticide resistance found in *Ae. aegypti* mosquitoes from Laos was the consequence of metabolic based resistance mechanisms with a minor role of the kdr mutations. Pyrethroids and temephos resistance was detected in most of the *Aedes* sp. populations tested (*Ae. aegypti* and *Ae. albopictus*), these findings have important implications for dengue vector control in Laos and highligh the urgent need to identify new insecticide families and new strategies to fight against arboviruses vectors.

The last frontier: origin and insecticide susceptibility of a recently introduced *Aedes albopictus* population from Portugal

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During the summer of 2017, two independent introduction events of the invasive mosquito *Aedes albopictus* were reported in Portugal. In the last week of September 2017, we have collected mosquito samples in the focus of Penafiel, in the north of the country, to study the genetic variation and insecticide susceptibility profile of the mosquito population. *Aedes albopictus* larvae were sampled mainly from storm drains and reared in the insectary to produce an F1 generation to be used in susceptibility bioassays. Adult mosquitoes were sampled by landing collections and kept for genetic analyses. Six insecticides were tested using WHO assays and discriminant doses: Permethrin (0.25%), Deltamethrin (0.03%), Cifluthrin (0.15%), Fenitrothion (1%), Bendiocarb (1%) and DDT (4%). Results showed susceptibility to pyrethroid insecticides (mortality >99%) but suspicion of reduced susceptibility to Fenitrothion (mortality rates: 89.7%-99.0%) and Bendiocarb (mortality rates: 69.9%-96.5). The analysis of 16 microsatellite loci was carried out in order to assess the genetic variation and putative geographic origin of this recently introduced mosquito population. Preliminary data suggest two independent origins for the introductions of *Ae. albopictus* in north and south of Portugal.

Insecticide Resistance Monitoring in *Aedes aegypti* from Brazil after Zika outbreak

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Brazil is a large country in South America, composed of 5,570 municipalities, amongst which 5,170 has the presence of *Aedes aegypti*. The country registered more than 60 and 75% of probable cases of dengue and chikungunya in 2016, and more than 20% of Zika cases registered in Americas so far. Insecticide resistance monitoring (IRM) has been employed by the dengue national control program coordinated by Ministry of Health (MoH) as one of the WHO recommended strategies to enhance vector control. During 1999-2013 four laboratories with expertise in field and bench work activities in Entomology, especially on vector control, composed a national network to support IRM in *Ae. aegypti*. This network evaluated around 70 populations per year, in average. Based on its results resistance to the larvicide temephos and to the adulticide deltamethrin was detected in most of the evaluated localities. Currently, MoH is distributing the IGR pyriproxyfen larvicide and the organophosphate malathion to be used in campaigns against *Ae. aegypti* in all states. As there was no update in IR resistance data since 2013, and driven by the alarming recent arboviruses outbreakes, a vast IRM scheme was set up in order to achieve a rapid feedback about the susceptible status to currently used insecticides, in a vast geographical amplitude. In total 146 municipalities were elected for sampling during 2017/18, and the eggs shipped toward two reference laboratories, where are performed dose-diagnostic bioassays with pyriproxyfen and malathion, as well as kdr genotyping. Dose-response bioassays would be considered for those populations where resistance is detected. This is the largest simultaneous IRM evaluation done so far in Brazil. The results will orient the MoH on better evaluating strategies for chemical control of *Ae. aegypti* considering each region of the country.

Systematizing insecticide resistance testing for *Aedes* spp. in Latin America: What will it take?

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In response to the Zika epidemic in the Americas, the United States Agency for International Development (USAID) funded the Zika AIRS Project (ZAP) to support the emergency response in Latin America and Caribbean (LAC) countries. Zika, spread by the mosquito vector *Aedes aegypti*, prompted increased efforts to effectively manage the vector and revitalize efforts to build vector control and entomological surveillance capacity in the region.

A critical step in the control of vector-borne disease is systematic insecticide resistance monitoring. Prior to the Zika epidemic, insecticide resistance testing on *Aedes* mosquitoes in most LAC countries was typically nonexistent or sporadic. ZAP worked directly with staff from Ministries of Health in Guatemala, Honduras, El Salvador, Paraguay, Jamaica, Guyana and the Dominican Republic to identify gaps and address challenges inhibiting effective and systematic insecticide resistance testing. A needs assessment was conducted in each site, reviewing systems capacity (infrastructure, equipment and supplies), technical capacity (trained and skilled staff), resources and budget.

The main challenges identified across all the countries were: 1) inadequate technical knowledge on appropriate protocols and methodologies; 2) poorly equipped entomology laboratories; 3) lack of essential supplies and resources; 4) lack of coordination between government and academic institutions; and, 5) perceived unimportance of resistance testing among key decision makers. To support a comprehensive approach to institutionalizing resistance testing, ZAP identified 3 main needs: 1) training of skilled staff; 2) establishment of high quality entomology laboratories; and, 3) raising awareness on importance of resistance testing.

ZAP will share its experience in building systems, technical capacity, and promoting appropriate resourcing in support of insecticide resistance testing in each country. Results will be shared about the effects of specific interventions on countries' capacities to conduct insecticide resistance testing and interpret results, and generating the demand and skills to ensure that findings are actively used to inform vector control decisions. Challenges remain, but important elements are now in place to provide a foundation for sustained insecticide resistance testing in the region.

A systematic approach to insecticide testing in LAC is critical and attainable but often requires additional efforts and resources. The efforts of ZAP can inform other countries in the region how to best integrate insecticide resistance testing into integrated vector management efforts, and reinforce the importance of evidence based decision-making.

A geographical perspective on insecticide resistance and mechanisms in the major *Aedes* vectors of arboviruses infecting humans

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Aedes aegypti and *Ae. albopictus* are the major vectors of multiple major arboviruses and control using insecticides is a critical component of disease management and prevention but may be threatened by insecticide resistance. We reviewed available evidence to investigate and map the geographical distribution of *Aedes* insecticide resistance and underlying mechanisms. Resistance to all four main classes of neurotoxic insecticide has been detected in the Americas, Africa and Asia but distributions are not homogeneous, suggesting both challenges and opportunities for resistance management. Interacting target site mutations are especially important in *Ae. aegypti* and show evidence of geographical patterning. Recent findings show how continued discovery work is required to ensure the most informative target site diagnostic markers are employed. Resistance-linked detoxification gene overexpression appears widespread and to involve many genes. Meta-analysis enables prioritisation of candidate genes for functional assessment and diagnostic development, which are key steps in understanding their relative impact on phenotypes relative to target site mutations. Estimating insecticide resistance in unsampled locations is currently hampered by a lack of standardisation and diagnostic doses, but could be greatly assisted by calibration and predictive application of existing DNA diagnostics for resistance. Widespread resistance calls for the careful use of existing formulations and implementation of insecticides with alternate modes of action.

Identifying novel genomic markers of insecticide resistance in the dengue mosquito *Aedes aegypti* by next-generation sequencing

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Insecticide resistance threatens the control of mosquitoes transmitting arboviral diseases worldwide. Although alternative vector control tools are being developed, their global implementation will require some time and managing resistance to the few available chemical insecticides is crucial for sustaining vector control efforts for the next decades. In mosquitoes, insecticide resistance is mainly the consequence of modifications of the proteins targeted by insecticides (target-site mutations) and of the biodegradation of insecticides by detoxification enzymes (metabolic resistance). Target-site mutations are relatively well characterized and easy to monitor using molecular diagnostic tools, while the genetic factors controlling metabolic resistance are poorly characterized impeding their monitoring in natural mosquito populations. In this context, we adopted an integrative approach combining experimental evolution, quantitative genetics and next-generation sequencing to identify novel genetic markers of insecticide resistance in the dengue mosquito *Aedes aegypti*. This study identified copy number variations and non-synonymous polymorphisms in genes encoding detoxification enzymes specifically associated with resistance to different insecticide families. These results pave the way for the development of novel diagnostic tools able to concomitantly track the whole range of insecticide resistance mechanisms in order to improve resistance management.

First detection of a Vssc allele V1016G in *Aedes albopictus* collected from Asia and Europe: A new emerging threat to controlling arboviral diseases

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We sampled 32 *Aedes albopictus* populations from 5 countries including Vietnam, Italy, Singapore, Brazil and Taiwan. Susceptibility of adult mosquitoes to a pyrethroid insecticide (permethrin) was conducted by topical application and WHO filter paper methods. Most populations of *Ae. albopictus* tested were highly susceptible to permethrin but few from Italy, Vietnam and Singapore, exhibited resistance. The target site for pyrethroids, a voltage-sensitive sodium channel (Vssc) was sequenced in surviving (resistant) populations after bioassays. Genotyping studies resulted in the detection of a knockdown resistance (kdr) allele V1016G in the Vssc for the first time from *Ae. albopictus*. The V1016G allele was detected in *Ae. albopictus* collected from Vietnam and Italy. The two other previously reported kdr alleles were also detected: F1534C in Vietnam and Singapore; and F1534S in Vietnam. Three resistant sub-strains each harbouring homozygous resistance alleles were established and susceptibilities to three different pyrethroids (with and without a cytochrome P450 inhibitor) were assayed. The bioassays indicated that the strain homozygous for the V1016G allele showed much greater levels of pyrethroid resistance than other strains harbouring F1534C or F1534S. Given that the V1016G allele was detected in *Ae. albopictus* collected not only from Asia, but also from a European country, it is possible that the mutated Vssc has already spread widely in the world. This study strongly emphasises the necessity for the frequent and regular monitoring of the V1016G allele in *Ae. albopictus*, particularly in temperate regions where this mosquito species is the major vector of arboviruses.

Insecticide resistance and its operational implications for dengue control on the Indonesian island of Bali

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Dengue is endemic on the Indonesian island of Bali and imposes a heavy economic burden. In the major outbreaks of 2010 and 2015 there were 12,574 and 10,704 reported cases with 35 and 28 fatalities. There are no effective vaccines or drugs available for the treatment of dengue and the only way to combat transmission is to target the mosquito vector, *Aedes aegypti*. In the capital, Denpasar, outbreak areas are treated with insecticidal fogs and space-sprays but little is known about the operational efficacy of the insecticides currently in use. Moreover, there is a poor understanding of how the results of standard resistance diagnostics might be interpreted in the context of insecticide efficacy in the field.

We investigated the phenotypes and genotypes of two pyrethroid-resistant *Aedes aegypti* strains. One originated from eggs collected in Denpasar in 2018 [DEN], the other from a lab-derived colony from Mexico [JP]. We characterised their phenotypes using WHO tube tests and cone tests and used "free-flight" rooms to explore the relationship between the results of these small-scale assays and more realistic insecticide-exposure.

In WHO tube tests, both DEN and JP showed low mortality rates when exposed to diagnostic concentrations of permethrin (5.2% and 14.3% respectively) and alpha-cypermethrin (14% and 22.3%, respectively). Malathion was far more effective but there remained evidence of resistance (60.4% and 76.4% mortality). As expected, mosquitoes surviving pyrethroid exposure exhibited high kdr frequencies. A reduced-genome (RAD-seq) analysis of those mosquitoes implicated other potential resistance mechanisms. The presence of enhanced oxidative activity was further examined using simple respirometers.

WHO cone tests, using recommended field-rates of permethrin (0.2g/m2) and malathion (2g/m2) showed that the forced exposure of mosquitoes to treated surfaces resulted in the death of all mosquitoes, regardless of strain. However, "free-flight" tests in patchily treated rooms demonstrated that, although susceptible Australian *Ae. aegypti* [AS] were well-controlled by field-rates of permethrin (94% mortality), the DEN strain was far less affected (48% mortality). Field-rates of malathion continued to be highly effective (100% and >98% death for the AS and DEN strains).

We demonstrate that small-scale resistance assays do not accurately predict survival or mortality under more realistic conditions. Importantly for the dengue control effort in Bali, we demonstrate that the pyrethroids are compromised and that malathion is a pragmatic choice of insecticide for local *Ae. aegypti* control.

Experimental evaluation of the impact of household aerosolized insecticides on pyrethroid resistant *Aedes aegypti*

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The extensive reliance on insecticides to control *Aedes aegypti* mosquitoes and disrupt transmission of dengue, chikungunya and Zika has fueled the emergence of widespread resistance to insecticides. Mismatch between the frequency of pyrethroid resistance in mosquitoes and the occurrence of pyrethroid-based insecticide applications for vector control is often hypothesized to be due to household use of commercial insecticide products. We experimentally quantified phenotypic and genotypic responses of four *Ae. aegypti* strains (three field, pyrethroid resistant, and one laboratory, pyrethroid susceptible) after exposure to two commonly used household aerosol insecticide products (a space spray and a residual spray formulation) containing pyrethroid active ingredients. Experiments were performed within homes of Mérida, Mexico. After exposure to the products, all three pyrethroid resistant field *Ae. aegypti* strains had significantly lower mortality rates (averaging 41% and 50% for the two products, respectively) than the controls (99%). Applying insecticides as surface sprays led to a significant increase in the frequency of I1016 kdr homozygotes in surviving *Ae. aegypti*, suggesting strong selection pressure for this allele. Given the large-scale use of household aerosol insecticide products in areas that are endemic for *Ae. aegypti*–transmitted diseases, their role as a pyrethroid resistance selection source, particularly when used as space sprays, should be taken into consideration when designing resistance management plans.

Pyrethroid-resistant mosquitoes compromise Australia's aircraft disinsection procedures

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The inadvertent import and release of exotic mosquitoes from aircraft is a threat to Australia's biosecurity. This menace has increased with the rise in global cargo and passenger movements and the subsequent escalation of *Aedes aegypti* and *Ae. albopictus* detections at Australia's ports. These mosquito imports pose three major incursion risks: 1) the establishment of competent disease vectors in new areas, 2) the establishment of insecticide-resistant variants in areas where targets are currently susceptible and 3) the release of infected mosquitoes that might transmit disease to passengers or airport workers.

Aircraft disinsection procedures for aircraft entering Australia are increasingly reliant on the residual treatment of cabins and holds with 200 mg / m^2 permethrin applied at 8 week intervals. That is a World Health Organization recommendation developed without consideration of the impacts of insecticide-resistance. That procedure needs to be re-evaluated given that examination of a subset of *Ae. aegypti* captured at Australian ports (2014-2016) confirms that 996P and 1023G knock-down resistant (kdr) mutations are common among intercepted samples (53/79 mosquitoes had heterozygous or homozygous mutations).

We explored the implications of this using permethrin-resistant *Ae. aegypti* (homozygous for 996P and 1023G) in our quarantine "free flight" facility, and bioassays conducted on a range of treated aircraft surfaces. The operational impact of residual permethrin was poor, particularly on carpets and seat covers (0-10% mortality). This was the result of insecticide resistance and the poor bioavailability of permethrin on absorptive surfaces (confirmed by High-Performance Liquid Chromatography [HPLC]). The 24h exposure of insecticide-resistant, free-flying mosquitoes to patchily-applied residues in a 20 m^3 flight chamber resulted in < 25% of the mortality seen for insecticide susceptible mosquitoes.

New disinsection chemistries and application methods are needed to protect our borders, our communities and the insecticide-susceptibility of our endemic mosquito populations.

Management of insecticide resistance in the major *Aedes* vectors of arboviruses: advances and challenges

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The landscape of mosquito-borne disease risk has changed dramatically in recent decades, due to the emergence and re-emergence of urban transmission cycles driven by invasive *Aedes aegypti* and *Aedes albopictus*. Insecticide resistance is already widespread in the yellow fever mosquito, *Ae. aegypti*, and is emerging in the Asian tiger mosquito *Ae. albopictus*, threatening the global fight against human arboviral diseases such as dengue, yellow fever, chikungunya and Zika. Since the panel of insecticides available for public health is limited, it is of primary importance to preserve the efficacy of existing and upcoming active ingredients. Timely implementation of insecticide resistance management (IRM) is crucial to maintain the arsenal of effective public health insecticides and sustain arbovirus vector control.

This review is one of a series being generated by the Worldwide Insecticide resistance Network (WIN) and aims at defining the principles and concepts underlying IRM, identifying the main factors affecting the evolution of resistance and evaluating the value of existing tools for resistance monitoring. Based on the lessons taken from resistance strategies used for other vector species and agricultural pests, we propose a framework for the implementation of IRM strategies for *Aedes* mosquito vectors.

Although IRM should be a fixture of all vector control programs, it is currently often absent from the strategic plans to control mosquito borne diseases especially arboviruses. Experience from other public health disease vectors and agricultural pests underscore the need for urgent action in implementing IRM for invasive *Aedes* mosquitoes. Based on a plan developed for malaria vectors, here we propose a roadmap towards a global plan for IRM in *Aedes* spp.

Session 3: New technologies and strategies for the control of invasive vectors or insecticide-resistant vectors

Neighbors help neighbors control urban mosquitoes

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The worldwide spread of invasive *Aedes* mosquitoes and arboviral diseases, have renewed the pressure for effective and sustainable urban mosquito control. We report on the success of a model we are confident will usher in a new era of urban mosquito control. The key innovation is the mobilization of neighbors guided by scientific advisors, an approach we termed Citizen Action through Science (Citizen AcTS). This approach was tested in a NE US town of approximately 1,000 residential yards infested with the invasive Asian tiger mosquito, *Aedes albopictus*, a major nuisance arboviral vector. We report a highly significant reduction in biting pressure that was maintained over time, and establish the thresholds needed for success. The Citizen AcTS model rejects the top-down approach consistently associated with intervention failures. Instead, it works through respectful exchanges among scientists and residents that lead to trust and individual 'buy-in' and transferring program ownership to the community.

Gravitrap as a surveillance and control tool

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The Gravitrap is a black cylindrical contraption, with the inner surface lined with a layer of weather-proof non-repellent adhesive lining that traps mosquitoes. Developed by the Environmental Health Institute when no good *Aedes* trap was available, it uses aged hay infusion as a lure to attract female *Aedes* mosquitoes that are seeking water to lay their eggs. Since 2013, an *Aedes* sentinel surveillance was established to understand the spatio-temporal dynamics of *Ae. aegypti* and *Ae. albopictus*, the factors that are associated with high mosquito abundance, and the effect of mosquito removal on dengue-case ratio. The surveillance was conducted in 34 high-rise residential estates in Singapore where Gravitraps are deployed in a systematic grid layout along public access areas within residential blocks. The weekly monitoring showed that *Aedes* mosquitoes are heterogeneously distributed among blocks and among floors within the block. The abundance of *Ae. aegypti* is positively associated with the age of the blocks, and is a lead indicator of dengue cases. Assessment of the effect of mosquito removal by Gravitraps using the before-after-control impact (BACI) analysis to compare the dengue-case ratio between estates that with and without Gravitraps, indicated a 30% reduction in case burden in estates with Gravitraps. Clearly, an adult *Aedes* surveillance approach is valuable in providing a temporal and spatial indication of *Aedes* mosquito population. In addition, the direct removal of the adult females has been shown to have an epidemiological impact. In Singapore, more than 50,000 Gravitraps are currently being monitored in public housing where a risk-based approach is adopted for targeted vector control operations on an island-wide scale.

Promising new tools to fight *Aedes* mosquitoes in Thailand

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Aedes aegypti is the most important arthropod borne viral disease vector which affects human health in tropical areas. Due to changes in mosquito behavior and an increase in insecticide resistance, it is crucial to develop new *Ae. aegypti* control techniques. Our goal is to investigate the efficacy of new tools implemented in vector control under field conditions in Thailand. We conducted field experiments to determine the efficacy of a pyriproxyfen/spinosad-treated device, the effect of these insect growth regulators on fecundity and fertility in female *Ae. aegypti* and sperm production in male *Ae. aegypti*. We also assessed the use of non-insecticidal sticky gravid traps, and ULV applications of spinosad larvicide as a residual in a tropical environment against *Aedes* mosquitoes. These investigations show promise as effective tools for *Ae. aegypti* control. Recent work conducted in the department of Entomology, AFRIMS will be discussed in regards to the *Aedes* mosquito control in Thailand.

Alarming *Aedes aegypti* resistance in Cambodia: Skirting resistance with an integrated vector management in an entomological/epidemiological approach

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The Ecomore2 project in Cambodia aims to estimate the effectiveness of an integrated vector control strategy targeting schools combined with an educational program to mitigate peaks of dengue and dengue-like syndromes (DLS) and to reduce subsequent overcrowding of health centers. A cluster randomized controlled trial was implemented in Kampong Cham province in 24 clusters (12 under integrated vector control and 12 without), each including one school, with an active surveillance of DLS in neighboring villages.

Recent results showed very high resistance of *Aedes aegypti* to temephos, the only larvicide used in Cambodia, and two adulticides (permethrin used for net treatment, and deltamethrin for insecticide fumigation). Our current research program was also implemented to determine if an integrated vector management program could decrease the number of *Ae. aegypti* mosquitoes in schools by using other insecticides : (i) Bacillus thruringiensis var. israelensis as a larvicide, (ii) pyriproxyfen for the dissemination of insect growth regulator larvicide and (iii) spores of Beauveria bassiana for slowly killing adults; all these methods were combined with education and sensitization of children.

Monitoring of mosquito populations during the first year, 2017, showed the presence of at least 58 mosquito species, including dengue virus vectors such as *Ae. aegypti* (N=515) and *Ae. albopictus* (N=298), but also vector species of malaria Plasmodium, and Japanese encephalitis, Rift Valley Fever, West Nile Fever, Zika, Chikungunya and Ross River viruses. *Ae. aegypti* and *Ae. albopictus* were present in all 24 schools, but in different proportions between schools and months of capture. Nevertheless, there was no difference in the relative abundance of *Ae. aegypti* and *Ae. albopictus* between intervention and control clusters. In parallel, epidemiological data neither showed any baseline difference of DLS.

Entomological preliminary data during the second year, following interventions, showed a decrease of 50% of *Ae. aegypti* relative abundance in treated clusters compared to untreated clusters. The latter showed an average relative abundance of *Ae. aegypti* similar to the first year. The difference for *Ae. albopictus* relative abundance was not significant between the 2 areas. Epidemiological data acquisition are still in progress. Between May and 15 August 2018, 485 DLS were detected, 320 in the control area, and 165 in the treated area. In conclusion, the integrated vector management worked in school. Actually, no difference of dengue incidence has been tested between the 2 clusters.

Integrated *Aedes* management for the control of Aedes-borne diseases

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Diseases caused by *Aedes*-borne viruses, such as dengue, Zika, chikungunya and yellow fever, are (re)emerging globally. The causes are multifactorial and include global trade, international travel, urbanisation, water storage practices, lack of resources of intervention and an inadequate evidence base for the public health impact of *Aedes* control tools. National authorities need comprehensive evidence-based guidance on how and when to implement *Aedes* control measures tailored to local entomological and epidemiological conditions.

This review is one of a series being conducted by the Worldwide Insecticide resistance Network (WIN). It describes a framework for implementing Integrated *Aedes* Management (IAM) to improve control of diseases caused by *Aedes*-borne viruses based on available evidence. IAM consists of a portfolio of operational actions and priorities for the control of *Aedes*-borne viruses that are tailored to different epidemiological and entomological risk scenarios. The framework has four activity pillars: i. integrated vector and disease surveillance, ii. vector control, iii. community mobilisation, and iv. intra- and inter-sectoral collaboration; and four supporting activities: i. capacity building, ii. research, iii. advocacy, and iv. policies and laws.

IAM supports implementation of the World Health Organization Global Vector Control Response and provides a comprehensive framework for health authorities to devise and deliver sustainable, effective, integrated, community-based, locally adapted vector control strategies in order to reduce the burden of *Aedes*-transmitted arboviruses. The success of IAM requires strong commitment and leadership from governments to maintain proactive disease prevention programs and preparedness for rapid responses to outbreaks.

Alternative Strategies for Mosquito-borne Arbovirus Control

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International public health workers are challenged by a burden of mosquito-borne arboviral diseases despite best efforts in control programmes. Arboviruses transmitted by *Aedes* mosquitoes represent major international public-health concerns that will surely require a range of integrated interventions to be effectively controlled. Well-documented successes indicate that rigorously applied vector control using existing interventions can reduce arbovirus transmission and disease; however, despite existing interventions epidemics and spread of arbovirus diseases continues. The reasons for this are complex but include ineffective coverage; lack of human, financial and infrastructural capacity; insecticide resistance, and inability to scale. As the scope of arboviruses continues to grow, development and evaluation of alternative vector control products and strategies are critical to pursue. Alternative strategies will provide additional options for arbovirus control and potentially add value to existing strategies. This presentation will focus on an overview of alternative strategies being evaluated and developed mainly for control of *Aedes aegypti* and *Aedes albopictus* with the objective of outlining key considerations to their success such as: What is the evidence-base required for endorsement of an alternative strategy? How best can alternative strategies be locally adapted to take into account the biology of the vector, virus transmission intensity as well as human and financial resources? As well as, when and where will such strategies/products offer greatest public health value and contribute to managing insecticide resistance?

Overview of auto-dissemination technology for mosquito controls

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Arboviral vector-borne diseases are highly fatal causing millions of deaths and generating long-term disability. Dengue, Zika and chikungunya are transmitted by *Aedes aegypti* and *Aedes albopictus*. These mosquitoes are peridomestic, highly anthropophilic and day-biting. Their presence in cryptic habitats and their skip-oviposition behaviour help to make these species difficult to detect, access, and eliminate. The behaviour of these *Aedes* vectors makes conventional mosquito management strategies fail. The precision targeted delivery of the control agent is the main requirement of vector control. The autodissemination strategy precisely is targets breeding habitats with small insecticide doses in an eco-friendly manner which exploits the oviposition behaviour of gravid females. Mosquitoes are attracted to a chemical source, contaminated with the control agent and then employed to disseminate that chemical to the larval habitat. Autodissemination technology mainly utilizes two concepts: 1) pull-and-push technology and 2) skip-oviposition behaviour. We developed and evaluated autodissemination devices using pyriproxyfen, an insect growth regulator and used this alongside an oviposition attractant system. The efficacy of this strategy depends on 1) design of the device, 2) oviposition attractant, 3) formulation adherence and persistence on the mosquito, and 4) insecticidal efficacy. Pyriproxyfen has tremendous larvicidal efficacy against both vectors (LC50 = 0.012 ppb). We developed several designs of stations for use against *Aedes albopictus* involving shape, size and mode of entry and exit. These were designed to utilise powder and/or oil. These devices caused 100% mortality under laboratory conditions. Our study assessed the station efficacy in residential areas infested with *Ae. albopictus* in New Jersey, USA. We found that the stations efficiently contaminated gravid females that subsequently contaminated ovicups placed at various distances from the device. Our stations also showed effective delivery of pyriproxyfen in cryptic habitats which we were unable to penetrate using conventional insecticide sprayers. Autodissemination stations produced negative impacts on field populations of eggs, larvae, and adult mosquitoes. Other autodissemination studies have exploited skip-oviposition to disseminate pyriproxyfen or Beauveria bassiana and showed the effective transfer of these control agents in sentinel habitats. In the present scenario where dengue cases are increasing unstoppably, the autodissemination technology may provide a tool for integrated vector management against the container-inhabiting mosquitoes. This study also provides future opportunities to explore oviposition attractants that can enhance the efficacy of the contamination device and stable formulations that can work for longer duration in different climates.

Targeted Indoor Residual Spraying (TIRS) for the control of *Aedes aegypti*

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Challenges in maintaining the coverage and efficacy of classic vector control methods in urban areas has renewed interest in indoor residual spraying (IRS) as a promising approach for *Aedes* borne disease prevention. Although conventional IRS has many benefits, application time and disruptive indoor spray applications make its scalability in urban areas problematic. Modifying and optimizing IRS to account for *Ae. aegypti* resting behaviors has resulted in the development of targeted IRS (TIRS) and resting site IRS (RS-IRS). The former consists of spraying walls below 1.5 m and dark areas under furniture. In the latter, only the areas under furniture are sprayed. These methods clearly reduce application time but may compare poorly with conventional IRS in terms of entomological efficacy.

Teams in Merida, Mexico compared the residual efficacy of conventional IRS (as developed for malaria control) with two TIRS methods using a carbamate insecticide against a pyrethroid-resistant, field-derived *Ae. aegypti* strain. The study was performed within real houses (n = 9) with similar layouts and standardized contents.

Conventional IRS was compared to TIRS and RS-IRS. Mosquito mortality was measured at eight intervals during a six month post-application period. One hundred *Ae. aegypti* females were released in each house on each sampling occasion. Live and dead individuals were collected after 24 hrs exposure.

Compared to conventional IRS, TIRS and RS-IRS took less time to apply (31% and 82% reduction, respectively) and used less insecticide (38% and 85% reduction, respectively). The mortality of pyrethroid-resistant *Ae. aegypti* did not differ significantly among the three IRS application methods for up to two months post-application, and did not significantly differ between conventional IRS and TIRS up to four months post-application.

These data illustrate that optimizing IRS to more efficiently target *Ae. aegypti* can both reduce application time and insecticide volume without reducing entomological efficacy.

New developments in the use of the Sterile Insect Technique (SIT)

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While many countries are increasingly exposed to the health risks (Dengue virus, Chikungunya, and Zika vector) associated with the invading tiger mosquitoes, *Aedes albopictus*, few tools are available to efficiently prevent or control this threat. Following the devastating chikungunya outbreak which affected 270,000 people on La Réunion in 2005-2006, there have been strong interest and political will to develop effective alternatives to the existing vector control strategy. A significant effort towards this goal was initiated in 2009 by the Research and Development Institute (IRD) and its national and international partners with the aim to develop a new control strategy against *Aedes albopictus* vector based on the Sterile Insect Technique (SIT). A first phase feasibility study of SIT application against *Aedes albopictus* was implemented on La Réunion Island during 2009-2014, and the main achievements have been the collection of relevant entomological baseline data on *Ae. albopictus* distribution, ecology, dispersal, mating behaviour, reproductive fitness, population genetics structure and gene flow. A second phase started in 2016, is ongoing and focused on sterile male mass rearing, field site characterization (spatial and temporal distribution of population) and Social mobilisation and communication for the development of field trials on two pilot sites on the island.

This Reunion project is one of numerous programs that are implemented in the world to control mosquitoes with the SIT. To help these programs, standardised mass rearing and releasing procedures for both of *Aedes* and *Anopheles* mosquitoes have been developed by the FAO-IAEA Insect Pest Control Laboratory, in strong collaboration with partners from Member States. Here we present some recent developments of the SIT package such as mass rearing cages, larval and adult diet, larval counters and irradiation procedures.

Gene editing population sex determination pathway for vector control

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The recent development of CRISPR/CAS9 based gene drive technology has unlocked the possibility to selectively edit a mosquito population for developing novel vector control measures. Genetic modifications designed to either impair female fertility or interfere with mosquito ability to transmit the malaria parasite have been spread from few laboratory individuals to large caged mosquito populations. These laboratory experiments have also supported mathematical modelling predicting how gene drive technology has the potential to eradicate malaria transmission in a span of few years from vast regions of Africa. With the aim of developing a gene drive population suppression strategy we targeted a highly conserved sequence within the sex determining gene *double sex* (*dsx*). In the human malaria vector *Anopheles gambiae* the gene *doublesex* (*Agdsx*) encodes two alternatively spliced transcripts *dsx-female* (*AgdsxF*) and *dsx-male* (*AgdsxM*) that in turn regulate the activation of distinct subordinate genes responsible for the differentiation of the two sexes. The female transcript, unlike *AgdsxM* contains an exon (exon 5) whose coding sequence is highly conserved in all *Anopheles* mosquitoes so far analysed. CRISPR-Cas9 targeted disruption of the intron 4-exon 5 sequence boundary, aimed at blocking the formation of functional *AgdsxF*, did not affect male development or fertility, whereas females homozygous for the disrupted allele showed an intersex phenotype characterised by the presence of male internal and external reproductive organs and complete sterility. A CRISPR-Cas9 gene drive construct targeting this same sequence was able to spread rapidly in caged mosquito populations reaching 100% prevalence within a span of 8-12 generations while progressively reducing the egg production to the point of total population collapse. Notably a variety of non-functional Cas9 resistant variants were generated in each generation at the target site, they all failed to block the spread of the drive. The potential of this gene drive solution for field applications will be further evaluated as part of a phased approach in large confined spaces that more closely mimic native ecological conditions, in accordance with the recommendations of the American National Academy of Sciences. Furthermore, given the conserved functional role of *dsx* for sex determination in all insect species so far analysed and the high degree of sequence conservation amongst members of the same species we propose that similar gene drive solutions could be developed to target other vector species and insect pests.

Session 4: Public/Private initiatives to foster innovation in public health

Filling the resistance management gap: the power of intrinsic resistance management in *Bacillus thuringiensis israelensis* strain AM65-52 (Bti AM65-52) protects public health with effective control of organophosphate and pyrethroid resistant vectors

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Integrated vector management programs have relied on the use of Bti strain AM65-52 to control disease vectors while managing resistance for more than 30 years. Starting in the 1980's, this unique strain was first used to manage temephos resistance in black flies for the WHO onchocerciasis control program. Its use has continued through today to control a broad range of mosquito species in disease prevention programs worldwide. Bti strain AM65-52 offers a unique mode of action (MOA) against mosquitoes and black flies based on the complex of δ-endotoxins found in its Insecticidal Crystal Protein (ICP). The synergy of Bti's Cyt-1a toxin with its four cry toxins provides intrinsic resistance management in one active ingredient. Commercial AM65-52 formulations of Bti are very effective against mosquito vectors that have documented resistance to commercial active ingredients including pyrethroids. This has allowed public health professionals to prevent disease transmission while managing resistance to both mosquito larvicides and adulticides. Wide Area Larvicide Spray (WALS™) technology was developed to cover wide areas with Bti strain AM65-52 WG. This approach has cost-effectively prevented disease transmission. WALS technology is cost effective because wide areas can be covered in a short time to prevent mosquito borne transmission while minimizing labor costs. Examples of IVM programs based on Bti strain AM65-52, both alone and in combination with other active ingredients, will be presented. Insects managed include vectors of dengue, chikungunya, Zika and West Nile virus; malaria, filariasis; and onchocerciasis. Specific examples of impacts on disease transmission will be presented.

Fludora Co-Max®: A new space spray combination for mosquito resistance management programs

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Mosquito resistance to conventional insecticides is becoming an increasing threat to the fight against vector borne diseases. We have developed a new product for space spray application combining two active ingredients with different modes of action. One of Active Ingredient is of a new chemistry class for space spray applications while the other one is a pyrethroid with special characteristics. It is based on a new proprietary formulation. The unique features of the active ingredients and efficacy results will be discussed.

Effective *Aedes* Control based on resting behaviour & New IRS Tools

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Historically, the control of *Aedes aegypti* has been based on fumigation (space spraying) and reduction in the number of mosquito breeding sites. However, the result of many studies suggests that houses provide a suitable environment for man-vector contact, as well as suitable resting sites for adult mosquitoes. Recently, emergency control measures for *Aedes aegypti* have been reviewed and reported that Indoor Residual Spraying (IRS) could be a suitable intervention, not only for emergency interventions, but also for routine vector control programs based on after feeding and oviposition resting behavior of *Aedes aegypti*.

This presentation will show the main conclusions of these studies and describe new IRS tools that improve significantly the efficiency of IRS operations for *Aedes* Control, reduce spray operator's fatigue and contamination risk, reduce costs and simplify operations.

Innovative formulation for long lasting residual insecticides

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Indoor residual spray (IRS) remains one of the core methods in the prevention of vector borne diseases such as malaria. However, over reliance of pyrethroid based interventions for many years has led to rapid spread of resistance in many parts of the world. Other challenges like insufficient of skilled staffs and logistic difficulties are also become the stumbling blocks to success in eliminating the malaria. One approach to ease these problems is to employ a non-pyrethroid based IRS with long residual effect. Actellic 300 CS is equipped with pirimiphos-methyl and a unique formulation, microcapsule suspension which has been proven to be able to control pyrethroid resistant strains with long lasting residual activity of more than 8 months. In addition, some studies had showed that the product application has high impact in reducing the mosquito biting rate as well as the malaria transmission.

Managing *Aedes* spp. with push-pull tactics: how non-repellent chemistries may mitigate behavior and vectorial capacities

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Associative learning in mosquitoes has demonstrated specific preferences for hosts, host-seeking, oviposition, resting-behaviors, and a range of other behaviors influential in the transmission of vector-borne diseases like Zika and Dengue. Exploiting these behaviors with insecticides which afford a push-pull paradigm of control is relatively new to mosquito disease interventions, whereas in other market segments it has been established for years. This is largely a function of the almost complete reliance on neuro-toxic chemistries which unfortunately share both spatial and irritant components; Non-repellent chemistries such as chlorfenapyr have the promise to allow mosquitoes to spend protracted time intervals on surfaces which may optimize exposure to afford improved efficacy in combatting these important disease vectors. Moreover, opportunities to use new or repurposed active ingredients may engender more strategic control approaches of these pests that wasn't available in the past. Integration of both repellent and non-repellent chemistries, adoption of attractive toxic sugar baits, with conventional approaches (i.e., long lasting insecticidal bed nets like Interceptor® G2 and larvicides like Abate®) may create new opportunities to quell outbreaks and reduce the impact of mosquito-borne diseases that take into account both the physical-chemical properties of newer insecticides (to public health) and with regards to managing incidence clusters to stem the spread of emerging outbreaks with special attention to resistance concerns and maximal protection to the public in general. The presentation herein represents, in concept, an approach that has not been adopted yet, but is well-supported from a range of studies on chlorfenapyr and published field studies which extoll these benefits.

Friendly™ mosquitoes for efficient and safe vector control

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As a primary vector of dengue, chikungunya, yellow fever and Zika viruses, *Aedes aegypti* mosquitoes are responsible for infecting millions of people every year. To date, controlling the spread of these diseases has been largely dependent on reducing the abundance of this mosquito. One species-specific and environmental friendly solution for reducing *Ae. aegypti* populations is the use of Friendly™ *Aedes*. Friendly™ *Aedes* are male *Aedes aegypti* that are unable to father offspring due to an engineered trait - their offspring die before adulthood. This genetic solution has been evaluated for effectiveness and safety by numerous regulatory agencies worldwide. Since 2010, over half a billion Friendly™ *Aedes* have been released into the environment, where they have been successful in achieving vector reductions. Recently, Oxitec launched a new Friendly™ *Aedes* in which only female offspring die. This provides further benefits including increased vector control performance and potential synergisms with existing management approaches.

Innovation to Impact: Update on progress and future priorities

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Innovation to Impact (i2i) was launched in 2013 to address challenges in the vector control value chain to promote innovation, streamline evaluation and accelerate impact of new tools as approaches. To achieve this, i2i has engaged a range of stakeholders encompassing the gamut of vector control partners to address specific priority areas. Since the inception of i2i, this partnership has worked to implement significant changes to the evaluation of vector control tools, most notably with the transition of WHO product evaluation from WHOPES to the Prequalification team and the requirement for data to be produced at GLP certified sites. While significant progress has been made, much still remains to be done particularly pertaining to quality control and, most importantly, supporting endemic countries to register and implement new tools effectively. This presentation will review the progress to data and highlight areas of focus for the i2i initiative in the next phase of its implementation, while discussing some remaining issues to be resolved to further optimize the evaluation and deployment of new vector control technologies.

Overview of the prequalification of vector control products

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Prequalification of vector control products (PQT-VC) is now a stream within the Prequalification Team of Regulation of Medicines and other Health Technologies (RHT) of the World Health Organization (WHO). The WHO function for evaluation of public health pesticides was transitioned to RHT in 2017 in order to harmonize approaches with the existing PQ product evaluation streams (Medicines, Vaccines, and Diagnostics). The mandate of PQT-VC is to: Increase access to safe, high quality, efficacious vector control products. PQT-VC will present an overview of the prequalification process and provide updates on the progress to date.

Building a vector control toolbox for IVM

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IVCC is a Product Development Partnership (PDP) that works with funders, innovators, academic groups, testing facilities and international organisations to develop new solutions for vector control. IVCC has supported the development and launch of several new tools for tackling malaria vectors and has major collaborative projects with leading companies for the development of novel insecticide active ingredients for use in public health. A key goal is the development of a toolbox of solutions that can be deployed as part of an integrated vector management programme, with the overall goal of malaria eradication. Insecticide resistance is a major threat to the success of the global malaria initiative. IVCC supports not only the development of new tools but also the streamlining of their route to market through the involvement of the Innovation to Impact (I2I) initiative, capacity strengthening for laboratory and field testing, as well as supporting the practical adoption of new resistance-breaking products through the IVCC NgenIRS programme. This programme, involving next generation products for indoor residual spraying, is now active in 18 African countries and is due to be followed up with a similar approach to support the introduction of novel LLINs, again funded by Unitaid. With the support of the Bill and Melinda Gates Foundation, IVCC and leading agrochemical companies signed a 'ZERO by 40' declaration at the Commonwealth Heads of Government Meeting in London in April 2018 to continue to develop innovative vector control tools in order to help eradicate malaria by 2040. IVCC is supported by the Bill & Melinda Gates Foundation, DFID, USAID, DFAT, SDC and Unitaid.

IVCC is now expanding its work beyond solutions for tackling malaria vectors in sub-Saharan Africa to malaria vectors in the Indo-Pacific region and products for the management and control of *Aedes* mosquitoes. Since 2017, IVCC has collaborated with USAID to support nine projects within their Grand Challenge for Combatting Zika and Future Threats initiative. Considerable progress has been made by these projects towards the early development of novel spatial repellents, biorational insecticides and enhanced lure and automated trapping systems. In May 2018, IVCC was awarded a grant by the Australian Government's Department for Foreign Affairs and Trade (Indo-Pacific Center for Health Security) to support the development and adoption of new technology for tackling vectors of malaria and other deadly mosquito-borne diseases in the Indo-Pacific region, including *Aedes* mosquitoes. Outline plans for this work will be presented.

Legislative framework for vector control: the situation for European countries, including overseas territories

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At the global level, countries which have agreed and signed the WHO International Health Regulations (IHR) document are recognizing some definitions and obligations on vectors of infectious agents constituting a public health risk, vector surveillance and control activities. However, these national vector-related activities may or may not be included into a formal legislative framework. In each of the WHO regions, the situation of vector control activities is very different, since there is a great variability at the region and country level of the Ministry in charge of vector surveillance and control. For some countries, it is the Ministry of Health, for others, Ministry of Agriculture and for others, Ministry of Environment. These differences are impacting on the approaches for methods and authorized products, as well as for resources to implement vector control operations. Only the European Region has a collective approach within the European Community (EU). The decisions of the European Parliament regarding communicable diseases and vector-borne diseases are the basis for the European legislative framework, then adapted by each European country of EU. For most of the EU countries, the vector surveillance and control are under the Ministry of Health, but the vector control products are under both Ministry of Health and Ministry of Environment, with authorizations based on the EU legislation. In EU countries, the activities are included into a national legislative framework and can be performed either by public health services, either by private services contracted by public health authorities. The plans for responding to vector-borne diseases emergency are under the government responsibility, but can be adapted to local situations and again activities can be performed by either public or private agencies. However, the absence of a harmonized legislative framework at regional and more largely global level is posing problem not only for responding to emergency in border situations, but also for testing and deploying new vector control tools and products. In some countries, new tools such as traps, chemicals and biological organisms can be included into the existing legislative framework, but in most countries the tools using genetically modified materials do not have the adequate legislation to authorize large-scale testing. As an example, for EU countries, mosquitoes hosting *Wolbachia* sp. bacteria through transfection are considered Genetically Modified Organisms (GMO) and have to be discussed under the rules of the Cartagena Protocol. Consequently, there is a strong need for a global legislative framework for vector-related activities.

VectorBase.org's PopBioMap: An online resource for insecticide resistance data

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Emerging resistance of disease vectors to chemical pesticides poses a serious threat to arbovirus and malaria control. To successfully mitigate this risk, it is critical to understand where resistance occurs, which vectors display resistance, which resistance mechanisms are conferring that resistance, and to what chemicals the resistance has been observed. VectorBase.org's PopBioMap is a graphical, map based, online tool for visualizing all of these pieces of information (and other non-resistance) surveillance data. Data is submitted directly by data generators or extracted from publications by our curators. We will highlight this resource including the significant amounts of genotypic and phenotypic data we have assembled, demonstrate how to access this data, and share details on how to participate in our efforts.

Toward a global partnership for the control of insecticide resistant Arbovirus vectors: The WIN initiative

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Arbovirus transmitted by *Aedes* mosquitoes, such as dengue, zika, chikungunya and yellow fever viruses have re-emerged globally. Vector control, mainly through the use of insecticides, plays a key role in the prevention and control of those diseases. However, the recurrent use of the same chemicals for decades, together with the dissemination of vectors resulted in the global spread of insecticide resistance. To reduce the burden and threat of *Aedes*-borne diseases, it is crucial to identify countries and regions where resistance represents an obstacle to vector control and to provide national authorities with robust evidence to deploy safe and locally adapted vector control tools. Managing resistance to public health insecticides will require global, integrated and coordinated actions and strong engagement of scientists, decision-makers, stakeholders and donors. Supported by the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Department of Neglected Tropical Diseases (NTDs) since 2016, the **Worldwide Insecticide resistance Network** known as "WIN" (http://win-network.ird.fr/) brings together 19 internationally recognized institutions to track insecticide resistance in arbovirus vectors worldwide. The WIN aims to support the implementation of the WHO-Global Vector Control Response (GVCR) by strengthening mosquito control and surveillance efforts and guide decision-making for resistance management. The network has gained international recognition for its role in mosquito resistance and is now expanding to a membership organization aiming at gathering all actors involved in vector-borne disease control (i.e. academia, International organizations, NGOs and not for profit organization, private sector). This presentation will provide a brief overview of WIN achievements and present the future scheme of the network to mitigate insecticide resistance and reduce the global burden of *Aedes*-transmitted arboviruses.

Abstracts for poster presentation

Scientific session 2: Insecticide resistance, operational consequences and options for management

Poster 1

A novel salivary gland protein that affects blood feeding behavior in *Aedes aegypti*

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Female mosquitoes express a plethora of proteins that helps them to locate a host and acquire a blood meal, thereby transmitting vector borne diseases. Previous comparison of sialome between male and female *Aedes aegypti* mosquitoes identified several salivary gland transcripts that were uniquely expressed in female mosquitoes, thus were suspected to play roles in blood acquisition, blood digestion and causing alterations to host physiology. Of the several female specific salivary proteins, a hypothetical protein was uniquely expressed thirty times more in the case of female mosquitoes as compared to males. We hypothesized that the protein has a role in blood feeding behavior of mosquitos. To test our hypothesis, we depleted the protein by RNAi knockdown in mosquitoes and studied their blood feeding behavior using a mouse model. Interestingly, our data showed that there is a drastic impairment in the blood feeding behavior of knockdown mosquitoes as compared to control, stating that this novel protein might play a role in mosquito host feeding behavior. By exploring the structure of this novel mosquito protein using NMR spectroscopy, we aim to characterize its function with the objective of understanding overall mosquito behavior and design novel vector control strategies.

Poster 2

Bioactive microbial quorum sensing molecule for mosquito repellent product development against mosquito vector *Aedes aegypti*

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There is a need for target specific new molecules for controlling and preventing the deadly disease caused by mosquito vectors and to enhance the efficiency of the existing insecticide arsenal, wherein most of them are structural and functional derivatives of plants and microbial compounds. In this study we focused on quorum sensing molecules produced by bacteria which are important in biofilm formation. These molecules produced by bacteria were isolated, identified and evaluated for their mosquito repellent activity. Out of 253 bacteria evaluated, five showed a significant repellent property against the *Aedes aegypti* mosquito adults. These bacteria were taxonomically identified as *Bacillus amyloliquefaciens* MSSRF S2, *Enterobacter cloacae* MSSRFS8, *Exiguobacterium mexicanum* MSSRF S9, P*roteus hauseri* - MSSRFS11 and *Pseudomonas aeruginosa* - MSSRFS20. Further studies are undergoing in order to purify and characterize these active molecules precisely.

Poster 3

Biochemical and molecular detection of insecticide resistance in sand-fly fever vector (*Phelebotomus papatasi*)

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Background: Sand fly fever is also known as 'papatacci fever,' 'Phlebotomus fever,' or 'three-days'. It was discovered that the agent is a filterable organism which is transmitted by infected sand flies. Phlebotomus papatasi is considered as a vector of disease.

Material & Method: Sand flies were collected from outdoor near their breeding sites from Matin-Abad Bdrood, Esfahan provinces of Iran. A selection was performed by exposing individuals to a sublethal dose of DDT4% until a homogeneous DDT-resistant strain can be obtained for subsequent experiments. In order to look for mutations in the sodium channel, genomic DNA was extracted using the QIAamp DNA Micro kit. Biochemical activities of α- and ß-esterases, mixed function oxidases (MFOs) and glutathione-S-transferases (GSTs) were tested for a *P.papatasi* strain with a resistance ratio of 2.52 to DDT.

Results: Results showed that the mean activity of α- EST, β- EST, GST and MFO enzymes were altered suggesting the importance of metabolic mechanisms in resistance of *P.papatasi* to DDT. .Enzyme activity ratios calculated between a tolerant and a susceptible populations were 3.78, 3.72, 3.21, 1.59 for α-esterases, β-esterases, MFOs and GSTs respectively.

Discussion: The comparison of the sodium channel genomic regions at locus 1014 showed a TTA sequence in all three populations suggesting that metabolic resistance play a central role in DDT resistance in *P.papatasi*.

Poster 4

Biochemical and Molecular Resistance Mechanisms to DDT and Some Pyrethroid Insecticides in vector of West Nile virus, *Culex pipiens*

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Mosquito belonging to the *Culex pipiens* complex act as important vectors of several diseases such as filariasis, West Nile virus, Japanese encephalitis and bird malaria. This study aimed at clarifying the molecular and biochemical resistance mechanisms of Cx. pipiens to DDT and some pyrethroid insecticides from Tehran, capital of Iran. Biochemical activities of α- and ß-esterases, Mixed Function Oxidase (MFO) and Glutathione-S Transferase (GST) were tested for a Cx .pipiens strain displaying a resistance ratio of 85.75 to Lambdacyhalothrin and for a DDT-resistant strain in comparison with a susceptible Laboratory strain. Genotyping of Kdr mutations involved in pyrethroid and DDT resistance (L1014F and L1014S) was also performed. This study showed a significant different ($p<0.05$) between the mean activity of α- , ß-esterases and (MFO), in both lambacyhalothrin- and DDT-resistant strains in comparison with the susceptible Laboratory Strain, while no significant difference ($p>0.05$) were found for GST activities in the both strains. Molecular study did not evidence the presence of Kdr mutations associated with resistance to pyrethroids and DDT. Overall, this study suggests that insecticide resistance in *Cx pipiens* from Teheran, Iran is mainly due to increase insecticide metabolism (metabolic resistance) with Kdr mutations playing a minor role in resistance. This research provides essential data for the planning of future chemical control program in the region.

Poster 5

Characterization of insecticide resistance in the dengue vector *Aedes aegypti* in Khon Kaen district, northeastern Thailand

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Mosquito-borne diseases are an important public health concern in many countries worldwide. Vector control by insecticides is the mainstay of prevention and control of these diseases. However, intensive use of the same chemicals for decades has selected for insecticide resistance in mosquitoes, resulting in a reduced efficacy of vector control interventions. Monitoring the susceptibility of local mosquito populations to insecticides is key to guiding decision-making for vector control. The objective of this study was to determine the level of resistance of *Aedes aegypti* to public health insecticides in Khon Kaen district, northeastern Thailand.

Aedes aegypti larvae were collected in 18 sites in Khon Kaen district to propagate adult mosquitoes for susceptibility bioassays. WHO tube tests were performed on the F1 generation of adult *Ae. aegypti* using 0.25% permethrin, 0.03% deltamethrin and 0.03% cypermethrin following WHO guidelines. In addition, eight larval populations from Khon Kaen district were assessed for temephos resistance using WHO larval bioassays on the F1 generation. Results indicated strong resistance to pyrethroids in all tested populations with mortality ranging from 0% to 37.5%, 57% to 81%, and 12% to 31.9% for permethrin, deltamethrin, and cypermethrin respectively. The Knock-Down time 50 (KDT50) and KDT90 suggested higher resistance to permethrin compared to deltamethrin and cypermethrin. Larval bioassays showed variable levels of susceptibility to temephos among the eight populations, with resistance ratios ranging from 1 to 28. Three mutations in the voltage-gated sodium channel gene (i.e. S989P, V1016G, and F1534C) are being investigated in field caught mosquitoes. Copy number variants of six genes implicated in insecticide resistance are being estimated to assess the role of detoxification pathway in insecticide resistance in *Ae. aegypti* from Northeastern Thailand.

Poster 6

Characterization of resistance to pyrethroids in *Aedes aegypti* populations from the South Pacific region

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Pacific island countries and territories are regularly affected by dengue fever outbreaks, and have recently experienced emergence or re-emergence of other arbovirus diseases like chikungunya and Zika. *Aedes aegypti* is the main arbovirus vector in the South Pacific region. Use of pyrethroids in the last decades has resulted in selection of insecticide resistance in mosquito populations. We investigated resistance to pyrethroids in *Aedes aegypti* populations in the French overseas territories of New Caledonia (18 populations) and French Polynesia (3 populations) through biological, biochemical and molecular assays.

A low to moderate resistance to deltamethrin was observed in wild populations of *Ae. aegypti* in both territories, with high geographical heterogeneity within and among islands. The lowest mortality rate was 45%, median value 79% (at the WHO diagnostic dose) in New Caledonia. The French Polynesian populations showed higher mortality rates in accordance with limited use of insecticides on Tahiti Island. Modifications in detoxification enzyme activities or quantities (metabolic resistance) was observed for oxydases (P450) and esterases in *Ae. aegypti* populations in both territories. Target-site mutations (knockdown resistance - kdr) were recorded in populations of *Ae. aegypti* from New Caledonia. Analysis of correlation between the kdr mutations and the resistance phenotypes showed a significant association between the most frequent mutation I1011M and deltamethrin resistance in New Caledonia, suggesting the contribution of the kdr mechanism to the pyrethroid resistance observed in the studied populations. While new vector control tools are emerging, innovative strategies are being evaluated on both territories, including the use of *Wolbachia* for either dengue transmission blocking (New Caledonia) or population suppression (French Polynesia, release of incompatible males).

Poster 7

Characterizing mechanisms associated with pyrethroid resistance in *Aedes aegypti* in Saudi Arabia

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Pyrethroids are routinely used as a control measure for dengue fever spread by *Aedes aegypti* in Saudi Arabia. Effectiveness of control may be impacted by resistance but there is limited information on insecticide resistance in *Ae. aegypti* in the middle eastern region and less still on mechanisms underlying resistance phenotypes. *Aedes aegypti* from Jeddah and Makkah, the two primary dengue-endemic areas of Saudi Arabia, were assayed for resistance phenotypes; their target site mutations investigated using sequencing and genotyping; and metabolic resistance mechanisms investigated using microarrays. Both the Jeddah and Makkah field strains were resistant to multiple insecticides, with especially high deltamethrin resistance in Makkah. Three kdr mutations were detected (S989P, V1016G, F1534C), two of which were previously only identified in Asia. The S989P and V1016G mutations were in perfect linkage disequilibrium (LD) and strongly predicted deltamethrin resistance. Microarray analysis sought to identify genes consistently differentially expressed between susceptible strains and both Saudi Arabian strains. Results showed enrichment of P450s, some previously identified as pyrethroid metabolisers. However, the lead candidate gene statistically, CYP9J7 has not previously been functionally-investigated and we investigated its metabolic capacity via in vitro insecticide metabolism assays. No depletion of either deltamethrin or permethrin was observed, but surprisingly CYP9J7 metabolised the organophosphate malathion. Mosquitoes from Jeddah and Makkah are highly resistant to pyrethroids. The gene expression and target site mutation data suggest that the Jeddah strain relies on both metabolic and target site resistance mechanisms, with evidence suggesting the latter may be more prominent at present. Further work is required to identify whether the organophosphate metabolism detected represents detoxification or activation, which have opposing implications for resistance management.

Poster 8

Current Updates on the Insecticide Resistance Mechanisms in Malaysian Populations of *Aedes* Mosquitoes

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Dengue fever (DF) and dengue haemorrhagic fever (DHF) are the most rapidly spreading vector-borne diseases with approximately 50 million cases of infection worldwide. By the end of 2017, in Malaysia 83000 cases were reported with 171 deaths. The issue with the available/existing insecticides is the universal emergence of resistance towards these chemicals. The mosquitoes are becoming tolerant towards the chemicals used, hence causing the failure of control programmes despite continuous efforts done by the Ministry of Health (MoH). Few in-depth molecular-based studies have been conducted. Results showed that there are knockdown resistance (kdr) mutations V1016G and F1534C in *Aedes* populations in Kuala Lumpur and these were from samples collected in 2010. Currently, there are a few updates on the insecticide resistance mechanisms in different strains of *Aedes aegypti* and *Ae. albopictus* of Malaysia. A recent and new study was conducted and samples were collected from Penang, Selangor and Kelantan state and WHO adult susceptibility bioassays were conducted to determine the resistance status of the mosquitoes with most strains of *Ae. aegypti* being highly resistant towards permethrin 0.75% and pirimiphos-methyl 0.25%. *Aedes albopictus* mosquito showed moderate to high susceptibility towards permethrin 0.75% but is highly resistant towards pirimiphos-methyl 0.25%. The samples were further analysed for mutations in the Voltage Gated Sodium Channel (VGSC) gene and most of the samples in Selangor and Kelantan showed homozygous resistant and heterozygous genotypes of the V1016G and F1534C kdr mutations. For samples from Penang however, in addition to samples with the V1016G and F1534C kdr mutations some samples only had the S989P kdr mutation without the presence of the V1016G mutation. Biochemical enzyme assays also showed elevated levels of mixed function oxidase enzyme (MFO) and the up-regulation of CYP9J32 gene (*Ae. aegypti*) and CYP6N3 gene (*Ae. albopictus*) from quantitative real-time PCR (qPCR) analysis.

Poster 9

First report of insecticide resistance in *Aedes aegypti* mosquitoes in Bangladesh

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Aedes aegypti is the most important vector of human arboviruses including Zika, dengue and chikungunya. Chemical control of Ae. aegypti is key to preventing and controlling arboviral disease transmission. For many years in Bangladesh, the city corporations, especially in Dhaka, have relied primarily on pyrethroids for space spraying to control mosquitoes, including *Ae. aegypti*. Chemical insecticides are also used in the private sector. Despite the widespread use of chemicals for mosquito control, the insecticide resistance status of *Ae. aegypti* has never been assessed in Bangladesh. Aedes eggs were collected using ovitraps baited with grass infusion from six districts throughout the country plus nine neighborhoods of Dhaka. Selection of areas was based on historic records of *Aedes*-borne arbovirus transmissions and intensity of current chemical vector control measures. Susceptibility to permethrin, deltamethrin, bendiocarb, and malathion was tested on Fo-F2 individuals using CDC bottle bioassays. High levels of resistance to permethrin were observed for all *Ae. aegypti* populations, with mortality ranging from 0 – 14.8%. There was also substantial resistance in populations tested with higher (2X) doses of permethrin (5.1 – 44.4% mortality). Susceptibility to deltamethrin and malathion varied between populations. Complete susceptibility to bendiocarb was observed in all populations. This is the first comprehensive survey of insecticide resistance in any mosquito species in Bangladesh. These data support a switch from the reliance on pyrethroid insecticides to insecticides with different modes of action. Routine monitoring of insecticide resistance in *Ae. aegypti* in Bangladesh should be conducted to understand susceptibility trends over space and time and to plan vector control interventions accordingly.

Poster 10

Insecticide resistance of *Aedes aeqypti* and *Aedes albopictus* populations from Central America: First regional report

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Extensive and inappropriate insecticide use favors the emergence and evolution of insecticide resistance (IR) in *Aedes* populations, thereby threatening efficient vector control. IR among *Aedes* populations has been well studied in different parts of the world. However, information on *Aedes* IR is almost non-existent for Central America. Therefore, we performed the first comprehensive IR assessment of main insecticide classes used in Central America. *Aedes* mosquito eggs were collected using ovitraps located at 12 sentinel sites across Central America. We identified phenotypic resistance using CDC bottle bioassays for adults and WHO dose-response assays for larvae. Underlying molecular resistance mechanisms were determined by biochemical assays and genotyping of knockdown resistance loci associated with pyrethroid and DDT resistance. We found complete susceptibility to bendiocarb and confirmed resistance to DDT at all sites. We also confirmed pyrethroid resistance in adult populations at seven sites. For larval populations, resistance to temephos was only found at two sites, with resistance ratios (RR50) ranging from 7.0 to 50.7. Elevated activity was present among multiple enzyme families associated with insecticide resistance to organophosphates, pyrethroids and DDT. We found that glutathione-s-transferase was either altered or incipiently altered, at all sites with confirmed permethrin resistance, except one. The genotyping of kdr region showed fixation of the mutant 1534C allele at most of the sites, and 1016I frequencies varied from 0.47 to 0.88. This is the first report of co-occurrence of V1016I/F1534C mutations coupled with elevated enzymatic activity, suggesting multiple mechanism of insecticide resistance in *Aedes* in Central America. Although insecticide-based vector control is not used systematically in the region, resistance is clearly present. Further research will be essential to identify key mechanisms of pyrethroid resistance, including transcription studies of genes encoding enzymes that are important to insecticide detoxification.

Poster 11

Point mutation V1016I in the voltage gated sodium channel (VGSC) gene of *Aedes aegypti* and cypermethrin resistance in dengue endemic areas in Medan City, North Sumatra province, Indonesia

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Background: Medan City is still fighting to reduce dengue. Their annual incidence rate is above the national target. Cypermethrin insecticide is commonly used in dengue vector control and can lead resistance to *Ae. Aegypti.* Major mechanism of cypermethrin insecticide resistance is the presence of mutation in voltage gated sodium channel (Vgsc) gene of *Ae. aegypti*.

Objective: Identifying resistance status of *Ae. aegypti* againts cypermethrin and detection of kdr mutation in *Ae. aegypti* VGSC gene.

Method: *Ae. aegypti* eggs were collected using 400 ovitraps (indoor and outdoor) in Tanjungrejo Village Lk. XIX and Gedung Johor Village Lk. XIII. Resistance status of *Ae. aegypti* againts cypermethrin was determined based on the value of resistance ratio (RR99) using diagnostic doses of cypermethrin (10µg/bottle) according to CDC bottle bioassay method. Detection of kdr mutation was performed in Ae aegypty survivor from two fold diagnostic dose of cypermethrin (20µg/bottle) exposure. Determining of kdr mutation based on PCR amplification and sequencing and the results were analyzed by Mega7 software.

Results: Resistance ratio (RR99) of *Ae. Aegypti* from indoor and outdoor location in Tanjungrejo Village Lk. XIX were 2,27 and 0,82 whereas in Gedung Johor Village Lk. XIII were 1,43 an
d 0,93 after exposed to diagnostic dose of cypermethrin respectively. Knockdown time (KT99) of Ae. aegypti from indoor and outdoor location in Tanjungrejo Village Lk. XIX were 79.64 minutes and 28,89 minutes and in Gedung Johor Village Lk.XIII were 50.17 minutes and 32,6 minutes respectively, whereas KT99 of *Ae. aegypti* control was 35.14 minutes. Parallel of Kdr Mutation in VGSC domain II segmen 6 and domain III segmen 6 were found in *Ae. aegypti* from Tanjungrejo Village Lk. XIX (S989P+V1016G and F1534C) and Gedung Johor Village Lk. XIII (V1016G and F1534C). Knockdown percentage of Ae aegypty from Gedung Johor Village Lk. XIII showed lower at two fold diagnostic dose compared to diagnostic dose of cypermethrin after 2 hours exposure due to the existance of avoidance behavior.

Conclusion: Kdr Mutation were found in population of *Ae. aegypti* resistant and susceptible to cypermethrin and it can be use as an early warning to the potential spread of Kdr mutation gene in *Ae. aegypti* population in Medan.
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Poster 12

Pyrethroid resistance and haplotype diversity of the voltage-gated sodium channel gene of *Aedes aegypti* populations from Costa Rica

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Pyrethroids are the main insecticides used for the vector control of *Aedes aegypti*, the primary vector of Zika, Dengue, and Chikungunya virus. In the last years, an increasing use of pyrethroids in public health interventions has been observed worldwide in response to Dengue and Chikungunya outbreaks and the Zika epidemic, including Central America. Using the CDC bottle bioassay, phenotypic resistance to deltamethrin, permethrin, DDT, and malathion was detected in the *Ae. aegypti* populations from El Limon and Puntarenas, Costa Rica. Moreover, preliminary results showed the emergence of mutation 1534C and 1016I on the voltage-gated sodium channel (Vgsc) gene in the populations from Costa Rica. Therefore, we aimed to establish the role of polymorphisms on the Vgsc gene in the pyrethroid resistance. For this, mosquitoes previously classified as resistant and susceptible to permethrin and deltamethrin were subjected to DNA extraction. A real-time PCR was used to genotype the codon 1016 and 1534 in the resistant and susceptible individuals. Subsequently, two fragments of the Vgsc gene spanning the codon 989, 1011, 1016 and 1534 were amplified and directly sequenced. Genotyping results indicated that mutation 1534C was presented in all individuals, but, mutation 1016I was only observed in deltamethrin-resistant individuals. Haplotype diversity analysis detected the emergence of specific haplotypes for each location, suggesting different selection pressures. Furthermore, low genetic diversity was observed for the Vgsc gene suggesting that this gene is under selection in the two sites from Costa Rica. Based on these results, further research is needed to determine the geographical distribution of mutation 1534F and 1016I across Costa Rica. Overall, findings revealed the complexity of the underlying genetic bases of insecticide resistance in *Ae. aegypti* populations from Costa Rica, and highlighted the importance to determine the insecticide resistance mechanisms to develop an action plan for insecticide resistance management and effective vector control.

Poster 13

Spread of a trio of Pyrethroid-resistance Associated Target-site Mutations in Northeast Brazilian Aedes aegypti and its Dynamic on Evolving Insecticide Resistance

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In Brazil and elsewhere *Aedes aegypti* is the main vector of four fatal and/or debilitating arboviruses (dengue, chikungunya, yellow fever and Zika). Despite the crucial importance of targeting *Ae. aegypti* for disease control, our understanding of evolving mechanisms under-pinning insecticide resistance across Brazilian *Ae. aegypti* mosquitoes and its threat to effectiveness of vector control interventions remain limited. Herein, to infer the likely impact of three pyrethroid-resistance associated target-site mutations (Val410Leu, Val1016Ile and Phe1534Cys – the voltage-gated sodium channel gene (Vgsc) in reduced effectiveness of anti-vector Brazilian`s programmes, the spread of these resistant-alleles was inferred in four Brazilian`s Northeast State (Paraíba-PB, Pernambuco-PE, Rio Grande do Norte-RN and Sergipe-SE). *Ae aegypti* were collected between 2016 and 2017 from 13 municipalities spanning urban to rural locations and screened for each target-site mutation using TaqMan assays. The resistant-alleles for each marker are widely disseminated across populations, with fixation of one or more mutations in some locations. Moreover, contrasting patterns in genotype frequencies among the four Brazilian Northeast regions, suggest contrasting strength of selection across geographic locations. Across the three mutant positions we identified a total of 13 haplotypes, including triple mutants (410Leu, 1016Ile and 1534Cys), which were most strongly associated with resistance in pyrethroid-selected mosquitoes. Therefore, co-evolution of the triple resistant allele in the Vgsc gene in *Ae. aegypti* mosquitoes is a concern and might impact sustainability of pyrethroid-based anti-vector interventions.

Poster 14

Status and the mechanisms of insecticide resistance of dengue vectors, *Aedes aegypti* and *Ae. albopictus* populations in Sri Lanka

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Dengue is an important health problem in Sri Lanka and 186101 cases, including 320 deaths, were reported due to dengue fever and dengue hemorrhagic fever during the year 2017. Control of the vector species *Aedes aegypti* and *Ae. albopictus* is mainly done through thermal fogging of adulticides and use of larvicides. Status of insecticide resistance and underlying resistance mechanisms of the vector populations were studied using *Ae. aegypti* and *Ae. albopictus* mosquitoes collected from nine administrative districts of Sri Lanka i.e. Badulla, Batticaloa, Colombo, Gampaha, Jaffna, Kandy, Kurunegala, Ratnapura and Puttalam. Mosquitoes were exposed to permethrin (0.25%), deltamethrin (0.025%), etofenprox (0.1%), λ-cyhalothrin (0.03%), cyfluthrin (0.15%), DDT (4%), malathion (0.8%) and propoxur (0.1%) using WHO bioassays. Efficacy of 1ppm temephos was tested against mosquito larvae in cement tanks. Biochemical and PCR assays were carried out to determine the activity levels of insecticide detoxifying enzymes and the insensitivity of insecticide target sites according to WHO guidelines.

All the populations were susceptible to organophosphates and carbamates with sensitive target site AChE except for Gampaha, Puttalam and Ratnapura populations where high AChE insensitivity was detected. Although the activity of esterases were elevated in all the populations, both species were susceptible to propoxur and malathion except for Jaffna populations where a malathion carboxylesterase resistance mechanism was present. Very high DDT resistance with high glutathione S-transferase activity levels were present in both species collected from all the localities. Among the four pyrethroids tested, deltamethrin was the most effective giving high mortalities in both species, followed by λ-cyhalothrin. *Aedes aegypti* populations, but not *Ae. albopictus*, were resistant to etofenprox. All populations of both species were resistant to permethrin except *Ae. albopictus* from Batticaloa and Colombo. Three 'kdr' type mutations i.e. S989P and V1016I in domain II, and F1534C in domain III were found in pyrethroid resistant *Ae. aegypti* mosquitoes for the first time in dengue vector mosquitoes from Sri Lanka. Pyrethroid resistance in dengue vector mosquitoes were mainly due to elevated monooxygenases and `kdr' type mutations. Efficacy of larvicide temephos (1 ppm) was very high and gave 100% larval mortality for ten months after application under laboratory conditions.

Poster 15

Status of resistant and knockdown of West Nile vector, *Culex pipiens* complex to different pesticides in Iran

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Insecticide resistances have been selected in most groups of arthropod transmitting human diseases, however the trends of these diseases are increasing in the world. Knockdown and susceptibility status of field and laboratory strains of *Cx. pipiens* was determined against DDT 4%, bendiocarb 0.1%, propoxur 0.1%, malathion 5%, fenitrothion 1.0%, permethrin 0.75%, deltamethrin 0.05%, lambdacyhalothrin 0.05%, etofenprox 0.5% and cyfluthrin 0.15% using bioassays. In addition to the use of discriminative doses, various exposure times were also employed for estimating lethal times (LT50 and LT90) and Knockdown time (KD50 and KD 90). The results of knockdown and susceptibility tests indicated that both field and Lab strains were resistant to the all above mentioned insecticides. DDT and malathion showed the higher and lower lethal time values (LT50) for the field strain respectively, while the higher and lower lethal times were observed for DDT and delthamethrin respectively in the SPH strain.. Knockdown tests showed that the higher and lower KD50 were observed for DDT and deltamethrin respectively in the field strain while the higher and lower KD50 were observed for DDT and lambdacyhalothrin respectively in the laboratory strain. Overall, this study confirms the considerable development of resistance of *Cx. pipiens* against most insecticide families in Iran and confirms the need for monitoring insecticide resistance in this vector species.

Poster 16

Targeted capture and sequencing of voltage-gated sodium channel gene in *Aedes* mosquitoes for detecting pyrethroid resistance mutations

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Pyrethroids are currently the most frequently used chemical insecticides to control adult mosquitoes. Pyrethroids target the ion-channels of voltage-gated sodium channels (VGSC) in insect nervous systems. Among many insect species, several amino-acid changing mutations on VGSC have been shown to be responsible for pyrethroid resistance; known as knock down resistance (kdr). The VGSC gene is highly conserved across many insect orders and usually has more than 30 exons. The entire gene region including introns often exceeds 100 kb. Due to the complex structure of VGSC gene, no simple PCR-based method can determine its whole coding sequence from genomic DNA. This precludes genotyping without a priori knowledge of mutation sites and the discovery of new amino-acid substitutions potentially conferring resistance. In this study, we designed biotin conjugated oligo DNA probes corresponding to the whole coding exons sequences of the Asian tiger mosquito, *Aedes albopictus* for a hybridization capture experiment. The probes effectively concentrated (approx. 30,000x) the whole targeted coding sequences of the VGSC gene from indexed and pooled next generation sequencing (NGS) libraries generated from fragmented whole genomic DNA of *Ae. albopictus*. Owing to the high nucleotide homology of the VGSC gene among evolutionally close species, the probes also captured corresponding coding regions of the VGSC gene from NGS libraries of the dengue-yellow fever mosquito, *Ae. aegypti*, with comparable efficiency and even from library of *Culex* mosquitoes but with reduced efficiency. With this targeted capture technique, we can genotype individual *Aedes* mosquitoes for VGSC in a highly multiplexed manner (>96 individual) from a single run using a relatively low throughput NGS platform and an Illumina kit (e.g. MiniSeq or iSeq). We also developed an automated pipeline tool, MoNaS (Mosquito Na+-channel mutation Search), for genotyping the VGSC gene in mosquitoes using NGS data. The MoNaS conducts NGS read-mapping to the reference, calling variants, annotating amino-acid changes, and finally showing results along with corresponding amino-acid positions in the reference VGSC sequence of the house-fly, Musca domestica. Our new targeted-capture technique and bioinformatic pipeline allow genotyping of whole VGSC gene in *Aedes* mosquitoes at low cost from the genomic DNA of field-caught mosquito samples and strongly enhances the detection of novel resistant mutations in mosquitos.

MoNaS: https://gph.niid.go.jp/mo-na-search/index.html

Poster 17

West African Aedes Surveillance Network (WAASuN): Strengthening the capacity of West African countries to control arboviral diseases

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Arboviral diseases such as dengue, Zika and chikungunya transmitted by *Aedes* mosquitoes have been reported in 34 African countries. Available data indicates that over the years there have been a number of dengue and chikungunya outbreaks in the West Africa sub-region including Cote Ivoire, Burkina Faso, Gabon, Senegal and Benin. Viral diseases infect about five million people a year in West Africa, impeding poverty reduction and sustainable development. However, *Aedes* surveillance in West Africa, which is the key to reducing prevalence of viral infections. remains very weak. Recently, the U.S. Centers for Disease Control and Prevention as well as World Health Organization has identified West Africa as a priority region for surveillance strengthening. This is because increased surveillance will provide information essential for the formulation of future vector control strategies and the prediction of disease outbreaks. In line with this objective, the West African *Aedes* Surveillance Network (WAASuN) was launched in Sierra Leone on the 13th of December 2017 at a meeting of scientists working on *Aedes* mosquitoes in the West African sub-region with 9 West African countries represented. The network aims to facilitate collaboration between countries on various aspects of *Aedes* surveillance. The network currently has a membership of 24 scientists in West Africa. The specific objectives of the network are to 1) help strengthen existing linkages in *Aedes* surveillance both inside and outside countries, 2) to bridge the gap between existing surveillance systems in the sub-region, 3) to help build capacity of member countries in surveillance activities and outbreak preparedness including monitoring and management of insecticide resistance and 4) to advocate for funding in *Aedes* surveillance activities in member countries.

Session 3: New technologies and strategies for the control of invasive vectors or insecticide-resistant vectors

Poster 18

New strategy and new insecticides for larval control of the insecticide resistant dengue vector *Aedes aegypti* in Lao PDR

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Background. Dengue is reemerging in Laos and the most important recent outbreak occurred in 2013, with 44,098 cases. The organophosphate temephos (Abate®) has been the only larvicide used for vector control for decades in Laos and recent studies showed that *Ae. aegypti* and *Ae. albopictus* populations from Vientiane capital were resistant to this insecticide. In this context of dengue endemicity combined with high rates of urbanization, there is an urgent need to provide the public health authorities with new strategies, including new insecticides to control the larval stage of the vectors. We tested the efficacy of new insecticides with different modes of action versus temephos and conducted field trials to assess the efficacy of an innovative program using the auto-dissemination technic aimed to treat *Aedes* larval cryptic habitats.

Methods and results. We tested the insecticide susceptibility of a population of *Ae. aegypti* from Vientiane against temephos, Bacillus thuringiensis israelensis (Bti), diflubenzuron, pyriproxyfen and, spinosad. Residual efficacies of Bti and diflubenzuron formulations were then evaluated under simulated field conditions using 200L plastic containers, largely used in Vientiane households for water storage. The larval bioassays showed that the wild *Ae. aegypti* strain was moderately resistant to temephos and spinosad (Resistant Ratio < 5) and fully susceptible to the other insecticides (RR=1). The simulated field trial showed that all of the insecticides (Bti and diflubenzuron) tested remained effective against the local strain after 28 weeks. The auto-dissemination method using In2Care® traps containing pyriproxyfen, was investigated on the premises of the Institut Pasteur du Laos and showed a significant reduction of larvae and adult *Aedes* abundance during the period of test.

Conclusions. These results suggest that Bti and diflubenzuron may be promising alternative larvicides for controlling temephos resistant vectors in water-storage containers in Laos. These results are of great importance for the Public Health authorities of the country and recently, they have taken into account these findings and have modified their National Strategic Plan to fight against dengue by the use of these new alternative insecticides in yearly rotation (effective in 2019). The field trials using the pyriproxyfen auto-dissemination strategy is undergoing, and if effective may represent a potential tool to be used in combination with the new larval control strategies in Laos.

Poster 19

New technology of Nano-emulsion: Promising of Azadiracta india and Pongamia pinnata oils the larvcidal agents for the control major vectors, Anopheles stephensi and *Aedes aegypti*

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Vector borne disease are transmitted to humans through the bite of the *Anopheles, Aedes* and *Culex* mosquitos leading to transmission of disease parasites in countries of Southeast Asia including India. The problem of persistent toxicity along with the growing incidence of insect resistance, has led to the shift from synthetic to green pesticide. The aim of this study was to evaluate the nanoemulsions of essential oils from *Azadiracta india* and *Pongamia pinnata* plants for the control of medically important vector mosquitos. Continuous use of chemical larvicides causes resistance in many species around the world and also environmental pollution. Nanoemulsions of herbal larvicides (such as essential oil based larvicides) are suitable alternatives for this purpose. However, no work so far has investigated the stability of a nanoemulsion after 100–200 fold dilution and its effect on larvae of *Anopheles stephensi* and *Aedes aegypti*. The essential oil of neem and ponamia has effective larvicidal activity. Different nanoemulsions of neem and pongamia essential oils were prepared using various amounts of surfactants and co-surfactants. Neem and pongamia oil nanoemulsions were screened for their efficacy against *Aedes aegypti* and *An.stephensi* using method by WHO, 2005. The LC50 and LC90 for neem and pongamia oil nanoemulsion were evaluated in preliminary study observation for 24h. The nanoemulsion was diluted in distilled water at various concentrations expressed as this essential oil content on aqueous media. A botanical nano-formulation has been developed which is biodegradable, environment and user friendly approaches for the vector control programme.

Poster 20

Potential use of combined sterile insect technique (SIT) and *Wolbachia* based approach for the control of the dengue vector *Aedes albopictus* in Sri Lanka

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The research in this study focused on the possibility of using a novel and environmental friendly strategy of combined sterile insect technique (SIT) and a *Wolbachia*-based approach in *Aedes albopictus* population control efforts in Sri Lanka. The above strategy uses the intracellular endosymbiont bacteria *Wolbachia*, in insects which is vertically transmitted from mother to offspring. Releasing male mosquitoes harboring a *Wolbachia* strain different from that in the wild population will produce reproductive incompatibilities. This study adopted the strategy for suppression of *Ae. albopictus* wild mosquito population by releasing sexually incompatible *Wolbachia* triple infected male mosquitoes on a large scale to the natural environment. Thus the work involved assessment of the density, species abundance and genotyping of *Wolbachia* strains naturally present in wild mosquito populations, introgression and characterization of *Wolbachia* triple infected *Ae. albopictus* line and determination of minimum sterility radiation dose for the males and females of introgressed line. Results revealed that *Ae. albopictus* mosquito was the dominant vector species in the study area (Narangodapaluwa PHI area) with an average ovitrap index of 75.25% (range; 56.9% - 94.7%) over the study year. The frequency of *Wolbachia* infection among the mosquito population was ~17% (13/78). *Ae. albopictus* was naturally infected with both strains A and B while *Ae. aegypti* was infected with strain A with sample infection frequency of 2.5%. All sequencing data were deposited in the Genbank database. Successful transformation of *Wolbachia* triple infection to *Ae. albopictus* with Sri Lankan genetic background was achieved through introgression experiment. Characterization of new *Wolbachia* triple infected *Ae. albopictus* Sri Lankan line revealed competitive fitness cost for fecundity, fertility and longevity compared with wild type while there was no cost for pupation rate, adult emergence and sex ratio. Complete sterility due to CI was not achieved for the new introgressed males. Therefore, integration of an irradiation (30Gy) is recommended to achieve complete male and female sterility prior to mosquito release (minimum sterility dose for males and females was 28 Gy and 30 Gy respectively). This minimum sterility dose is lower than the irradiation dose applied for SIT (>55 Gy) and thereby reduced the effect on male competitiveness significantly. Therefore, a combined SIT and *Wolbachia* based approach in *Ae. albopictus* dengue vector control is recommended as an additional tool in integrated vector management in Sri Lanka.

Session 4: Public / Private initiatives to foster innovation in public health

Poster 21

Fludora® Co-Max: Bayothrin® and its special features

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Given the challenge of resistance, each carefully selected insecticide must play a unique role and be of very high quality. Bayothrin® is one of two active ingredients in Fludora® Co-Max. It is a pyrethroid with special characteristics. We will show you why Bayothrin® is a pyrethroid like no other.

Poster 22

Fludora® Co-Max: Combination of Insecticides to tackle Resistance

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The approach of using combinations of different modes of action is new in Vector Control diseases management. Fludora® Co-Max is based on this principle with unique and complementary active ingredients: Bayothrin® and Flupyradifurone; two unrelated modes of action acting on different target sites; the resulting complementary activity improves robustness and performance. The combinaison approach against resistant pest groups will be discussed.

Poster 23

New matrix-release formulation, SumiLarv®2MR containing pyriproxyfen for long lasting control of *Aedes aegypti* larvae

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Aedes aegypti is a vector of human viral diseases, such as dengue, Zika, Chikungunya, and Yellow fever. Indoor and outdoor water storage containers are the main breeding habitats for this species in Southeast Asia, Central and South America, and also in some savannah areas of Africa. A new long lasting "matrix-release" formulation, SumiLarv®2MR, containing 2% pyriproxyfen has been developed to control container-breeding Ae. aegypti. Pyriproxyfen is an insect growth regulator with a very low mammalian toxicity that inhibits the emergence of adult mosquitoes. Pyriproxyfen is recommended for use in drinking water by the World Health Organization (WHO, 2007). The key feature of SumiLarv®2MR is the controlled slow release of pyriproxyfen so that an effective concentration of active ingredient is maintained in treated water. This concentration is sufficient to inhibit mosquito larvae/pupae from emerging as adults for at least six months after treatment, regardless of the frequency of water replacement. We evaluated the long-lasting efficacy of SumiLarv®2MR under simulated-field conditions in Tanzania and the effectiveness of mass treatment of the formulation to suppress Ae. aegypti populations in a rural village in Lao PDR. The results show that the matrix-release formulation maintains the active ingredient at an effective concentration in treated water for 6 months in domestic water containers. The long-lasting efficacy of SumiLarv®2MR demonstrated in these trials will reduce the number of treatments required per year and will therefore enable significant reductions in operational costs. These results are promising for the future long term control of Ae. aegypti and the diseases that this insect transmits. After extensive small-scale (Phase II) and large-scale (Phase III) trials in Tanzania, Thailand, Malaysia, Mexico and India, it is now recommended by WHO Pesticide Evaluation Scheme (WHO, 2017).

Poster 24

Sylando® - A novel IRS insecticide for mitigating resistance to malaria vectors

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Indoor spraying of walls and ceilings with residual insecticide remains a primary method of malaria control. Insecticide resistance in malaria vectors is a growing problem. Novel insecticides for indoor residual spraying (IRS) which can improve the control of pyrethroid resistant malaria vectors are urgently needed. Sylando® (chlorfenapyr 240 g ai/L SC) is a pyrrole insecticide that has shown potential to provide improved control of pyrethroid resistant An. gambiae. It works by targeting the oxidative pathways in the insect's mitochondria thus disrupting ATP production. Chlorfenapyr shows no cross resistance to existing classes of public health insecticides, hence its novel mode of action makes it a suitable candidate insecticide for targeting malaria vectors that are resistant to current public health insecticides.

Poster 25

The WIN Initiative: A Global Network to Combat Insecticide Resistance in Arbovirus Vectors

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Arbovirus transmitted by *Aedes* mosquitoes, such as dengue, Zika, chikungunya and yellow fever have been re-emerging all over the world. Vector control, mainly by the use of insecticides, play a key role in disease prevention but the use of the same chemicals for decades, together with the dissemination of vectors resulted in the global spread of insecticide resistance. A coordinated approach is imperative to detect and manage insecticide resistance and to deploy alternative strategies for vector control. Initiated with the support of the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Department of Neglected Tropical Diseases (NTDs), the **Worldwide Insecticide resistance Network, WIN** (http://win-network.ird.fr/) brings together 19 internationally recognized institutions in vector research to track and combat insecticide resistance in mosquito vectors of arboviruses at a global scale. The missions of WIN are i) to establish a global resistance surveillance system for arbovirus vectors, ii) to fill **knowledge gaps** and identify **research priorities** on insecticide resistance, and iii) to assist WHO and national authorities in **decision-making** on insecticide resistance management and deployment of resistance-breaking tools. Since its creation in March 2016, the WIN has organized an international conference on vector resistance in Brazil and produced in-deep reviews to support the development of a global plan for insecticide resistance management in arbovirus vectors. The WIN is now entering into a new era by developing a membership organization open to new academia, public health agencies, international organizations, industries, NGOs, etc. to put insecticide resistance on the vector control agenda.

2nd WIN International Conference on **"Integrated approaches and innovative tools for combating insecticide resistance in arbovirus vectors"**

October 1-3 2018, Grand Copthorne Waterfront hotel, Singapore

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