E-SOVE

the 21st conference

2018

Arthropod Vector Science for the benefit of society: Educate, Empathize, Engage

22"d- 26th October 2018
Palermo, Italy

PROGRAM AND ABSTRACT

The European Society for Vector Ecology would like to thank the following organizations for their contribution and support to the E-SOVE 2018 conference:

Valent Biosciences

Enbiotech

Biogents AG

Comune di Palermo

Istituto Zooprofilattico Sperimentale
della Sicilia "A.Mirri"

European Biological Control Laboratory
– USDA ARS

Università degli Studi di Palermo

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WELCOME ADDRESS

Dear Friends and Colleagues,
On behalf of the European Society for Vector Ecology it is my great pleasure to welcome you to the 21st Euro SOVE conference in beautiful Palermo, Sicily.

The primary goal of the Euro SOVE conferences has always been to bring together scientists from the field of vector ecology and control, in order to share current research development, enhance networking and increase visibility of everyone's accomplishments. The meetings are always highly informative with diverse presentations, exceptionally great quality of talks, with ample opportunities to socialize with each other while enjoying the local beauties and traditions of our host country. So, I am hopeful and confident that the Palermo meeting will meet and surpass everyone's expectations!

We - scientists - have made remarkable progress in the recent years to communicate and network with each other, as it is evident by the increasing number of collaborative international projects and networking activities. But how active are we in engaging with the public and the policy makers? And when we do, are we using the right "language"? If someone looks at some of the public's perspective on vaccination, GMOs, and global warming it becomes clear that there is a certain measure of doubt and even fear of science and technology And how is this relevant to our field? What about the release of sterile and/or transgenic mosquitoes? Or what about the emergency response plans to WNV outbreaks in Greece and Texas with aerial ULV space spray applications? How do the public and the regulators perceive these technologies? We say "transgenic mosquitoes" people think "mosquito mutant/monsters". We say "ULV space sprays" they think "spray with carcinogenic chemicals".

Some of this doubt is based on misinformation or may be a consequence of an important communication gap between scientists and society. We are often so caught up in our scientific agendas and the need to excel through publishing that we may neglect or simply not know how to reach out to the public. So, how can we strengthen our links with our communities in order to improve their understanding and trust in science and, also, to better prioritize our scientific goals based on societal needs? I look forward to address these important questions with all of you.

I would like to extend my deepest gratitude to Alessandra Torina, Major Dhillon, Norbert Becker, Bill Van Dyke and Michalis Miaoulis for their tremendous contribution in organizing the conference and to all members of the local organizing committee for being such wonderful hosts. My heartfelt thanks to our scientific committee for their help in shaping a highly informative scientific program but most importantly to all of you, dear conference participants and speakers, for making all of this possible.

Enjoy the conference!

Alex Chaskopoulou,
ESOVE Director

PROGRAM AT A GLANCE

	Sunday 21st
16.00-19.00	Participants registration
	Monday 22nd
07.30-08.30	Participants registration
08.30-10.00	**Conference opening address and Welcome**
10.00-10.30	Coffee Break
10.30-11.00	**Keynote Lecture:** Andrea Crisanti
11.00-13.00	**SYMPOSIUM 1 - Vector Ecology & Behaviour I** Chairs: Bulent Alten & Alessandra Torina
13.00-14.30	Lunch
14.30-16.30	**SYMPOSIUM 2 - Vector Ecology & Behaviour II** Chairs: Francis Schaffner & Ioannis Giantsis
16.30-17.00	Coffee Break
17.00-19.00	**SYMPOSIUM 3 - Vector Pathogen Interactions** Chairs: Eva Veronesi & Chantal Vogels
20.00-21.30	**WELCOME COCKTAIL**
	Tuesday 23rd
08.30-09.00	**Keynote Lecture:** John Vontas
09.00-11.00	**SYMPOSIUM 4 - Reaching out to Society: Bridging Research, Policy and Practice for Solving Real-life Vector-borne Problems** Chairs: Marieta Braks & Roxanne Connelly
11.00-11.30	Coffee Break
11.30-12.30	**SYMPOSIUM 5 - Novel Vector Control Tools I** Chairs: Romeo Bellini & Alexandra Chaskopoulou
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14.00-15.00	**SYMPOSIUM 6 - Novel Vector Control Tools II** Chairs: Romeo Bellini & Alexandra Chaskopoulou
15.00-15.30	Coffee Break
15.30-17.30	**POSTER SESSION**
17.30-18.30	**EMCA General Meeting**
18.30-20.00	**ESOVE Board Meeting**

	Wednesday 24th
	EXCURSION DAY
	Thursday 25th
08.30-9.00	**Keynote Lecture:** Jose de la Fuente
9.00-10.30	**SYMPOSIUM 7 - Vector Networks and Projects: An update** Chairs: Alessandra della Torre & Simon Carpenter
10.30-11.00	Coffee Break
11.00-12.00	**ROUNDTABLE - Vector Networks and Projects** Moderators: Eva Veronesi & Simon Carpenter
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13.30-15.00	**POSTER SESSION**
15.00-15.30	Coffee Break
15.30-17.30	**SYMPOSIUM 8 - Emerging & Re-emerging Vector-borne Diseases** Chairs: Olivier Briet & Dusan Petric
20.00 onwards	**GALA DINNER**
	Friday 26th **Joint Program with the ECM National Course on the Control of Vectors in Europe**
8.30-09.30	**Welcome address by Local Public Health Authorities**
09.30-10.00	**Keynote Lecture:** David Roiz
10.00-11.30	**SYMPOSIUM 9 - Invasive vector species: Surveillance & Management Strategies I** Chairs: Adolfo Ibanez-Justicia & Andreas Rose
11.30-12.00	Coffee Break
12.00-13.30	**SYMPOSIUM 10 - Invasive vector species: Surveillance & Management Strategies II** Chairs: Adolfo Ibanez-Justicia & Andreas Rose
13.30-14.00	**Concluding Remarks**
14.00-15.30	Lunch

SCIENTIFIC PROGRAM

	Sunday 21st	
16.00 - 19.00	**Participants registration**	

	Monday 22nd	
07.30 - 08.30	**Participants registration**	
08.30 - 09.30	**Welcome Address**	**Alexandra Chaskopoulou** - *E-SOVE Director* **Alessandra Torina** - *Local Organizing Committee* **Salvatore Seminara** - *General Director of Istituto Zooprofilattico Sperimentale della Sicilia* **Regional Authorities** **Major Dhillon** - *SOVE Executive Director*
	Presidential Address	**Lal Mian** - SOVE President
09.30 - 10.00	**Conference Central Theme**	**Vector Control for The Benefit of Society: Educate, Empathize, Engage** **Bill Van Dyke** - *Northwest Mosquito & Vector Control District*
10.00 - 10.30		Coffee break
10.30 - 11.00	**Keynote Lecture** **Andrea Crisanti**	**Gene editing population sex determination pathway for malaria vector control**

SYMPOSIUM 1
Vector Ecology & Behavior I

Chairmen: **Bulent Alten, Alessandra Torina**

11.00 - 11.12	**Carlos Barceló**	**The latitude determine the *Culicoides* species composition and its seasonal pattern in mainland Spain**
11.12 - 11.24	**Michela Bertola**	**Occurrence of potential malaria vectors in north-eastern Italy**
11.24 - 11.36	**Maria Bourquia**	**Towards a risk mapping of *Culicoides*-borne diseases in Morocco**
11.36 - 11.48	**Aislinn Currie-Jordan**	**Field-based Analysis of the Biting Rates of *Aedes detritus* and other UK Mosquito Species on Humans**
11.48 - 12.00	**Chantel J. de Beer**	**Development of a standardised protocol for the in vitro feeding of field collected *Culicoides imicola* (Diptera: Ceratopogonidae) from South Africa**

12.00 - 12.12	**Kamil Erguler**	Longitudinal surveillance datasets inform about the environmental dependence of vector populations: a case study on sand flies
12.12 - 12.24	**Yannick Grimaud**	Modelling temporal dynamics of *Culicoides* populations on Reunion Island (Indian Ocean) vectors of viruses of veterinary importance
12.24 - 12.36	**Jerome A. Hogsette**	Stable fly and house fly studies at the European Biological Control Laboratory, Thessaloniki, Greece.
12.36 - 12.48	**Ann-Christin Honnen**	*Aedes albopictus* in its native range: NGS-based analysis of the population structure of the Asian tiger mosquito
12.48 - 13.00	**Julia J. López**	Bionomic aspects of *Aedes (Stegomyia) albopictus*: strong evidence of sexual dimorphism in Mallorca
13.00 - 14.30		Lunch

SYMPOSIUM 2

Chairmen: Francis Schaffner, Ioannis Giantsis

14.30 - 14.42	**Gioia Capelli**	Entomological surveillance for West Nile virus in north-eastern Italy and its importance for public health
14.42 - 14.54	**Disa Eklöf**	The effect of grazing and mowing on the abundance of *Aedes sticticus* eggs in soil samples in Central Sweden
14.54 - 15.06	**Jenny C. Hesson**	Blood-meal analysis of Sindbis virus vectors in Scandinavia
15.06 - 15.18	**Kadidiata Ilboudo**	Effect of irradiation on the quality in the males of tsetse flies (Glossina palpalis gambiensis Vanderplank) at CIRDES laboratory, BURKINA FASO
15.18 - 15.30	**Javid Kashefi**	Cattle Fever Tick (CFT) resistance to acaricides and the quest for its natural enemies
15.30 - 15.42	**Jeanine Loonen**	Malaria vector studies to determine Médecins Sans Frontières (MSF) strategy for malaria vector control in the Democratic Republic of Congo (DRC)
15.42 - 15.54	**Renke Lühken**	Mosquito microhabitat temperatures and the influence on the extrinsic incubation period of different pathogens

SCIENTIFIC PROGRAM

15.54 - 16.06	**Soeren Metelmann**	**Climatic suitability for *Aedes albopictus* in the UK - The role of diurnal temperature cycles in current and future climates**
16.06 - 16.18	**Sandra Oerther**	**Sand flies in Southwest Germany**
16.18 - 16.30	**Gert J. Venter**	**The effect of site selection on light trap efficiency for the collection of *Culicoides* midges**
16.30 - 17.00		Coffee break

SYMPOSIUM 3

Vector Pathogen Interactions

Chairmen: Eva Veronesi, Chantal Vogels

17.00 - 17.13	**Luis M. Hernández-Triana**	**Vector competence of *Aedes detritus* (Diptera: Culicidae) for Batai virus**
17.13 - 17.26	**Stephanie Jansen**	**Experimental transmission of Zika virus by mosquitoes from Central Europe**
17.26 - 17.39	**Tereza Lestinova**	***Leishmania mexicana* amastigotes of various origin, proteomic and developmental comparison**
17.39 - 17.52	**Andrei Daniel Mihalca**	**Comparative microfilariaemia of *Dirofilaria* spp. in the capillary and peripheral venous blood in naturally infected dogs assessed using an unusual blood collection method**
17.52 - 18.05	**Tim W.R. Möhlmann**	**The role of gut bacteria in vector competence of mosquitoes and biting midges for arthropod-borne viruses**
18.05 - 18.18	**Roberto Rosà**	**Early warning of tick-borne encephalitis: climatic variables and rodent density successfully explain *Ixodes ricinus* co-feeding transmission in northern Italy**
18.18 - 18.31	**Charlotte Sohier**	**Experimental evidence of mechanical transmission of lumpy skin disease virus by *Stomoxys calcitrans* biting flies**
18.31 - 18.44	**Ahmet Hakan Unlu**	**Genetic and antigenic diversity in *Theileria annulata* populations after sexual recombination**
18.44 - 19.00	**Chantal Vogels**	**Vector competence of biting midges and mosquitoes for Shuni virus**

20.00 - 21.30	**Welcome cocktail**

	Tuesday, 23rd	
8.30 - 9.00	**Keynote lecture** **John Vontas**	**Insecticide resistance and novel technologies for improving the efficiency and sustainability of mosquito control**

		SYMPOSIUM 4 **Reaching out to society:** **Bridging research, policy and practice for solving real-life vector-borne problems** Chairmen: **Marieta Braks, Roxanne Connelly**
09.00 - 09.15	**Andy Adams**	**Let's make science make sense**
09.15 - 09.30	**Marieta Braks**	**Public health as icing on the science cake**
09.30 - 09.45	**Rubert Bueno-Marì**	**The triangle between scientists, society and authorities: a necessary structure to address the vector management challenges of the XXI Century**
09.45 - 10.00	**Roxanne Connelly**	**Communicating hazard vs risk in mosquito control**
10.00 - 10.15	**Ewelina Czwienczek**	**Risk assessments on vector-borne diseases of animals and plants by EFSA and the EFSA role of supporting risk assessments by Member States**
10.15 - 10.30	**Grey Frandsen**	**Accelerating public acceptance of groundbreaking public health interventions**
10.30 - 10.45	**Eva Veronesi**	**Infravec2 and the role of integrating scientific strategy with governance**
10.45 - 11.00		**Discussions**
11.00 - 11.30		Coffee break

		SYMPOSIUM 5 **Novel Vector Control Tools I** Chairmen: **Romeo Bellini, Alexandra Chaskopoulou**
11.30 - 11.42	**Fabrizio Balestrino**	**Quality control methods for *Aedes albopictus* sterile male production**
11.42 - 11.54	**Sarah Carlin**	**Management of mosquito populations using two methods to prevent infestation of catch basins**

11.54 - 12.06	Sylvie Cornelie	**Evidence of cuticular resistance to pyrethroids in *Anopheles gambiae*, new targets for vector control?**
12.06 - 12.18	Andrea Drago	**Laboratory Evaluation of Mosquito Repellents with different methods**
12.18 - 12.30	John Invest	**Indoor Residual Spraying for Malaria control: past, present and... does it have a future?**
12.30 - 14.00		Lunch

		SYMPOSIUM 6 Novel Vector Control Tools II Chairmen: Romeo Bellini, Alexandra Chaskopoulou
14.00 - 14.12	Fabrizio Balestrino, Arianna Puggioli	**Integrated sterile insect technique (sit) to suppress *Aedes albopictus* populations in urban areas**
14.12 - 14.24	Dan Kline	**Use of novel spatial repellent delivery devices/ methods for the management of peridomestic mosquitoes**
14.24 - 14.36	Edwige A. Koffi	**Does complementary vector control strategies provide additional protection to the massive use of long-lasting insecticidal mosquito nets against malaria in areas with pyrethroid-resistant vectors in rural Burkina Faso and Ivory Coast?**
14.36 - 14.48	Lison Laroche	**Assessment of the boosted SIT approach using pyriproxyfen on tsetse flies in laboratory conditions**
14.48 - 15.00	Farooq Tanveer	**Repelling vector mosquitoes with electric fields**
15.00 - 15.30		Coffee break

15.30 - 17.30	POSTER SESSION

17.30 - 18.30	**EMCA General Meeting**

18.30 - 20.00	**E-SOVE business meeting** Alexandra Chaskopoulou, Major Dhillon

Wednesday 24th

FULL DAY EXCURSION

Thursday 25th

8.30 - 9.00	**Keynote lecture** **Jose de la Fuente**	**Tick-pathogen molecular interactions and implications for the control of vector-borne diseases**
	Chairmen: **Alessandra Della Torre, Simon Carpenter**	**SYMPOSIUM 7** **Vector Networks & Projects: An Update**
09.00 - 09.12	**Thierry Baldet**	**Vectopole Sud, a French Network of platforms for research in medical, veterinary and agricultural entomology**
09.12 - 09.24	**Dominique Bicout**	**VeCA (Vector Control Analysis) application**
09.24 - 09.36	**Simon Carpenter**	***Culicoides* and the emergence of arboviruses in Europe: the issues of creating and retaining expertise on neglected vectors**
09.36 - 09.48	**Alessandra Della Torre**	**The *Aedes* invasive mosquito COST Action**
09.48 - 10.00	**Andrei D. Mihalca**	**EurNegVec COST Action: the aftermath**
10.00 - 10.12	**Antonis Michaelakis**	**LIFE CONOPS: Management Plan for *Aedes albopictus***
10.12 - 10.24	**Eva Veronesi**	**The Infravec2 Infrastructure Project: Providing Vector Researchers with No-cost Resources, Services and Facility Access**
10.30 - 11.00		Coffee break
11.00 - 12.00		**OPEN ROUND TABLE** Moderators: **Eva Veronesi, Simon Carpenter**
12.00 - 13.30		Lunch
13.30 - 15.00		**POSTER SESSION**

15.00 - 15.30		Coffee break

		SYMPOSIUM 8 **Emerging and Re-emerging Vector-borne Diseases** Chairmen: **Olivier Briet, Dusan Petric**
15.30 - 15.42	**Olivier Briet**	Emerging and re-emerging vector borne diseases in the EU
15.42 - 15.54	**Catherine Bouchard**	Toward social-behavioral/ecological informed responses against Lyme Disease in Canada
15.54 - 16.06	**Desmond H Foley**	Global maps of dominant *Aedes* arbovirus vectors
16.06 - 16.18	**Janet Foley**	Gauging the status of a Rocky Mountain spotted fever epidemic emerging from Mexico across the US border
16.18 - 16.30	**Max Fotakis**	Detection of high leishmania infection rates and insecticide resistance mutations in sand fly populations from Greece and Turkey
16.30 - 16.42	**Claire Garros**	The epidemiology of *Culicoides*-borne diseases in the Indian Ocean: Examples of the Bluetongue and Epizootic Hemorrhagic Disease fever viruses in La Reunion Island
16.42 - 16.54	**Anna Heitmann**	Experimental risk assessment for chikungunya virus transmission based on vector competence, distribution and temperature suitability in Europe, 2018
16.54 - 17.06	**Jan O. Lundström**	Sindbis virus polyarthritis outbreak signaled by virus prevalence in the mosquito vectors
17.06 - 17.18	**Valerie Redant**	Spread and persistance of Japanese encephalitis virus in experimentally infected pigs
17.18 - 17.30	**Maarten M. Schrama**	Human practices promote presence and abundance of disease transmitting mosquito species
17.30 - 17.42	**Laura Vavassori**	Sentinel surveillance through travelers

20.00 - onwards		**GALA DINNER**

Friday 26th
Joint Program with ECM National Course on the Control of Vectors in Europe in the XXI Century

08.30 - 09.30	**Welcome Address**	**Silvio Borrello** - *Chief Veterinary Officer, Italian Ministry of Health* **Salvatore Seminara** - *General Director of Istituto Zooprofilattico Sperimentale della Sicilia* **Santo Caracappa** - *Health Director of Istituto Zooprofilattico Sperimentale della Sicilia* **Alexandra Chaskopoulou** - *E-SOVE Director* **Regional Authorities**
09.30 - 10.00	**Keynote Lecture** **David Roiz**	**Integrated *Aedes* management for the control of *Aedes*-borne diseases**

SYMPOSIUM 9
Invasive vector species: Surveillance & Management Strategies I

Chairmen: **Adolfo Ibanez-Justicia, Andreas Rose**

10.00 - 10.12	**Alessandro Albieri**	**Quality control measures in the management of *Aedes albopictus***
10.12 - 10.24	**Norbert Becker**	**Dispersal and control of *Aedes albopictus* in Southwest Germany**
10.24 - 10.36	**Romeo Bellini**	**Private expenditures for self-protection against *Aedes albopictus* in Emilia-Romagna, Italy**
10.36 - 10.48	**Beniamino Caputo**	**Pilot validation of mosquito nuisance assessment by ZanzaMapp, a mobile application to involve citizen in mosquito monitoring**
10.48 - 11.00	**Francesca Marini**	**The dispersal of *Aedes albopictus* females in temperate areas and its relevance in the containment of exotic arbovirus outbreaks**
11.00 - 11.12	**Eleonora Flacio**	**Swiss Reference Network for invasive mosquitoes**
11.12 - 11.24	**Tessa Visser**	**Optimization of odour-baited trapping systems for the surveillance & control of *Aedes aegypti* in Paramaribo, Suriname**

SCIENTIFIC PROGRAM

11.30 - 12.00		Coffee break
		SYMPOSIUM 10 **Invasive vector species:** **Surveillance & Management Strategies II** Chairmen: **Adolfo Ibanez-Justicia, Andreas Rose**
12.00 - 12.12	**Mattia Manica**	**Estimating mosquito population abundance from standard monitoring devices**
12.12 - 12.24	**Andreas Rose**	**Automatic identification of the species and sex of captured mosquitoes in an advanced, remotely operated mosquito trapping system: technical background and results from the laboratory and field**
12.24 - 12.36	**Pedro Sanchez-Lopez**	**Coordinated regional strategy for invasive mosquito surveillance in Southeast Spain**
12.36 - 12.48	**Laurence Vial**	**Update in the geographical distribution of the invasive tick *Hyalomma marginatum* in South of France: first attempts to identify factors favoring its establishment**
12.48 - 13.00	**Patrizia Visentin**	**The tiger mosquito told to the children**
13.00 - 13.12	**Adolfo Ibañez-Justicia**	**Pathways for introduction and dispersal of invasive mosquito species and risk-based surveillance and control: an integrated approach**
13.12 - 13.24	**William Wint**	***Aedes aegypti* in Europe: Past, Present and Future**

13.30 - 14.00	**Concluding Remarks**

14.00 - 15.30	Lunch

ABSTRACTS

ORAL PRESENTATIONS

Gene editing population sex determination pathway for malaria vector control

A. Crisanti

Department of Life Sciences, Imperial College, London

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 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.

Insecticide resistance and novel technologies for improving the efficiency and sustainability of mosquito control

***J. Vontas*[1,2]**

[1] *Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas (IMBB-FORTH), 73100 Heraklion, Greece*

[2] *Agricultural University of Athens (AUA), 11855 Athens, Greece*

After having been eliminated from Europe more than five decades ago, vector borne diseases have recently re-appeared sporadically in several countries, while mosquito nuisance is dramatically expanding too. Environmental management and use of insecticides are the most effective means to control mosquitoes and the pathogens they transmit. Some well-documented successes indicate that insecticides can substantially reduce mosquito populations and diseases, but vector control has also failed to prevent epidemics and expansion of invasive species in other occasions. Among main reasons for that, insecticide resistance which evolves as a real threat for vector control, particularly given the limited availability of insecticides for Public Health. Contemporary status of insecticide resistance in major "European" mosquitoes species, as well as research advents in the field, including molecular diagnostic tools which can facilitate evidence based integrated vector management, will be summarised. A brief overview of alternative vector controls product and tools under development will be also introduced, including highlights how each could offer innovative public health value, mitigate insecticide resistance and improve the efficiency and sustainability of mosquito control.

Tick-pathogen molecular interactions and implications for the control of vector-borne diseases

J. de la Fuente

SaBio. Instituto de Investigación en Recursos Cinegéticos IREC-CSIC-UCLM-JCCM, Ciudad Real, Spain.

Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, USA.

Tick-pathogen interactions evolved as conflict and cooperation. Our model pathogen is *Anaplasma phagocytophilum*, an obligate intracellular bacterium vectored primarily by *Ixodes* spp. that causes various forms of granulocytic anaplasmosis in humans and animals. During tick feeding, *A. phagocytophilum* initially infects midgut cells and then subsequently develops in other tissues including hemocytes and salivary glands for transmission to susceptible hosts. As other intracellular pathogens, *A. phagocytophilum* develops within membrane-bound inclusions in the tick cell cytoplasm, where they evade and manipulate host cell response to limit pathogen infection and multiplication. In an ongoing evolutionary arms race, both *A. phagocytophilum* and tick cells have developed molecular mechanisms to ensure tick survival and pathogen infection and transmission. Recent insights into these mechanisms have shown that *A. phagocytophilum* induces tick proteins that increase tick survival, feeding fitness and vector competence while causing global tissue-specific changes in tick cell interactome (protein-protein physical and functional interactions), regulome (transcription factors-target genes interactions), and miRNAome (miRNA-target gene interactions) among other processes. Molecular and functional studies have shown that *A. phagocytophilum* uses pathogen-tick protein-protein interactions for infection and causes remodeling of the cytoskeleton, inhibition of cell apoptosis, subversion of carbohydrate metabolism and oxidative stress, manipulation of the immune response and control of cell epigenetics to facilitate infection. Simultaneously, tick cells activate molecular mechanisms such as apoptosis, immune response and different metabolic pathways to limit pathogen infection and preserve fitness. Furthermore, recent research has demonstrated that *A. phagocytophilum* uses common strategies but affecting different molecular mechanisms for infection in both vertebrate and tick cells. Targeting some of these mechanisms may results in new strategies for the control of *A. phagocytophilum* and other vector-borne diseases.

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

D. Roiz

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Diseases caused by *Aedes*-borne viruses, such as dengue, Zika, chikungunya and yellow fever, are emerging and 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 (WHO GVCR) 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.

The latitude determine the *Culicoides* species composition and its seasonal pattern in mainland Spain

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Bluetongue as well as Schmallenberg are examples of infectious diseases of ruminants caused by viruses transmitted by species from genus *Culicoides* (Diptera; Ceratopogonidae). These insects are frequently age graded for monitoring purposes in surveillance programs for BTV. *Parous* females (PF) are the only fraction that could effectively transmit the virus; therefore, the study of the seasonal variation of PF would be of great interest for evaluating the risk of transmission of BTV. Data of the Spanish Entomosurveillance National Program from 2008 to 2010 were used to analyze the seasonal pattern of the maximum catches of nulliparous (NF) and PF of the vector species *C. imicola*, *Obsoletus complex*, *C. newsteadi*, and *C. pulicaris*. In addition, latitude variation on the seasonal pattern of PF has been analysed following a North-South axis in mainland Spain. Results showed that total percentages of PF were always higher in summer. The analysis of the latitudinal seasonal variation of NF and PF in Spain has demonstrated that Northern provinces showed an absence of *C. imicola* while the *Obsoletus* species were more present at Northern areas. In fact, there were provinces with periods of the year free of vector species. The species *C. newsteadi* and *C. pulicaris* were less abundant than the other two species, showing the highest population in Toledo demonstrating the inland preferences of these species. These findings would be of interest for a better understanding of the periods of risk of transmission of BTV in Spain and calculate the Seasonally Vector Free Period (SVFP). Further analysis including more provinces and especially other Mediterranean countries would be of interest by observe the interaction between the proximity from the coast and the *Culicoides* seasonality and better understanding the role of the seasonal trend of PF in BTV occurrences.

Occurrence of potential malaria vectors in north-eastern Italy

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Malaria is still one of the most important infectious diseases worldwide. Italy is malaria free since 1970, but in 2017 malaria cases were reported in people with no travel history in endemic areas. The aim of our study was to determine the occurrence, seasonal density and activity and distribution of *Anopheles* species potential or proven malaria vectors in north-eastern Italy. These data will be the basis to assess the malariaogenic potential and the risk of re-emergence of autochthonous cases in Veneto and Friuli Venezia Giulia Regions. Entomological surveys were carried out in different sites from May to September (2017-2018) located in suitable environments for *Anopheles* species. Mosquitoes were captured by aspiration techniques and using BG-sentinel and CDC traps baited with CO2. Larval samplings were also performed. Specimens were identified to complex level by morphological features and to species level by molecular analysis. At present, 35 municipalities and 49 sites were monitored for larvae and/or adults. In total, 41/42 sites were positive for adult of *Anopheles maculipennis* s.l. (97.6%); larvae of species belonging to *maculipennis* complex were found in 4/6 sampled sites (66.7%) and seven sites were positive both for adults and larvae. The breeding sites were mainly paddy fields (89.4%) but they were also found in artificial containers near animal shelters (8.5%). A total of 3,454 *Anopheles maculipennis* s.l. were collected mainly in animal shelters and farms (85.6%), less in dwellings as abandoned house (9.5%) or private garden (2.8%). To date, 252 specimens were identified as *An. messeae* (196; 77.7%) and *An. maculipennis* s.s. (56; 22.2%); molecular analysis are still ongoing. According to these preliminary data, *Anopheles* are still present and are locally abundant but the main vectors of malaria (*An. labranchiae* and *An. sacharovi*) are absent or present at very low density. Funding: Veneto and Friuli Venezia Giulia Regions

Towards a risk mapping of *Culicoides*-borne diseases in Morocco

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Vector abundance maps are a necessary step in mapping the risks associated with vector-borne diseases to help target surveillance and control actions. Risk mapping requires sampling vector species at different scales, which is made difficult by heterogeneity in vector spatial distribution and dynamics. In Morocco, *Culicoides* were responsible for African horse sickness (AHS) outbreaks in the 1960s and late 1980s. Since 2004, Morocco is facing an endemic situation of bluetongue virus (BTV) transmission. Our study aimed to produce first abundance maps for the most abundant *Culicoides* species, and risk maps for BTV and AHSV, across the country. We conducted a national cross-sectional survey using a stratified sampling strategy based on eco-agronomic zoning, for which we hypothesized the homogeneity of *Culicoides* diversity and dynamics. Historical collection time series were used to determine maximum abundance periods based on climate zonation. This strategy allowed to plan a national sampling of *Culicoides* abundance during a single collection season. Collections were conducted twice a year with 144 farms trapped. *Culicoides* were sampled for consecutive 48h using a suction light trap. We obtained a total of 262 samples under identification process. The results so far have shown that *C. imicola* was the most common species (present in at least 70% of the sites), followed by *C. circumscriptus* (absent at mild altitudes) and *C. kingi* (mostly present in the south). *Culicoides obsoletus/C. scoticus* have been collected frequently in the country, including in the south. This highlighted the wide and unexpected spatial distribution of these species from Scandinavia to North Africa reinforcing the existence of sibling species. Other species have also been identified, such as *C. puncticollis*, *C. newsteadi*, *C. catanei/C. gegjelensis* (rather present in the north on the coast), *C. punctatus* (rare in the south), *C. pulicaris/C. lupicaris* and *C. kurensis*. Interestingly, although *C. paolae* is mentioned in the literature as being associated with prickly pears in the Mediterranean basin, this species was absent in many areas heavily vegetated by this fig tree. This original dataset will be used to determine the ecological factors of the abundance and distribution of *Culicoides* of veterinary interest in order to provide accurate mapping of vector-related risks across Morocco.

Field-based analysis of the biting rates of *Aedes detritus* and other UK mosquito species on humans

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Mosquito-borne viruses pose a global threat with increasing numbers of cases being recorded in Europe. Several mosquito species in the United Kingdom could act as vectors for viruses such as West Nile and Japanese encephalitis. However, despite the risk posed, our knowledge of their feeding behaviour and preferences is limited. Gaining such knowledge will improve our ability to quantify risk and provide a basis for rational development of appropriate vector control strategies. We analysed the distribution and abundance of mosquitoes on the Dee estuary of NW England, particularly *Aedes detritus* which breeds in salt marshes and is a potential vector of pathogenic viruses. We collected adult and larval stages of mosquitoes at weekly intervals using a network of 12 traps ('Mosquito Magnets') and 20 natural water bodies to assess seasonal variation in mosquito abundance. Larval habitats were also monitored using a drone flown at weekly intervals across the study area. The results show how environmental drivers, particularly tidal flooding, result in seasonal fluctuations in mosquito populations: mosquito catch increases from <1 per night in summer to >10 per night in autumn following a series of high tides flooding mosquito breeding sites. To allow us to relate catches from traps to biting risk, we compared mosquito catches from Mosquito Magnets with Human Landing Catches and a novel Human Decoy Trap that exploits responses of mosquitoes to the visual, olfactory and thermal cues produced by humans. There was no significant difference between the mean nightly catches from a Mosquito Magnet (2.7, +2.5/-7.2, 95%CI) and Human Landing Catch (4.2, +1.3/-5.5) and a Mosquito Magnet (54.4, +25.2/-117.9) and Human Decoy Trap (72.4, +40.5/-129.6). Our results, combined with laboratory studies of virus development in *Aedes detritus*, provide improved measures and methods of monitoring risk of arbovirus transmission by European mosquitoes.

Development of a standardised protocol for the in vitro feeding of field collected *Culicoides imicola* (Diptera: Ceratopogonidae) from South Africa

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In the absence of laboratory colonies, in vitro feeding of field collected vector species, such as *C. imicola*, will form an indispensable part of the research on bluetongue in sheep and African horse sickness in horses. Due to their small size and a number of unidentified factors that may influence the feeding response the in vitro feeding of field collected *Culicoides* can be challenging. The identification of factors that may affect the feeding rate will be essential for colony establishment and comparable vector competence studies. In an effort to optimised and standardized feeding protocols a commercial available Hemotek® feeding system with a Parafilm® membrane, as used in mosquito and *Culicoides* colony maintenance, was used to pinpoint factors that may affect feeding rates. Factors evaluated included blood source, anticoagulants, blood and room temperature, feeding orientation, exposure time to the blood and the time of day of feeding. The Hemotek feeding system was also compared to existing feeding systems. Feeding rate, blood meal size and survival rates were compared. Except for the time of the day of the feeding event, all of the factors evaluated did influenced feeding success. Preliminary results indicated the optimum feeding rates would be obtained on defibrinated bovine blood at 34.5°C at a room temperature of 24°C. A downwards feeding time of at least 35 minutes is recommended. Feeding rates as obtained with the Hemotek system (53.7%) was comparable to that of existing feeding systems (44.2%). Although feeding rates of up to 89.7% were obtained in midges that were fed on blood soaked cotton wool pledgets immediately after collection, this feeding rate falls to 29.7% in midges sustained for 24 hours. Despite large variation between feeding events it was possible to identified factors that may contribute to the standardization of feeding protocols.

Longitudinal surveillance datasets inform about the environmental dependence of vector populations: a case study on sand flies

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As continued climate warming is predicted, expansion of suitable habitats and distribution ranges of various vectors and vector-borne pathogens is anticipated. There is a pressing need for mathematical models to predict vector spreading and support management, yet phenotypic diversity and complex environmental dependence hamper model development for many important vector species. Here, we present the principles for developing species-specific and environment-driven vector dynamics models based on a generic structure informed by data. We explore the extent and type of data needed and demonstrate that the longitudinal surveillance datasets inform not only about the environmental dependency of population dynamics but also about the underlying biological processes, even in the absence of laboratory experiments. As a case study, we focus on the *Phlebotomus* species due their role in the transmission of about 20 *Leishmania* species. Leishmaniasis, which manifests itself in various forms, is a neglected tropical disease claiming more than 50,000 lives annually. Due to humanmade changes in the environment and evolving resistance to the existing therapies, leishmaniasis is becoming a raising nuisance not only in tropical and Mediterranean countries but also in the temperate regions of North America and Europe. We develop a generic model structure adaptable for several important sand fly species and perform parameter inference to calibrate the model for different species using a surveillance dataset from Greece and Cyprus. The emerging model successfully replicates the observations and demonstrates high predictive capacity for sand fly populations in Turkey. We present the methodology as an extensible tool to predict vector abundance and distribution, and control local, regional, and eventually global populations and diseases transmitted by them.

Modelling temporal dynamics of *Culicoides* populations on Reunion Island (Indian Ocean) vectors of viruses of veterinary importance

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Reunion Island regularly faces outbreaks of epizootic haemorrhagic disease (EHD) and bluetongue (BT), two viral diseases transmitted by haematophagous midges of the genus *Culicoides* (Diptera: Ceratopogonidae) to animals of economic importance such as cattle, sheep and goats. To date, five species of *Culicoides* are recorded in Reunion Island: *Culicoides bolitinos*, *C. enderleini*, *C. grahamii*, *C. imicola*, and *C. kibatiensis*. Although epizootics and *Culicoides* diversity are already well documented, abundance and seasonality of the five species are not. According to a recent viral screening of local *Culicoides* populations (unpublished data), at least four species are involved in the transmission of each virus. Therefore, characterizing the risk period by modelling the temporal dynamics of the five *Culicoides* species is a key step to better understand BT and EHD epidemiology and improve their control.

Between 2016 and 2018, 55 biweekly *Culicoides* catches using OVI traps were set up in 11 sites. A hurdle model (*i.e.* a presence/absence model combined with an abundance model) was developed for each species in order to determine climatic and environmental drivers of presence and abundance of *Culicoides*.

Regarding abundance, average *Culicoides* catch per site ranges from 4 to 45,875 individuals. Also, diversity differ between sites with *C. imicola* being dominant at low altitude and *C. kibatiensis* at high altitude. A marked seasonality is observed for the 3 other species. Eleven meteorological and environmental determinants were used to model presence and abundance of each species: temperature, humidity, rain, wind, global radiation, vegetation index, eco-climatic area, land use, farm density, animal density and length of nearby watercourse. The association of these determinants to explain presence and/or abundance depends on the species, but each plays a role in at least one species.

This is the first study to model *Culicoides* population dynamics in Reunion Island. In the absence of vaccination and vector control strategies, determining periods of high abundance of *Culicoides* is a crucial first step towards identifying periods at high risk of transmission for both viruses.

Stable fly and house fly studies at the European Biological Control Laboratory, Thessaloniki, Greece

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In the summer of 2017, studies were begun at the European Biological Control Laboratory (EBCL) in Thessaloniki, Greece, to document the population dynamics and seasonality of stable flies and house flies. This is the first time an annual study of this type has been conducted in Greece and most likely in Eastern Europe as well. The EBCL facilities are located on the American University where dairy cattle are housed in open corrals and closed structures with feed and water supplied ad libitum. Manure is removed from housing areas and piled nearby on a concrete surface. HD Cedar Box traps and Terminator/Captivator traps were evaluated for trapping house flies. After a week, the Terminator/Captivator traps were selected for the study because of the ease of use. Knight Stick sticky traps were selected for stable fly sampling. This trap consistently catches more stable flies than the Olson sticky trap, a former standard trap, and is very portable. Traps were placed in the field in early June 2017 and were serviced weekly through May 2018. Stable fly populations peaked from April through August, while house fly populations were high from May through September and then began to decrease. The purpose of the 2017 project was to monitor fly populations and no management techniques were implemented for adult or immature populations. However, in June 2018 a management project was initiated at a small farm near the EBCL with the EBCL dairy site used as a control. Aspects of both studies will be discussed.

Aedes albopictus in its native range: NGS-based analysis of the population structure of the Asian tiger mosquito

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The Asian tiger mosquito (*Aedes albopictus*) is an invasive species establishing new populations in previously un-infested areas every year. In order to manage populations of invasive mosquitoes it is essential to determine the source population, and thus the likely routes of introduction. The native range of *Aedes albopictus* covers a wide geographic area ranging from temperate China and Japan to tropical climates in India and Indonesia. A range of this size suggest either an enormous flexibility in habitat choice or that there a two geographical forms that differ in their ability to exploit the different habitats. One long standing hypothesis suggests that there are indeed two forms: a diapausing temperate form and a non-diapausing tropical form . There is some evidence suggesting a northern and a southern group in the native range (Kotsakiozi et al. 2017). One way to tackle these questions is the use of information on genetic relatedness between individuals and populations. To further our understanding with respect to the genetic relationship of the different populations in the native range we undertook a study covering the entire native range of this mosquito using a whole-genome next-generation sequencing (NGS) approach. The wealth of data obtained through whole-genome sequencing allows a fine scale analysis of the amount of genetic relatedness and also exchange between populations. Additionally, we will further investigate the Northern and Southern group and whether that correlates with a predominant temperate or tropical climate.

Bionomic aspects of *Aedes (Stegomyia) albopictus*: strong evidence of sexual dimorphism in Mallorca

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Aedes (Stegomyia) albopictus (Skuse, 1894), commonly known as the Asian tiger mosquito, is an exotic highly invasive species that was recorded for the first time in Spain in 2004 and in Mallorca in 2012. Currently, it is widely present in the Mediterranean east coast of mainland Spain and all the islands of the Balearic archipelago (Spain). Most studies involving this species in the region have been focused in surveillance and control methods, consequently there is a lack of microevolutionary studies for *Ae. albopictus*. Unveil patters of sexual dimorphism in *Ae. albopictus* populations can provide value information about their ecology and behavior and how they react to the unique selective pressures found in the Balearic Islands. Taking that into account, the goal of this study was to investigate the patterns of sexual dimorphism and bionomic parameters of *Ae. albopictus* populations from the Balearic Islands and determine if males are reacting to the selective pressures differently than females. For this, mosquito eggs where collected from the field using oviposition traps and reared in laboratory conditions where different bionomic variables were daily measured (i.e. % egg hatching, larvae and pupae development). . In order to study morphometry of males and females, once adults emerged from pupae, the left wing of each adult mosquito was removed and mounted on a microscope slide with a cover slip. The wings were then photographed under 50x magnification and 18 landmarks were digitized using TpsDig V1.40 software. Subsequently, to explore the degree of wing shape dissimilarity between males and females were analyzed using MorphoJ 1.06d software. Our preliminary results indicated a clear pattern of sexual dimorphism on the wing shape of *Ae. albopictus* specimens when analyzed by the cross-validated classification test. Males were correctly distinguished from females with an accuracy of 84% and females from males 75% ($P = 0.0009$). These results are in agreement with the canonical variance analysis showing a total segregation of males and females. Collectively, our results are suggesting that selective pressures may be affecting males differently than females.

Entomological surveillance for West Nile virus in north-eastern Italy and its importance for public health

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West Nile Virus (WNV) is a public health concern and can infect mosquitoes, animals, and humans. The virus is endemic in north-eastern Italy where it has been found to circulate every year since its introduction in 2008. Therefore, surveillance activities in humans, animals and mosquitoes are ongoing. Here we report the results of the entomological surveillance (2010-2017) and its significance in a One Health perspective. Carbon dioxide-baited CDC trap were activated fortnightly from May to November. The collected specimens were sorted in pools according to species, site and date of collection and submitted to biomolecular analysis. More than one million (1,221,578) of mosquitoes were collected belonging to 18 species. *Culex pipiens* was the main species (~82%), followed by *Aedes caspius* (~8%) and *Ae. albopictus* (~2%). In total 22,095 pools were tested for Flaviviridae and WNV was detected in 147 pools (lineage-1= 27; lineage-2= 120) while Usutu virus (USUV) in 391 pools of *Cx. pipiens*. The first WNV-positive pools are usually detected in mid-July and the last one at the end of September. The entomological and veterinary monitoring detect WNV circulation before human cases and positive blood donors. Therefore the finding of WNV in mosquitoes and animals currently acts as a trigger for the start of the blood donor screening at a province level, provided that the monitoring plan is compliant with a minimum set of rules. The cost/ benefit ratio of this system linked to the human surveillance was positive or negative depending on the intensity of the WNV circulation. The results herein reported show the importance of the entomological surveillance for public health. In addition, they show the temporal circulation and introduction of different WNV strains as well as circulation of other mosquito-borne viruses, as USUV.

The effect of grazing and mowing on the abundance of *Aedes sticticus* eggs in soil samples in Central Sweden

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The floodplains around the southern/eastern parts of river Dalälven in central Sweden is flooded more or less yearly. This results in periodically high amounts of floodwater mosquitoes, *Aedes sticticus*, a nuisance to the inhabitants of the area. Currently mosquito production in the worst affected areas are controlled with *Bacillus thuringiensis israelensis*, Bti, but more sustainable methods to control mosquito production has been requested by Swedish authorities. It has been suggested that management of meadows around the river through grazing or mowing can reduce the number of hatching eggs. Previous studies have indicated a lower number of larvae at grazed/mowed areas. In this survey we investigated the effect of meadow management type as well as the history of management on the abundance of the floodwater mosquito *Aedes sticticus* and other floodwater mosquito species in soil samples. Soil samples were collected during June and July from floodwater meadow sites managed by mowing or grazing, as well as non-managed sites. Both meadows treated with Bti when necessary, and non-treated, were sampled. 25 samples, 20x20cm, were collected from each site. Samples were dried in room temperature before flooded with tap water. Mosquito larvae production was measured by counting hatched larvae, and larvae were identified to species by morphological keys and/or molecular methods. An inventory of the plant species at the sample sites were conducted by using a frame measuring 50x50 cm in order to determine plant diversity at the different sites. Results from this study will be presented at the conference.

Blood-meal analysis of Sindbis virus vectors in Scandinavia

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The vector status of a mosquito species is determined by several parameters; including its infection rate (IR) in the field, transmission competence, and importantly its host preference and abundance in proximity to viremic hosts. Sindbis virus (SINV) is the most northern mosquito-borne virus in Europe that causes outbreaks of human disease. It has been isolated from several places in Europe, but only linked to disease in Fennoscandia. Earlier studies have shown very high IRs for *Culex torrentium* (36/1000), and moderate IRs for *Cx. pipiens* (8/1000), *Culiseta morsitans* (6/1000) and lower IRs for *Aedes cinereus* and *Ae. rossicus*. In vector competence studies, *Cx. torrentium* was a highly efficient transmitter of SINV, while Cx. pipiens and *Ae. cinereus* were moderate transmitters. Thus, the prevailing hypothesis is that *Cx. torrentium* is the main responsible vector for the enzootic bird to bird transmission and *Ae. cinerus* for the epidemic bird to human transmission. This is based on host-attraction studies of the species, showing *Culex* to be strictly ornithophilic and *Ae. cinereus* to be opportunistic. However, recent studies in other parts of Europe, utilizing molecular methods to analyse the blood-meal DNA, have shown a more complex picture of host choice. Therefore, we investigated the blood-meals of the five potential SINV vectors in Sweden. We used degenerate vertebrate primers to amplify the barcoding region of the host mitochondrial DNA in the blood-meal, which was then sequenced and blasted against GenBank. The results show that both *Cx. torrentium* and *Cx. pipiens* in Sweden mainly take blood from Thrushes, the main host of SINV, but also from humans. Both *Aedes* species are more opportunistic, taking blood from a variety of hosts. Thus, our results support the *Culex*-Thrush connection in the enzootic transmission cycle, and adds that also opportunistic *Culex* may transmit SINV to humans.

Effect of irradiation on the quality in the males of tsetse flies (*Glossina palpalis gambiensis* Vanderplank) at CIRDES laboratory, Burkina Faso

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In order to assess the effect of irradiation on male pupae of *Glossina palpalis gambiensis*, we assessed quality parameters of the irradiated male pupae, notably the hatching rate of the pupae, the flying ability of emerged tsetse flies and survival rate of young flies in starving condition following an available quality control protocol. The fertility of male flies from irradiated pupae was also assessed through female productivity and the insemination ability of irradiated males. For this purpose 30 virgin females of 3 days old were mated with 10 irradiated males aged 6 days in breeding cages. Productivity (number of pupae) was compared to a control batch. Simultaneously to this test, other cages of 30 virgin females of 3 days old, mated with 10 irradiated males of 6 days old were followed for the insemination capacity of the irradiated males. Dissections were performed to evaluate the filling level of spermatheca of females mated with irradiated males and those mated with control males. Irradiation had no effect on pupae hatching ($p=0.083$). The fly ability rate was significantly greater in the control group than in the treated lot ($p<0.001$). Male survival from control pupae was significantly longer ($p<0.001$), with an average of 5 and 4 days respectively for the control and irradiated treatment. Female productivity was 1.67 ± 1.55 pupae and 18.93 ± 3.38 pupae, respectively, for females mated with irradiated males and females mated with control males, respectivily. The sterility rate induced in females was 89.67%. Most females had full spermatheca and spermatheca fill level was generally similar for both treatments. Ultimately, irradiation does not affect the viability of the pupae. However, it has an effect on the performance of male flies without affecting their biological quality.

Cattle Fever Tick (CFT) resistance to acaricides and the quest for it s natural enemies

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Cattle fever tick (CFT), *Rhipicephalus annulatus,* is a hard tick native to Mediterranean region with several hosts such as cattle and white tailed deer. It is a competent vector of several protozoan parasites that can cause serious diseases, such as piroplasmosis and babesiosis, which can be of great financial damage to the cattle industry, especially in areas with restricted vector control. *R. annulatus* is an invasive species in the US, with significant impact on cattle production especially in the state of Texas. The development of resistance of the CFT to acaricides due to their extensive use has resulted in treatment failures and a subsequent increase in the tick populations, which were once eradicated from Texas. In the quest to discover its natural enemies special attention is given to the Balkan region, since molecular analysis of CFT showed that the Texas populations were similar to those of Bulgaria. Extensive travelling and cooperation with various institutions and small family farms from Greece, Albania, and Bulgaria in regions where no acaricide treatments are applied resulted in the creation of a wide network of potential tick exposure sites. Classical biological control using specialist parasitoid, predators and nematodes from the native ranges of CFT could complement other existing control strategies and seems to be the most sustainable control method which can result in the management of the tick populations. During our quest for discovery of natural enemies of CFT, recently an entomopathogenic nematode was isolated from one of the soil samples collected at animal resting places using wax moth larvae, *Galleria mellonella*. Furthermore, *Ixodiphagus* sp., a known tick parasitoid, has been collected by trapping methods in Greece. The current status of the project, future research plans and all challenges associated with exploration of natural enemies of CFT will be discussed.

Malaria vector studies to determine Médecins Sans Frontières (MSF) strategy for malaria vector control in the Democratic Republic of Congo (DRC)

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Malaria is an ever-increasing public health problem in the Democratic Republic of the Congo (DRC) and is compounded by recurrent conflicts in the country. Médecins Sans Frontières (MSF) developed a vector-control study, a novel way for MSF to get insight in insecticide resistance levels and to determine the best strategy to control malaria vectors. This study evaluated the main malaria vector species, their susceptibility towards insecticides and effectiveness of Long Lasting Insecticide-treated Nets (LLINs) measured by their killing power. The focus was particularly on malaria risk for internally displaced people living in conflict areas. Methods Mosquitoes and LLINs were collected in Shamwana (Katanga, 2014), Baraka (South Kivu, 2015) and Kashuga (North Kivu, 2017). Standardized WHO insecticide susceptibility tests were performed and effectiveness of new LLINs was tested with WHO cone bioassays. Mosquito species determination and detection of knockdown resistance (kdr) mutations was done by PCR. ELISA-tests were performed to determine *Plasmodium falciparum* infection rates. Effectiveness of used LLINs was tested with a non-resistant *An. coluzzii* lab strain.

The main malaria vectors were *Anopheles gambiae* s.s. and *Anopheles funestus*. Plasmodium falciparum infection rate was 1.8% (46/2,547) in Shamwana, 3.3% (39/1,178) in Baraka and 13.9% (45/324) in Kashuga. Molecular tests showed the presence of kdr mutations, indicative of resistance towards pyrethroids and DDT. Insecticide susceptibility tests showed resistance development towards pyrethroids. Effectiveness of LLINs varied substantially between brands but also within brands.

This is the first study to report on insecticide resistance in local malaria mosquitoes and effectiveness of LLINs in conflict areas of DRC. Insecticide resistance was confirmed by several tests. Our results triggered a careful reconsideration of the existing vector control strategy, including the reinforcement of correct usage of LLINs, ownership and maintenance to maximize not only the mass effect of LLINs but also the individual protection.

Mosquito microhabitat temperatures and the influence on the extrinsic incubation period of different pathogens

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The extrinsic incubation period (EIP) of vector-borne pathogens in cold-blooded insects directly depends on the environmental temperatures. Thus, transmission models are generally based on data from weather station. However, these probably do not represent the actual temperatures present in the microhabitats of the vectors. Therefore, we studied the resting sites of mosquitoes and corresponding temperature conditions to identify the potential consequences for the EIP. In total, 288 artificial resting sites of different sizes (0.4 l, 76 l, 162 l) were attached to trees in different heights (0 m, 1.5 m, 4.5 m) at 16 anthropogenic study sites in Northern Germany. Each resting site was equipped with a data logger to record hourly temperature data. Mosquitoes were collected biweekly from April to October in 2016 and 2017. The numbers of collected mosquito specimens increased with decreasing size of the resting site and decreased with the height of the placement. Resting sites can have a significantly higher temperature range compared to the nearest weather station. This results in shorting of the EIPs for pathogens like Dirofilaria, West-Nile Virus or Usutu virus. The study demonstrates that data from weather stations do not necessarily represent the temperatures in the microhabitats of vectors. Therefore, transmission models include microclimate data to give a reliable estimation of pathogen transmission.

Symposium 2: Vector Ecology & Behavior II

Climatic suitability for *Aedes albopictus* in the UK - The role of diurnal temperature cycles in current and future climates

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The Asian tiger mosquito *Aedes albopictus* is extending its northern range in Europe. In 2016 and 2017, eggs and larvae have been found in the UK for the first time. As this mosquito is an important vector species, able to transmit various pathogens of animals and humans there is a major interest in whether this originally sub-tropical mosquito could become established in the temperate climate of the UK. Previous studies have analysed the mosquito's climatic suitability, using seasonal or daily rainfall and temperature data. However, none of these studies considered the impact of the diurnal temperature cycle. This cycle represents the full temperature range experienced by the mosquito in field conditions and demonstrably has a big impact on the mosquito's development. Here, we suggest a dynamical model for the life cycle of *Ae. albopictus* that explicitly takes into account the mosquito's dependencies on the diurnal temperature range, precipitation, and human population density. We derive a new metric for habitat suitability and drive our model with current and future climate data sets to analyse the chances for a long-term establishment in the UK. Contrary to most other published studies, we find only a low suitability in the UK, except for some warmer and densely populated regions such as Greater London and cities along the south coast of England. Still, mosquito surveillance will be necessary for most of England, as we can show how the suitability will increase with climate change.

Sand flies in Southwest Germany

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Vector-borne diseases are of growing global importance. The spread of phlebotomine sand flies and other dipterans is a major risk for the introduction and spread of emerging infectious diseases favored by globalization and climate change. At present, sand flies are the vectors of the genera Phleboviruses (family Bunyaviridae) including Toscana virus, Sicilian virus, Sand fly fever Naples virus, and *Leishmania* spp. parasites in Europe. Phlebotomine sand flies occur throughout the tropics and sub-tropics, as well as in temperate zones. In Europe, phlebotomine sand flies have been typical present particularly in the Mediterranean region. However, in recent years, their range has increased and in July 1999, sand flies could be detected for the first time in southwestern areas of Germany. Previous studies have proven the natural occurrence of sand flies in Germany. Up to now two phlebotomine species were recorded: *Phlebotomus perniciosus* and *Phlebotomus mascittii*. Therefore, our field study started in 2015 to assess the abundance of sand flies in Southwest Germany. In 2017, 52 individuals could be identified as *Phlebotomus mascittii* and 10 new positive sites for this species in the southwestern Rhineland-Palatinate could be detected for the first time.

The effect of site selection on light trap efficiency for the collection of *Culicoides* midges.

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Certain species in the genus *Culicoides* (Diptera: Ceratopogonidae) are considered the biological vectors of a number of viruses of veterinary importance, including some OIE listed diseases such as bluetongue and African horse sickness. Comparable monitoring of not only species diversity but also vector abundance will be of primary importance in defining transmission risk and for the evaluation of control measures. To determine the effect of site selection on light trap efficiency, 220 V Onderstepoort UV light traps were synchronously operated at four seemingly comparable sites within relative close proximity to each other. Although the traps were 60 m to 70 m apart, they were not in direct sight from each other. Traps were operated overnight underneath the eaves of open sided and semi closed stables housing between 20 and 40 cattle each. Four evaluations, representing seasonal variation, were conducted over a year. Although species diversity was similar between sites statistical significant differences were observed in the numbers collected as well as the proportional representation of the dominant species, *Culicoides imicola* Kieffer. Parous rates as well as the numbers of blood engorged and gravid females and males differ between sites. The site with the highest abundance differ between seasons. Variation between sites were, however, less conspicuous than seasonal variation. The position of the trap in relation to physical structures proofs to play a fundamental role in trapping efficiency. Due to the relative short range of attraction of the trap, trap visibility over extended distances may be of less importance than that of the randomised movement of nearby hosts in relation to the trap. *Culicoides* abundance is not homogeneously in an area and can varies significantly over short distances. The study adds to the factors that contribute to the variability in light trap results and the reliable comparison between geographical areas.

Vector competence of *Aedes detritus* (Diptera: *Culicidae*) for Batai virus

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Currently a number of zoonotic mosquito-borne viruses are present in Europe, and the presence of at least six invasive mosquito species has been reported. Coupled with climate change and increased international travel, these factors have heightened the risk for spread of such viruses within Europe. Identification of potential vectors for mosquito-borne viruses is critical to predicting disease emergence and control. Batai virus is an *Orthobunyavirus* widely distributed throughout Africa and Asia, which has been associated to cause mild illness in sheep and goats, and influenza-like febrile illness in humans. In Europe it has been reported across Eastern and Central Europe, and has recently emerged in Germany where it has been recorded in *Anopheles* and *Aedes* mosquitoes. In this study, we assessed the susceptibility of *Aedes detritus*, a species that feeds on humans and livestock, to infection with Batai virus. Mosquito larvae were sampled from Northern England, reared to maturity and identified morphologically. A total of 112 females (F0) *Ae. detritus* were fed horse blood containing Batai virus (10e6 PFU/ml, strain Batai 53.2 Germany). They were maintained in a Bugdorm insect cage at 20°C, and groups were sampled at 0, 7 and 15 days post infection (dpi). Infection rate of more than 90% was detected at dpi 0. Dissemination rates, the percentage of mosquitoes that blood fed containing virus in their legs of 41.6% and 30.7% at dpi 7 and 15, respectively, were observed. Batai virus was detected in the saliva of five specimens at 7 dpi (41.6%) and a single mosquito at 15 dpi (7.7%). This evaluation of vector competence of a UK population of *Aedes detritus* for Batai virus demonstrates that this species is susceptible to infection and suggests it is a potential mosquito vector of Batai virus.

Symposium 3: Vector Pathogen Interactions

Experimental transmission of Zika virus by mosquitoes from Central Europe

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In 2015, Zika virus (ZIKV) emerged in Columbia and Brazil and spread rapidly across the American continent and the Caribbean, causing an epidemic with notable numbers of associated clinical cases of microcephaly and Guillain Barré syndrome. Mosquitoes of the species *Aedes aegypti (Ae. aegypti)* and *Aedes albopictus (Ae. albopictus)* are considered the primary and secondary vectors of ZIKV. Due to the rapid spread of *Ae. albopictus* in Europe, it is of particular interest to assess the vectorial capacity of European *Ae. albopictus* species. Furthermore, the question remains whether other *Aedes* species as well as other common mosquito species such as *Culex* spp. play a role in transmission of ZIKV. In addition, to assess the risk of possible spread to regions with temperate climate such as Central Europe, information is required on ZIKV vector competence of mosquitoes under reduced temperature conditions. This study aimed to evaluate the vector competence of central European mosquito species for ZIKV. The invasive species *Ae. albopictus* and *Aedes japonicus japonicus (Ae. japonicus)* as well as various taxa of the *Culex pipiens* complex were collected in Germany and challenged with ZIKV at 18°C, 21°C, 24°C and 27°C. *Ae. aegypti* and Italian *Ae. albopictus* were used as positive controls. As expected, *Ae. aegypti* showed positive infection and transmission rates for ZIKV. None of the *Culex* taxa showed vector competence for ZIKV. In contrast, saliva of both investigated *Aedes* species from Germany was positive for infectious virus particles, whereby the number of saliva-positive mosquitoes increased with increasing temperatures. Notably, none of the tested *Aedes* populations were susceptible to ZIKV at 18°C, which limits the spread of ZIKV in Central Europe to summer periods with higher temperatures.

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Leishmania mexicana amastigotes of various origin; proteomic and developmental comparison

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All *Leishmania* species share a digenetic life cycle characterized by motile promastigote stages that develop in the gut of phlebotomine sand flies and by non-motile amastigotes residing inside mononuclear phagocytic cells of vertebrate hosts. Thus, *Leishmania* parasites had to adapt to these two host environments that extremely differ mainly in pH and temperature. In natural conditions, amastigotes are permanently under the host´s pressure and therefore they had to evolve strategies to avoid host innate immune response, such as complement system, or intra-macrophage defense mechanisms like nitric oxide and reactive oxygen intermediates. In laboratory conditions, however, most studies on amastigote biology are done using in vitro macrophage infection systems (at vertebrate-reminiscent conditions) or using axenic amastigotes generated from promastigotes in cell-free medium by decrease of pH and increase of temperature. However, under these conditions, the pressure of host´s environment is incomplete or even missing and this fact could be reflected in a regulation of gene expression and protein translation. Therefore, our main aim was to study to what extent are axenically or macrophage-differentiated amastigotes similar to amastigotes originating from an infected mouse and if all types of aforementioned amastigotes are equally useful as a model for study of amastigote biology. To answer these questions, we used proteomic approach with quantification based on isobaric labeling (TMT 10 plex label) to characterize and compare proteome of all three types of *Leishmania mexicana* amastigotes. In addition, we compared these amastigotes in relation to the development in *Lutzomyia longipalpis*. Females of *Lu. longipalpis* were fed trough a chick-skin membrane on blood with 3x106 amastigotes from different sources. Infection rate and localization of leishmania infection in the sand fly midgut were studied by light microscopy. Morphological forms were determined and compared from midgut smears sampled at 1, 2, 3, 4 and 8 days post blood-meal.

Comparative microfilariaemia of *Dirofilaria* spp. in the capillary and peripheral venous blood in naturally infected dogs assessed using an unusual blood collection method

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Dirofilaria immitis and *D. repens* are mosquito-borne nematodes, infecting mostly dogs, but also other carnivores and accidentally even humans. They have a great impact on human and animal health, and the transmission of both species has been extensively studied. The microfilariemia (i.e. number of microfilariae in the blood) is known to have a circadian variation for both *D. immitis* and *D. repens*. The circadian variation of the microfilariemia was studied so far only in blood collected from large, accessible veins, routinely used for blood collection during clinical procedures. However, the mosquitoes feed superficially, on capillary blood. Our study aimed to evaluate the feasibility of using triatomine bugs for the collection of capillary blood in dogs and to comparatively evaluate the level of microfilariemia and its circadian variation in capillary blood vs. peripheral venous blood in a dog naturally co-infected with *D. immitis* and *D. repens*. The feeding success of triatomines on dogs was 50%, with large variations in the volume of ingested blood during the meal. The relative values of microfilariaemia (mf/bug) were strongly correlated with the volume of blood recovered. In the evening samples, the more blood recovered from each bug, the higher values of microfilariemia. In the morning samples, the results were opposite. The counting of microfilariae showed the dominance of *D. immitis* in all the samples, with significantly higher microfilariemia in the peripheral venous blood. For *D. repens*, the higher counts were in the capillary blood samples. Our study showed that triatomine bugs can be used as a model for the collection and study of microfilariaemia in the capillary blood in mammals.

The role of gut bacteria in vector competence of mosquitoes and biting midges for arthropod-borne viruses

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Arthropod-borne viruses enter the midgut of an insect vector before disseminating throughout the insect body. Arboviruses and gut bacteria can interact, which can determine the infection success of these viruses. We tested the impact of gut bacteria on vector competence of *Aedes aegypti* mosquitoes for Zika virus (ZIKV) and chikungunya virus (CHIKV), and of *Culicoides nubeculosus* and *C. sonorensis* biting midges for Schmallenberg virus (SBV). First, we mapped the gut bacteria of *Ae. aegypti, C. nubeculosus*, and *C. sonorensis*. Each species was treated either with or without antibiotics. The 16S rRNA of gut bacteria was sequenced using Illumina MiSeq, and the relative abundance of bacteria was compared. Second, we determined the vector competence for both antibiotic-treated and untreated groups for each vector species. Mosquitoes were fed on human blood mixed with ZIKV and CHIKV, and biting midges were fed on cattle blood mixed with SBV. After a ten-day incubation period at 25°C, individual insects were tested for presence of virus in their body. For all species we showed that the bacterial composition in the gut was significantly changed after antibiotic treatment. Interestingly, the percentage of infected midges increased with at least 75% in the antibiotic treated group compared to the untreated group. However, we did not observe any effect of antibiotic treatment on infection of *Ae. aegypti* mosquitoes exposed to ZIKV or CHIKV. We conclude that the gut bacterial community suppresses the infection of arboviruses in *Culicoides* biting midges for SBV, whereas no evidence for this interaction was found in mosquitoes. These findings implicate that the gut bacteria of biting midges might be exploited as a novel control strategy for the prevention of virus transmission. Future research should focus on the mechanism behind this interaction, and how this knowledge may be applied in novel vector control strategies.

Early warning of tick-borne encephalitis: climatic variables and rodent density successfully explain *Ixodes ricinus* co-feeding transmission in northern Italy

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Tick-borne Encephalitis (TBE) is an important zoonosis in many parts of eastern and central Europe. The most efficient transmission route for the TBE virus (TBEv) seems to be the saliva-activated non-viraemic transmission between *Ixodes ricinus* ticks co-feeding on rodent hosts. During the period 2001-2014, a population of the yellow-necked mouse, (*Apodemus flavicollis*), was intensively live-trapped in a known TBE focus in the Province of Trento (northern Italy). The total numbers of feeding ticks at each life stage, as well as the number of co-feeding groups were recorded. In addition, a serological test for the detection of TBEv antibodies was carried out for each trapped rodent. First, a combined effect of climatic conditions and rodent density on the number of co-feeding groups was observed. Specifically, co-feeding occurrence on rodents during the season was affected by autumnal cooling of the previous season and was associated with rodent density in a humped (concave-down) relationship. Rodent weight and gender also affected co-feeding occurrence with the heaviest males carrying more co-feeding tick groups. In addition, we found that the overall number of co-feeding tick groups on rodents positively affected TBEv infection in rodents the following year. This is presumably due to a higher probability of feeding larvae to acquire TBEv infection during the season through co-feeding transmission; these larvae would moult and feed on rodents as infected nymphs the following season, thus increasing TBEv circulation and transmission risk. In conclusion, specific climatic conditions in conjunction with specific range of rodent densities are the principal drivers of co-feeding ticks on rodents and could be used to provide an early warning indicator for TBE risk. These simple predictors will potentially allow targeted public health actions to be implemented promptly, with implications for prevention and control of TBEv circulation.

Experimental evidence of mechanical transmission of lumpy skin disease virus by *Stomoxys calcitrans* biting flies

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Lumpy skin disease (LSD) is caused by a *Capripoxvirus* of the family Poxviridae. LSD is a devastating disease of cattle characterized by fever, nodules on the skin, internal pox lesions and lymphadenopathy. The disease is present in many African countries. Since 2012, it has been spreading from the Middle East to south-east Europe, affecting EU Member States (Greece and Bulgaria) and several other countries in the Balkans. Several haematophagous arthropod species like dipterans (e.g. mosquitoes, *Culicoides*, biting flies, horseflies) and ticks are suspected to play a role in the transmission of LSDV. Few conclusive data are however available on the importance of each of these potential vectors in LSDV transmission. Therefore an in vivo transmission study was carried out to investigate the efficiency of LSDV transmission by *Stomoxys calcitrans* biting flies, *Haematopota pluvialis* horseflies and *Dermacentor reticulatus* ticks from experimentally infected donor cows to receptor cows. During the viremic period determined by qPCR after experimental inoculation, field collected *Dermacentor reticulatus*, *Stomoxys calcitrans* and *Haematopota pluvialis* were allowed to partially feed on the donor cows before being transferred to the receptor animals. These were then daily monitored for clinical signs and virological and serological tests were performed. No LSDV transmission was found after placing partially fed *Haematopota pluvialis* and *Dermacentor reticulatus* on the receptor cows. In contrast, LSDV transmission by *Stomoxys calcitrans* was evidenced in two independent experiments, both by induction of nodules and virus detection in the blood of acceptor animals. Since there was a maximum of three days between the partial blood feeding on the donor cow and the continued feeding on receptor animals, our results are suggestive for a mechanical transmission of the virus by this vector. Further work is in progress to further clarify the exact nature of the transmission.

Genetic and antigenic diversity in *Theileria annulata* populations after sexual recombination

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The genetic and antigenic diversity within and between field parasite populations is an important epidemiological parameter that needs to be investigated and quantified for the development and deployment of novel control strategies. The effective role of recombination for generating genetic and antigenic diversity in natural parasite populations has been proposed. These recombined and genetically diverse parasite populations can give rise to new risk factors, such as drug resistance, reduced vaccine protection, occurrence of highly pathogenic isolates. Previous data indicated that genetic and antigenic diversity in *T. annulata* populations vary not only between different geographical regions but also within a single host and this polymorphism is presumed to be due to the high level of genetic exchange occurring in parasite populations. The occurrence of a sexual cycle has been confirmed in a closely related orthologue parasite, *T. parva*, however in *T. annulata*, an evidence for the occurrence of mating has came from population genetic studies and no experimental data is available yet regarding to the role of recombination after transmission of *T. annulata* to ticks. In this study, a panel of 23 micro and minisatellite markers were used to identify, characterise and analyse two different clonal and nonclonal *T. annulata* isolates after recombination. We have measured the frequency of recombination in *T. annulata* isolates for each of four chromosomes. The analysis using polymorphic markers demonstrated the existence of genetic exchange between *T. annulata* isolates and this evident recombination was experimentally shown for the first time. The highest level of recombination was found to be located on the fourth chromosome. However, there was no correlation between recombination rate and chromosomal length. It should be pointed out that none of the recombined populations detected in the present study showed a detectable level of antigenic diversity. This study was financially supported by TUBITAK-110O718.

Vector competence of biting midges and mosquitoes for Shuni virus

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Shuni virus (SHUV) is a neglected virus with zoonotic characteristics that was recently associated with severe disease in livestock and wildlife. Isolations from field-collected biting midges and mosquitoes suggest that SHUV may be transmitted by these insects. However, the ability of biting midges and mosquitoes to become infected, maintain, and transmit SHUV has not been tested before. To gain first insight into the potential involvement of biting midges and mosquitoes in SHUV transmission, we selected four main vectors that transmit other arboviruses to test their susceptibility to SHUV. *Culicoides nubeculosus*, *C. sonorensis, Culex pipiens pipiens*, and *Aedes aegypti* were orally exposed to SHUV by providing an infectious blood meal. Biting midges showed high infection rates of approximately 40%-60%, whereas infection rates of mosquitoes were only 0-2%. Moreover, SHUV was able to successfully disseminate in both biting midge species, whereas no evidence of transmission by neither of the mosquito species was found. To gain more insight into the barriers against SHUV infection in mosquitoes, we intrathoracically injected both mosquito species with SHUV. Injections of SHUV into the mosquito thorax revealed that SHUV was able to successfully replicate in *Ae. aegypti*, whereas no sufficient virus replication was observed in *Cx. p. pipiens*. Furthermore, SHUV was able to disseminate in injected mosquitoes, suggesting the existence of a midgut and salivary gland barrier. The results of this study suggest that SHUV can be transmitted efficiently by at least two *Culicoides* biting midge species, whereas the involvement of mosquitoes has not been supported. Recent outbreaks of other midge-borne viruses showed the ability of these viruses to rapidly emerge in new areas, and their devastating impact on animal health. SHUV should therefore be considered as an important arbovirus which may expand into Europe in the near future.

Let's make science make sense

A. Adams

Bayer S.A.S

If you have been trained as a scientist, a researcher, you almost take it for granted that the fundamentals of hypothesis-driven research, with written protocols, controls, replicates, statistics, traceability and repeatability, are universally recognized. This is work that can be peer-reviewed and published in scientific journals. This is good stuff. If we look at it a slightly different way, the researcher has the courage to recognize that we don't know everything and tries to find out. He or she provides "hope". This is how we progress. The alternative leaves us afraid of the unknown. Today there are those who view scientific progress with suspicion. It is easy for them to play with doubt and appeal to deeper fears. Simple messages meet emotional needs and tweet, easily. Emotion trumps logic, every time. Just look at the debate about vaccination. It takes a measles epidemic and dead people before we wake up to the reality. As scientists, we need to face the fact that we've lost the plot. We have a duty to do better than that. We must communicate the benefits of what we do, the importance of progress, of knowledge over ignorance...but we must do it in a way that reaches the audience. We must stand up for science, not just in our discussions with each other, but in an active and sensitive engagement with the outside World!

Biographical sketch of the Author: Andy completed a PhD in insect physiology at Imperial College London before post-doctoral research in spray application technology and insecticide resistance in UK and US. All good so far. And then he moved to the dark side as a biologist with Wellcome Environmental Health. During nearly 30 years in the industry, many of those years in the non-ag businesses of AgrEvo, Aventis and Bayer, he has led Biology and Regulatory Groups at Global and European level and Chaired the European Biocides Forum, a sector group of the European Chemicals industry association. For the last 5 years, Andy has been a member of the Global Public and Governmental Affairs group at Bayer Crop Science where his main responsibilities are as "issue lead" for endocrine disruption and neonicotinoid insecticides, known to some as the "bee killers". His work often brings him into contact with Government officials, European Commission staff, politicians and representatives of NGOs.

Public health as icing on the science cake

M. Braks

Dutch National Institute of Public Health and the Environment (RIVM)

The government, as the provider of the funding, makes certain demands of academia, one of which is to produce and transfer knowledge for the benefit of society. We – scientists in medical entomology, usually, have no problem addressing this knowledge valorization in our research proposals. After all, we study vectors, which are a topic of public health concern. Right? Well… not per definition. While attaching great value in knowing and complying with the principles of science, we often apply the principles of public health rather loosely in these proposals, often, because we do not exactly know what public health practice entails. Of course, the reverse is true too; in many instances government struggles to understand the researcher's reality. The frustration of both the government and the scientist that valorization often fails in practice is due to the misunderstanding of each other needs and challenges. As a medical entomologist working in public health field for more than a decade, I often serve as an Intermediary translating either public health needs into scientific research or scientific data into the public health practice.

Biographical sketch of the Author: is a medical entomologist with the Dutch National Institute of Public Health and the Environment (RIVM), the Netherlands. After studying biology at the University of Utrecht, she obtained her Ph.D. in Entomology from the Wageningen University in 1999. Subsequently, she worked seven years in the USA first as a postdoc at the Florida Medical Entomology Laboratory and at the Entomology Laboratory of the University of California at Riverside and later as an employee of the Department of Vector - borne diseases of the California State Department of Public Health in Sacramento. With extensive experience in the surveillance and control of vectors and diseases, she returned to the Netherlands to work at the Centre for Zoonoses and Environmental Microbiology of the RIVM in 2007. In addition to national activities, she takes predominantly part in international networks and projects related to vector-borne diseases. She is project leader of Mobocon, a program strengthening mosquito borne disease control through capacity building in sustainable vector control in Dutch Caribbean Municipalities funded by the Dutch Ministry of Public Health (2017-2020).

The triangle between scientists, society and authorities: a necessary structure to address the vector management challenges of the XXI Century

R. Bueno-Marí
Laboratorios Lokímica, Spain

Nowadays a good construction of the triangle between policy makers, scientists and society is essential to address properly the issues linked to vectors and vector-borne diseases (VBD). In this triad of actors, scientists and technicians daily involved in research, design and implementation of vector surveillance and control programs undoubtedly play a key role since they are the real source of the problem knowledge. However, they cannot work alone. The engagement and active participation of society is imperative in many cases (high degree of domiciliation in some vectors, adoption of individual good practice codes in terms of anti-vectorial). As occur in all sheds of science, public engagement with vector control builds on a foundation of a clear and concise communication, so the adoption of good communication skills by medical entomologists is mandatory. On the other hand, politicians, policy makers and regulatory authorities are also indispensable interlocutors in this framework. They are key not only the obtainment of needed economic resources, but also what is even more important: incorporate the surveillance and control programs of vectors and VBD as primordial parts of our public health systems, either at national, regional or local level. Once again, to achieve properly regulations on VBD, scientists and technicians are responsible to transmit to authorities all scientific and technical aspects related (risks, needs, etc.), and this new communication channel requires different type of language with different communication skills as well. We will provide information about different tools to improve communication routes between scientists, society and authorities, as well as different projects related to vectors with strong component on society engagement that have been recently implemented in Spain.

Biographical sketch of the Author: he finished his PhD in 2010 focusing his research on biodiversity, bioecology and health impact of mosquitoes, and obtained the Special Doctorate Award by the University of Valencia (Spain) in the field of Natural Sciences two years later. He is an active member of several scientific associations at national and international level, highlighting his role as current President-Elect of the European Mosquito Control Association (EMCA). He also collaborates with several scientific journals as member of the Editorial Board, is an expert advisor of the Iberoamerican Society of Scientific Information (SIIC) and has published more than 60 scientific publications in journals related with the fields of medical and veterinary entomology, applied zoology and public health. He currently cooperates with several universities and other academic institutions in superior teaching issues related with his field of expertise (medical entomology, vector control and vector-borne diseases) and since 2012 is the head of the Research and Development (R&D) Department of a leading company in environmental health called Laboratorios Lokímica. One of his most important tasks today is the active participation in the design and implementation of vector surveillance and control programs in some of the most important cities of Spain, as well as to be in close contact between society and authorities in terms of divulgation and transmission of information related to vectors and VBD.

Communicating hazard *vs* risk in mosquito control

R. Connelly

Centers for Disease Control & Prevention (CDC), USA

The modern implements of social media, the internet, and transparency in government have led to an increase in public awareness of mosquito-borne diseases and mosquito control activities. Ideally, increased awareness would lead to behavior changes that reduce the risk of exposure to mosquito-borne pathogens. Unfortunately, readily available scientific information can be taken out of context and used in ways that are detrimental to mosquito control activities. The use of insecticides in mosquito control is one tool that is often criticized. While the hazards of insecticides are noted and easy to find on-line, there is rarely an accompanying rational discussion of risk to provide reason and balance. One example of this lack of balance is the use the hazard data reported on Safety Data Sheets (SDS) for insecticides as evidence for banning their use. The information on the SDS is provided for safe use and handling of insecticides and reducing risk of exposure to the chemical. Because the SDS provides only a straight-forward list of technical information about the chemical, it is often used without knowledge of the training, experience, and emphasis on risk that is part of the job of the mosquito control professional. We will discuss ways to communicate hazards in the context of risk to the public to assist public health professionals with doing their job effectively and in a timely manner.

Biographical Sketch of the Author: Dr. Roxanne Connelly is the Chief Entomologist, Entomology and Ecology Team Lead for the Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-borne Diseases, Arboviral Disease Branch in Fort Collins, CO. She leads a team of experts focused on public health applied research, control and response in the area of vector surveillance and control. Prior to moving to Fort Collins, Dr. Connelly spent 17 years on the faculty of the University of Florida working directly with mosquito control programs and county extension agents throughout Florida addressing mosquito research needs as well as training and extension programming centered on mosquito biology and control and medical entomology. She mentored graduate students and taught Mosquito Biology, Mosquito Control, Insecticide Resistance, Mosquito Morphology and Identification, and Integrated Mosquito Management.

Risk assessments on vector-borne diseases of animals and plants by EFSA and the EFSA role of supporting risk assessments by Member States.

E. Czwienczek

European Food Safety Authority (EFSA)

The European Food Safety Authority (EFSA) is a European agency that provides independent scientific advice and communication on risks associated with the food chain. It is supported in this task by 10 Panels of scientific experts, including the Panel on Plant Health (PLH) and the Panel on Animal Health and Welfare (AHAW). Amongst other diseases, the PLH Panel assesses the risk of vector-borne diseases transmitted to plants by arthropods such as spittlebugs, sharpshooters, aphids, thrips, beetles. e.g. *Xylella fastidiosa* related projects (pest categorisation, grants etc.), risk assessment of Flavescence doree, Tospoviruses categorisation, risk assessment of Bemisia tabaci, just to mention a few. The AHAW Panel has assessed the risk for several vector-borne diseases in the EU affecting animals. Some of which of these diseases are zoonotic. A recent example is the production of digital story maps communicating the important aspects of 36 vector-borne diseases, and the potential risks they may pose for animal or public health. These storymaps are the result of a collaboration with the European Centre for Disease Control, VectorNet (a network sharing information on the distribution of vectors), and DACRAH (a consortium involved with the data collection for risk assessments on animal health).

Biographical sketch of the Author: Ewelina obtained her PhD in Forest Sciences in Italy (University of Padua). The PhD research project was focused on the interactions between different forest insects and their host plants along the altitudinal gradients in a pan-European study. She started to work in the European Food Safety Authority (EFSA) as a trainee on the pest risk assessment of the vector-borne pathogen *Xylella fastidiosa*, and then continued her work on the *Xylella* project in Belgium (Université catholique de Louvain and Université Libre de Bruxelles) as postdoctoral fellow. In between she was also involved in teaching and research activities as an assistant professor at the Bialystok University of Technology in Poland (Faculty of Forestry). Nowadays, as scientific officer at EFSA's Animal and Plant Health Unit (ALPHA), her main interest is in vector-borne diseases of plants and animals.

Accelerating public acceptance of groundbreaking public health interventions

G. Frandsen

Oxitec, LTD

Our generation of leaders in public health and vector control are presiding over a growing paradox – we're driving the rapid advancement of significant technological breakthroughs in vector control, but we have yet to make the necessary advancements in how we forge genuine partnerships with those we intend to serve. The lack of investment in robust public engagement, and the narrow toolset used by our field, are limiting our ability to pave the way for successful interventions at precisely the time they are needed the most.

We need to form a new public engagement framework for how we inspire and empower the public globally about novel vector control innovations, and it doesn't start with yet another set of informational brochures to be handed out at community events. To keep pace with technological innovation, we must view public engagement as an urgent prerequisite to success, and one that requires significant energy, attention, and expertise not typically found within our space. To help pave the way for new solutions to advance to the field quickly and efficiently, we must transform how we view public engagement and generate a new, shared set of responsibilities and standards not currently defined.

How we do this is an open question. Who will do it is potentially more pressing.

Biographical sketch of the Author: Grey is the CEO of Oxitec, Ltd. Grey is also an adjunct faculty member at the University of California San Francisco (UCSF) and is advisor to UCSF's Global Health Group. Grey recently authored the Caribbean Regional Public Health Agency's (CARPHA) new framework for public engagement in vector control, and he advised the US CDC and the new Puerto Rico Vector Control Unit during the Zika crisis. Grey also served in the U.S. Government in a range of leadership roles in conflicts and post-conflict stabilization operations globally.

Infravec2 and the role of integrating scientific strategy with governance

E. Veronesi

Institute of Parasitology, University of Zürich (Switzerland)

Infravec2, the four years EU Horizon2020 "Research and innovation programme" (grant agreement n.731060) has a dedicated work package developing and deepening relationships among the scientific community and relevant stakeholders (governmental and non-governmental policy makers, commercial sectors, international funding bodies in research and public health) to control the spread of vector-borne diseases and their vectors. Lack of communication both among scientist and with stakeholders seems to be the major concern in science affecting the sustainability of achievements with the consequent loss of resources and funding. With this project, we are working to strengthen communication among networks of researchers, including non-European partners in endemic countries that are on the front line of vector surveillance, disease emergence, and outbreak control. Fullest utilization and implementation of Infravec2 resources (available at no-cost to worldwide researchers) in the public health mission for surveillance and prevention, and for response to epidemic outbreaks including transfer of new tools and technologies to appropriate partners and stakeholders is one of the main goals. Moreover, the integrated and harmonised access to resources at European level can facilitate the use beyond research and contribute to evidence-based policy making. As part of this project, common European standard of biosecurity for infected arthropod containment infrastructures will be developed and presented at the European and International level as a recognised model of quality control and assurance in order to discuss feedback and comment form public bodies with eventual adoption. An overview of different approaches aiming to improve capacity building, such as questionnaires and interviews enabling to identify strengths, gaps and needs among laboratories for their infrastructures and technical expertise on vectors and vector disease-related topics especially in developing regions, will be here discussed.

Biographical sketch of the Author: Eva has been working as field and laboratory entomologist since 1996, focusing on various subjects of medical and veterinary entomology. After large experience on control, surveillance, identification and rearing of mosquitoes and sandflies in Italy (CAA), she moved to the UK (The Pirbright Institute) as researcher scientist working on vector competence for arboviruses (Bluetongue, African horse sickness & Schmallenberg) in *Culicoides* species. She is currently working as Senior Scientist at the National Centre for Vector Entomology (Zürich University) focusing on the interaction between viruses (Zika, Dengue, Chikungunya, West Nile, Sindbis, Bluetongue) and mosquitoes or *Culicoides*. She is Infravec2 Stakeholder networking Coordinator. She is particularly interested in implementing and applying her knowledge and experience in field situations especially in developing African countries. This includes capacity building, knowledge transfer (laboratory set-up and quality control, teaching and training), project management, research planning and execution.

Quality control methods for *Aedes albopictus* sterile male production

F. Balestrino

C.A.A. Giorgio Nicoli S.r.l., Via Argini Nord 3351, 40014 Crevalcore (BO), Italy

The capacity of the released sterile males to survive, disperse, compete with wild males and inseminate wild females is an essential prerequisite to be evaluated in any area-wide integrated pest management (AW-IPM) programs including a sterile insect release method. Adequate quality control tests supported by standardized procedures need to be developed to measure these parameters and to identify and correct potential inappropriate rearing or handling methods affecting the overall male quality. In this study, we report results on the creation and validation of the first quality control devices designed to infer the survival and mating capacity of radio-sterilized *Aedes albopictus* males through the observation of their flight capacity under restricted conditions (flight organ device) and after stress treatment (aspirator device). Results obtained consistently indicate comparable flight capacity and quality parameters between untreated and 35 Gy irradiated males while a negative impact was observed with higher radiation doses at all observation time performed. The male flight capacity registered with the proposed quality control devices can be successfully employed, with different predictive capacities and response time, to infer the adult male quality. These simple and cost-effective tools provide a valuable method to detect and amend potentially sub-standard procedures in the sterile male production line and hence contribute to maintaining optimal quality and field performance of the mosquitoes being released.

Management of mosquito populations using two methods to prevent infestation of catch basins

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The mosquito control has long been part of the spending of the public administrations. Mosquito control can be undertaken in different ways but it is a primary concern to find effectiveness and eco-friendly methods. As an example of this way of action, in a village of Veneto (Ponte San Nicolò, 13.5sq Km, and 13,500 inhabitants) the mosquito control is carried out in two ways. Half of the catch basins in the public areas are treated by larvicide (Diflubenzuron) and half by a device named ZanzaraStop® (ZS); the latter works as a top that is opened only by water weight. The aim of this work is to compare the effectiveness of this system by monitoring both areas with ovitraps and BG-Sentinel traps. Ten ovitraps and 2 BG-sentinel were placed in the area with ZS and ten ovitraps and 1 BG-sentinel in the area treated by larvicide (TL). Occasional checks were performed to verify the integrity and functioning of the catch basins of both areas. The monitoring started in June and is still ongoing. Ovitraps are checked bi-weekly whereas the BG-Sentinel run for 24 hours every week. To date, the average number of eggs per collection is higher, although not significant, in ZS area (192.18) compared to TL area (151.7). There is a tendency to reach significance in June ($p=0.086$). The average numbers of adult mosquitoes per capture caught with BG-sentinel are similar in both areas (7.92 vs 7.94). During the survey, we found many ZS slightly open because of the presence of leaves or debris and a consequent occurrence of larvae into the catch basins, while those clean were negative. Concluding, ZS can be effective for mosquito larval control only by a constant service. On the contrary, routine larvicidal treatment works better to reduce larvae in catch basins.

Evidence of cuticular resistance to pyrethroids in *Anopheles gambiae*, new targets for vector control ?

S. Cornelie[1], G.A. Yahouédo[1], M. Rossignol[1], C. Ginibre[1], V. Balabanidou[2], N. Garcia Albeniz Mendez[3], O. Pigeon[3], J. Vontas[2] and F. Chandre[1]

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Vector control remains the only barrier against vector borne disease transmission worldwide. Our understanding of resistance mechanisms contribute to highlight new targets and innovative vector control strategies. Cuticular resistance is an overlooked mechanism in malaria mosquitoes mostly because target site mutations are widely distributed in African populations and accounted for an important part of the resistant phenotype. Here, we investigated the role of the cuticle in the resistance to pyrethroids using a resistant strain of *Anopheles gambiae* free of *kdr* mutations (MRS). We selected an *An. gambiae* resistant strain with deltamethrin and without kdr mutations. Susceptibility of this strain against the four classes of insecticides was assessed by WHO test tubes. Ultrastructure of cuticle was investigated by transmission electron microscopy. We developed an original protocol to measure penetration of insecticide inside the mosquito s body by gas chromatography coupled with electron capture. Expression of cuticle and detoxification genes was assessed by RT-qPCR and global protein expression was analyzed by quantitative label free proteomic techniques. MRS strain, is resistant to several pyrethroids and pseudo pyrethroids with resistance ratio greater than two. Results showed MRS cuticle is significantly thicker than the susceptible one. Penetration of deltamethrin measured inside mosquito body was significantly reduced in the resistant strain. Transcriptomic analysis revealed that detoxification genes mainly from p450 oxidases family, cuticular genes from CPAP and CPR families were strongly expressed in MRS strain. Proteomic data confirmed the overexpression of CPAP proteins. In conclusion, these results highlight the implication of cuticle in resistant mosquito phenotype. CPAP proteins family are potentially implicated in this process. Once their exact role will be elucidated, this family may represent new target for vector control. Targeting cuticle of malaria mosquito by genetic approaches may represent a new strategy of vector control against malaria.

Symposium 5: Novel Vector Control Tools I

Laboratory evaluation of mosquito repellents with different methods

A. Drago and P. Visentin

Entostudio srl, Ponte S. Nicolò (PD), Italy

Nowadays the international guidelines (EPA, WHO, ECHA) for efficacy testing of mosquito repellents for human skin recommend different methods to calculate the protection time: First Bite or 95% reduction in bites, First Bite and First Confirmed Bite. Furthermore, the number of volunteers that should be involved in the test are not specified, just EPA guidelines recommends five treated test subjects for a label claim of less than five hours of repellency and ten treated test subjects for a label claim of five or more hours of repellency. A study was conducted testing the efficacy of different commercial products containing IR3535 (ethylbutylacetylaminopropionate) against different 3 mosquitoes species, *Aedes albopictus Skuse*, *Anopheles gambie* and *Culex quinquefasciatus Say* and performing 10 replications per each test. The duration of protection provided by each product was tested by means of arm-in-cage studies, in which volunteers insert their repellent-treated arms into a cage with unfed mosquitoes. First Bite, First Confirmed Bite and 95% Reduction in Bites was used to assess the protection time. The purpose of this study was to find out which method provides a more reliable evaluation of the products and which is the lower number of replications needed to have a statistically-valid number of test subjects, keeping in mind that this parameter is strongly affecting the costs of the test.

Indoor residual spraying for Malaria control: past, present and ... does it have a future?

J. Invest and J. Lucas

Sumitomo Chemical Company

The invention and deployment of long-lasting bed nets (LLINs) on a massive scale in the last 15 years has resulted in dramatic falls in malaria across Africa and the rest of the world. A predominant reliance on this tool containing a single class of chemistry has however resulted in widespread pyrethroid resistance. There is mounting evidence that nets have lost biological efficacy especially in Africa, leading to concerns that bed nets are now starting to lose their effectiveness and malaria rates could rise and challenge the WHO malaria elimination goals. Pyrethroid +PBO LLINs have recently been shown to be more effective than pyrethroid only nets in reducing malaria transmission in areas of high resistance, and so offer some hope for the future. As a solution however, as these PBO containing nets still rely on pyrethroids, they are seen more as a temporary solution while LLINS treated with novel modes of action chemistry are developed. Olyset Plus® containing permethrin and PBO is the first `PBO` LLIN to show epidemiological impact in reducing malaria compared to a standard permethrin only net in an area of pyrethroid resistance in Muleba, Tanzania.

Due to the complexity in the way bed nets function, providing both personal protection and a community effect, it is recognised that epidemiological data will be needed to support such products, with the result that it will take many years before LLINs treated with alternative modes of action chemistry to come to market. However Indoor Residual Spray (IRS) products effectively kill mosquitoes but have to some extent fallen from favour in malaria control programmes and been replaced by LLINs. However, there are already high levels of resistance reported in many malaria endemic countries to IRS products containing DDT, pyrethroids and carbamates with worrying signs that resistance is now growing to more recently introduced organophosphate product. New chemistry with different modes of action are desperately needed to allow rotation in IRS and help preserve insecticide susceptibility.

Thankfully SumiShield® 50WG, which is based on the neonicitinoid insecticide clothianidin has recently been reviewed under the new WHO Prequalification system and has obtained a PQ listing. This is the first new mode of action IRS product for almost 40 years!

While we wait for new mode of action bed nets to come to market new IRS tools like SumiShield will become essential in areas where there is evidence that LLINs are failing as there are currently no other tools available that provide such good control. IRS also has the benefit over LLINs that the treatment requires no user compliance as nets do and works during early evening and morning when people may not be under their LLIN but relaxing in their homes.

Sumitomo is one of several manufacturers working with the IVCC NGenIRS program that seeks to bring products like SumiShield into the market at scale and in a cost-effective manner. This allows IRS to play a critical role in insecticide resistance management and the journey on the road to malaria elimination and ultimate eradication.

Integrated sterile insect technique (SIT) to suppress *Aedes albopictus* populations in urban areas

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Genetic control methods, such as the SIT, are under development for their potentiality to achieve better results than currently available *Aedes albopictus* control measures. To become cost-effective, SIT programs should be able to produce large quantity of good quality sterile males with very low residual presence of females and to distribute them at low cost in the urban areas. The dose of sterile males to be released depends from the density of wild males, as, in order to introduce sufficient rate of sterility into the local population, a certain ratio of sterile to wild males should be achieved and maintained for a sufficient time. Thus, in areas where the wild population is high, such as in the urban areas of Northern Italy, it might be convenient to strongly reduce the target population before and during the sterile males release, allowing a more convenient sterile to wild males ratio. To explore and possibly quantify this ratio, field trials have been conducted during the summer 2018, in some pilot localities. Door-to-door control campaigns have been conducted regularly to reduce the *Aedes albopictus* density; monitoring has been done by ovitraps, BG-Sentinel and HLC; males irradiated with 35 Gy were released weekly; two mark-release-recapture sessions were also managed. Induced sterility rate was estimated by hatching the eggs in SIT treated versus control urban areas. Results will be presented and critically discussed.

Use of novel spatial repellent delivery devices/methods for the management of peridomestic mosquitoes

D. Kline

Center of Medical, Agricultural And Veterinary Entomology, USDA-ARS, Gainesville, FL, USA.

Spatial repellents are chemical compounds which are effective at low vapor phase. Their release as an airborne vapor can produce a range of behavior responses in the mosquito, which results in reduction of human-vector contact. This paper will report on our laboratory and semi-field studies conducted to determine the impact of two vapor-active pyrethroids, metofluthrin and transfluthrin, on host-seeking and oviposition behaviors of *Aedes aegypti*. A major objective of these studies was to develop/evaluate passive release devices that can be used in the peridomestic environment.

Does complementary vector control strategies provide additional protection to the massive use of long-lasting insecticidal mosquito nets against malaria in areas with pyrethroid-resistant vectors in rural Burkina Faso and Ivory Coast?

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Malaria vector control in West Africa relies mainly on the use of mosquito nets treated with pyrethroids. However, the widespread pyrethroid resistance in malaria vector populations threatens the efficacy of this mono-strategy. We aimed to assess whether complementary vector control strategies to long-lasting insecticidal mosquito nets (LLINs) provide additional protection in areas with pyrethroids-resistance vectors in rural Burkina Faso and Ivory Coast. A cluster randomized controlled trial (RCT) was run in 28 and 27 villages in Ivory Coast and Burkina Faso, respectively. The tested strategies were 1) Indoor residual sprayings of insecticide; 2) intensive communication for human behavioral changes; 3) larviciding with natural toxins of *Bacillus thuringiensis israelensis*; 4) Use of Ivermectin both in small ruminants and cattle. These tools are at late-stage of development or are already available within the arsenal of vector control tools in order to complement the current massive LLNs distribution scaled up by national malaria control programs (NMCPs). In this conference, we will present the early results of the RCT regarding the impact of the combination of each of these 4 strategies with LLINs on the malaria transmission and incidence.

Assessment of the boosted SIT approach using pyriproxyfen on tsetse in laboratory conditions

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Tsetse flies (*Glossina*), are African hematophagous Diptera, main vectors of animal and human trypanosomoses in Africa. The Sterile Insect Technique (SIT), has been successful to control tsetse when conducted on isolated populations. A new approach, called boosted SIT, combining SIT with the contamination of wild females by sterile males coated with pyriproxyfen (PP) has been proposed for large-scale control of vector populations. The aim of the study was to determine in laboratory conditions the transfer dynamics of PP from a sterile male to a female during mating, as well as the impact of PP on female fertility. To do this, we developed a method of treating sterile males by impregnating them with a powder containing 40 % of PP. The results showed that the PP had no impact on the survival of sterile males. With respect to persistence, PP persisted on sterile males up to 10 days at a dose of 100 ng. In addition, we showed a horizontal transfer of PP from a sterile male treated to a female. During mating, females received an average of 50 ng PP and by simple contact without mating more than 10 ng PP. Ongoing experiments will verify the impact of the transferred PP on female fertility. These initial results must be confirmed under field conditions. Eventually, the large-scale application of the boosted SIT approach against tsetse flies could be implemented in Africa.

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Repelling vector mosquitoes with electric fields

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An electric field is formed between two electrically charged conductors (electrodes). The strength of the electric field depends on the electric potential difference (voltage) and the distance between the electrodes. To observe the effects of electric fields on mosquitoes, we performed experiments in which two cube-shaped plastic insect cages (Bug Dorm-1 Insect Rearing Cage) were fixed at the two sides of a glass tunnel. Parallel plate electrodes were positioned in the middle of the glass tunnel and were connected to an adjustable high voltage source, with which a wide range of strong electric fields could be generated between the electrodes. During the experiments, hungry female mosquitoes (*Aedes aegypti*) were released in cage1. A test person then introduced his hand into cage2, while a ventilator produced a gentle air flow through the glass tunnel in the direction of the mosquitoes in cage1. Mosquitoes lured into the direction of the hand had to pass through the electric fields between the plates. It was observed that the electric field of strength 1 kilovolt per centimeter (1kV/cm) prevented mosquitoes from passing through. As the field strength decreased, more and more mosquitoes passed through the barrier. When the electric field was turned off, mosquitoes passed freely. These results demonstrate that strong electric fields can be used to effectively repel mosquitoes. Additional work in this project includes the study of the potential of electric fields to protect human habitations and out-door resting places or enclosures against mosquitoes. The field testing of initial prototypes is going on in Regensburg (Germany) during the summer of 2018. (This research is made possible through the generous support of the United States Agency for International Development, USAID (Grant No: AID-OAA-F-16-00092)).

Vectopole Sud, a French Network of platforms for research in medical, veterinary and agricultural entomology

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Six institutional partners, CIRAD, CNRS, EID Méditerranée, INRA, IRD and the University of Montpellier have been working together since 2010 on the issue of arthropod vectors of pathogens that cause infectious diseases in humans and animals, and insect pests responsible for damages to crops. This collaboration for over 8 years has resulted in the creation of "Vectopole Sud", a Montpellier network of platforms dedicated to researches on arthropods of medical, veterinary and agricultural importance.

The network draws from the infrastructure and expertise of each partner to improve early warning systems, monitoring, control of pests and vectors in order to protect human health, agriculture and livestock production. The network has received continuous funding by the French Government and the Occitanie Region enabling it to modernize its different laboratories and rearing facilities and support the research activities.

Vectopole Sud includes four experimental platforms, confined insectariums and associated laboratories, which are open to national and international partners. Overall, the Vectopole Sud has a network of partners in Europe and tropical regions worldwide, notably through IRD and CIRAD, two French institutions supporting research for development.

Vectopole Sud brings together over 400 researchers and technicians from five research units belonging to the Montpellier University of Excellence and Agropolis International scientific communities. It conducts multidisciplinary research and expertise on biology, public & animal health, ecology, environmental science, genetics, and evolutionary medicine. It contributes to a better environment, animal health and human wellbeing through an integrated One Health approach, by fighting human and animal diseases to ensure more productive and sustainable farming systems (food security).

We will present the different platforms of the Vectopole Sud network, its various activities (infrastructures, research, services and training) as well as its different perspectives.

www.vectopole-sud.fr

VeCA (Vector Control Analysis) application

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Vector management is the primary action for preventing and controling outbreaks of vector-borne diseases like WNV infections. However, there is always an uncertainty when the vector control strategies have to be adopted in cost/effective evidence based way. The aim of this work is to develop a tool allowing appraising and comparing the efficiency and cost effectiveness of different vector control strategies against main vectors of West Nile fever in Europe. To this end, we have developed a mechanistic and climate-driven modeling of the population dynamics of mosquito vectors (mainly, *Culex spp.*) coupled with mosquito control measures. The model allows assessing the likely impacts and cost of various strategies of vector control interventions (larviciding, adulticiding and source reduction) alone and in combination as well as to aid singling out best strategies.

Culicoides and the emergence of arboviruses in Europe: the issues of creating and retaining expertise on neglected vectors

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In the past two decades, the importance of arboviruses transmitted by *Culicoides* biting midges in Europe has risen dramatically, leading to a ‘golden age’ in research on this vector group. I will also identify current bottlenecks in research imposed by the biology of *Culicoides* which are among the smallest haematophagous flies known and discuss recent research carried out in the Pirbright Institute. This links to the second part of my talk, where I will discuss a project recently funded by the BBSRC, which seeks to bring together workers on *Culicoides*, sandflies and blackflies and place research on these groups in context with each other. This project will act as a focus for collaboration and also enable research through catalyst grants for study in countries receiving Official Development Assistance from the UK government. The aim is to create a community that is more robust to rapid changes in funding and to address issues in working on some of the most challenging subjects in vector pathogen interactions.

The *Aedes* invasive mosquito COST action

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COST (CO-operation in Science and Technology) is a pan-European intergovernmental framework dedicated to networking activities for European researchers, engineers and scholars to jointly develop new ideas and initiatives across all scientific disciplines through trans-European coordination of nationally funded research activities. COST funds networking activities (Meetings, Training Schools, Short-Term Scientific Missions and Dissemination Activities), but does not fund research or equipment purchase (www.cost.eu/).

The surveillance and control of *Aedes albopictus* and other *Aedes* Invasive Mosquito (AIM) species and the management of the risk of introduction and spread of Exotic AIM-Borne Viruses (dengue; yellow fever; chikungunya; Japanese encephalitis and Zika) in Europe requires multidisciplinary research, cost-effective conventional/innovative methods, strong international and cross-sectorial linkage, as well as better integration of public and private sector initiatives and a better informed and aware society. The AIM-COST Action (www.aedescost.eu; www.cost.eu/COST_Actions/ca/CA17108) aims to remove the fragmentation in expertise and inconsistencies in the implementation of surveillance and control of AIM in European countries. The Action will also promote innovation in the fields of AIM research, prevention and control (and more generally of medical/veterinary entomology and epidemiology of AIM-borne diseases) by developing synergies between scientists, decision-makers, the private sector and civil society necessary to harmonise and rationalise sustainable approaches - both conventional and novel – for the surveillance, control, analysis and dissemination throughout Europe and beyond.

The AIM-COST Action kick-off Management Committee meeting on September 6th marked the official starting date of the 4-year Action with the participation of two Management Committee members from each of the 27 COST-Member States (out of 37) that have already joined the Action.

The talk will briefly present AIM-COST Action structure, objectives and working groups and planned activities. We remind the audience that new Participants as well as Members and Cooperating-States can still join the Action, and would be more than welcome.

EurNegVec COST Action: the aftermath

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COST Actions are networking tools aiming to gather researchers to cooperate and co-ordinate nationally funded activities. They are in fact bottom-up networks with a standard duration of four years. The four financing instruments available during a COST Action are meetings (management meetings, conferences, workshops), short term scientific missions (individual mobility for researchers), training schools and publications. EurNegVec (European Network for Neglected Vectors and Vector-Borne Infections) was a network comprising over 400 scientists from 44 countries (35 COST countries, 5 near neighboring countries, four overseas countries). The network organized five meetings (Brussels, Cluj-Napoca, Izmir, Zaragoza, Chania), six training schools (France, Italy, Belgium, Romania, Spain, Portugal) and granted 84 short term scientific missions. Various publications have been prepared under the frame of EurNegVec, including 1 monograph and two special journal issues. The network was for many participants the launching pad for their participation in various international projects and networks. As one of the main principles of EurNegVec was to support your researchers, the network significantly contributed to the advanced training of human resource. One year after the Action ended, the network is still actively communicating via emails, the participants have ongoing and long-lasting collaborations, new publications are being produced and new research ideas and projects are developed.

Symposium 7: Vector Networks & Projects - An Update

LIFE CONOPS: Management Plan for *Aedes albopictus*

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Aedes (Stegomyia) albopictus (Skuse1894) (Diptera: Culicidae), also known as the Asian Tiger Mosquito , is an invasive mosquito species already well established in many EU countries, causing high concern in public health for the serious nuisance pest it generates and for its vectorial capacity for pathogens causing human diseases such as dengue, chikungunya and Zika viruses. It is, therefore, necessary that the responsible authorities evaluate the situation in their territory and eventually implement specific management program aimed at the reduction of the mosquito population density, possibly below the noxious and the epidemiological thresholds. In the framework of LIFE CONOPS project (www.conops.gr) a detailed management plan to control the invasive mosquito species *Aedes albopictus* was developed. The management plan includes coordinated actions such as standardized control measures and quality control activities, monitoring protocols, the involvement of local community and stakeholders and an emergency vector control plan to reduce the risk of an epidemic in case of detection of infected persons. Others invasive mosquito species present in limited areas or at risk of introduction such as *Aedes aegypti* may deserve specific and different approaches.

The Infravec2 Infrastructure Project: Providing Vector Researchers with No-cost Resources, Services and Facility Access

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The Infravec2 infrastructure project provides insect vector resources and facility access to researchers worldwide at no cost. The project is funded by the European Commission Horizon 2020 Research Infrastructure Program (INFRAIA). The 24 Infravec2 partners operate major European biosecure insectaries for experimental infection and containment of insect vectors and other key insect vector technology platforms including front-line field sites in Africa, the Pacific, and the Americas. Researchers can shop online and request resources from an extensive product catalog (www.infravec2.eu). Infravec2 is also developing innovative new research tools, providing training courses, and is networking the community with activities such as common experimental standards and protocols to obtain reproducible vector infection results across different facilities. Infravec2 is a source of EU research support for vector researchers. The Infravec2 goal is to accelerate European innovation in basic and translational insect vector biology, and to consolidate a high-quality insect vector infrastructure with long-term perspectives for improving global public health.

Emerging and re-emerging vector borne diseases in the EU

O. Briet

European Centre for Disease Prevention & Control (ECDC), Sweden

The European Centre for Disease Prevention and Control (ECDC) aims to strengthen Europe's defences against infectious diseases, by providing evidence for effective and efficient decision-making, strengthening public health systems, and supporting the response to public health threats. This presentation gives a brief overview of the Emerging and Vector-borne Disease programme at ECDC, giving illustrative examples of some of its activities against currently emerging and re-emerging vector-borne diseases, such as disease surveillance, the production of risk assessments and the development of model-based tools for the assessment of disease risk and evaluation of cost-effectiveness of intervention options.

Toward social-behavioral/ecological informed responses against Lyme disease in Canada

C. Bouchard, C. Aenishaenslin, E.E Rees, S. Kotchi, Y. Pelcat, F. Milord, C. Russel, C. Jardine, J.K. Koffi, L.R. Lindsay, P.A. Leighton and N.H. Ogden

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The risk of contracting Lyme disease (LD) varies greatly due to spatial heterogeneity in ecological risk factors and risk-associated behaviors. LD risk is expected to be higher in areas where ecological characteristics favour a higher density of infected ticks and when human behavior enable transmission of LD. Few studies have jointly addressed the human social and behavioral risk factors and have tried to quantify their relative importance, which is representing a critical gap. Here we present the geographic hotspots of LD risk identified by a social-behavioral/ecological risk assessment in order to provide 1) useful targets for geographic prioritization of public health interventions to address the areas and populations at greatest risk and 2) an assessment tool to examine the relative contribution of different types of risk factors in relation to emerging hotspots for human LD cases. Our study focuses on two provinces where LD is an endemic disease (Quebec and Ontario). LD knowledge and behaviors in the population were measured using web survey data collected in 2014. These data were used as a proxy for the social-behavioral component ofrisk. For the ecological risk analysis, we developed a tick abundance model by integrating data from ongoing long-term tick surveillance programs and high-resolution data derived from Eatih Observation. Social-behavioral and ecological components of risk were combined with human population density to create integrated risk maps. Map predictions were validated by testing the association between high-risk areas and the current spatial distribution of human LD cases. This study highlights a new area of research on the development and validation of integrated social/behavioral-ecological informed response. Our approach is widely applicable to other vectorborne or zoonotic diseases and to different epidemiological context where the geographic patterns of risk are driven by the interplay between social-behavioral and ecological factors.

Global maps of dominant *Aedes arbovirus* vectors

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Arboviruses transmitted to humans by mosquitoes (Diptera: Culicidae) are a major health concern globally. Global maps of the dominant *Anopheles* vectors of malaria are available as a simple visual aid to understand the distribution of vector hazard. We aimed to produce similar maps for the major *Aedes* mosquito vectors of arboviruses to humans. A total of 48 *Aedes* species and the arboviruses they transmit to humans were ranked in importance, and their geographic distribution mapped by use of ecological niche models and rasterized country of occurrence information. The resulting global maps of the highest ranked, and combinations of dominant species highlighted the complexity of and gaps in knowledge about the distribution of vector and arbovirus species. The combination of *Ae. aegypti/Ae. albopictus* were predicted to co-occur with humans most frequently. These simplified maps will evolve as new information becomes available and should benefit those wanting to see a list of the most medically important arbovirus vectors effecting humans in a given area.

Gauging the status of a Rocky Mountain spotted fever epidemic emerging from Mexico across the US border

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Sporadic Rocky Mountain spotted fever epidemics have occurred in Mexico for almost a century. Two large, ongoing epidemics are now serious threats in the northern Mexican states of Sonora and Baja California. They are associated with emergence and northward introgression of a unique tropical lineage of brown dog tick that has been found across Mexico and Central and South America but is now being found in Florida, western Arizona, and southern California. This tick may harbor highly virulent strains of *Rickettsia rickettsii* and may be an aggressive human biter. This talk will summarize large epidemiological studies of RMSF emergence at the US-Mexican border. One study aimed to use canine sentinels at differing distances north of the border to evaluate possible spread into the US. The second examines distribution of tick infestation, risk factors, and exposure to *R. rickettsii* in Mexicali in Baja California. Climate, geography, and many cultural attributes are very similar across both sides of the border, and many people (and their animals) cross the border every day. However, reduced public health capacity, dog licensing and control, and tick preventive measures in Mexico, together with differing socio-economic contributing factors may change epidemic patterns on each side of the border. Tying these studies together can help us to understand how to manage the shared risk.

Detection of high leishmania infection rates and insecticide resistance mutations in sand fly populations from Greece and Turkey

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Leishmaniasis is a parasitic disease caused by protozoans of the genus *Leishmania* and is transmitted by sand flies of the genus *Phlebotomus*. The disease poses a serious public health threat in the Mediterranean basin, a region currently in the epicenter of a major refugee crisis. Greece and Turkey are both endemic for visceral (VL) and cutaneous leishmaniasis (CL). *Leishmania infantum* is the most common etiological agent responsible for cases of VL and CL in the two countries while Leishmania tropica is responsible for many CL cases in Turkey. Vector control programs rely mainly on insecticides yet a worldwide problem associated with their intensive use is the development of resistance. Knowledge of vector sand flies insecticide resistance status, species composition, geographic distribution, and pathogen load is essential for the design and implementation of effective and efficient sand fly control programs but such information is scarce. By using modern molecular diagnostic tools we conducted analytical sand fly monitoring studies in selected sites of increased epidemiological importance. High *Leishmania* infection rates (43 & 52%) were recorded in sand flies collected from refu- gee camps in Northern Greece, indicating these foci as putative disease transmission hotspots. Target site pyrethroid resistance mutations (KDR) were detected in popula- tions of the major leishmaniasis vector *P. papatasi* from Sanliurfa Turkey highlighting the potential risk of pyrethroid resistance in *leishmania* vectors in the Mediterranean basin. These findings pose serious challenges for leishmaniasis control and highlight the necessity for entomological monitoring in support of evidence based vector man- agement strategies.

The epidemiology of *Culicoides*-borne diseases in the Indian Ocean: Examples of the Bluetongue and Epizootic Hemorrhagic Disease fever viruses in La Reunion Island

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Bluetongue and epizootic hemorrhagic disease viruses are recognized worldwide as important vector-borne viruses with major consequences on livestock trade and breeding. They are both transmitted by certain small hematophagous biting midge species belonging to the *Culicoides* genus, to cattle, sheep and goats. If the epidemiological situation in epizootic territories has been recently widely studied after massive outbreaks in Europe, endemic areas have been neglected. In this presentation, we will review three studies implemented between 2016-2018 in La Réunion Island to give better insights to the local situation: (i) a virological and serological survey on cattle to identify the two virus dynamics and prevalence, (ii) an entomological study on *Culicoides* species diversity and seasonal dynamics, and (iii) a virus screening in *Culicoides* species to identify and characterize vector species of both viruses, the level of infection in wild species populations and virus dynamics in *Culicoides* populations. Altogether, these three studies helped to have a better understanding of the epidemiological situations in the island. Five *Culicoides* species are described on the island, presenting an altitudinal gradient and 4 species are implicated in both virus transmissions. Seasonal dynamics is particularly marked for 3 species. Therefore, bluetongue might be considered as an endemic disease with continuous virus circulation, limited clinical cases and high serological prevalence while Epizootic hemorrhagic disease appeared as an epizootic disease with seasonal transmission patterns.

Experimental risk assessment for chikungunya virus transmission based on vector competence, distribution and temperature suitability in Europe, 2018

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Chikungunya virus (CHIKV) is a mosquito-borne Alphavirus (Togaviridae family), causing chronic arthralgia. The establishment of the Asian tiger mosquito *Aedes albopictus* in South and Central Europe and the regular import of CHIKV by infected travellers have resulted in at least 5 local outbreaks of CHIKV in France and Italy. The ongoing spread of *Ae. albopictus* to Central Europe and regular virus introductions to European countries, indicate the need of a comprehensive analyses of the Europe-wide spatial risk of CHIKV transmission. Therefore, this study aimed to construct European CHIKV risk maps, conducting vector competence studies with two *Ae. albopictus* populations (Germany, Italy) at three different temperatures (18°C, 21°C, 24°C) representative for the recent distribution of the mosquito species in Europe. Analyses of mosquito saliva for infectious virus particles demonstrated transmission rates of >35% independent of the *Ae. albopictus* population or temperature. The analysis of the temperature data of the current European vector distribution indicated, that most of these areas including the sites with suspected established German populations potentially allow CHIKV transmission, i.e. time periods with preceding 14 days having a mean daily temperature >= 18°C. Thus, the current risk of CHIKV transmission in Europe is not restricted by temperatures allowing extrinsic incubation of the virus, but rather by the vector distribution. Accordingly, all European countries with established populations of *Ae. albopictus* must implement respective entomological surveillance and monitoring systems, allowing suitable control measures.

Sindbis virus polyarthritis outbreak signaled by virus prevalence in the mosquito vectors

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Polyarthritis and rash caused by Sindbis virus (SINV), was first recognized in northern Europe about 50 years ago, and is known as Ockelbo disease in Sweden and Pogosta in Finland. This mosquito-borne virus occurs mainly in tropical and sub-tropical countries, and in northern Europe it is suggested to cause regularly reoccurring outbreaks. The seven-year cycle of SINV outbreaks in northern Europe has been commonly referred to in scientific journals, although the hypothesis is based solely on reported human cases. In the search for a more objective signal of outbreak risk, we evaluated mosquito abundance and SINV prevalence in vector mosquitoes from an endemic area in central Sweden. Vector mosquitoes collected in the River Dalälven floodplains during the years before, during, and after the hypothesized 2002 outbreak year were assayed for virus on cell culture. Obtained isolates were partially sequenced, and the nucleotide sequences analysed using neighbour joining and median joining network analysis. Only one SINV strain was recovered in 2001, and 4 strains in 2003, while 15 strains were recovered in 2002 with significantly increased infection rates in the bridge-vectors, and also increased in the enzootic vectors. Late summer infection rates were 12.1/1000 in the enzootic vectors *Culex torrentium/pipiens*, and 0.7/1000 in the bridge-vector *Aedes cinereus*. Sequence analysis showed that all isolates belonged to the SINV genotype I and that they had a single clonal origin. The genetic analysis revealed a local maintenance of SINV in the River Dalälven floodplains over the years, with a single introduction responsible for the current endemic situation. Our findings suggest that increased SINV prevalence in vector mosquitoes constitutes the most valuable outbreak markers for further scrutinizing the suggested seven-year cycle of Ockelbo/Pogosta outbreaks and the mechanisms behind.

Spread and persistance of Japanese encephalitis virus in experimentally infected pigs

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Pigs are important amplification hosts of Japanese encephalitis virus (JEV), a mosquito-borne zoonotic flavivirus that is currently endemic in Asia. Little is however known about the (neuro) pathogenesis of JEV in this host. We addressed this knowledge gap via an in vivo infection experiment since recent studies reported vector-free JEV transmission between pigs and vector competence of European *C. pipiens* mosquitoes for JEV, indicating that a JEV introduction in Europe could have more serious consequences than previously anticipated. 9-week-old pigs were inoculated intradermal via a needle with 105 TCID50 of JEV genotype 3 Nakayama strain. On day 0, 1, 2, 3, 5, 7, 14 and 21 post infection (pi), three pigs were euthanized and blood and tissue samples collected to study the pathogenesis in detail. No clinical signs were observed during the experiment. A short viremia was detected by qRT-PCR between 3 and 5 days pi. Unexpectedly, all tested brain tissues (cerebrum, cerebellum, brain stem, thalamus, olfactory bulb and trigeminal ganglion) were found positive at 3 dpi and low levels of JEV RNA (copy number ≈ 102 TCID50/g) remained detectable in all neurological tissues, except for trigeminal ganglia, till 21 dpi. Higher viral loads were observed in lymphoid tissues like spleen (positive between 3 and 14 dpi; copy number ≈ 103 TCID50/g) and prescapular lymph nodes (positive between 2 and 21 dpi; copy number ≈ 104 TCID50/g). Remarkably, even higher viral loads (copy number ≈ 106 TCID50/g) were found in the tonsils till the end of the experiment at 21 dpi. Ongoing virus isolations will shed light on the infectiousness of the persisting viral RNA in the different tissues. These findings indicate a generalized virus entry in the brain between 2 and 3 dpi and advocate for an in depth study of the role of lymphoid tissues in JEV persistence and spread.

Human practices promote presence and abundance of disease transmitting mosquito species

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Although widely acknowledged that large scale mosquito population dynamics are driven by climatic conditions (temperature, precipitation), there is a growing awareness that mosquitoes inhabit complex ecosystems that can be strongly affected by local human-induced landscape changes. Understanding how mosquitoes communities respond to such changes is essential to assess the consequences of local development for disease spread or vector control strategies. Here, we evaluated whether and how 1) human induced landscape changes induce shifts in communities of mosquito species and 2) how these differ between disease vectors and non-disease vector species. To this end, we sampled a 300 km transect on the border of KNP in South Africa to compare mosquito communities within the park to those in the rural areas outside the park. we show that, at paired sites inside and outside the park with very similar climatic conditions, human induced changes in the landscape are associated with a consistent shift in the community composition of mosquitoes. There was a clear and consistent pattern in the shifts in community composition, with disease vectors species responsible for >75% of the observed shift at all locations. Moreover, the total mosquito abundance increased on average threefold across a wide range of habitat types. These shifts were associated with an increased level of anthropogenic stressors in the landscape including an increase in nutrient and pesticide pollution of local water bodies, an increase in ungulate biomass, a decrease in vegetation biomass and an increase in human population density. This study shows significant changes in mosquito communities associated with human-induced landscape change, even between nearby sites with similar climatic conditions. Our results thus underline the importance of anthropogenic stress as a key driver of mosquito communities and abundance, and emphasize the importance of local landscape-level processes in shaping the composition of disease and non-disease vectors.

Sentinel surveillance through travelers

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(Re-) emerging viruses transmitted by mosquitoes are increasingly threatening human and animal health and the number of travellers returning to Europe, infected with arboviruses (arthropod-borne viruses) such as dengue, chikungunya and Zika, is increasing. Within the ZikaPlan research initiative, an arbovirus surveillance system using travellers as sentinels has been launched. Sugar-baited nucleic acid preserving Flinders Technology Associates (FTA) cards kits have been developed for the collection of mosquito saliva and given to travellers travelling to Brazil, a country with a high prevalence of arbovirusus. The mosquitoes, while sucking the sugar solution, expectorate saliva onto the preserving filter paper that is eluted and sequenced in order to identify potential mosquito-borne arbovirus. The pocket-size mosquito saliva kits are linked to a smartphone app developed to document arboviral infections related symptoms in travellers. Data collected by the smartphone app are synchronized with data from the corresponding mosquito saliva kits, in order to associate symptoms of potential arboviral infections with the presence of arboviruses in the mosquito populations at the travel destination. In the presentation we will report preliminary data from the evaluation of the mosquito saliva kit and discuss how this new combined tool may be implemented as an early warning system for the surveillance of arboviruses.

Toward social-behavioral/ecological informed responses against Lyme disease in Canada

C. Bouchard, C. Aenishaenslin, E.E Rees, S. Kotchi, Y. Pelcat, F. Milord, C. Russel, C. Jardine, J.K. Koffi, L.R. Lindsay, P.A. Leighton and N.H. Ogden

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The risk of contracting Lyme disease (LD) varies greatly due to spatial heterogeneity in ecological risk factors and risk-associated behaviors. LD risk is expected to be higher in areas where ecological characteristics favour a higher density of infected ticks and when human behavior enable transmission of LD. Few studies have jointly addressed the human social and behavioral risk factors and have tried to quantify their relative importance, which is representing a critical gap. Here we present the geographic hotspots of LD risk identified by a social-behavioral/ecological risk assessment in order to provide 1) useful targets for geographic prioritization of public health interventions to address the areas and populations at greatest risk and 2) an assessment tool to examine the relative contribution of different types of risk factors in relation to emerging hotspots for human LD cases. Our study focuses on two provinces where LD is an endemic disease (Quebec and Ontario). LD knowledge and behaviors in the population were measured using web survey data collected in 2014. These data were used as a proxy for the social-behavioral component ofrisk. For the ecological risk analysis, we developed a tick abundance model by integrating data from ongoing long-term tick surveillance programs and high-resolution data derived from Eatih Observation. Social-behavioral and ecological components of risk were combined with human population density to create integrated risk maps. Map predictions were validated by testing the association between high-risk areas and the current spatial distribution of human LD cases. This study highlights a new area of research on the development and validation of integrated social/behavioral-ecological informed response. Our approach is widely applicable to other vectorborne or zoonotic diseases and to different epidemiological context where the geographic patterns of risk are driven by the interplay between social-behavioral and ecological factors.

Quality control measures in the management of *Aedes albopictus*

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Quality controls measures might be conveniently adopted in mosquito control programs to evaluate the performances of control operations. In the case of *Aedes albopictus* management program developed in the Emilia-Romagna region, Italy, three quality controls procedures have been developed and currently applied. 1) The quantitative regional monitoring *Aedes albopictus* network conducted by ovitraps produces biweekly data during the summer, which are submitted to a four steps validation procedure before being stored in the database; 2) in the Emilia-Romagna region about 1.3 million public drains are regularly treated by larvicide in the period from April to September. A protocol has been developed to check the efficacy of these treatments; 3) in case of Zika, Dengue and Chikungunya imported human case notifications the regional protocol requires the immediate adoption of mosquito control treatments to prevent the start of endemic transmission. Following the conduction of this emergency treatments, quality control inspections are performed to evaluate their efficacy in mosquito suppression. The three quality control operations must be performed by skilled medical entomologists without conflict of interest with the entities charged of mosquito monitoring or control. Results and critical issues raised during the application of quality control procedures will be presented and discussed.

Dispersal and control of *Aedes albopictus* in Southwest Germany

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The Asian tiger mosquito *Aedes albopictus* has undergone a dramatic expansion of its range during last years in Southwest Germany. Since its first detection in 2007 in Germany at the motorway A5 coming from Italy via Switzerland to Germany, it has been continuously introduced by vehicles and trains, most probably from Italy. Since 2014 up to date established populations have been recorded in Freiburg (2014), Heidelberg (2015), Sinsheim (2016), Lörrach and Karlsruhe (2017). Without control the container indices ranged between 20 and >30%. After the first detection of the mass developments of *Ae. albopictus* immediate and comprehensive control measures were initiated to reduce or even eliminate the *Aedes* population. Citizen awareness was increased by providing thorough information about the biology and control of *Ae. albopictus* via various tools. Beside environmental management (elimination and modification of breeding sites), during door-to-door inspections on a bi-weekly interval all accessible breeding sites have been successfully treated with Bti - resulting in a significant reduction to almost elimination of the *Aedes* populations. However, due to cryptic breeding sites and non-cooperative citizens a certain percentage of the *Aedes* population survived the actions. Therefore, in some areas sterile males (SIT) which are provided by Drs Romeo Bellini and Arianna Puggioli, CAA, Crevalcore, are released on a weekly basis to support effectively the ongoing control activities. The results of 2018 will be discussed in the presentation.

Symposium 9: Invasive vector species - Surveillance & Management Strategies I

Private expenditures for self-protection against *Aedes albopictus* in Emilia-Romagna, Italy

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While some studies on the costs supported by public administrations for *Ae. albopictus* control activities already exist, much less information is available about expenditure of private citizens. Within the framework of the Life Conops project (www.conops.gr), a telephone survey was conducted on the Emilia-Romagna region households, with the aim of collecting information about: the level of nuisance caused by this mosquito species, the health concern of population, the measures adopted by households for self-protection and the related expenditure. From a sample of about 2,500 households, we obtained 412 valid questionnaires from the territory covered by eight health districts/sub-districts. The mean expenditure resulted about 48 per household (SD 68.97), while the median cost resulted about 26 per household, which allows an estimation of the total private expenditure for protection against the mosquito in Emilia-Romagna. The study investigated the influence on household expenditures from factors such as type and location of dwelling houses, number of household members and their level of education, the presence of children, etc.

Pilot validation of mosquito nuisance assessment by ZanzaMapp, a mobile application to involve citizen in mosquito monitoring.

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The global spread of invasive tropical mosquito species such as *Aedes albopictus* has expanded the risk of transmission of arbovirus (e.g. dengue and chikungunya) to previously unaffected European regions, as highlighted by the ~500 chikungunya cases reported from Italy in 2017. Wide-scale monitoring of mosquito nuisance and of risk of arbovirus transmission is difficult to implement and current monitoring schemes are limited by lack of cost-effective tools and adequate budget. To overcome these limits we developed a mobile application that allows users to report geo-localized mosquito presence by filling in a short questionnaire. In 2016 breeding seasons, we gathered >24.000 records, which represent a very encouraging feature, giving the limited effort invested in publicizing the app.

We here present results from a pilot small-scale study carried out in Procida island (Naples) to validate this novel monitoring approach by comparing data obtained from citizen via Zanzamapp with data obtained by expert entomologists via Human-Landing Catches (HLCs).

Zanzamapp data collection and daily-HLC were carried in a single week in September 2016. The relationship between the two sets of data was analysed in different spatio-temporal windows (i.e. Zanzamapp data were compared to mosquito collections at different intervals before HLC and in different buffers around HLC sites).

Results showed a positive relationship between the weighted mean of Zanzamapp records and HLC data. The strongest correlation was found when considering Zanzamapp records obtained within 3-days from HLC and in a 100-m buffer from the HLC-site. These results support the possibility to obtain valuable information by a citizen science approach and encourage further studies to optimize the analytical approach needed to exploit Zanzamapp records for production of "real-time mosquito nuisance maps" which could complement and reinforce traditional monitoring schemes and could be applied beyond regional and national borders.

The dispersal of *Aedes albopictus* females in temperate areas and its relevance in the containment of exotic arbovirus outbreaks

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The Chikungunya outbreak occurred in 2017 in central and southern Italy, with about 500 human cases, raises the need to understand the potential dynamics of transmission and spread of exotic arboviruses (such as Dengue, Chikungunya and Zika) in temperate regions stably colonized by *Aedes albopictus*, as well as to optimize the mosquito adult control interventions in the presence of human viremic cases. We here present the results of three mark-release-recapture experiments conducted in a peri-urban area of North-East Italy, with a view to estimate the *Ae. albopictus* female flight range associated to the search of oviposition sites and provide evidence-based suggestions to implement the control treatments in case of autochthonous transmissions of exotic arbovirus. In particular, 90% of marked *Ae. albopictus* females were recaptured within 250 m from the release site, distance which some mosquitoes may reach in less than 3 days. In fact, the results from our modelling showed that the female dispersal can be so rapid that insecticide sprayings within a 200 m radius around a potential viremic case could fail to target the 10% of the mosquito population Therefore, even if insecticide treatments are carried out after only 2-3 days since the arrival of an infected person, the probability that a potentially infected mosquito escapes from the treatment area is not null. This suggests insecticide treatments aimed to prevent risk of autochthonous outbreaks of exotic arboviruses in temperate regions should be carried out in a buffer of at least 250 m, even if they are promptly accomplished. Indeed, if insecticide sprayings are carried out with a longer delay since symptom onset, a larger buffer should be more appropriate to effectively targeting the large majority of potentially infected mosquitoes and thus successfully reducing the risk of further autochthonous cases.

Swiss Reference Network for invasive mosquitoes

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In Switzerland surveys on invasive mosquito species started in the south most region bordering with Italy, where the first *Aedes albopictus* were found. North of the Alps Ae. japonicus was first discovered in 2008. *Ae. koreicus* appeared in the South part in 2012 and since 2013 all the three invasive *Aedes* mosquitoes are regularly found with surveys along the motorway. In fact these mosquitoes are spreading through Switzerland: *Ae. albopictus* is moving northwards, *Ae. japonicus* Southwards and *Ae. koreicus* appears mostly in the southern part of the country but could be found North of the Alps along the motorway. Over a decade the federal government is supporting different surveys on invasive mosquito and a specific national guide line on survey and control methods in order to help regions newly touched by the thematic. In 2017 it was settled the base for a national reference network (NRN) on invasive mosquito to coordinate surveillance systems and to help Cantons in the control measures to be taken (according to the released ordinances Art. 51 and Art. 52). To the NRN belongs the National Invasive Mosquito Competence Network: a higher-level organizational unit comprising the National Survey Program on Invasive Mosquito, four Regional Reporting Centers and the National Coordination Center for the Survey and Control of Invasive Mosquitoes and. The National Survey program on Invasive Mosquito surveys IM on the main traffic routes. The Regional Reporting Centers receive notifications from the population of their region and respond to relevant inquiries, triage the messages according to probability and forward them to the Coordination Center. The Coordination Center, based in Canton Ticino, where the very first survey on IM started in 2000, collects and validates all the data concerning invasive mosquito, standardizes all the methods and procedures to be taken on IM in Switzerland, coordinates the Regional Reporting Centers, advises interested authorities, develops and disseminates information material on the topic, and validates all the data before forwarding to the National Center of Faunistic Mapping.

Pathways for introduction and dispersal of invasive mosquito species and risk-based surveillance and control: an integrated approach

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In the last decades, invasive mosquito species (IMS) originating from (sub)tropical regions have arrived to Europe, introduced via different pathways. The identification of pathways for introduction of IMS is critical to decide on surveillance strategies needed to reduce the risk of future introductions and/or potential outbreaks of mosquito-borne diseases. This presentation reviews the state-of-the-art in our knowledge of pathways associated with the introduction and dispersal of IMS in Europe. Four main routes for introduction and dispersal of IMS have been identified: the trade on used tires, the import of Lucky bamboo plants from Asia, the passive transport of IMS in vehicles (traffic by road, airplanes, and sea), and the natural dispersal of IMS. It is concluded that the trade on used tires remains the main pathway for long distance transportation of IMS into and within Europe, and passive transport in ground vehicles represents the main threat for dispersion from already established IMS populations. In order to detect introductions and avoid possible proliferation of IMS, a risk-based and adaptive surveillance has been implemented in The Netherlands in: used tire companies, lucky bamboo greenhouses, airports, harbours, and flower auctions. Results show that the surveillance strategy yielded early detection of introductions of IMS and allowed promptly mosquito control actions, preventing the possible establishment. However, new unexpected *Ae. albopictus* introductions, notified by citizens, occurred outside our risk categorized locations in the surveillance, gave a chance to this invasive species to proliferate. Fortunately, these incursions have been contained. Prompt communication on the presence of IMS to the authorities is crucial because of the possible increase of these introductions facilitated by the ability of IMS to travel with human passive transport (e.g. cars, trucks, trains), and the presence of established populations of IMS in neighbouring countries.

Estimating mosquito population abundance from standard monitoring devices

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Estimates from standard monitoring devices inform public health authorities about the relative abundance of vector species, a critical parameter to guide control interventions. At present, the estimation of the absolute abundance of a vector species is challenging and relies mostly on laborious methods such as Mark-Release-Recapture. However, estimates of absolute abundance are needed to estimate and quantitatively characterize the risk of pathogen transmission from vectors to hosts. In this study we discuss the potential use of the N-mixture model statistical approach to the estimation of population abundance of vector species, which may not otherwise be estimated by Mark-Release-Recapture methods due to ethical concerns arising from the potential exposure of people to health risks deriving by the field release of disease vectors. Therefore, we applied the N-mixture model to a case study in Rome (Lazio Region, Italy) where monitoring data on the mosquito vector *Aedes albopictus* (Tiger mosquito) are routinely collected. The invasive species *Ae. albopictus* has been the vector for a recent outbreaks of Chikungunya in the Lazio Region in the 2017 and estimates of the species abundance are essential to avoid future outbreaks. Results show that the N-mixture model, when properly informed by prior knowledge on the species biology and trapping methods, could be put in use to estimate vectors population abundance. Therefore, even if ethically questionable methods (when pathogen transmission risk occurs) such as Mark-Release-Recapture still are the golden standard for estimating population abundance, the N-Mixture model approach could be exploited to provide an assessment of vectors population abundance when competing methods are not feasible.

Automatic identification of the species and sex of captured mosquitoes in an advanced, remotely operated mosquito trapping system: technical background and results from the laboratory and field

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The current state of the art of remote-controlled, automatic mosquito traps (BG-Counter /w BG-Sentinel) uses a light barrier to detect and identify the catch. The wingbeat of insects pulled by the trap s air stream through this (essentially two-dimensional) light barrier yields short, but typical patterns that can be used to distinguish mosquitoes from other insects. However, the data set produced by this set-up is insufficient for species identification. Automatic traps are therefore typically used for real-time measurement of the population sizes and activity times of nuisance mosquitoes in their typical environment, to determine the best point in time for control efforts, and to monitor their success. For the advanced automatic surveillance and monitoring of mosquitoes of special interest (such as invasive species and/or specific disease vector), we designed a new type of optical system, the heart of which is a three-dimensional opto-electronic senor field. Mosquitoes passing this array produce data sets that are detailed enough for a precise determination of wingbeat patterns and other characteristics. In addition, the system's machine learning capabilities can be trained to further identify and count mosquitoes from groups of special interest. These could not only be different species and their sexes, but also marked individuals, age groups, etc. We present different use case, such as the separation of simultaneously present species (*Cx. tarsalis*, *Cx. pipiens* and *An. freeborni*), and the differentiation of female and male *Ae. albopictus* and *Ae. aegypti*, respectively. We also trained the system with ca. 280.000 recordings obtained from 6 mosquito species (*Ae. albopoictus*, *Ae. aegypti*, *An. gambiae*, *An. arabiensis*, *Cx. pipiens*, *Cx. quinquefasciatus*) and were able to distinguish even closely related species in this matrix with accuracies from 85 to more than 95%. (The work presented here was being supported by the European Union's Horizon 2020 programme; grant 691131, project REMOSIS).

Coordinated regional strategy for invasive mosquito surveillance in Southeast Spain

P. Sanchez-Lopez

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In response to the chikungunya and Zika outbreaks that spread through the American continent for the first time from 2013 to 2016, and the presence of a competent vector, the Asian tiger mosquito (*Aedes albopictus*) in large parts of the country, the Spanish Ministry of Health launched in 2016 a first Preparedness and Response National Plan against Dengue, Chikungunya and Zika. In the Autonomous Region of Murcia, in the southeast of Spain, the tiger mosquito was first identified in 2011, and has spread through many municipalities since then. However, no coordinated effort had been implemented so far to study the species distribution in the whole region. In 2018, with the support of a national grant from the Spanish Ministry of Environment, through Fundación Biodiversidad, the Service of Environmental Health of the Regional Health Council implemented the first Regional Tiger Mosquito Surveillance System (RTMSS). This system consists of a new central Laboratory of Vectors located at the Service of Microbiology and Parasitology facilities, of the Hospital Clínico Universitario Virgen de la Arrixaca in the city of Murcia, and an informatics tool called VecMap, used to design a sampling strategy based on ovitraps, to communicate with the municipalities of the region through a mobile application, and to produce distribution maps. The RTMSS involved all the 45 municipalities of the Murcia Region, and supplied 538 ovitraps to the city councils, who collect and deliver them to the Laboratory of Vectors on a biweekly basis. The RTMSS gives feedback reports to the municipalities, containing maps and pest management recomendations. Additionally, the RTMSS has also collected adult mosquitoes for arbovirus surveillance. Within the first months of samplings 24 out of the 45 municipalities resulted possitive to *Ae. albopictus* eggs, being this the first comprehensive regional effort in mosquito and arbovirus surveillance in the Murcia Region.

Update in the geographical distribution of the invasive tick *Hyalomma marginatum* in South of France: first attempts to identify factors favoring its establishment

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Hyalomma marginatum, the main tick vector of Crimean-Congo Hemorrhagic Fever virus (CCHFv) in the Mediterranean Basin, has been reported for several decades in Corsica Island. However, its establishment in continental France is much more recent. Viable tick populations were observed for the first time in 2015; previous mentions only concerned isolated specimens that could have been likely introduced through bird migrations or terrestrial ungulate translocations. In order to investigate the current geographical distribution of this tick species in south of France, two sampling campaigns were conducted at spring in 2017 and 2018. More than 80 horse structures were visited, since recent study conducted in Corsica demonstrated that horses are the most likely hosts for adult parasitic stages *of H. marginatum* and constitute good sentinels to detect its presence. We targeted structures with shrubby and grassy pastures for horses as they are considered suitable habitats for free-living stages of *H. marginatum*. During visits, the sampling pastures and the surrounding natural habitats were described and animal owners were questioned concerning their tick control practices, as well as animal movements as possible pathway for tick introduction. Combined with climatic conditions (at least temperature and humidity), all these parameters were considered as explanatory variables for modelling the occurrence and relative abundance of *H. marginatum*. Against all expectations, *H. marginatum* was detected among the entire Mediterranean region from the Pyrénées-Orientales (Spain border) to Var at the east. Local heterogeneity was highlighted with main presence hotspots around Perpignan, Montpellier, and Hyères, where the tick was fully absent from Camargue and Plain of Crau. Modelling analyses are under progress and results will be presented at the E-sove conference. As *H. marginatum* may have not filled yet its entire ecological niche, possible approaches to monitor this invasion process will be discussed.

Symposium 10: Invasive vector species - Surveillance & Management Strategies II

The tiger mosquito told to the children

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Education of citizens is paramount as part of an integrated mosquito control. It is usually carried out by public administrations through disinfestation plans in public areas. However, it is known that in urban areas most mosquito breeding sites are located in private areas, in particular in medium-sized villages characterized by houses with backyards. Therefore, a community-based approach is recommended to reduce/eliminate the breeding sites in private areas. For this reason, an information campaign addressed to kids has been carried out by several municipalities in the province of Venice. A training activity has been held during five kids summer camps (more than one hundred children participating). The entomologists taught them on mosquito biology, monitoring and how to get rid of mosquitoes from their backyards, focusing on *Aedes albopictus*. A game (mosquito hunting) was conducted and live insect pets were shown to them. Finally, summary brochures were provided to the kids. A footage of the activity has been filmed and it is available on the IZSV e website and on the social networks and herein presented. The activity has been successful and other municipalities have requested to propose the event again. The alliance with children can be an effective way to spread in families the knowledge of mosquito control.

Symposium 10: Invasive vector species - Surveillance & Management Strategies II

Optimization of odour-baited trapping systems for the surveillance & control of *Aedes aegypti* in Paramaribo, Suriname

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Our aim was to test odour-baited traps as an alternative method for mosquito surveillance and control. Blends of synthetic chemical attractants, derived from human skin emanations, can be used to attract host-seeking female mosquitoes. These blends gave rise to the development of odour-baited traps for mass mosquito trapping and control. Although tested to reduce malaria in Africa, these odour-baited traps might also offer a solution to diseases like dengue, chikungunya and Zika, which are amongst others prevalent South American countries, including Suriname. For our study, we ran three Latin-square trials of BG traps of different design and in different configurations (with/without odour bait and with/without CO_2) across eight different locations in urban Paramaribo.

This study demonstrates the effectiveness of the BG-Sentinel when baited with a combination of CO_2 and the MB5 blend for trapping *Ae. aegypti* females, and surprisingly also males. The MB5 blend outperformed the commercially available BG-Lure in the BG-Sentinel. Although to a lesser extent than the latter trap type, the BG-Bowl also attracted *Ae. aegypti* when baited with either the MB5 blend or the BG-Lure. Moreover, the results show that CO_2, in our case provided by sugar-yeast fermentation, is an indispensable component of the attractive blend. The next step would be to focus on research that makes these traps feasible for usage in mass-trapping systems by searching for CO_2 alternatives and evaluating the effect of trap placement and coverage.

Aedes aegypti in Europe: Past, Present and Future

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The global distribution of *Aedes* aegypti is the subject of considerable attention because of its pivotal role as a vector of several high profile diseases including dengue, yellow fever, Zika, chikungunya, and others. There is also a lot of interest in the projected future distribution of the species. Little effort has however been focused on its historical distribution, which has changed substantially over the past 100 years, especially in Southern Europe where it was once widespread, but largely disappeared in the middle of the 20th century. This work uses historical records of the vector distribution in southern Europe, the Near East and North Africa from the late nineteenth Century until the 1960s to construct a spatial distribution model using matching historical environmental and demographic data. The resulting model is then implemented using current climate and demographic data to assess the potential distribution of the vector in the present day. The models were rerun with several different assumptions about the thresholds that determine habitat suitability for *Ae. aegypti*. The historical model matches the historical distributions well. When applied to current covariates, the predicted present day distribution is somewhat broader than it used to be particularly in north-west France, North Africa and Turkey. Though it is beginning to reappear in the eastern Caucasus, this potential distribution clearly does not match the actual distribution of the insect, which suggests some other factor is responsible for its absence lack of breeding sites due to the more or less universal availability of piped water is often cited. The inferred distribution also doesn't match those projections for the coming decades based on models trained on the current distribution which predict little or no presence in the Mediterranean Region. At the same time, the vector is spreading fast in the USA, which also has widespread piped water, and is predicted to consolidate its range there in future. These contradictions and their implications for possible re-invasion of Europe are discussed.

ABSTRACTS

POSTER PRESENTATIONS

P1 Changes in seasonal dynamic of *Culicoides* in ovine farms in Sicily: effect of altitude, temperature and rainfall

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Culicoides (Diptera: Ceratopogonidae) represent a major threat for zootecnics due to their role as bluetongue virus vectors. Diffusion of *Culicoides* in the territory may be influenced by a range of environmental factors, making correlation studies of importance for vector control. During January-December 2016, catches were performed weekly in 3 farms at altitude 244, 417 and 485m above sea level (farm A, B, C) *via* Onderstepoort-type traps. *Culicoides* monthly median abundance were registered and correlation with Montly Mean Temperature and Rainfall (MMT – MMR) performed *via* Pearson coefficient ($\alpha=0.05$). Considering farm A, median abundance of *Culicoides* spp. increased from January to July (Median=579), *C. obsoletus* complex showed cyclic peaks at May, August, October and *C.newsteadi* was constantly present with a peak at May (Median= 160). At farm B, *Culicoides* spp. abundance was very low, although a peak in July (mainly *C. obsoletus* complex, median=19). *Culicoides* abundance was higher at farm C than B, with peaks of *C. newsteadi* at April (Median=27), June, September although low presence of *C. obsoletus* complex. Abundance of *Culicoides* spp. in farm A showed a significant positive correlation with MMT ($r=0.7$, $p<0.05$), with a tendency both for *C. obsoletus* and *C. newsteadi*, and negative correlation of *Culicoides* spp. and *C. newsteadi* with MMR ($r=-0.59$, $p<0.05$). In farm B significant negative correlation was maintained between *C. newsteadi* and MMR ($r=-0.61$, $p<0.05$), whilst in farm C positive correlation was present between *Culicoides* spp. and MMT ($r=0.61$, $p<0.05$), with tendency for *C. newsteadi*. Data obtained showed a reduced abundance of *Culicoides* at higher altitudes, as well as a main influence of temperature and rainfall on *Culicoides* diffusion, especially for the species *C. newsteadi*. Authors thank Mr. Pippo Bono, Mr. Nicola Galati and Mrs. Francesca Marino for technical collaboration. Funded by Italian Ministry of Health RC IZS SI 03/15 and 16/16.

Vector Ecology & Behavior category

A New *Culicoides* Surveillance Network in Mainland France

P2

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Following the re-emergence of the bluetongue virus serotype 8 (BTV8) in September 2015 and in accordance with the European Commission's regulatory requirements, a new entomological surveillance network has been set up throughout mainland France to monitor the activity of *Culicoides*, which are vectors of BTV. The new *Culicoides* surveillance network uses suction light traps that operate one night per week in one site per eco-climatic zone. Twenty-four eco-climatic zones were determined in mainland France by analyzing the data collected by the first surveillance network set up from 2009 to 2012 during the emergence of BTV8. Each eco-climatic zone, regrouping one or more French departments, is homogeneous, taking into account the diversity of *Culicoides* species, the duration of vector inactivity and the week of the beginning and the end of inactivity. This entomological surveillance active from November (Y0) to April (Y1) has made it possible to determine successfully periods without *Culicoides* vectors for the different eco-climatic zones defined on the continental territory. The information provided by this network, coupled with the surveillance of viral circulation, made it possible to report during winters 2015 to 2018 several French departments in BTV seasonally free zones, thus lifting restrictions on livestock movements. Research impact highlights: Following the re-emergence of BTV8 in September 2015, a new *Culicoides* surveillance network is operational throughout mainland France to determine the periods without vectors in each eco-climatic zone. The information provided by this network, coupled with viral circulation monitoring, allows BTV's seasonal free zones to be declared each winter, thus lifting restrictions on livestock movements.

P3 Exploring *Culicoides* species communities at continental scale: a unique collaborative approach

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Culicoides biting midges were responsible for continental scale epizootics of viruses, including several bluetongue virus (BTV) serotypes. The existence of epi-zones has been suggested to explain the geographical range of the spread of BTV serotypes. The structure of *Culicoides* populations may contribute to the definition of these epi-zones, whereas their movements may be involved in their connections. One of the objectives of the PALE-Blu project (Understanding pathogen, livestock, environment interactions involving bluetongue virus) is to determine if the composition of *Culicoides* species communities may be involved in the existence of these epi-zones. For this purpose, an extensive dataset of abundance data, focusing on the most common *Culicoides* species, was established. We used as long as possible longitudinal *Culicoides* follow-ups carried out with UV light-traps. The yearly maximum abundance (i.e. the largest single night trap catch in the collecting period) was used to aggregate data against time and to produce an index of the species abundance. If the collection period covers several years, then we used the average of the yearly maxima of abundance for *Culicoides obsoletus*/*Culicoides scoticus*, *Culicoides imicola*, *Culicoides chiopterus*, *Culicoides dewulfi*, *Culicoides pulicaris*/*Culicoides lupicaris, Culicoides punctatus, Culicoides newsteadi*, and *Culicoides kingi*. The gathered data may have been shared by National surveillance systems or have been produced by VectorNet (2014-2018), which is a joint initiative of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). This project supported the collection of data on vectors, related to both animal and human health, to map the presence/absence of vectors in Europe (in the geographical sense) and around the Mediterranean basin. The VectorNet data were obtained by a formal request to ECDC (reference number 18-1421). The final dataset contains a total of 639 sites from 27 countries (from Senegal to Scandinavia, and from Iceland to Turkey). The existence of this database was only possible by a collaborative approach, involving 29 partners. The purpose of this poster was to thank contributors and enhanced future collaborations at continental scale.

Vector Ecology & Behavior category

P4

Rickettsia species in Italy: an update on main vectors and clinical importance for public health

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Rickettsiosis in Italy is still a threat for public health, often due to a lack of proper awareness on the role of tick vectors, *Rickettsia* species and related diseases, leading to underestimation of the need for vector control strategies, misdiagnosis and clinical complications. The present work aimed to provide an overview on distribution of *Rickettsia* species in Italy, *via* the integration of data from humans, animals and vectors (ticks and fleas) collected by IZS SI, *via* molecular analysis targeting *ompA*, *ompB* and *gltA* genes, with bibliographic data. Apart from the detection of *R. conorii conorii*, the causative agent of Mediterranean Spotted Fever (MSF), in ticks of *Rhipicephalus* genera, main reports of other species within the *Rickettsia* Spotted Fever Group include *R. massiliae* (from ticks *Rh. sanguineus/Rh. turanicus*), *R. aeschlimannii* and *R. slovaca* (*Hyalomma* ticks), *R. israelensis* (from *Rh. sanguineus*), *R. helvetica* and *R. monacensis* (*Dermacentor marginatum, Ixodes ricinus*). Clinical manifestations associated to the *Rickettsia* infections in humans in Italy varies from classical MSF triadic symptoms (fever-exanthema-tache noire) to MSF-like symptoms with severe complications (i.e. multiple organ failure, hepatitis associated to *R. aeschlimannii* infection) and SENLAT (Scalp Eschars and Neck Lymphadenopathy) disease associated to *R. slovaca* infection. In addition, *R. felis* was detected in *Ctenocephalides felis* fleas and *Rh. turanicus*, with a possible risk for human transmission of murine typhus-like disease. The finding of other Rickettsia species including *R. hoogstralii* and Candidatus *R. barbarie*, with unreported pathogenicity, suggests the need for additional efforts for a proper diagnosis and identification of *Rickettsia* species in order to ensure preservation of public health.

Thanks to Francesca Marino, Pippo Bono. Funded by Italian Ministry of Health RC IZS SI 15/15.

P5 Hands on Course on Arthropods of Medical and Veterinary Significance: A global perspective, from theory to practice

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The Parasitology and Entomology team of the National Veterinary School of Toulouse, France, organizes an entomology course to be held next August 2019. The course will highlight topics such as arthropod-borne diseases, resistance in arthropod populations, control tools, principles of laboratory rearings, and morphological identification of arthropods of medical and veterinary importance. The course will encompass several arthropod groups, namely sand flies, mosquitoes, *Culicoides* spp., Tabanidae, Muscidae, fleas, bedbugs, ticks, Dermanyssidae and Sarcoptidae. Lectures will take place in the morning, while the afternoon will be dedicated to laboratory and field work. Target participants will be: postgraduate (MSc, PhD) students, Post-Doc, research fellows, entomologists, laboratory staff and residents of the European Veterinary Parasitology College. Key note-speakers will include Prof Richard Wall (University of Bristol, UK), Dr Anna-Bella Failloux (Institut Pasteur, Paris, France) and Prof Steve Torr (Liverpool School of Tropical Medicine, UK). The course will take place in Toulouse from the 26th to the 29th of August 2019.

Impact of weekly feeding frequency on the performance of *Glossina palpalis gambiensis* at the CIRDES laboratory, Burkina Faso

P6

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The International Center for Research and Development on Livestock Production in the Subhumid Zone (CIRDES), Bobo-Dioulasso, Burkina Faso possess the bigger mass rearing insectary of *Glossina palpalis gambiensis* Vanderplank, 1949 in West Africa. Flies are currently fed 6 times per week that leads to high cost. In the context of cost optimization of the mass rearing facility, we evaluated the impact of three blood-feeding frequencies on fly's performance. Three blood feeding frequencies were tested: 3 blood meals per week (F3), 4 blood meals per week (F4) and 6 blood meals per week (F6, control) with fresh bovine blood collected at the slaughterhouse of Bobo-Dioulasso. The performance parameters evaluated were: tsetse fly survival, female productivity, pupal quality defined as the pupae weight hatching rate and flight performance of teneral flies. These parameters are combined in a formula that calculates a quality factor, a proxy to evaluate the colony performance. For each frequency, three cages of 50 flies were studied and it was replicated 4 times. Tsetse flies survival was significantly better for F4 than F3 and F6. Female's productivity and pupal quality were similar between F4 and F6 and significantly better than F3. The quality factor was similar between F4 and F6 and both higher than F3. The results showed that the feeding frequency of 4 days per week could be adopted for tsetse feeding at CIRDES without an impact on the colony performance while limiting production costs.

P7 Distribution and blood feeding preferences of *Culex pipiens* complex mosquitoes in areas of North Greece with a recent history of WNV transmission

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Culex pipiens sensu lato species are considered the most widespread mosquitoes in temperate latitudes. They are of great medical importance in Europe, since they constitute the principal vectors of several arboviruses, such as the West Nile Virus (WNV). *Cx. pipiens* sensu lato comprises of 2 morphologically indistinguishable species, namely *Cx. quinquefasciatus* and *Cx. pipiens*, with the latter being further subdivided into two biotypes, *pipiens* and *molestus* which can form hybrids. Despite their morphological similarity, the members of the *Cx. pipiens* complex display distinct behavioral profiles. More specifically, regarding the host preference of the two biotypes, the pipiens form has been characterized as ornithophilic, whereas the molestus form as mammophilic. Due to its preference for bird hosts, the pipiens form is known to play an important role in the enzootic transmission cycle of WNV. Further more investigating the feeding patterns of the hybrid forms is of high importance as they may display an intermediate host preference which makes them ideal bridge vectors of WNV from birds to mammals. While the two biotypes are found in distinct habitats in several regions, in Southern Europe they occur sympatrically. In the present study we investigated the distribution and seasonality of *Culex* mosquitoes with a focus on *Cx. pipiens* complex collected from different environments (urban, agricultural, natural wetlands) of Northern Greece with a history of WNV transmission. Their blood feeding preferences were also investigated. On account of their indistinguishable morphological features, species identification was conducted based on PCR diagnostic assays targeting on polymorphisms of the second intron of the acetylcholinesterase-2 locus of *Cx. quinquefasciatus* versus *Cx. pipiens* and of a specific microsatellite region for the two biotypes. For the blood meal analysis a combination of different molecular methods were applied in order to reliably identify the host source.

Defining ecological regions for vector-borne diseases in Italy **P8**

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Vector-borne diseases (VBD) are a significant human and animal health concern. Their spread is largely constrained by their vectors habits, which are heavily influenced by climate and environment. In this paper a map of the eco-climatic regions of Italy was developed adopting a data-driven spatial clustering approach using recent and detailed spatial data on climatic and environmental factors. Seven variables, relevant for a broad set of human and animal VBDs were selected: the standard deviation of altitude, the mean daytime land surface temperature, the mean amplitude and the peak timing of the annual cycle of land surface temperature, the mean and amplitude of the annual cycle of the greenness value, and the daily mean amount of rainfall. Principal Component Analysis followed by multivariate geographic clustering using the k-medoids technique were used to group the pixels with similar characteristics into different ecoregions, and at different spatial resolutions (250 m, 1 km and 2 km). The resulting maps highlight regions in which vectors and VBDs are expected to similarly spread or persist and they show that the spatial structure is generally maintained at different spatial resolutions. The potential applications of the ecoregion map with Bluetongue vectors and West Nile Disease (WND) outbreaks in Italy was investigated. *Culicoides imicola* was found to prefer two out of 22 ecoregions, one of which was also preferred by *C. newsteadi*. *Culicoides obsoletus/scoticus* occupy all sampled ecoregions, according to its known widespread distribution across the peninsula. WND outbreak locations strongly cluster in 4 ecoregions, dominated by human influenced landscape, with intense cultivations and complex irrigation network. This approach could be a supportive tool in case of VBDs, defining pixel-based areas that are conducive environment for VBD spread, indicating where surveillance and prevention measures could be prioritized in Italy.

P9 The influence of seasonal variation on geometric morphometrics of *Glossina brevipalpis* Newstead and *Glossina austeni* Newstead from South Africa

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Geometric morphometrics represents a simple and economic tool that can be used in any laboratory to study tsetse population structure. Since the correlation between morphometrics and genetic analysis is only 0.5, genetic analysis is considered more reliable than morphometric analysis. The discrepancy between the two techniques can be ascribe to the potential influence of environmental factors on morphometric analysis results. The potential influence of seasonal variation on geometric morphometrics analysis was evaluated. *Glossina brevipalis* and *Glossina austeni* were collected on a monthly basis over a year at three sites with high tsetse abundance but with dissimilar climatic conditions in north-eastern KwaZulu-Natal, South Africa. The right wings of males and female flies were removed and nine landmarks (Cartesian coordinates) were defined by vein intersections using the COO program of the CLIC software package. All subsequent analyses on the Cartesian coordinates were done using the MorphoJ integrated software package. Seasonal variation and sexual dimorphism in centroid size and shape of the two species were observed at all three sites. For both species, the variation in centroid size between localities was similar to the seasonal variation in centroid size at each of the sites. The multivariate regression of the first relative warp against centroid size was significant in most of the analyses. This indicated an allometric effect that can be ascribed to seasonal influences. It is evident that environmental factors such as humidity and temperature can influence both shape and size of *G. brevipalpis* and *G. austeni* to a certain extend. These results confirmed that the potential influence of environmental factors need to be taken in consideration when geometric morphometrics is selected as a tool to study tsetse population structure.

Vector Ecology & Behavior category

Resting population of mosquitoes in Belgrade

P10

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The Institute for Biocides and Medical Ecology conducts surveillance and control of mosquito population on the territory of Belgrade, the capital of Republic of Serbia. Mosquitoes survive winter in different stages of development. *Culex pipiens* is the most numerous species in Belgrade and it rests during the winter in the form of fertilized females. During January and February 2018, the Institute received numerous calls from citizens who complained about the presence of mosquitoes in their apartments. High number of mosquitoes was detected through monitoring in spaces below the buildings, from where they entered the apartments. Two treatments were carried out with a two week interval in between. Larvicide treatment was carried out with piriproxifen and adulticide treatment with etofenprox and deltametrin. After the second treatment, the problem was solved in the settlement that has 24 buildings. Monitoring shows no more mosquitoes in spaces below the buildings. Similar procedures were applied in other parts of the city where the same problem was observed.

P11

Phlebotomine sand flies of Peloponnese Peninsula

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Phlebotomine sand flies (Diptera: Psychodidae) are important vectors of leishmaniases in Mediterranean countries including Greece. However, data about their presence, species composition, feeding preferences and role in *Leishmania* transmission in regions of Peloponnese Peninsula are scarce. To fill this gap of knowledge, entomological survey was conducted at 32 localities in regions of Arcadia, Elis, Laconia and Messenia. Using CDC light traps, 1982 specimens were collected and identified by combination of morphological and molecular approaches (MALDI-TOF protein profiling, DNA barcoding, multiplex PCR and PCR-RFLP). Nine species were recorded: 7 of the genus *Phlebotomus* (*P. killicki*, *P. neglectus*, *P. papatasi*, *P. perfiliewi*, *P. simici*, *P. tobbi*, *Paraphlebotomus* sp.) and 2 of the genus *Sergentomyia* (*S. dentata*, *S. minuta*). *Phlebotomus neglectus* was the most abundant species, present at all surveyed localities, followed by *P. perfiliewi* and *P. tobbi*. *Sergentomyia minuta* was rare at most localities while it was a dominant species in Elis and occurred sympatrically with *S. dentata*. The finding of *P. killicki* is a first record of this species in Greece mainland and outside Crete where originally described. *Paraphlebotomus* specimens showed intermediate morphological features between *P. sergenti* and *P. similis*, provided unique protein profiles by MALDI-TOF typing and distinctive sequences of ITS2, cytB and COI gene markers, suggesting existence of a cryptic species within the *P. sergenti* complex.

To understand their feeding preferences, bloodmeals of engorged females were identified by sequencing of vertebrate-specific segment of mitochondrial cytochrome b gene as well as a novel approach of peptide mapping, showing affinity to feed on sheep, goats and chickens. In addition, unfed females of 3 *Larroussius* species (*P. neglectus*, *P. perfiliewi, P. tobbi*), *P. simici* and *S. minuta* were pooled and tested for presence of *Leishmania* by PCR amplification ITS-1 region. All 115 females (23 pools, 5 females per pool) were found negative.

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Ixodid tick bite incidence on humans in Eastern Spain

P12

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Climate change and movement of people, animals and goods create conditions that favor the rapid expansion of arthropods that can act as vectors and cause zoonoses. Ticks are currently considered to be, second only to mosquitoes, one of the most important groups as vectors of human infectious diseases in the world. An unusual increase in the number of people treated because of tick bites in health centers in the province of Castellón (Valencian Community, Spain) was detected in the year 2017. This trend has continued throughout the year 2018. First data obtained from the incidence of ticks as well as the characteristics of the cases in the eastern part of Spain are presented and discussed. The identification of ixodid ticks detected in medical assistance centers from the central territory of Castellón has resulted in three species: *Dermacentor marginatus*, feed on birds and small mammals and has affinity for humans; *Hyalomma lusitanicum*, feed on rodents, rabbits and wild carnivores or herbivores and has affinity for humans; and *Rhipicephalus sanguineus*, feed on dogs but has low affinity for humans. Of these, the most abundant species has been *H. lusitanicum*, with a rate of 85% of reported cases. The estimation of the incidence of tick bites attended in the emergency services of a regional health center is provided. The overall cumulative incidence is 2-3 cases per 1000 inhabitants in the region. The medical information is completed with an epidemiological survey in which recorded data are related to the sex and age of the person assisted, number of bites, anatomical place where the tick was removed, symptoms and signs due to the bite, and potential contact of people with animals.

P13 Review on sheep ticks in Tunisia

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In Tunisia, the population of sheep exceeded 7 millions, they are distributed all over the country with important mobility in North – South and West – East axis but also random movements. This population consists of several sheep breeds (mainly Barbarine, Queue Fine de l'Ouest, Thibar) that have a documented difference in sensitivity to ticks. Different tick species infesting sheep, their seasonal activity and the pathogens they potentially transmit in Tunisia are presented. The authors begin by presenting the general context in Tunisia (geographic, climatic and breeding characteristics) then they present a review on sheep ticks of Tunisia; they insist on the regional and breed differences that have been well documented. Two tick species were collected from animals: *Rhipicephalus sanguineus* sensu lato (98.6%) and *Hyalomma excavatum* (1.4%). The infestation peak was observed in August for *R. sanguineus* s.l. (83%) and in May for *H. excavatum* (6.6%). The highest infestation intensity (number of ticks/number of infested sheep) was 3.7, in August.

A novel molecular tool for the detection of Phlebotomine larval DNA in soil substrates

P14

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Phlebotomine sand flies constitute a group of haematophagous insects of great medical and veterinary importance. Despite the significant knowledge on the biology and behavior of adult sand flies in the wild, there is little information available on the ecology of their larval stages due to difficulties of detecting them in the soil. So far, investigations on sand fly breeding sites have been based on methods to catch emerging adults or on microscopic examination of the soil for the presence of immatures, neither of which is very precise or efficient for studying immatures in the field. Thus, there is a need for a better method to detect, identify and quantify immatures in soil samples. To address this issue we developed a novel molecular genetic approach for the detection of sand fly larval DNA within soil samples. Different numbers of live larvae of *Phlebotomus papatasi* and *P. tobbi* (alone or in combination), were mixed with soil and analyzed to identify and quantify the sand flies. By modifying already existing soil DNA extraction protocols in combination with a real-time PCR using species-specific primers, we successfully detected as little as one larva/40 ml of soil. We could also distinguish the two species of sand fly. This method should be very useful for increasing our knowledge of sand fly larval ecology in the field, and thus help develop more efficient, targeted control strategies against *Phlebotomus* sand flies.

P15 Vector competence and feeding behaviour of Italian populations of *Culicoides obsoletus/scoticus* and *C. imicola* under laboratory conditions

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Midges belonging to the genus *Culicoides* are notorious pests of domestic and wild an- imals, capable to act as biological vector of several arboviruses. Besides their ability to transmit bluetongue virus (BTV), some species of *Culicoides* may play an important role as vector of emerging or re-emerging arboviruses, such as African Horse Sickness virus (AHSV), Epizootic Haemorragic Disease virus (EHDV), Equine Encephalosis virus (EEV), Akabane virus (AKV), Schmallenberg virus (SBV), Bovine Ephemeral Fever virus (BEFV), in case they reach Italy. In this context, the vector competence of the Italian populations of *Culicoides imicola* and/or *Culicoides obsoletus/scoticus* for BTV, AHSV, SBV and EHDV has been investigated under laboratory conditions. Different methods have been success- fully used to feed on blood wild-caught *Culicoides imicola* and *Culicoides obsoletus/scoti- cus*: blood-feeding device with magnetic stirrer (as described by Venter et al. 1991, with one-day-chicken skin or stretched parafilm as feeding membranes), the Hemotek de- vice, and cotton pledgets. Differences in terms of feeding response between methods, vector species and vector populations are described and discussed. Regarding the ab- dominal pigmentation after the blood meal digestion, it has been noted that in a vari- able percentage of both *C. imicola* and *C. obsoletus/C. scoticus*, including midges result- ing positive to virus detection, the abdomens were still unpigmented on day 10 post infection (dpi). This finding supports the hypothesis that more than one blood meal is needed to pigment the abdomen, and that unpigmented females collected in the field do not have the same epidemiological value of males or immature stages, in which de- tecting arboviruses implies transovarian transmission. Incubation period length was also compared, and the possibility that testing midges at different dpi may affect the infection rate is discussed.

Vector Ecology & Behavior category

Modelling temporal dynamics of *Culicoides* populations on Reunion Island (Indian Ocean) vectors of viruses of veterinary importance

P16

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Reunion Island regularly faces outbreaks of epizootic haemorrhagic disease (EHD) and bluetongue (BT), two viral diseases transmitted by haematophagous midges of the genus *Culicoides* (Diptera: Ceratopogonidae) to animals of economic importance such as cattle, sheep and goats. To date, five species of *Culicoides* are recorded in Reunion Island: *Culicoides bolitinos*, *C. enderleini*, *C. grahamii*, *C. imicola*, and *C. kibatiensis*. Although epizootics and *Culicoides* diversity are already well documented, abundance and seasonality of the five species are not. According to a recent viral screening of local *Culicoides* populations (unpublished data), at least four species are involved in the transmission of each virus. Therefore, characterizing the risk period by modelling the temporal dynamics of the five *Culicoides* species is a key step to better understand BT and EHD epidemiology and improve their control.
Between 2016 and 2018, 55 biweekly *Culicoides* catches using OVI traps were set up in 11 sites. A hurdle model (*i.e.* a presence/absence model combined with an abundance model) was developed for each species in order to determine climatic and environmental drivers of presence and abundance of *Culicoides*.
Regarding abundance, average *Culicoides* catch per site ranges from 4 to 45,875 individuals. Also, diversity differ between sites with *C. imicola* being dominant at low altitude and *C. kibatiensis* at high altitude. A marked seasonality is observed for the 3 other species. Eleven meteorological and environmental determinants were used to model presence and abundance of each species: temperature, humidity, rain, wind, global radiation, vegetation index, eco-climatic area, land use, farm density, animal density and length of nearby watercourse. The association of these determinants to explain presence and/or abundance depends on the species, but each plays a role in at least one species.
This is the first study to model *Culicoides* population dynamics in Reunion Island. In the absence of vaccination and vector control strategies, determining periods of high abundance of *Culicoides* is a crucial first step towards identifying periods at high risk of transmission for both viruses.

P17 Identification of bloodmeal sources in phlebotomine sand flies using a novel MALDI-TOF MS-based method

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Hematophagous females of phlebotomine sand flies (Diptera: Psychodidae) are exclusive vectors of diseases such as leishmaniases. Identification of their blood sources is therefore crucial for understanding transmission cycles and knowing disease reservoirs. It can also help with a setting proper control measures in endemic areas. Nevertheless, conventional methods for host blood determination are known as laborious, expensive and facing some limitations related to blood degradation and tiny volumes of bloodmeals. A promising approach employing MALDI-TOF protein profiling was reported recently, however it was applied solely for freshly engorged females until 24 hours post-bloodmeal (PBM). In the present work we introduced a novel method for bloodmeal identification, which is based on peptide mass mapping (PMM) using MAL- DI-TOF mass spectrometry. Host blood from homogenized abdomen was digested by exogenously added trypsin and the resulting peptides, typically specific fragments of host haemoglobins, were detected and sequenced using MALDI-TOF MS. The approach was first tested on females experimentally fed on rabbit and several rodents yielding
100% correct host identification until 36 h PBM. Moreover, 80% samples gave proper assignment of blood origin at 48 h PBM. When investigating females fed on two hosts, PMM successfully revealed both bloodmeals for 60% specimens until 36 h PBM. In a blind study using field-collected females PMM method correctly identified host blood for 52 of 54 (96%) fresh females from Greece and 10 of 24 (42%) one-year old specimens from Armenia as was verified using traditional cyt B gene sequencing. MALDI-TOF peptide mass mapping was proven as an accurate and robust approach for host blood identification, especially useful during field surveys when high numbers of samples are processed. The method is cost-effective and fast with a minimal sample input. Al- though tested on phlebotomine sand flies, it might be applied to other blood-sucking insects as well. Funding: This work was supported by the Czech Science Foundation (15-04329S), the Institute of Microbiology (RVO61388971) and VectorNet, a European network for sharing data on the geographic distribution of arthropod vectors, trans- mitting human and animal disease agents (Contract).

Low effective population sizes in *Amblyomma variegatum* in West Africa: implication for the sustainability of acaricide-based control programs

P18

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Effective population sizes have rarely been estimated in ticks despite the importance of this parameter for evaluating the evolutionary and adaptive potential of tick populations. This work was aimed at evaluating the effective population sizes of *Amblyomma variegatum*. In addition to the direct losses it imposes on livestock, this tick is the main vector of *Ehrlichia ruminantium*, the agent of heartwater (cowdriosis) that induces up to 80% mortality in susceptible sheep and goats. The usage of acaricide footbaths seems as the most accurate way to protect livestock from all the deleterious effects of *A. variegatum*. The durability of such a protection would depend on the potential of *A. variegatum* to evolve acaricide resistances. We developed microsatellite markers to estimate the effective population sizes of *A. variegatum* in three neighbor villages from Burkina Faso. As sampling involved two tick generations, effective population sizes were independently estimated by two methods insensitive to heterozygosity: the first one is based on linkage disequilibrium analysis within sampling while the second uses the changes in allele frequencies across generations. Both methods estimated the number of reproducing ticks as ranging from two to a few tens reproductive adults per village and cohort. Such small estimates plead for low probabilities of both apparition and selection for acaricide resistance mutants, a result congruent with the rarity of records of acaricide resistance in *A. variegatum*. This situation will be compared with that of the southern cattle tick *Rhipicephalus microplus* that show much larger effective population sizes and numerous reports of acaricide resistances. Meanwhile, we will also examine how the biology of *A. variegatum* can explain such low estimates in effective population sizes.

P19 Studies on Culicinae diversity and impact of ecological changes on its major vector species from Punjab (India)

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The ecology of agricultural state of Punjab in India has tremendously changed during the last five decades. The intensive cultivation of paddy crop during summer and monsoon months has made the availability of water throughout the state in abundance. Mosquito transmitted diseases are flaring up in different parts of the state due to this reason. The rapid development, population explosion, an excessive use of insecticides and pesticides and other ecological changes will obviously affect the mosquito species composition, their resistance to insecticide, breeding and resting habits, vectorial capacity etc. leading to quite different picture of mosquito fauna and the incidence of mosquito borne diseases. Nobody has done collection surveys and studied Culicinae diversity for the last 70 years or so in a proper way. The knowledge on vector species is scanty. Intensive and extensive collection surveys were carried out to study the Culicinae diversity by the author. As many as 26 species referable to 13 genera have collected and studied in detail. An effort has been made to collect the data on temperature, humidity, rainfall, prevalence of species in order to study various kinds of variations among mosquito species. Studies on ecological succession of certain vector species have also been accomplished. Four species have been recorded for first time from the state. The data collected from last 2-3 decades suggested that out of 22 districts of Punjab, Ludhiana and Patiala are the main pockets for the prevalence of major vector species.

Membrane feeding assays with the human body louse *Pediculus humanus humanus* **P20**

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The German Environment Agency has the statutory responsibility to test the efficacy of head lice products according to the German Infectious Diseases Protection Act (Infektionsschutzgesetz). Therefore, the human body louse, which serves as a substitute for the head louse, has been fed on rabbits for more than 40 years. In order to fulfill the 3R for animal, various successful attempts have been made to change the rearing method for blood-sucking arthropods to membrane feeding. However, up to now, no membrane feeding method is described to maintain human lice cross-generational in the laboratory. To replace the rabbit host, equine, porcine, ovine and bovine blood and different membranes were tested. Due to ethic concerns, human donor blood was not used, and neither was blood from small mammals (e.g. rabbits). Animal blood was defibrinated or anticoagulated by heparin or sodium citrate. The most promising results in engorgement and mortality were achieved with double-stretched parafilm and defibrinated equine and porcine blood. Further comparative experiments with these two blood sorts showed that both adults and freshly hatched juveniles survived significantly more feedings with equine blood. Adults fed 8 times on equine blood (LT50: 3 feedings) and 4 times on porcine blood (LT50: 2 feedings); juveniles fed 8 times on equine blood (LT50: 2 feedings) and 2 times on porcine blood (LT50: 1 feeding). With both blood sorts egg laying and hatching was observed. Descendants of the membrane fed adults survived up to two feedings with porcine blood and four feedings with equine blood (both LT50: 2 feedings). However, no molting occurred. Among the examined blood sorts, equine blood yielded the highest survival rates and will be used for further studies. However, the adaptation to the rabbit host for over 40 years seems to be the most challenging part for a successful change to membrane feeding.

P21 Comparative analysis of the distribution of Ixodidae tick vectors in sheep and goat farms in the Sicilian territory

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Tick infestations cause relevant economic damage in zootecnics, due to vector transmission of Theileriosis, Babesiosis, Anaplasmosis. Few studies have been conducted on the diffusion of tick species in sheeps/goats. Ticks were harvested from 10% of sheeps/goats in 33 georeferenced farms throughout Sicily. Tick were identified at the species level by morphological keys or biomolecular assays. The Mann-Whitney/Kruskal-Wallis test were used for statistical analysis ($\alpha=0.05$). In total, 1,600 ticks were collected, including *Rhipicephalus sanguineus* (40.12%) in 6 provinces, *R. bursa* (34.18%) in 3 provinces, *R. turanicus* (21.62%) in Agrigento, *Haemaphysalis. sulcata* (2.31%) in Ragusa, *Hyalomma lusitanicum* (1.68%) and *Ixodes ricinus* (0.06%) in Messina. *R. sanguineus* is especially present at 200-400 m and 600-800 above sea level (a.s.l.), whilst *R. bursa* at high altitudes (600-1200 m a.s.l.). *R. turanicus* has been recorded at 49-600 m a.s.l, similarly to *H. lusitanicum*. Specimens of *Hae. sulcata* and *I. ricinus* were found at moderate altitude (784 m s.l.m). A significant difference was revealed between *R. bursa* specimens found in ranges 0-200 m/200-400 m a.s.l. compared to 1000-1200 m a.s.l., as well as for *R. turanicus* at 0-600 m a.s.l. compared to 600-1200 m a.s.l. A predominance of *R. sanguineus* and *R. turanicus* over *R. bursa* was observed in sole sheep/goat farms, compared to mixed breeding farms. Data collected showed the presence of different tick vector species infesting sheeps/goats in Sicily, related to the provinces, altitudes and type of farms, with a predominance of *R. sanguineus*, *R. bursa* and *R. turanicus*. Deeper analysis will allow to evaluate the distribution of vector ticks in relation to local geoclimatic factors in the selected farms.

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The fleas in Lithuania: the diversity and vector-borne pathogens

P22

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Fleas (Insecta: Siphonaptera) are common ectoparasites of mammals and birds in different habitats throughout the world. Currently, 16 families with 246 genera and about 2500 species of fleas are known in the world. Only five flea families occurring on birds and all other species parasitize mammals. Flea-borne pathogens (for example *Rickettsia* sp., *Bartonella* sp., *Yersinia* sp.) are widely distributed over the world in endemic-disease foci, and these diseases could acquire an epidemic form due to changes in vector-host ecology. Human susceptibility to zoonotic infection is not clear due to changes in climatic conditions, increased human migration and animal transportation. Furthermore, due to the rapid development of international relations the threat of an infection being carried to another country remains. In this study, we investigated the diversity of fleas from different hosts and flea-borne pathogens in Lithuania. A total 13 fleas species were identified: *Ctenophthalmus agyrtes, Ct. assimilis, Ct. uncinatus, Ceratophyllus sciurorum, Ctenocephalides felis, C. canis, Hystrichopsylla talpae, H. orientalis, Megabothris turbidus, M. walker, Palaeopsylla soricis, Peromyscopsylla bidentate, Nosopsyllus fasciatus.* We used real-time PCR, nested-PCR, multiplex PCR and vector-borne bacteria flow chip for different pathogens detection. Genus of *Bartonella, Rickettsia* and *Borrelia* were detected. Pathogens found in fleas are causing zoonoses (*Bartonella grahamii, B. rochalimae, B. henselae, B. clarridgeiae, B. washoensis, Rickettsia helvetica, R. felis, R. monacensis*).

P23 Veterinary and health importance blackfly species (Diptera: Simuliidae) from Valencian Autonomous Region (Spain) and risk maps.

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In the last years, blackflies (Diptera: Simuliidae) have been the subject of attention in different Spanish media such as television, newspaper and radio owing to the annoyance occasioned to the citizens. So, it is very important to gain awareness of their dispersion patterns in order to predict which human settlements could suffer their painful bites and their derivative symptomatic reactions. Valencia is one of the Spanish regions affected by this insect. Nonetheless, there is a lack of knowledge about its bioecology in this place. It has prompted this study in order to provide information about which species are important on the Valencian Autonomous Region. A total of nineteen sampling points of twelve rivers have been studied. As a result, seven species of blackfly have been determined as worrying from veterinary point of view, whose females bother both, wild and domestic animals biting them to get blood. Indeed, some of them are reported to be vectors of important pathogenic agents as protozoa and filarial nematodes causing of diseases. On the other hand, five of this mentioned species also use to annoy to humans. Fortunately, neither of them are vectors of pathogenic agents causing of illnesses so far, as other species do in some places of the world. But, where are these worrying simuliid species for Spanish citizens? We provide information about their provincial distribution around the country, as well as their European one. Moreover, we pay special attention to *S. erythrocephalum*, a disturbing specie which can origin dangerous outbreaks in which females tend to form swarms and attack to someone massively. Finally, have been created several maps in which are showed the breeding points and the nearby human settlements whose citizens could be affected by the females of this simuliid specie in a gradient of danger, based on adult flight range.

Vector Ecology & Behavior category

Influence of temperature on the biology of *Aedes koreicus*: an experimental and modelling study

P24

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Aedes koreicus, an Asian mosquito species, was detected in Europe for the first time in Belgium in 2008 and in Italy 2011. It has since established in northeastern Italy (Trentino and Veneto regions), thanks to its adaptation to temperate weather conditions. Since it is a relatively new invasive species, few studies have been conducted so far to investigate its biology and dynamics. Using laboratory colonies established from larvae and pupae collected in Trentino region (our study area), we carried out standard laboratory experiments to assess how different temperatures (T=4, 8, 13, 18, 23, 28, 33°C) affect immature stages (eggs, larvae and pupae) development and survival. Moreover, we estimated adult survival and the gonotrophic cycle length at 18 and 23°C. We then designed a mechanistic mathematical model, based on our experiments findings, to fit the adult captures recorded in 2016 and 2017 in the considered region. We found that the egg hatching rate is lower at 8°C, while it is about 50% for the other considered temperatures. Larval survival is quite high and seems little affected by temperature changes, while pupae mortality increases exponentially at 33°C. Our mosquito population model fits the time series of adult mosquitoes captured over the season quite well, and thus it provides a good framework to investigate how different climatic conditions might affect *Ae. koreicus* dynamics during its breeding season. Our findings can help at defining the future geographical expansion of this species, also by taking into account possible climatic changes.

P25 Larval superiority of *Culex pipiens* to *Aedes albopictus* in a replacement series experiment: prospects for coexistence in Germany

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The Asian tiger mosquito *Aedes albopictus* is an extremely invasive, globally distributed and medically important vector of various human and veterinary pathogens. In Germany, where this species was recently introduced, its establishment may become modulated by interspecific competition from autochthonous mosquito species, especially *Culex pipiens* (s.l.). While competitive superiority of *Ae. albopictus* to *Cx. pipiens* (s.l.) has been described elsewhere, it has not been assessed in the epidemiological conditions of Germany. The present study aimed to determine if such superiority exists under the physicochemical and microclimatic conditions typical for container habitats in Germany.
Methods: In a replacement series experiment, the larval and pupal responses of *Ae. albopictus* and *Cx. pipiens* (s.l.) (mortality, development time, growth) to interspecific interaction (five larval ratios) at (sub-)optimal temperatures (15, 20 and 25 °C) and differing food supply (3 and 6 mg animal-based food larva-1) were investigated using a randomized split-plot design. In addition to physicochemical measurements of the test media, natural physicochemical conditions were determined for comparative analyses in mosquito breeding sites across the Rhine-Main metropolitan region of Germany.
Results: Under the physicochemical and microclimatic conditions similar to the breeding sites of the Rhine-Main region, competitive superiority of *Cx. pipiens* (s.l.) to *Ae. albopictus* in terms of larval survival was more frequently observed than balanced coexistence. Food regime and multifactorial interactions, but not temperature alone, were controlling factors for interspecific competition. Larval food regime and the larval ratio of *Ae. albopictus* influenced the physicochemistry and algal growth at 15 °C, with increased *Ae. albopictus* mortality linked to a decreasing number of *Scenedesmus*, *Oocystis* and *Anabaena* algae.
Conclusions: Under the present environmental conditions, the spread of *Ae. albopictus* from isolated foci in Germany may generally be slowed by biotic interactions with the ubiquitous *Cx. pipiens* (s.l.) (and potentially other container-breeding mosquito species) and by limnic microalgae in microhabitats with high resource levels. Detailed knowledge of the context dependency in temperate mosquito ecology, and interrelations of physicochemistry and phycology may help achieve a better understanding of the upcoming *Ae. albopictus* colonization processes in central and northern Europe.

Distribution of sand fly species collected in Portugal from 2014 to 2017: data from the national vector surveillance programme

P26

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Phlebotomine sandflies have a worldwide distribution and are common in the Mediterranean Basin. Despite their limited dispersal ability, there are reports that recent climate changes resulted in the expansion of geographic areas where sandflies circulate. This represents a Public Health problem since sandflies are vectors of pathogens. In the Mediterranean area, sandflies can transmit leishmania parasites and also phleboviruses. In Portugal there are five sandfly species described: *Phlebotomus ariasi, P. papatasi, P. perniciosus, P. sergenti* and Sergentomyia minuta. With the purpose of contributing to the knowledge of sandfly species distribution in Portugal, sandflies collected in a national vector surveillance programme were studied.

Sandflies were identified morphologically with taxonomic keys and population diversity was analyzed through a partial sequence of mitochondrial cytochrome oxidase gene subunit (COI) using DnaSP software. Two hundred and fourteen counties were surveyed from 2014 to 2017. One hundred and sixty nine males (n= 126 *P. perniciosus*; n= 33 *P. ariasi*; n=6 *P. sergenti*; n= 1 P. papatasi; n=3 *S. minuta*) and 20 females *(P. perniciosus*) were identified morphologically. COI sequences were analysed in 77 females, (n= 43 *P. perniciosus*; n= 24 *P. ariasi*; n= 1 *P. sergenti*; n= 9 *S. minuta*). *P. perniciosus* was the most ubiquitous species present in 46 counties, followed by *P. ariasi* in 15 counties. COI sequence diversity analysis showed that *P. perniciosus* presented the less diverse population with only six mutations, all singletons (in 637 positions), exhibiting low haplotype diversity (Hd= 0,2625). *P. ariasi* and *S. minuta* populations are more diverse with higher haplotype diversity (Hd= 0,9312 and Hd= 0, 9167, respectively). The known sandfly distribution and population diversity was updated and the importance of vector surveillance was reinforced since the most prevalent species are the main vectors of phleboviruses (*P. perniciosus*) and leishmania (*P. perniciosus* and *P. ariasi*) in Portugal.

P27 Genetic costs associated with the presence of kdr and ace.1 insecticide resistance genes in *Anopheles gambiae* s.s.

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The microbiome diversity is under genetic control of mosquito and influences the Plasmodium transmission. In addition, the resistance management strategies require good understanding of the evolution processes of insecticide resistance genes. Female mosquito usually mates only once, so resistant males more likely contributing to the spread of insecticide resistance genes. This study investigated the microbiome diversity level of females and the mating performance of males of *An. gambiae* s.s bearing *kdr* and *ace-1* insecticide resistance genes.

The meta-genomic diversity of the microbiome was explored using four mosquito strains (acerkis, acerkiskdr, kdrkis and kisumu), by sequencing the 16S-rDNA region of bacteria using Pyrosequencing technology. Male sexual performance was assessed through crossment experiments using 10 virgin females kisumu and 10 virgin males of each these four strains which were maintained in the cage during 72 hours. Then batches of larvae coming from the progeny of individualized female were tested for *kdr* L1014F and *ace-1* G119S genes to identify the genotype of the male that copulated the female Kisumu's strain.

Our results show that the kdrkis strain has a greater microbiome diversity than all other strains. While acerkiskdr strain has the lowest microbiome diversity. And the probability that a male acerkis mates with a female kisumu is 10.28 times higher than that of male kisumu taken as reference. The different level of gut microbiome diversity observed with these strains bearing different insecticide genes resistance may probably link to the differences in Plasmodium transmission in natural populations of *An. gambiae* s.s. While the role of the *ace-1* gene in mating success of males would contribute with insecticides selection to its spread in the natural populations of *An. gambiae* s.s. This seems to compromise resistance management strategies such as insecticide rotation.

Vector Ecology & Behavior category

Spread of diflubenzuron resistance in the mosquito *Culex pipiens* in northern Italy

P28

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Chemical control, by the use of insecticides, remains the main tool to control mosquito populations and prevent mosquito-borne diseases. In this context, the use of larvicides is highly recommended. Indeed, compared to adulticides, larvicides can have a longer effect, be focused on the breeding sites and be more safe for the environment. However, the limited number of larvicides currently available for control programmes along with their intense use has caused a rapid selection of insecticide resistance in several species, that is seriously threatening the efficacy of this tool. In the mosquito *Culex pipiens*, insecticides resistance was documented against pyrethroids, organophosphates and, more recently, also to chitin synthesis inhibitors, such as diflubenzuron. Resistance against diflubenzuron has been shown to involve a direct interaction with the chitin synthase 1 (CHS1) and be due to the occurrence of two non-synonymous mutations at the site 1043 of this gene. In Italy, in 2015, first evidences of resistant phenotypes to diflubenzuron in *Cx. pipiens* have been found in Ravenna (Emilia-Romagna region), highlighting the importance to assess the diffusion of resistant alleles across this region and implement effective management strategies. Here, we present the results obtained by the monitoring activity of the diflubenzuron resistance in *Cx. pipiens* populations conducted in the 2017 year. About 1500 individuals from thirty localities across the Emilia-Romagna region were sampled and analysed for the occurrence of resistant alleles by PCR-based molecular assays. The diffusion pattern of the resistant alleles since their first occurrence in 2015 is discussed in relation to its implications on the safety of human health and on vector control strategies.

P29 Entomological Study for the Detection of Tos-V on Sand-flies Samples Collected in a Leishmaniosis Endemic Area of Lampedusa Island from Sicily

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Phleboviruses are common human pathogens diffused on the Mediterranean area, known to cause a mild three-days fever. Nevertheless, *Toscana Virus* has a great concern since its infection could provoke also central nervous system disorders like meningo-encephalitis. Furthermore, the phlebotomine may represent the only reservoir for the sand-fly viruses, as well as being recognized carriers for Leishmania. Then, with the purpose to monitor the phleboviral spread, the present study was conducted as an entomological investigation in areas of Lampedusa previously reported for leishmaniosis infection cases. The survey was carried through a primary collection of the sand-flies, by means of CDC light traps, and a second step of molecular screening test to detect the viruses, employing the RT-PCR on the genomic S-segment of Toscana Virus. The outcomes demonstrated a TOSV positivity in five out of seventeen pools, which were subsequently processed by sequencing and identified by using BLAST on the NCBI data base. In conclusion, the data presented in this study, presumably, demonstrate the presence of TOSV in sand flies collected in Lampedusa (Sicily). Even though epidemiological data are, usually, the main source for a regional surveillance, the entomological survey on the vectors have been an alternative but useful way to reveal the viral presence, as other researchers have also proven. Then, it could eventually constitute a complementary support to monitor the phleboviral dissemination.

A study on mosquitoes (Diptera, Culicidae) in the Třeboň Basin (Bohemia, Czech Republic) with emphasis on the presence and behavior of *Culex modestus* (Fic.), a potential WNV vector.

P30

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The mosquito fauna has been monitored in the Třeboň Basin – a UNESCO protected biospheric area with a unique system of more than 450 fish ponds built around the Třeboň city (49°N, 14°46´E). The area of the Třeboň basin is 1360 sq. km, with average altitude of 453 m a.s.l. In 2012 – 2018 different mosquito collection methods were applied (catching larvae and female trapping using EVS traps with CO_2 as attractant being used regularly). Mosquito larvae collections were carried out in approx. 30 various natural biotopes in the inundation zones of the Lužnice River and adjacent wetlands and flood plain forests, in coniferous forests, peat bogs and inundated meadows. EVS CO_2 traps were placed mostly on the edges of about 50 fish ponds, especially in their reed (*Phragmites australis*) zones. Twenty-eight native mosquito species have been identified in the study area since 2012. After floods, larvae of the floodwater mosquito species, such as *Aedes vexans, Ae. sticticus,* and *Ae. cinereus,* predominated. In years with no major floods, larvae of the snow-melt mosquito species, such as *Ae. cantans or Ae. punctor*, prevailed. On the edges of fish ponds, females of *Culex modestus,* a potential vector of the West Nile virus, collected by EVS CO_2 traps were very abundant in some years. Daily variation in the biting activity of *Cx. modestus* was studied in detail in summer 2018. No WNV was isolated from *Cx. modestus* in 2016-2017. Using EVS CO_2 traps, two females of *Uranotaenia unguiculata* were trapped in the reed zones of the Černičný pond (49°4´41´´N, 14°45´20´´E) and the Malý Tisý pond (49°3´15´´N, 14°44´57´´E) on 2nd and 3th August 2017. Two more females of the species were trapped in the area on 26th July 2018. They were the first records of *Ur. unguiculata* in Bohemia and in one of the northernmost points of distribution of this thermophilic species in Europe. Another new species for the area under study was *Anopheles daciae* (Blažejová, Šebesta, Rettich et al., 2018). The study was supported by MH CZ-DRO (National Institute of Public Health-NIPH,75010330).

P31 Molecular detection of *Wolbachia pipientis* in natural populations of *Culex pipiens* in Morocco

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Background: *Culex pipiens* mosquitoes are naturally infected by *Wolbachia*, an endosymbiotic bacterium able to manipulate host reproduction through several mechanisms such as cytoplasmic incompatibility and also interfere with pathogens transmission. The *Cx. pipiens* complex is subdivided in two different forms, *molestus* and *pipiens*, morphologically identical but genetically different. We aim to complete the geographic distribution of the different forms of *Cx. pipiens* in Morocco and to determine the *Wolbachia* types that they harbored.

Methods: A total of 459 specimens collected in three cities of Morocco were processed for DNA extraction. Mosquitoes were identified using a multiplex PCR assay with diagnostic primers designed from the flanking region of microsatellite CQ11. To characterize the *Wolbachia* types, samples were tested by PCR-RFLP based on two ANK *Wolbachia* markers ank2 and pk1.

Results: Three different *Wolbachia* types were identified in *Cx. pipiens*. All specimens from Tangier and Casablanca were infected by type I. However, in Marrakech, there was a great diversity of wPip infection; we found 56 mosquitoes (55%) infected with wPip-V group, 39 (39%) were infected with wPip-I group, and only six were infected with wPip-IV group. The results showed that the wPip-I group was dominant in the rural area (67%), whereas type V was dominant in the urban site (91%) compared with rural area (28%). In contrast, wPip-IV was detected only in three specimens in rural and in urban areas. Also, we found that 65% of the pipiens form was infected by wPip-V, 28% by wPip-I, and 7% by wPip-IV, whereas 67% of the molestus form were infected by wPip-I, and the remaining 33% were infected by wPip-V. Of hybrids, 50% and 43% were infected by wPip-V and wPip-I, respectively. Conclusion *Cx. pipiens* were infected with a variety of *Wolbachia*. These findings show that the diversity of wPip depends on the location.

A survey on ectoparasites and vector-borne pathogens in south-west Madagascar

P32

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Since there is an exponential growth of the human population which highly depends on forest resources, human encroachment of natural habitats is a rising concern in Madagascar. Subsequently, livestock and synanthropic animals like rats and mice get into closer contact with the endemic wildlife. With this study we screened ectoparasites from mammalian hosts for their potential for interspecific transmission of vectors and pathogens.

The field work took place in the Mahafaly region in the dry season (Jun - Aug 2016) and in the rainy season (Feb - Apr 2017). Trapping of small mammals was conducted with baited Sherman live traps placed as line transects in habitats of different status of degradation: 1) Relatively pristine forest of the National Park, 2) degraded forest, 3) cultivated and shrub land. Rats and mice were trapped in villages. Ectoparasites were removed from the hosts using a comb and forceps. Cattle, sheep and goats were additionally screened for ectoparasites. The specimens were stored in 99% ethanol and, after identification to species level, the DNA of individual and pooled parasites was extracted. A combination of real time and conventional PCR assays was used to detect Rickettsiae, Borreliae, Bartonellae and Yersiniae as vector-borne pathogens.

We identified 13 species of ectoparasites (1192 individuals of ticks, lice and fleas) collected from 14 host species. A bigger portion of the parasites were found to live strictly host-specific. In contrast, one flea species (*Echidnophaga gallinacea*) was collected from 6 different hosts. Rickettsiae were found in all parasite species, Bartonellae only in lice, Borreliae in ticks and *Yersinia pestis* in four pools of fleas from one rat. Phylogenetic analyses indicated close relationship to pathogenic bacteria in all screened genera, exhibiting a potential vector capacity of the respective ectoparasites.

P33 Reduced blood feeding and infectivity to dengue and Zika viruses in the Thai *Aedes aegypti* females being exposed to radiation by the Sterile Insect Technique

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Arboviral diseases such as dengue and Zika virus infection are considered a global public health problem. The diseases are transmitted mainly by *Aedes aegypti* mosquitoes, which distributed through out tropics and sub-tropics. So far most vector control programs have not yet demonstrated sustainable and significant impacts on *Ae. aegypti* vector populations and the diseases they transmit. Recently, sterile insect technique (SIT) has been attempted to suppress *Ae. aegypti* vector populations in several countries. In this study, we preliminary assessed and compared blood feeding success and infectivity of irradiated and non-irradiated *Ae. aegypti* females when they were infected with either DENV-1 or ZIKV. Our results demonstrated that, after radiation, *Ae. aegypti* females reduced their ability to feed on blood meals, either non-infected blood mixed with only medium (p=0.020) or blood infected with DENV-1 (p=0.025) and ZIKV (p=0.002). However, RT-PCR detection of DENV-1 and ZIKV in irradiated mosquito samples showed that both DENV-1 and ZIKV infections were only found in the abdomen but not in the head and thorax or legs in contrast to non-irradiated ones where infections were detected in all mosquito parts. In addition, the infection rates of both DENV-1 and ZIKV were significantly reduced (p<0.05) when compared between irradiated and non-irradiated samples. It is speculated that high radiation dosage, i.e., 70 Gy, may have negative impact on *Ae. aegypti* females and hence reducing their vector competence. These results should reduce the concerns related to an accidental release of some irradiated *Ae. aegypti* females together with irradiated sterile males during implementation of an SIT-based vector control programme since these small numbers of irradiated *Ae. aegypti* females were found to be less competent as the vectors of both dengue and Zika diseases.

Novel Mosquito Insect-Specific Flavivirus from Czechia

P34

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Insect-specific flaviviruses (ISFs) with unknown vertebrate host are described almost all over the world, however, the majority of ISFs have not been discovered yet. There´s only limited information about their ecology but several studies demonstrate that some ISFs can influence replication of other medically important flaviviruses (e.g., Dengue) in mosquito cells and probably represent an ancestral lineage of the family Flaviviridae. In our surveillance study the ISFs presence in nearly 9,700 mosquitoes captured in Czechia was investigated and RNA sequences representing four novel ISFs related to previously described ISFs were detected in almost all mosquito species. One of these viruses was isolated on C6/36 mosquito cell culture. The combination of NGS and conventional PCRs with specifically designed primer pairs provides the complete genome of this ISF. Several interesting observations were noticed. (i) In contrast to the majority of ISFs described till now, no cytopathic effect was detected and quantification of virus particles was impossible by most regular methods. The infection of cell culture was confirmed by PCR, with Flavivirus universal primer pairs, as late as 19 days post inoculation, not earlier. (ii) Four different methods were tested to experimentally infect laboratory reared *Culex quinquefasciatus* and *Culex pipiens molestus*, but none of them led to persisting infection in mosquitoes. Several recent studies showed enhancing effect of Wolbachia for flaviviruses infection and propagation, but either Wolbachia-positive mosquitoes remained negative. (iii) Experimentally infected mosquitoes were tested by specific PCR and, interestingly, one locus of their mRNA related to ISFs was amplified, which has never happened in non-infected control specimens. We suggest some non-described interaction between flaviviruses entering the cells and flaviviruses-related endogenous viral elements in mosquito genome, which lead to transcription and PCR detection of these products.

P35 Preliminary assessment of the role of mosquitoes diversity in West Nile Virus transmission dynamics

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The effects of biodiversity on infectious diseases dynamics are known, especially for vector borne diseases (VBD). Biodiversity may influence VBD transmission in several ways including reduction of the fraction competent vectors in the community (competition) and reduction of the fraction of the susceptible host population (dilution). We studied the mosquito population at a single location where WNV was detected in 2017 only hypothesizing the potential role of vector species diversity on West Nile Virus (WNV) circulation. Mosquitoes were collected in 2010-2017 at a WWF Reserve in Veneto Lagoon, as part of the entomological surveillance for WNV. Mosquitoes were bi-weekly captured from May to October using CDC-CO2 traps, identified and tested for WNV using RT Real-Time PCR. Biodiversity variations were assessed by comparing the total Species Richness (S), the Shannon-Wiener s Entropy Index (H), and the Equal Number of Species (ENS). An average of 11.25 captures were performed per year (range: 8-13); the overall S ranged between 6 and 13. The trend of H indicated non-significant fluctuations during the eight years, accounting for observation at the capture-level, with an overall low level of biodiversity (average value: 0.78; S.E. 0.04). The ENS resulted markedly lower than the number of species observed, indicating the presence of few predominant species. The most abundant species was *Culex pipiens* (60.1%). Preliminary assessments of variation in environmental variables (temperature, precipitation, and relative humidity) did not revealed significant differences between 2017 and the previous years. Although an overall reduced biodiversity in the last year was observed, our results did not allowed the formulation of hypotheses on the effect of mosquitoes biodiversity on WNV circulation in the study area. Further studies on underlying factors are needed to unravel the disease dynamics, also including the effect of diversities of host species in the area. Funding: Ministry of Health (project RC IZSVE-03/15)

Human exposure to tick bites: alert in Piedmont (north western Italy) during 2018 vector season

P36

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Humans have been highly exposed to tick bites in Piedmont Region (North Western Italy) during 2018 vector season. We collected ticks removed from bitten humans, thanks to a communication campaign and an information network between veterinary services and local human health authority.

Ticks were morphologically identified using standard taxonomic keys. Ticks removed from individuals under 18 or over 70 years old and adults with suspected symptoms or immunocompromised were screened by biomolecular essays for the presence of *Borrelia burgdorferi* sl (Skotarczak et al. 2002), *Rickettsia* spp. (Choi et *al* 2005), *Anaplasma* spp. (Massung et al. 2003).

From January to July 2018 we collected 424 ticks from 326 bitten humans. According to the life stage, most frequently collected ticks were nymphs (N=300; 70,7%) and females (N=93; 21,9%). The tick most commonly retrieved was *Ixodes ricinus* (N=342; 80,7%). Biomolecular analysis was performed on 166 tick samples. Thirty-nine ticks (23,5%) tested positive to at least one pathogen. *Rickettsia* spp. was detected in 26 samples (15,7%), including *R. monacensis* (N=20) and *R. helvetica* (N=6). *Borrelia burgdorferi* sl was detected in 12 ticks (7,2%), including 4 genospecies (*B. afzelii*, *B. garinii*, *B. lusitaniae* and *B. valaisiana*). *Anaplasma phagocytophilum* was detected in 1 sample. In 2 cases, co-infection between *Rickettsia* spp. and *Borrelia burgdorferi* sl was observed.

In conclusion, during the first months of 2018 vector season in Piedmont an exceptional number of ticks (more than doubled with respect to previous 5 years) was observed, but data on tick population and pathogen prevalence was similar to previous years. The increasing number of humans exposed to ticks and the detection of pathogens in 23,5% of tested samples raises the attention on the importance of this surveillance as an indirect tool for evaluating the risk of tick-borne diseases in bitten individuals.

P37 Molecular investigation of ticks as potential vectors of *Mycoplasma agalactiae*

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Contagious Agalactia (CA) is an infectious disease of sheep and goats, included in the OIE list for its economic impact. In the Mediterranean, *Mycoplasma agalactiae* is the main etiologic agent. The disease is widely spread in Sicily, representing an unresolved problem for zootechnics. Despite some evidences, few studies investigated the potential role of hematophagous arthropods in maintaining and spreading CA infection. In this work, a preliminary study was carried out to investigate the potential role of ticks in *M. agalactiae* maintenance among flocks in Sicilian territory.

A total of 52 ticks were collected from 7 infected sheep displaying clinical symptoms during a confirmed CA outbreak, and morphologically identified *via* validated keys. DNA was extracted from 9 tick pools using a commercial kit, and *M. agalactiae* DNA was detected *via* Real-time PCR, targeting a region of P40 gene.

Identified Ixodidae ticks included *Rhipicephalus bursa* (n=42), *R. sanguineus* (n=4) and *Haemaphysalis punctata* (n=6). Two out of 9 pools were positive for the presence of *M. agalactiae* DNA. Pools comprised *R. bursa* adult females collected from two sheep positive for *M. agalactiae*, as resulted by the presence of antibodies and antigen excretion. Although further in-depth analysis are needed, obtained preliminary data suggest the potential role of hematophagous parasites in the maintenance and spread of CA.

A role of arthropods in *Mycoplasma* transmission has been confirmed for fleas and mites. Recently, hemotropic mycoplasms were reported in lice from goats and in *Ixodes* ticks. The possible involvement of these arthropods as vectors or reservoirs of *M. agalactiae* could explain peculiar epidemiological aspects related to the endemism of CA in the territory. Further investigations could evaluate the pathogen presence in the different tick developmental stages and its possible transmissibility to subsequent generations. Funded by Ministry of Health: RC IZS SI 06/15 and 15/15.

Do arboviruses manipulate the behaviour and change the physiology of *Aedes aegypti*?

P38

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Mosquito-borne viral diseases, such as Zika and chikungunya, pose an increasing threat to human health. Considering the concurrent outbreaks of these diseases and lack of fundamental knowledge on multiple virus infections, it is important to unravel the effect of arboviral coinfection on vector competence. To enhance their own transmission, some pathogens, such as malaria parasites, manipulate the behaviour of the mosquito. However, the effect of a virus infection on the host-seeking behaviour and physiology of mosquitoes, and hence disease transmission, remains largely unknown. Therefore, the objective of the proposed study is to unravel the effect of virus coinfection on vector competence and single virus vector manipulation by using a system that consists of the mosquito *Aedes aegypti*, and the Zika and chikungunya viruses. Our proposed study will integrate a behavioural, physiological and transcriptomic approach. The effects of Zika and chikungunya virus infection on *A. aegypti* will be studied in our unique Biological Safety Level 3 facility. We will focus on traits that influence disease transmission, including mosquito fitness, host-seeking and blood-feeding behaviour. We will test the effect of virus infection on odour perception (via electrophysiology) and odour processing in the mosquito brain by detecting virus location through confocal and electron microscopy, and by comparative transcriptomics. Finally, we will assess which behavioural and physiological factors or interactions have the largest impact on disease risk by incorporating these factors into disease transmission models. These models will help to better understand current and future outbreaks of mosquito-borne viruses.

P39 The bacterial TBD (Tick Borne Diseases) agents from *Ixodes ricinus* ticks surveyed with the 16 S Metagenomics techniques in Romania

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The Arthropods and in a particular way the ticks represent dangerous vectors that can be used as warfare agents with an insidious and an invasive behavior. That's why, all the national security programs take into account the potential of these organisms to act as an emergent vector and to be used as bioterrorist agents. Moreover the climatic changes can modify the known geographical distribution of the vectors. The present work is the first survey of the tick population in Romania in order to evaluate the bacterial microbiota outside and inside the *Ixodes ricinus* body (the most abundant and important ixodide vector in Romania) using the Next Generation Sequencing technique. Material and methods: The ticks (adults only) were collected with the flagging method from vegetation, from 2 sampling sites (Brateiu, natural foci for Tick Borne Encephalitis and Christian, both localities from the Sibiu county, in Romania) during the years 2006-2007. The sampled ticks grouped into pools (5-10 exemplars) were preserved in RNAlater® solution (Ambion) until whole DNA was isolated. After purification and quantification, DNA was used as template for 16S rDNA variable segments (V1- V9) amplification using 16S Metagenomic kit (Thermo Fischer Scientific). For the sequence assemblage and data analyze we used an IonTorrent PGM platform within Ion Reporter software (Thermo Fischer Scientific). The same DNA samples were tested by Real-Time PCR using TicKitqPCR® for Francisella tularensis (ISFtu2 target) and *Borrelia burgdorferi* sl. (fla gene target). Results: Analyzing 75% *I. ricinus* females and 25% males we found 37 bacterial species belonging to 25 bacterial genera and represented by the natural bacterial flora inside and outside the tick body. Among the TBD bacteria we found: *Francisella tularensis* (the causative agent of Tularemia, potential agent for bioterrorism), *Coxiella burnetti* (Q-fever agent, one of the most infectious known organism used as biological weapon) *Rickettsia tamurae* (since 2009 represent one of the Japanese Spotted Fever causative agent), *Spiroplasma eriocheiris* (possible an emerging arthropod borne pathogen associated with Transmissible Spongiform Encephalopathies for humans) and *Burkholderia* sp. (causative agents for severe diseases on humans and livestock). The 16S Metagenomics analysis didn't provide evidence the *B. burgdorferi* species presence but in the same samples the Real-Time PCR method did (*B. burgdorferi* sl.). Conclusion: The 16S Metagenomics survey is a powerful technique (associated with complementary Real-Time PCR analyzes) to identify the bacterial agents causative for TBD and or associated with the bioterrorist agents in the ticks populations.

Novel Vector Control Tools category

Characterization of resistance to fipronil in a laboratory strain of *Ctenocephalides felis* **P40**

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Fleas are considered as one of the major pests of domestic animals, especially *Ctenocephalides felis*, also known as the cat flea. They are responsible for irritating pets and their owners as their bites can induce pruritus and allergic reactions. They are also responsible for the transmission of pathogens of zoonotic and veterinary importance (i.e. *Bartonella* spp., *Rickettsia* spp.). Ectoparasiticide products represent the mainstay for preventing and halting insect bites and transmission of arthropod-borne infections in companion animals. Since the last 80s, fipronil has been one of the most used insecticides worldwide. In the past 10 years, veterinarians reported a lack of efficacy when using this molecule for flea control. So far, the resistance of fleas to insecticide has not been properly studied. The aim of our work was to investigate the mechanisms underlying resistance to fipronil in a laboratory flea strain originated from a field population resistant to fipronil and to compare to a reference susceptible strain. The phenotypic resistance was investigated via tarsal contact using filter papers impregnated with increasing doses of fipronil and dieldrin. The resistant strain showed a resistance ratio at LD50 of 7.6 and 62.8 for fipronil and dieldrin respectively. The activity of detoxification enzymes was investigated in adult fleas and third-stage larvae. The results showed a significant increase in the activity of β esterases and α esterases in the adults and larvae of the resistant strain. A significant decrease in the cytochrome P450 mono-oxygenases in the resistant larvae was also observed. The molecular assays confirmed the presence of Rdl mutation in resistant fleas. These results represent the first step in the characterization of the resistance mechanisms to fipronil in *C. felis.*

P41 Evaluation of the efficacy of Aquatain against *Culex pipiens* and *Aedes albopictus* larvae in catch basins using a floating monitor system

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Aedes albopictus and *Culex pipiens* are commonly distributed in Italy and represent the main species found in catch basins. The application of a silicon-based film product like Aquatain is a new tool recently introduced for catch basins treatment. The particular way of action of Aquatain makes the monitoring of larvae by dipping a not appropriate approach to evaluate its efficacy. The larval hypoxia caused by Aquatain makes larvae die or lay on the bottom therefore when samples of water are kept larvae can be collected but it doesn't mean they will develop in adult as the hypoxia could kill them before the conclusion of the development cycle. If larvae are not present in the samples, it doesn't mean there are no larvae in the catch basins because they may lay on the bottom. Aquatain was tested in field using a Floating Monitor System to evaluate the adult emerging. The test was performed applying the product in 25 catch basins, while 25 more catch basins were used as control. Two applications were performed, one dosage was tested. The floating system (EFMS) is done of a plastic cylinder 25 cm diameter and 6 cm high, close on the top by a mosquito net. Monitoring was performed every week. Heavy rain seems to affect the efficacy of the product even if it is not clear the reason. If the no intensive showers happen the product show to be affective for not less than 4 weeks.

The use of mass trapping for mosquito control operator: a review of different trials against salt marsh and urban mosquitoes on the French Mediterranean coast since 2013

P42

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EID Mediterranée is a public operator whose main mission is mosquito control in saltmarshes along the French Mediterranean coast since 1958. Strategy is based on larvicide control with *Bacillus thuringiensis* ser. *israelensis* (Bti). Even if this method is considered to be quite efficient and environmentally friendly, alternative or complementary methods are actively studied. The use of traps in mosquito control strategy is more than ever interesting for mosquito control operators as larvicidal treatments are not always possible or not effective enough. Traps are also ecologically friendlier than adulticidal insecticides, as the use of insecticidal products is often point at by citizen, politics and ecologists. Mass trapping is an interesting approach to protect areas such as camping sites or districts but experiments are need for wider barrier around villages or cities. Studies have shown that traps need to be fully integrated in a strategy instead of being use disparately. During the past 5 years, EID Mediterranée tested different commercially available traps and strategies in camping sites, districts and leisure bases. The Mosquito Magnet Independence trap has been tested against salt marsh mosquitoes in two places surrounded by mosquito breeding sites: in 2013, as a trap-barrier enclosing an hotel (2013) with no Bti treatment, and in 2015 around a camping site as a complement to Bti treatment. Other studies were performed against urban mosquito *Ae.albopictus*. In 2016, a barrier of Mosquito Magnet completed with a network of Gravid Aedes trap (BG-GAT) showed no significant results in a camping site, whereas results of the 2017 experiment in a leisure base were quite satisfying. In 2017, an experiment was carried out in a small residential area with BG-GAT only. Traps were settled in citizen houses and results showed a great efficiency in reducing *Ae. albopictus* population. All those results help EID Mediterranée to refine its strategy in rural and urban areas.

P43 Entomological evaluation of indoor residual spraying (actellic®300cs) on malaria transmission in the Diebougou district, South-West Burkina Faso

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The rapid spread of insecticide resistance in malaria vectors and the possibly recent increase in malaria cases in Africa require to develop and evaluate new vector strategies able to manage resistance. The combination of two insecticides targeting different time of the life cycle of the *Anopheles* vector is one possibility. In this study, we evaluated in communities, the effect of indoor residual spraying (IRS) with pirimiphos-methyl in combination with Long-Lasting Insecticidal Nets (LLIN) against LLINs alone on malaria transmission and the vectors' diversity, resistance and behaviour.

The study took place in 13 villages (5 sprayed and 8 unsprayed). We performed 4 surveys of hourly mosquito collection following the implementation of IRS using the human-landing collection technique. Malaria vectors species, *Plasmodium* infection, blood-meal source, Kdr-est and kdr-ouest target-site mutation were searched by PCR. Residual activity of IRS on susceptible and wild *Anopheles* strains was monitored for 7 months post spraying.

When compared to the unsprayed control villages (186 mosquitoes), malaria vector density was drastically lower in the sprayed villages (54 mosquitoes). This lead to an 8-fold difference in Entomological Inoculation Rate (0.84 to 0.14). The Endophagy (18.22%) and anthropophagy (15.28%) were significantly lower in the treated villages. Moreover, malaria vectors (both *An. gambiae* s.l. and *An. funestus*) were collected later in the night in the sprayed village than in control ones. The residual efficacy (mortality rate) of the IRS ranged from 98-100% within 7 months for both susceptible and wild *anopheles* strains.

Pirimiphos-methyl IRS in addition to LLIN allow to drastically reduce malaria transmission and is therefore promising complementary vector control tool to be used in high transmission areas of Burkina Faso. However, the vector population in the sprayed villages exhibits behavioural traits that might contribute to maintain residual transmission.

Modelling the effect of the sterile insect technique, alone or combined with insecticide auto-dissemination, on *Aedes albopictus*

P44

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The Sterile Insect Technique (SIT) is a demographic control procedure consisting in releasing males sterilized by ionizing radiations. A workaround technique, named boosted SIT, aims at contaminating the sterilized males with pyriproxyfen; the males would then transfer this insecticide to other compartments of the target population (females, breeding sites) through auto-dissemination. To anticipate the effect of SIT and boosted SIT on mosquito populations, they were included into an age-structured population model of *Aedes albopictus* in the geographical context of La Reunion Island. A sensitivity analysis shows that timing and duration of the male release events, as well as the quantity of sterile males released, are of key importance for both control methods. The persistence of pyriproxyfen in larval habitat appears to be the major determinant for boosted SIT to be more efficient than SIT. While the model appears a powerful tool to investigate release strategies, our findings specifically call for lab experiments towards a better assessment of pyriproxyfen transfer rates and persistence in breeding sites.

P45 Evaluation of larvicide treatments by drone against mosquitoes in wetland and agricultural areas of Po river delta, Italy.

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Conventional ground larval mosquito control operations have some limitations particularly in wetlands and agricultural areas, due to vegetation and accessibility constraints, which have a major impact on their effectiveness. This is why in some areas of Po river Delta (Municipality of Comacchio, Emilia-Romagna region, Italy), a preliminary test was conducted in September 2017 on the using of unmanned aerial vehicles (drones) to map flooded areas (*Aedes caspius* and *Ae. detritus* breeding sites) and to perform larvicide treatments by *Bacillus thuringiensis israelensis* (B.t.i.). New field tests were conducted in June 2018 by B.t.i. and by B.t.i. + *Lysinibacillus phaericus*, against *Ae. caspius* and *Culex pipiens* in agricultural areas. The possibility to automate the drone to independently perform larvicidal treatments only where is necessary has been also tested. The targets of 2018 trials were: ability of the larvicidal product to reach the water; larval mortality based on the technical parameters adopted for the drone flight (speed, flight height, dose of the formulation); the efficiency of the drone flight plan system based on the upload of pre-digitized breeding sites by GIS into memory drone and evaluation of productivity expressed in hectares/hour.

Novel Vector Control Tools category

Residual activity of pyriproxyfen against mosquito developmental stages inhabiting catch basins in southern California

P46

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A field study has been carried out on the season-long efficacy of the insect growth regulator, pyriproxyfen (Nylar 0.5% G) in comparison with methoprene (Altosid XRP Pellets) against mosquito developmental stages in catch basins in Riverside County, Southern California. Pyriproxyfen was applied at 75, 100, 125, 150, 175 g/catch basin and methoprene at 3.5 g /catch basin. A total of 80 catch basins (10 per each treatment and 20 for control) were used. Posttreatment observations of catch basins and pupal collections and rearing them to adults were carried out at weekly intervals. Mosquito species composition, consisting mostly of *Culex* species, was predominated by *Cx. quinquefasciatus*. Activity of both IGRs was expressed as percent inhibition of adult emergence (% IAE). Data on % IAE collected over the 50-week period are analyzed and discussed here.

P47 Long Lasting IRS: New test Method and Results

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Intradomicial Residual House spraying has been showed to be the most rapid method for reducing density of malaria mosquitoes biting indoor and has a rapid effect on malaria transmission. However, these applications are expensive to carry out and often only has a short effect, e g 3-5 months for bendiocarb. A new microencapsulated product from Syngenta has up to one year residual effect, but the product is very expensive and only used where special high donor support is obtained. Still, the product only covers one mosquito season. We have for years worked on 2 season IRS product by improving the micro encapsulation and formulation process. However, the standard WHO laboratory test method failed to correlate to semi field test methods, and a new screening method was developed where products are applied in test houses as done for bednets but constructed for testing IRS products. Test technology and results are presented.

Emerging & Re-emerging Vector-borne Diseases category

Entomological investigation around four cryptic malaria cases in Apulia region (Italy)

P48

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The presence of competent vectors combined with the changing climatic conditions could increase the risk of local transmission in vulnerable areas resulting from imported gametocyte carriers. Recent Plasmodium vivax malaria outbreaks in Greece and sporadic mosquito-borne cases in other receptive countries of Southern Europe confirm this concern. Accurate investigations are required to provide evidence on the origin of suspected autochthonous infections (induced, introduced, or cryptic malaria). In late September 2017, in Apulia region, health authorities reported four local Plasmodium falciparum cases with onset of symptoms within the same week, without reported travel history to malaria-endemic countries. No blood transfusion, tissue/organ transplantation, intravenous drug use were referred. To assess the presence of potential malaria vectors in Ginosa and Castellaneta rural areas where the four agricultural workers used to live/work, an extensive entomological survey was performed. Methods: From 4th October to 29th November, selected sites (6 livestock farms, 2 patients homes and a sand quarry) located between Apulia and Basilicata were monitored by collecting Anophelinae adults and larvae. When possible, mosquito species were identified morphologically whereas a molecular approach was performed for the *Anopheles maculipennis* complex. Results: 239 mosquitoes were collected from 8/9 sites: 144 (60,2%) were identified as *Anopheles labranchiae*; 69 (28,9%), as *Anopheles superpictus* and 26 (10,9%) were Culicinae. *An. labranchiae* occurred in four sites of Apulia (including around one patient s home). In Basilicata *An. superpictus* was recorded in 3 farms and, interestingly, it was found in sympatry with An. labranchiae in the two innermost farms. Conclusion: The survey showed the presence of *An. labranchiae* and *An. superpictus*, historical malaria vectors in Southern Italy before eradication. Susceptibility of these species to imported afrotropical *P. falciparum* remains poorly understood. These results, together with thorough molecular epidemiology investigations, excluded iatrogenic as well as local transmission modes, describing these cases as cryptic.

P49 Stilbene ST18 and the terfenyl TR4: in vitro activity against *Trypanosoma cruzi*

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Chagas disease and Leishmaniasis are life-threatening illnesses caused by the protozoan parasites Leishmania spp. and *Trypanosoma cruzi*, respectively. They are known as neglected diseases due to the lack of effective drug treatments and the scarcity of research work devoted to them. Therefore, the development of novel and effective drugs is an important and urgent need. Natural products are an important source of bioactive molecules for the development of new drugs. Recently, studies showed an interesting cytotoxic action of stilbene ST18 and the terfenyl TR4 compounds in Leishmania. In this study, we evaluated the in vitro trypanocidal activity of st18 and TR4 and Nifurtimox, drug used for the treatment of Chagas disease. In addition, we evaluated the compounds action in infected macrophages with *Trypanosoma cruzi*. Results showed that three compounds exhibited significant activity against *Trypanosoma* compared to Nifurtimox. St18 and Tr4 compounds inhibited *Trypanosoma* growth with IC50 values of 4.5 and 32 μM, respectively. The treatment of infected macrophages with trypanosomes with the IC90 compounds showed a reduction of infection compared to control: St18 reduced the infected cells to 52 %, Tr4 reduced the infected cells to 63%. In conclusion, these news compounds could be considered as promising lead drugs for the development of new therapies for the treatment of Chagas disease.

Emerging & Re-emerging Vector-borne Diseases category

Equid piroplasmosis in Italy: changes in seroprevalence according to geographical location

P50

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Equine piroplasmosis is a tick-borne disease caused by the protozoan parasites *Theileria equi* and *Babesia caballi*, still representing a major threat for public veterinary health due to difficulty in diagnosis and various degrees of clinical signs of infection. The present work aimed to provide an overview on the seroprevalence of such pathogens in serum samples from donkeys, horses and mules collected over 2012-2017 throughout Italian territory. A total of 1316 samples, including 887 horses, 422 donkeys and 7 mules, were assayed for the detection of IgG antibodies against *Babesia caballi* and *Theileria equi via* commercial kits. Samples were organized in 3 groups according to geographical location (Northern, Central, Southern Italy groups – n=284, n=54 and n=978, respectively), and prevalence values were compared among the three areas by Chi-squared test ($\alpha = 0.05$). Total prevalence for *T. equi* and *B. caballi* were 33.8 % and 11.7 %. Considering the three geographical locations, prevalence for *T. equi* were 40.7% Northern (n=22), 20.4% Central (n=58) and 37.3% Southern (n=365), whilst for *B. caballi* obtained values were 18.3% (n=52), 27.8% (n=15) and 8.9% (n=87). Chi-squared analysis revealed a significant difference among the three areas both for *T. equi* (χ^2= 29.289, df = 2, P< 0.0001) and *B. caballi* (χ^2= 32.961, df = 2, P< 0.0001) prevalence. Data from the study confirmed previous reports on the higher prevalence of *T. equi* over *B. caballi* in Italy, although values from Northern and Central Italy were lower than previously reported. Novel studies are needed to identify regional factors determining significant differences in *Theileria equi* and *Babesia caballi* prevalence throughout Italy.

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P51 Molecular characterization of k26 gene of *Leishmania infantum*, isolate by human patients from Sicily region.

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In the present work, we explored the genetic polymorphism of Leishmania isolates from twenty-five cases of human Leishmaniasis: two cases of visceral Leishmaniasis (VL) and twenty-three of cutaneous Leishmaniasis (CL). The characterization is carried out in comparison with twenty-five human isolates of *Leishmania* and one reference strain, *L. infantum* MHOM/TN/80IPT1 (MON-1). MON-1 is the most common zymodeme responsible for Leishmaniasis in Italy. The aim of the study is to genotype Leishmania isolates from Sicily by PCR, analyzing size polymorphism of K26 gene to discriminate between MON-1 and non MON-1 zymodemes. K26 is a protein belonging to the Hydrofilic acylated surface protein B (HASPB) family. It is characterized by repeated aminoacidal domains and shows polymorphisms. The k26 polymorphism of MON-1 zymodeme is determinate in the size of 626 bp. The analysis show that all the 25 isolates belong to the *L. infantum* species, in particular the product size of 626 bp is detect in six patients affected by cutaneous Leishmaniasis. The molecular tools applied in this study can constitute a helpful support for parasite tracking and for a better understanding of the epidemiological evolution of Leishmaniasis.

Emerging & Re-emerging Vector-borne Diseases category

Alkhurma hemorrhagic fever virus RNA in *Hyalomma* ticks infesting migratory birds

P52

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Alkhurma hemorrhagic fever virus (AHFV) was identified in 1995 after an outbreak of viral hemorrhagic fever in the Jeddah Province in Saudi Arabia. The clinical manifestation of Alkhurma hemorrhagic fever (AHF) includes malaise and influenza-like symptoms followed by encephalitis and ecchymosis. The case fatality is reported to be as high as 25% but could be lower since mild cases probably are undiagnosed. An African origin has been suggested for AHFV as well as ticks being potential vectors and goats and sheep potential reservoirs for the virus. AHF is endemic in several western provinces of Saudi Arabia and the case frequency is increasing in the region. Clinical cases have been reported in Africa near the Egypt-Sudan border and seropositive patients and ticks with AHFV RNA have been detected in Djibouti, indicating a possible wider geographical area for the virus. In the light of these findings, we investigated whether ticks infesting migratory birds en route from Africa to Europe and Asia during spring migration carry AHFV. Ticks were collected from northward migratory birds caught at eight bird observatories in the Mediterranean basin during the spring of 2009, 2010, 2014, and 2015 and screened for AHFV by qPCR. In total 36,893 birds were caught and 1,771 ticks collected. AHFV RNA was detected in six (n=6) *Hyalomma marginatum* sensu lato ticks (likely *H. rufipes*) collected from two Sedge warblers (*Acrocephalus schoenobaenus*), one Eastern woodchat shrike (*Lanius senator niloticus*), and one Western yellow wagtail (*Motacilla flava*) caught in Greece and one Common redstart (*Phoenicurus phoenicurus*) caught in Turkey, raising concerns for dissemination of the virus to novel areas. Increased surveillance and further investigations of the ecology and the modes of transmission of AHFV, including the role of *H. marginatum* s.l. ticks as vectors and passerine birds as reservoirs or distributors of potentially infected ticks, are needed.

P53 The role of small rodents in transmission of vector-borne pathogens in Lithuania

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Small rodents are considered reservoir hosts for vector-borne pathogens. However, there still is a lack of knowledge on the geographical distribution, genetic diversity and the reservoir hosts for these microorganisms in Baltic countries. This study aimed to investigate the prevalence and genetic diversity of vector-borne bacterial pathogens *Borrelia* spp., *Rickettsia* spp. and *Bartonella* spp. in small rodents. Small rodents of 8 species (*Apodemus flavicollis, A. agrarius, Mus musculus, Micromys minutus, Myodes glareolus, Microtus oeconomus, M. agrestis* and *M. arvalis*) were captured withlive-traps in different locations of Lithuania during 2013 2016.. DNR was extracted from the spleen samples of small rodents. Different genome regions of pathogens were amplified with subsequent sequencing of fragments obtained during PCR amplification. The overall prevalence of *Borrelia* spp. in rodent was 25%, Rickettsia spp. - 34.5 % and *Bartonella* spp. - 23.7%. *Borrelia* spp. were detected in six small rodent species (*M. arvalis, M. agrestis, M. glareolus, A. flavicollis, A. agrarius*). The genotyping of ospA gene and sequence analysis of partial 16S (rrsA) 23S (rrlA) ITS region of *Borrelia* indicated the presence of two *Borrelia* species: *B.afzelii* from the *Borrelia* burgdorferi sensu lato complex, and *B.miyamotoi* belonging to the relapsing fever group. The sequence of gltA and 17kDa protein coding gene analysis presence Rickettsia helvetica from *A. flavicolis, M. minutus*, and *M. glareolus*. Sequence analysis of gltA, rpoB, groEL and ITS region genes of Bartonella isolates demonstrated the presence of *Bartonella grahamii*, *Bartonella taylorii* and *Bartonella* sp. from the *Bartonella rochalimae* group. Phylogenetic analysis based on each of the targets demonstrated the presence of different *B. grahamii* and *B. taylorii* strains in small rodents. The findings of our study demonstrated the importance of small rodents as potential reservoirs of zoonotic pathogens in Lithuania.

Emerging & Re-emerging Vector-borne Diseases category

Investigation of tick-borne pathogens in *I. lividus* in Lithuania

P54

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Ixodes lividus (Koch, 1844) ticks are specific parasites of the sand martin *Riparia riparia* (L.). The distribution range of *I. lividus* covers Europe and Asia, and it can be found wherever there are sand martin nests. However, until now there have been no reports on the presence of this tick in Lithuania. The aim of this study was to analyze the prevalence of Rickettsia spp. and Borrelia spp. In *Ixodes lividus* ticks in Lithuania. A total of 47 nests were collected in 3 different colonies of sand martin in the central part of Lithuania in 2013 and 2015. In total, 2,770 ticks were found and identified as *I. lividus* based on morphological characteristics and sequence analysis of the tick mitochondrial 16S rRNA gene. Phylogenetic analysis of 16S rRNA sequences indicates four genotypes of *I.lividus* transported by sand martin in the Baltic region. Rickettsia pathogens were detected in 90.6% of pooled larvae samples and in all of examined 7 nymphs, 4 females and in one male specimen. Sequence analysis of partial gltA and 17kDa antigen genes showed 99-100% identity with the corresponding Rickettsia vini sequences obtained in Gen Bank. None of tested *I. lividus* ticks was infected with *B. burgdorferi* s.l. pathogens. This study represents the first record of *I. lividus* from sand martin nests in Lithuania. The presence of *R. vini* is reported for the first time in Lithuania.

P55 Lumpy skin disease: improvement of diagnostic methods and assessment of the vector potential of insects of Switzerland

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Lumpy skin disease (LSD) represents an OIE listed viral disorder of cattle as the consequences of infection lead to high economic losses. The occurrence of LSD was restricted to the African continent until 1989. In 2015, the first outbreak in Europe was reported in Greece, and subsequently several countries of the Balkan region, including Serbia, Kosovo, Bulgaria, Romania, Albania, Montenegro and FYR Macedonia, were affected in 2016. As a result of intensive vaccination campaigns, only Greece and FYR Macedonia were still reporting LSD cases in 2017, and Turkey reported cases in 2018.The role of arthropods in the transmission of LSDV has been postulated based on circumstantial evidence and mathematical models, with the biting fly *Stomoxys calcitrans* being the main incriminated vector. The explosive spread of LSD in the Balkan countries, however, was reminiscent to the recent spread of the *Culicoides*-transmitted bluetongue virus in Europe. In a two-year project (2018-2020) funded by the Swiss Federal Food Safety and Veterinary Office (FSVO grant nr. 1.18.d), the following topics are addressed: a) collection of clearly defined diagnostic material from experimentally infected cattle, b) development and evaluation of serological assays; c) assessment of current molecular biological methods as well as virus isolation; d) transmission efficiency (mechanical, biological) for LSDV of field-collected mosquitoes, *Culicoides* spp. and *Stomoxis calcitrans* under realistic climate conditions; d) population dynamics of biting flies (*S. calcitrans*, *Haematobia* spp.) which due to their feeding behaviour (very frequent and interrupted blood meals) can act as immediate mechanical vectors.

Zoonotic *Bartonella* species in arthropods collected from pet dogs in Sicily

P56

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Cat Scratch Disease (CSD) is an infectious disease whose etiologic agent is *Bartonella henselae*, although novel case reports demonstrated the possible involvement of other *Bartonella* species in CSD. Furthermore, apart from the detection of DNA of zoonotic *Bartonella* species in the classical vector *Ctenocephalides felis*, other studies suggested that various arthropods may also play a role in *Bartonella* transmission. The present study aimed to analyse the presence of *Bartonella* spp. DNA in ticks and fleas collected from dogs in the Sicilian territory. A total of 25 fleas and 112 ticks were collected from pet dogs, and identified at species level by morphological analysis; from each sample, DNA was extracted prior to analysis with conventional PCRs targeting heme-binding protein, 16S-23S ribosomal RNA intergenic spacer and 16S rRNA genes for detection of *Bartonella* DNA. PCR products were visualized on agarose gel and sequenced. Morphological analysis revealed 23 *C. canis* fleas, 2 *C. felis* fleas and 112 *Rhipicephalus sanguineus* ticks. Two PCR positive products were obtained only from one *C. felis* sample and one *C. canis* sample, whose sequencing revealed the *B. henselae* and *B. rochalimae* species, respectively. The presence of *B. rochalimae* in *C. canis* fleas species collected from dogs in Italy represent an important finding considering the emerging pathogenicity of such *Bartonella* species and that in the majority of reports only *C. felis* was found positive to *Bartonella* DNA. Results of the present work suggest the need for a potentiated vector control in the territory in order to determine the role of possible novel arthropod vectors in the transmission of *Bartonella* spp.

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P57 Do the LLINs really protect from malaria at community level in areas of high transmission?

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Long Lasting Insecticidal Nets (LLINs) are among the most effective strategies in malaria vector control, halving the population at risk in sub-Saharan Africa since 2010. Despite this success, in some hyperendemic African countries, such as Burkina Faso, the annual incidence and the entomological infection rates are still very high. Evidence from a hyperendemic LLIN-protected village of Burkina Faso (Goden, Ouagadougou area) in 2011-2012 showed high Sporozoite Rates in major vector species (SR>7%), despite very low Human Blood Index (HBI<16%). We here report a similar picture from another LLIN-protected village in the same area (Koubri), where *Anopheles funestus* shows consistently high SR (8.6%) associated to a very low HBI (5.8%) associated to a strong exophilic behavior, suggesting again lack of community level protection despite massive LLIN implementation. We thus carried out a second entomological survey in Goden in 2015 by Human Landing Catches at three different time points during the night (21-22; 00-01; 03-04) and collected a total of 1,955 *Anopheles gambiae* complex specimens (*A. coluzzii* 55%, *A. arabiensis* 44%, *A. gambiae* 1%, without significant differences indoors vs outdoor, nor among time points). The median number of host-seeking mosquitoes/hour/person was 23.5 and the SR among the 692 specimens analysed so far was 6.2% (without significant differences in indoors vs. outdoors), corresponding to an entomological inoculation rate in equal to 1.46 infective bites/hour. Overall, these results highlight that, despite the large-scale usage of LLINs in the area since 2010, the mosquito population is still present at high densities and highly infected, probably as a consequence of shifting in biting behaviour of vector species. This leads to a non-negligible risk of malaria transmission to people either sleeping unprotected by a bednet indoors or exposed to mosquito bites outdoors and may explain the reduced effect of LLIN implementation in hyperendemic regions of sub-Saharan Africa.

Mosquito fauna (Diptera: Culicidae) and its vector potential along the Danube and black sea shore in Romania

P58

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The WNV circulation is endemic on large territories in Romania. The human infections appear continuously as sporadic cases and occasionally severe outbreaks and represent an important public health problem. The investigations for the evaluation of the emergence risk with the aim to apply preventive control measures were carried out in eight counties along the Danube and Black Sea shore included in the territory where the epidemic of severe human neurological infections with WNV took place in 1996, having as vector *Culex pipiens* species. In the period 1997-2017, a proportion of 31.6 % from the cases of neurological infections registered in Romania appeared in only 8 counties along the Danube and Black Sea shore. These areas include various natural and anthropic ecosystems favourable for the development and spread of mosquitoes. There were detected 41 mosquito species in these areas, including species that could be vectors of WNV and are spread in abundant populations. Virus isolation from *Culex pipiens* females (by inoculation on suckling mice) and virus identification as WNV (by a multi-level protocol based on RT-PCR, nested and sequencing) were performed. The WNV was detected (by RAMP test and/or RT-PCR) in females, including hibernant ones, and males of *Culex pipiens*, and females of *Coquillettidia richiardii*, *Ochlerotatus caspius* and *Anopheles maculipennis* s.l. WNV is present in mosquito vectors in this territory, it can pass the winter inside the hibernant mosquitoes and can be transmitted from a mosquito generation to the next one. In addition, the high seroprevalence of specific IgG antibodies against WNV detected in its vertebrate hosts (domestic and wild birds and horses) by specific ELISA techniques confirmed the permanent enzootic circulation of the virus on territories along the Danube and Black Sea shore. It has been demonstrated the permanent risk of its transmission to humans. The permanent surveillance of WNV endemic circulation and the implementation of integrated mosquito control programmes in the key areas at risk in Romania are adequate decisions for public health.

P59 Midges and mosquitoes as potential vectors of arboviruses in Etosha National Park, Namibia

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An entomological survey aiming to determine presence and abundance of mosquito and *Culicoides* species in the Etosha National Park (ENP), and detect viruses that they could transmit, was carried out from 2014 to 2016. A total of 290 *Culicoides* and mosquito collections were made in 11 sites located in the ENP. Overall, 104 *Culicoides* catches were performed by using blacklight traps, and more than 240,000 biting midges were identified, using the wing morphology. *Culicoides schultzei* resulted the species most commonly found (61%), followed by species of Schultzei group (21%), *Culicoides nivosus* (7%), *Culicoides imicola* (4%), *Culicoides subschultzei* (3%), and other species (4%). A total of 929 pools (23,561 midges) were sorted and tested for Bluetongue virus (BTV), Epizootic Haemorragic Disease virus, African Horse Sickness virus (AHSV), Equine Encephalosis virus and Schmallenberg virus. Nineteen pools resulted positive to BTV: 11 were composed by *C. imicola*, 1 by *C. nivosus*, 5 by *C. schultzei* and 2 pools by species belonging to the Schultzei group. Moreover one pool of C. imicola was also found positive for AHSV. One hundred eighty-six adult mosquito collections were performed by using BG-sentinel and CO2-light traps. More than 15,000 *Culicidae* were identified to genus or species level, and a total of 605 pools were sorted and tested for West Nile virus (WNV), Usutu virus, and Rift Valley Fever virus. In addition 355 pools were also tested for Zika virus. Of the 605 pools tested, 13 were positive for WNV, composed by *Aedes*, *Anopheles*, *Culex pipiens* s.l. and *Culex* sp.. This study evidenced the presence of enzootic cycles of arboviruses which are recognized as significant agents of human and animal diseases, and suggests the importance of having in place an entomological surveillance system capable of monitoring the arbovirus circulation in the ENP.

Comparing molecular methods for diagnosing the infection of ticks by Crimean-Congo hemorrhagic fever virus

P60

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Crimean-Congo haemorrhagic fever virus (CCHFv) is the most widespread tick-borne virus in the world. This zoonotic agent circulates unnoticed in animals and constitutes a threat to public health with a fatality rate ranging 10 to 40% in humans. Since the first CCHFv sequence data were published, many publications have demonstrated the high diversity of CCHFv, being this fact a complication for its detection.

In the last two years new human cases have emerged in Spain. The two primary reported cases were linked to tick bites and had fatal outcomes. Furthermore, the detection of CCHFv in its main vector in southern Spain, *Hyalomma lusitanicum*, shows the importance of deepening on the epidemiological status of CCHFv as a first step to prevent transmission.

Therefore, with the objective of setting up the most appropriate molecular tools for diagnosing CCHFv in ticks and estimate the interference of potential endogenous nairoviruses, ticks were collected from 16 populations of wild ungulates in south-central Spain in 2017/2018. Two hundred and fifty-seven *Hy. lusitanicum* ticks were analysed by different PCRs: i) a generic RT-PCR for the genus *Nairovirus*; ii) two nested conventional RT-PCRs for CCHFv; and iii) a multiplex real-time RT-PCR designed for the universal detection of all currently known CCHFv genotypes.

From the 257 ticks analysed, 87 (33.9%; 95%CI: 28.3-39.8%) were positive to the generic *Nairovirus* genus RT-PCR whereas 5 (1.9%; 95%CI: 0.8-4.5%) were positive in the conventional PCRs for CCHFv and none (0%) was positive in the multiplex real-time RT-PCR. Although still pending on sequencing results, the prevalence of nested PCR positive ticks is within the reported values in central Spain. The low prevalence of PCR positive ticks is in agreement with to the low reported human exposure and the low incidence of cases reported in the country.

P61 Exposure of Iberian red deer to Crimean-Congo haemorrhagic fever virus

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Crimean-Congo haemorrhagic fever virus (CCHFv) has caused two fatal primary cases in Spain since it was reported for the first time in 2010. These cases may represent the tip of the iceberg of the emerging and silent circulation of CCHFv in Iberia where the virus may be more widespread than thought and threaten to emerge in new areas.

We herein approached the spatial distribution of CCHFv by analysing the exposure to CCHFv of one of the main hosts for its vectors, the red deer (*Cervus elaphus*). A cross-sectional survey was designed to analyse red deer sera collected from 64 Spanish and 5 nearby Portuguese locations. 1,444 sera were analysed by a competitive ELISA developed using a His-tagged recombinant N protein of CCHFv strain Kosovo Hoti as antigen. Sera that were clearly positive or showed an inconclusive result were retested. Finally, sera were classified as positive if: i) clearly positive twice; or ii) doubtful in one round and positive in the other. The rest were considered as negative.

373 sera (25.8%; 95%CI: 23.6-28.2) were clearly positive. 43 of the 69 (62.3%) surveyed locations had at least 1 seropositive individual. In 8 locations over 50% of the red deer were exposed whereas seropositivity ranged 30-50% in 11 additional locations. The highest rates were observed in the southwestern quarter of Iberia where *Hyalomma* ticks are abundant. Some few positive samples were found in northern Spain.

CCHFv seems to be more widespread in Iberia than expected but the rate of infected *Hyalomma* ticks is low in high seropositive locations and only two clinical human cases have been reported. The high exposure of deer to ticks over lifetime may underlie the observed high seroprevalence values. The wild ungulate-tick binomial needs to be further explored in the region in relation to the emergence of CCHFv.

Emerging & Re-emerging Vector-borne Diseases category

Toscana virus and *Phlebotomus perfiliewi* (Diptera: Psychodidae) parallel surveys in Serbia (Vojvodina Province); First serological study about phleboviruses in Serbia

P62

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Toscana virus (*Phenuiviridae*, *Phlebovirus)* (TOSV) is transmitted by the bite of infected Phlebotomine sand flies. *Phlebotomus perfiliewi* and *P. pernicious* are the main vectors although it is likely that TOSV can also be transmitted by other species such as *P. neglectus* for instance. TOSV causes meningitis during the warm season, and it is one of the most medically important vector-borne disease distributed in the Mediterranean area. Seroprevalence studies have also confirmed that TOSV was more actively circulating in the southern Mediterranean such as North Africa than in southern Europe. However there is very limited data in the Balkans. Our aim was to measure the presence of TOSV specific antibodies in human sera samples collected from Serbia (Vojvodina Province). At the same time, sand flies were collected and tested for viral RNA presence, and the results showed that there was no TOSV's RNA in the tested sand flies. A total of 862 human sera were collected from 31 different locations of Vojvodina province from different age and gender groups and tested with virus neutralization test (VNT): 1.04% were positive, corresponding to 6 of the 31 locations were the samples originated. The presence of P. *perfiliewi* was found in 3 of the 6 localities where TOSV antibodies were detected. In our study, there was no association between the exclusive presence of *P. neglectus* and sera containing antibodies against TOSV.

P63 Susceptibility of *Aedes aegypti* to essential oils from *Ocimum micranthum*, *Ocotea quixos* and *Piper aduncum*

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Aedes aegypti mosquito, widely spread in tropical and subtropical areas throughout the world is considered the main arbovirus vector of Yellow fever, Dengue, Chikungunya and Zika viruses. Control efforts have been implemented since decades worldwide aimed to reduce its impact on human health. The recent reduction of chemicals available for vector control due to their negative impact on environment and human health and the increase in mosquito resistance to insecticides have driven the research community to identify and evaluate sustainable alternatives to synthetic insecticides. In this study the potential larvicidal effect of essential oils extracted from the tropical plants *Ocimum micranthum*, *Ocotea quixos* and *Piper aduncum* were tested in vitro. GC and GC-MS analyses showed that the main compounds were eugenol (18.6%), 1,8-cineole (39.2%) and dillapiole (48.2%), respectively. Susceptibility to essential oils was carried out according to WHO protocol. After 24 hours the mean percentage mortality ranged from 2.7 to 100% for *P. aduncum*, from 2.2 to 100% for *O. micranthum*, and from 2.9 to 100% for *O. quixos*; after 48 hours a further slight mortality was observed. The highest potential was displayed by *P. aduncum*, followed by *O. micranthum* and *O. quixos*, with an LC50 of 25.73, 69.29 and 75.51 ppm, respectively. The rapid and effective larvicidal activity of all these three oils could be considered promising, considering also the easy possibility of local cultivation in tropical and subtropical areas and the simple technology for their large-scale preparation and production. Further studies are needed to evaluate the individual components and their single larvicidal activity in each oil.

Moreover these compounds should be tested as mosquito larvicides also against other species of health concern like *Ae. albopictus* and *Culex pipiens*.

Monitoring protocol in Chania Regional Unit and hatching behavior

P64

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Invasive mosquito species (IMS) threaten ecosystems, habitats or native mosquito species and they can also pose a big threat to human health. In Greece the only IMS that has been reported during the last fourteen years is *Aedes albopictus*. The first record was in 2004 at Corfu island, and over the following years *Ae.albopictus* expanded in several regions of Greece and in 2014 it was recorded for the first time in Crete by Hellenic Center for Disease Control and Prevention (HCDCP). LIFE CONOPS (LIFE12 ENV/GR/000466) project managed to establish a permanent oviposition monitoring system in Crete, from 2015 until now. In this context, a hatching behavior protocol was designed and implemented in 2017. The main objective of the first year (2017) of the protocol was to observe the density and the hatching behavior of the *Ae. albopictus* population in Chania Regional Unit. A monitoring system was developed with a total number of 52 ovitraps and 8 BG sentinel, operated weekly, placed at the airport of Chania and in villages approximately up to 3km far from the airport. All eggs were transferred weekly to Benaki Phytopathological Institute for hatching. At the same time, a geodatabase was developed to record the number of adult mosquitoes and eggs, which was updated weekly. In addition, ordinary Kriging was used to interpolate the monthly average number of eggs per ovitrap in the study area, from March 2017 to December 2017. In total 730 and 42.931 number of *Aedes albopictus* adults and eggs were collected respectively. The highest number of the adults and eggs of *Aedes albopictus* was collected in the period from August to September. According to our results we have a successful hatching protocol ranging from 95% to 100% depending on the sampling period. Our main objective of the future study is to continue to observe and compare the hatching behavior of *Ae. albopictus* from several populations allover Greece.

P65 The state of the art of the exotic mosquito species (Diptera: Culicidae) in Belgium: new invasion pathways detected for Belgium

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In July 2017, a three year monitoring project (MEMO) aiming at detecting and evaluating the occurrence and invasion phase of exotic mosquitoes (EMS) in Belgium started. Adult traps and ovitraps were placed and larval sampling was conducted at 23 locations. These locations were selected based on their high potential of introducing EMS. DNA-based techniques are used to verify the morphological identification of 5% of the collected mosquito specimens. In 2017, 2622 adult mosquitoes, 2916 larvae and 650 eggs were collected. In total 15 species (or complexes) belonging to 5 genera (*Aedes, Anopheles, Culex, Culiseta and Coquillettidia*) were identified. Morphological and DNA-based identifications were in agreement. Furthermore, DNA sequence data confirmed that adults of the *Anopheles maculipennis* complex were *Anopheles messeae* and that both *Culex pipiens* biotype *pipiens* and biotype *molestus* were collected. *Culiseta longiareolata* was for the first time observed in Belgium. EMS made up 2% of all collected specimens. Three EMS, *Aedes japonicus*, *Aedes koreicus* and *Anopheles pharoensis* were intercepted in 2017. In the first six months of 2018, *Aedes albopictus* was intercepted at four locations. Previous monitoring projects indicated that *Aedes* mosquitoes entered Belgium via import of lucky bamboo plants and of tyres. New for Belgium is the interception of *Aedes albopictus* and *Aedes japonicus* along the border with France and Germany, respectively. And the interception of *Anopheles pharoenis* in a cargo airport. In the first case, a possible natural spread from these countries may have occurred. In this perspective, the understanding of the invasion process of the three exotic *Aedes* species, based on field observations and population genetics, will provide information on their invasion phase (introduction, establishment or spread) and seasonality which will be essential to guide surveillance and control.

Exotic mosquito species surveillance in Belgium: an overview from 2007 – 2018

P66

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The introduction and establishment of exotic mosquito species (EMS) and sporadic outbreaks of autochthonous *Aedes*-borne disease cases in South European countries emphasize the importance of EMS surveillance. The Belgian government set-up a nationwide inventory of mosquito biodiversity (MODIRISK project) between 2007 and 2010. This was an essential step towards understanding the risk of diseases transmitted by mosquitoes in Belgium. In 2012, a next step was set by implementing EMS surveillance at 22 points of entry (PoE) to evaluate the guidelines of the European Centre for Disease Prevention and Control (ECDC) (EXOSURV project). The main aim was the early detection of EMS introductions into Belgium. This routine surveillance continued between 2013 and 2015 at 12 PoE and in 2016 at three PoE (FASFC project). Subsequently, a three-year active monitoring project of EMS (MEMO project) started in July 2017 at 23 PoE. One of the objectives is to make recommendations for a future, long-term, cost-effective monitoring plan for EMS in Belgium. In total four EMS were collected between 2007 and 2018. Two of them are locally established, *Aedes koreicus* in Maasmechelen (confirmed yearly between 2008 and 2018) and *Aedes japonicus* in Natoye (confirmed yearly between 2009 and 2014 and in 2017 and 2018). *Aedes japonicus* was also collected for the first time in Eupen at the border with Germany in 2017. The two other EMS do not seem established yet. *Aedes albopictus* was intercepted in 2013 2016 and 2018 alternately at five different PoE. *Anopheles pharoensis* was collected only in 2017. These results indicate that a permanent, structured, national EMS surveillance programme in Belgium is needed instead of recurrent short-term projects with different funding and limitations. Moreover, assistance of local partners and a multi-faceted approach (including e.g. citizen science) will be crucial to keep future surveillance sustainable and cost-efficient.

P67 The efficacy of a cheap adult trap to monitor *Aedes albopictus*

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The introduction of the Asian tiger mosquito in Italy caused the need of a completely different approach to mosquito control because of the different behaviour respect to the autochthonous species *Culex pipiens*. The struggle against the larval instars is the base of the fight against *Aedes albopictus* but in some cases the adulticidal intervention becomes necessary. Spraying on vegetation is an effective tool to suppress the population of tiger mosquito but it has strong side effect killing several non-target arthropod species, so this method should be used just in really necessary cases. The most commonly employed monitoring systems to evaluate the level of infestation are the ovitraps but it is the Human Landing Rate the method that provides more reliable results but meantime it is laborious since it should be performed always by the same person and at the same hour because mosquitoes considerably vary their activity during the day. Many different adult mosquito traps are available on the market but they are not effective against Asian tiger mosquito or very expensive and cannot be left working in public area for the risk of damaging or stealing. In this project the ovitrap and the human landing technique are compared to a cheap trap created by a spinoff of Mahidol University in Bangkok. It is a trap made of waterproof cardboard containing water as attractant and a glued paper to trap adults. This trap, which was created to monitor *Aedes aegypti*, is easy to assemble, lightweight to transport and has an affordable cost. These three monitoring systems are compared inside four public parks in Padua with weekly evaluations.

The Swiss national mosquito network: structure and data management

P68

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Since their appearance in Europe, invasive *Aedes* mosquito species represent a threat to public health and there is need for surveillance and control. Beside Canton Ticino, where invasive mosquitoes are surveyed since 2000, also other Cantons now start to face the problem. Due to the lack of capacities at local level in 2016 Guidelines for the surveillance and control of invasive *Aedes* mosquitoes in Switzerland were published. Following this publication a national reference network for the surveillance of invasive mosquitoes was set up, in order to coordinate the activities across Switzerland and link them with the neighbouring countries, as Mosquitoes are a cross-border issue. The network is founded by the federal government and consists in a coordinating office (SUPSI), the national surveillance programme (Swiss TPH), four regional reporting units (SUPSI, Swiss TPH, City of Zurich, University of Lausanne) and the Swiss Centre for Cartography of the Fauna (SZKF/CSCF). The aim of the network is that all data on the presence and distribution of invasive *Aedes* mosquitoes collected through surveillance programmes and by reports from the citizens are recorded in a systematic manner. All collected data pass through a quality check by the coordinating office before being entered in the national data base hosted by the SZKF/CSCF. These data are then made available to various stakeholders at three levels of resolution: a low resolution level will be accessible to the broad public, a higher definition level to the Cantonal and Federal authorities and the raw data to the owner who collected them. All data will be referenced, in order to be accessible for scientific purposes to third parties after approval of the owner. Here, we will present the structure of the national reference network and how the data flow will be organized and managed.

P69 The first record of *Aedes japonicus* in Posavina region, Bosnia and Herzegovina

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Aedes japonicus (Theobald, 1901) is widespread species, which invaded several European countries. So far, it has been detected in Austria, Belgium, Croatia, France, Germany, Hungary, Italy, Netherlands, Slovenia and Switzerland. It is considered a potential vector of West Nile Virus (WNV), which circulates through most of Balkan countries including Bosnia and Herzegovina. The present study was carried out to identify introduction and distribution of invasive mosquito species in the Posavina region, located in north-eastern part of Bosnia and Herzegovina during the summer part of the season 2017. Monitoring was conducted by use of ovitraps in five selected municipalities bordering Croatia. Additionally, ten tire repair services along with all other potential breeding sites in vicinity to the ovitraps were inspected (natural and artificial water recipients) and included in the monitoring program. Samples with immature stages were reared to adult mosquitoes. Morphological identification confirmed the presence of *Culex pipiens* complex and *Ae. japonicus* in the prospected region. Two municipalities were positive to the presence of *Ae. japonicus*. Although majority of samples collected by ovitraps consisted of *Cx. pipiens* complex, it was observed that immature stages of *Ae. japonicus* did not coexist with *Cx. pipiens*. *Aedes japonicus* was not recorded previously in Bosnia and Herzegovina. Considering the fact that this invasive species has already adapted it`s development to temperate climate conditions of Europe, including the neighbouring Croatia, the spreading of *Ae. japonicus* in a wider area of Bosnia and Herzegovina may be expected.

Updated distribution of invasive *Aedes* mosquitoes in the Upper Rhine Valley

P70

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The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), is one of the world's most invasive species. In addition to its remarkable invasive capabilities, it is an important vector of dengue and chikungunya viruses. The Upper Rhine Valley extends across three countries (France, Germany and Switzerland) and has been colonised by this species since 2014. Moreover, two additional invasive mosquitoes *Ae. japonicus* (Theobald) and *Ae. koreicus* (Edwards) have also recently been recorded. Daily movements of people and mosquitoes within this cross-border area led us to consider the Upper Rhine Valley as a region sharing the same potential health risk. Due to (i) the similar exposure to invasive mosquitoes, and (ii) the wish to consider the issue at the cross-border level as a good way to improve surveillance and prevention, experts from the three neighbouring countries initiated the TIGER project, the Tri-national Initiative Group of Entomology in Upper Rhine Valley. Within the framework of the operational programme INTERREG V Upper Rhine, the TIGER project aims to provide scientific and technical support to administrations and institutions for the evaluation and management of the health risk related to invasive mosquitoes. At the surveillance level, this means standardisation of a cross-border ovitrap network and real-time sharing of the collected data. While the project has just been launched, we will present here the latest data on the distribution of invasive *Aedes* species in the Upper Rhine Valley.

P71 Three years of Asian tiger mosquito (*Aedes albopictus*) monitoring in the region of Bohemia, Czech Republic

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In 2016-2018, the monitoring of possible introduction of an invasive mosquito species, the Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) (Diptera, Culicidae), was conducted in the Bohemia, Czech Republic. The focus was placed on local major traffic arteries (motorways D1, D3 and D5 and an expressway E49), which connecting Southwestern European and Balkan countries, infested by *Ae. albopictus*, with the Czech capital Prague. In total, more than 100 ovitraps were placed on 16 study sites - close surroundings of refueling gas stations and neighbouring parking lots. During 2016, no introduction of the tiger mosquito was proved. In 2017, totally eight specimens of *Ae. albopictus* were collected at the ovitrap site near Mezno/Mitrovice, Central Bohemia on D3 motorway and other two specimens were recovered more than 200 km far away at the gas station near Rozvadov, West Bohemia on D5 motorway, close to Czech-German cross border. Even though the introduction of this mosquito into the Czech Republic is known since 2012 from surroundings of Mikulov town (South Moravian Region), these records were the first in the region of Bohemia. Moreover, this study shows the potential of *Ae. albopictus* to occur basically anywhere within the area of the Czech Republic.

A survey for *Culicoides* biting midges in the Republic of Macedonia, 2016-2017

P72

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Despite the occurrence of several bluetongue epidemics, *Culicoides* fauna has been not investigated in the Republic of Macedonia. Within the framework of the Vector Net project and thanks to a collaboration between the F acuity of Veterinary Medicine-Skopje, the Istituto Zooprofilattico Sperimentale dell Abruzzo e de! Molise (IZSAM, Italy) and the Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD, France), an entomological survey was carried out to determine the *Culicoides* species composition and abundance. Light-trap catches were performed on cattle farms in seven municipalities between May and December 2016, and May and August 2017. More than 100 collections were analyzed according to the protocols of the OIE and National Reference Laboratory for Bluetongue (IZSAM), and a total of 34, 102 *Culicoides* were identified. *Culicoides obsoletus/scoticus* (79.13%), *C. punctatus* (9.49%) and *C. newsteadi* (3.32%) were the most abundant species found in all the monitored sites. *Culicoides pulicaris* and *C. lupicaris* were present with a relative abundance of less than 1%. Few specimens of *C. dewulfi* and *C. imicola* were also identified together with two more species, recently described the literature, namely *C. paradoxalis* and *C. bysta*. In the areas surveyed, *Culicoides obsoletus/scoticus* was the most widespread and abundant species observed (n=26,987). Four specimens of C. imicola were collected in Gevgelija, at a site close to the Greek border, in 2016. This finding suggests that *C. imicola* is present in southern Republic of Macedonia, but with a very low abundance. However, repetitive sampling (i.e. more collection sites and more collections per site) are needed to better define its distribution and abundance, during the seasonal peak of this species, in late summer. In conclusion, several *Culicoides* species could act as vectors of arbovirnses in the Republic of Macedonia, namely *C. obsoletus/scoticus*, *C. imicola*, C. *dewulfi*, *C. pulicaris, C. newsteadi, C. lupic.aris*, and *C. punctatus*.

P73 Monitoring vessels traveling from West Africa to Hamburg (Germany) for invasive mosquitoes and associated infectious agents

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Hamburg (Germany) is a designated Point of Entry in terms of the International Health Regulations (IHR, WHO 2005). Therefore, following Annex 5 of the IHR, vector control measures are recommended for conveyances from areas, which might potentially allow the introduction of vectors or associated pathogens from endemic countries. However, little information has been published on the species and the magnitude of the actual spread of mosquito vectors via transnational vessels. In a preliminary study in 2016, a Roll-O-Roll-Off-vessel was monitored while travelling from Hamburg via West Africa to Brazil and back to Hamburg. A total of 128 mosquito specimens was trapped, including 8 *Aedes aegypti* and 4 of *Anopheles gambiae*. In the here presented study, several Roll-O-Roll-Off vessels will be sampled after travelling from West Africa and to Hamburg with no stop after leaving the African continent. Biogents2 traps with CO2 are placed in the same locations on the ship, where the preliminary study already detected mosquito specimens (ramp, pantry, navigation bridge). Data logger will monitor the temperature and humidity conditions on the ships. After collection and identification of the trapped mosquitoes, all specimens are investigated for pathogens using molecular detection techniques. The study will be done during the peak of the rainy season in West Africa in August until end of September 2018, and the results are presented on the conference.

Current distribution of the invasive mosquito *Aedes japonicus* (Diptera; Culicidae) in Italy

P74

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The Asian bush mosquito *Aedes (Finlaya) japonicus japonicus* (Diptera: Culicidae) is one of the most invasive mosquito species worldwide and recently invaded several countries of Central Europe. In Italy, it was found for the first time in three sites in Udine province, Friuli Venezia Giulia (FVG) region in 2015. In the following years, a survey was carried out and is still ongoing, to better define its spread. Artificial and natural breeding sites were monitored for larval collection and BG-Sentinel traps for collecting adult mosquitoes. Identification was performed for larvae and emerging adults by morphological and molecular analysis. In total, 40 municipalities were monitored and *Ae. japonicus* was found in 28 (70%). Interestingly, it was found in 2018 in eight sites negative in 2016. *Aedes japonicus* larvae were found in every kind of artificial containers, tires and catch basins, often cohabitating with other species, i.e. *Culex pipiens*, *Cx. hortensis*, *Culiseta longiareolata* and in one case with *Ae. Albopictus* too. Several specimens were also caught by BG-Sentinel traps. The colonized area is hilly or mountainous with altitude ranging from 103 to 1263 m asl. The species is known to be a pest problem and having the vector competence for arboviruses such as Japanese encephalitis, West Nile, Dengue and Chikungunya viruses. Other two invasive species, *Ae. albopictus* and *Ae. koreicus*, are already established in FVG region. Thus, its establishment complicates the current surveillance system requiring well-trained personnel for identification. From a Public Health perspective, a new potential vector of pathogens to animals and humans may represent a challenge for the Health System.

P75 Detection and surveillance of the Asian tiger mosquito *Aedes albopictus* in Portugal, 2017-2018

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The Asian tiger mosquito *Aedes albopictus* is an invasive mosquito originating from the Asia-Pacific region, which is now widespread in the Mediterranean coast and is known to be dispersing in several European countries. This species is of major concern to public and veterinary health because of its vector role in the transmission of several pathogens. In 2017, two introduction events of *Ae. albopictus* were detected for the first time in Portugal. One in a tyre company located in the North of Portugal, Penafiel, and the second, nearby a golf resort in the South, Algarve. A prompt surveillance response to determine dispersal and abundance was locally implemented by the National Vector Surveillance Network (REde de VIgilância de VEctores REVIVE). In the north population, before winter setting (2017/2018), the last *Ae. albopictus* report, a larva specimen, was collected in a tyre on 3 November 2017. On 16 May 2018, the first new report was a larvae identified in a gutter, most probably resulting from the development of overwintering eggs. In Algarve s population, the survey activities started in September 2017 and the first *Ae. albopictus* sample was detected in July 2018. Studies on mitogenome sequence analysis are ongoing to determine population diversity and putative geographic origin. The risk of establishment of *Ae. albopictus* in Portugal is high, since for two consecutive years its presence has been reported at the same locations. Its establishment raises concern for autochthonous mosquito-borne disease outbreaks.

Surveillance and control of mosquitoes in the French Points of Entry under the International Health Regulation

P76

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As described in its revised version of 2005, the purpose of the International Health regulation (WHO, 2005) is to prevent, protect against, control and provide a public health response to the international spread of disease . Regarding vector-borne diseases, the aim is to prevent the dissemination of vectors by implementing surveillance and control measures at points of entry (ports and airports). In France, these measures have been transcribed in the national law in 2013. At the same time, the National Centre for Expertise on Vectors (CNEV) has been mandated by the Ministry of Health to provide guidelines aimed at helping platform managers to design their surveillance and control programs, which is mandatory in regions where *Aedes albopictus* is established. These guidelines focus mostly on mosquitoes which were identified as the vectors with the higher risk of dissemination or introduction. EID Méditerranée intervenes on behalf of six platform managers (four airports and two ports) in southern France. As recommended in the CNEV guidelines, the approach is composed of three steps: (1) description of the geographical and entomological characteristics of the platform, (2) implementation of a surveillance program and (3) definition of vector control measures. The description of the platform consists in the mapping of the potential breeding sites, the inventory of the species occurring by larval and adult sampling all along the year and the identification of potential safety or environmental constraints. Then, the surveillance is based on the use of traps targeting host-seeking and gravid females, with thresholds triggering the implementation of control measures. This surveillance system has allowed to identify the presence of *Aedes albopictus* in all the platforms and to reduce the risk of dissemination by implementing adequate actions. The trap network can also be useful to detect the introduction of invasive species, although no observation has occurred yet.

P77 A study for the effectiveness of education at reducing *Aedes albopictus* breeding sites in urban areas

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The Asian tiger mosquito, *Aedes (Stegomyia) albopictus* (Skuse, 1895) (Diptera: Culicidae), is considered one of the most invasive species in the world presenting a significant expansion in many parts of the globe. It tends to proliferate in small, often man-made bodies of water, largely present in urban private areas. Given the fact that private areas are inaccessible to prefecture s control measures and intense chemical control efforts are of low efficacy, resident-based management (i.e. breeding source reduction) is considered a most effective and affordable mean for controlling of mosquitoes. For this reason, education and community participation are considered crucial for source reduction and mosquito control. In the current study, we employed a KAP questionnaire (knowledge, attitudes and practices of residents), household surveys, and introduced an education campaign to evaluate changes in the abundance of breeding sites among the households of the respondents. The study site was the municipality of Palaio Faliro which is situated southwest of Athens city Centre (Greece). A primary aim of our study was to evaluate the effect of an education intervention on household source reduction. The results revealed that only a minority of residents knew where mosquitoes breed (18.6%) and only 46% felt that residents had any responsibility for managing breeding habitat. Findings from our study suggest that a single visit-inspection of a trained mosquito expert in the households and the inspection of the potential breeding sites in their yards can influence the residents behavior towards source reduction. However, educational interventions alone with printed education material cannot enhance significant community participation and source reduction.

Risk profiles and exotic *Aedes* control policies in the Netherlands

P78

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Four different exotic *Aedes* species have been introduced in the Netherlands to some effect. These species differ markedly in their ecology and hence their survival probabilities. *Aedes aegypti*, *Ae. albopictus*, *Ae. atropalpus* and *Ae. japonicus* all have their quite different introduction stories to tell. Nevertheless, based on their shared oviposition preference and egg survival, they share pathways into Europe to a certain degree. Also our policy responses have been determined to a large extent by these shared pathways. Especially the approach taken towards preventing establishment from *Aedes* species arriving as eggs with used tires, has been the 'golden' standard in *Aedes* prevention in the Netherlands.

In this work I will first focus on what we know about the different pathways over the years and the ease with which incoming mosquitoes spread within our country. Then i will review the response in the light of these differences and show the ways in which we are modifying current policies. In 2018 a risk assessment for *Ae. japonicus* has been written which has led to a modification in the approach that is taken towards this species. The assessment itself mostly constitutes of a literature review of the vector status in the Netherlands and resulted in a decision not to intervene any longer in our only big and permanent established population. While investing in containment (the prevention of population growth and spread) of the population around Lelystad cannot be justified based on the perceived, relatively low epidemiological risk, the quick extirpation of newly detected small introductions is considered warranted.

Pathways into the Netherlands are by no means fixed and their relative contribution is likely to change. The current rise in *Ae. aegypti* detections in the airport as well as recent small outbreaks of tiger mosquitoes in places that cannot be traced back to introductions of risk products, signify a change that necessarily also lead to discussion and an updated *Aedes* risk management policy. From the *Ae. japonicus* example as well as from other perspectives, the talk will highlight how this is being done in the Netherlands, hopefully providing useful data for any situation in which establishment did not already take place but given the risk of introductions, a timely and justified response is wanted.

P79 Evaluation of the urban risk of arbovirus transmission in Côte d'Ivoire by using immuno-epidemiological biomarker of exposure of man to *Aedes* bites

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Human exposure to *Aedes bites*, therefore to the risk of arbovirosis, is not uniform and current entomological tools are unable to measure this contact at the individual level. Identifying the most vulnerable people or neighborhoods would make the fight against *Aedes* more focused and effective. The aim of this study was to evaluate the heterogeneity of children's exposure to *Aedes* stings in urban areas in Africa using the specific salivary biomarker (anti-Nterm-34kDa IgG). Methodology: A cross-sectional study was conducted in November 2015 in three neighborhoods in Abidjan: Anoumambo, Bromakoté and Petit-Bassam. A total of 813 children aged from 9 months to 14 years were included in the study. The ELISA technique allowed to measure the IgG level against *Aedes aegypti* s Nterm-34kDa salivary peptide in the serum of a subpopulation of 194 children from 1 to 14 years old. Results: The density of *Ae. aegypti* varied according to the entomological method used and to the neighborhood. Interestingly, the children living in Bromakoté and Petit-Bassam had a high level of anti-Nterm-34kDa IgG responses compared to those of Anoumambo ($p<0.0001$). In addition, young children between 1 and 5 years old had higher anti-Nterm-34kDa IgG levels than those in the 11-14 age group ($p = 0.0031$). In conclusion, this biomarker appears to be an indicator that health authorities could use to assess population exposure and identify neighborhoods at risk of arbovirosis in urban areas.

Asymmetric introgression between *Anopheles gambiae* and *Anopheles coluzzii* and rhythms of locomotor activity of their hybrids

P80

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Background: *Anopheles gambiae s.s* Giles, the main vector of malaria in sub-Saharan Africa, undergoes a speciation process giving rise to two independent reproductive units. Originally designated as "M" and "S" molecular forms, these two units are now recognized as 'true' species respectively *Anopheles coluzzii* Coetzee and *Anopheles gambiae* Giles. The speciation between the two species proceeds by the establishment of a reproductive isolation which is manifested by the rarity of hybrids. However, in Guinea Bissau there is strong hybridization leading to asymmetric introgression of gene flow. The purpose of the study was to verify whether there was synchronization between the locomotor activity of hybrids and that one of two parental species.
Methods: *Anopheles gambiae* and *Anopheles coluzzii* were raised in the laboratory, and hybrids were produced using technique of forced mating between adults of both parental species. Locomotor activity of both species as well as this of virgin male and female hybrids was recorded using a Locomotor Activity Monitor (LAM) for one day at constant darkness. The Akaike Information Criterion (AIC) was calculated to assess whether forced mating had an effect on locomotor activity.
Results: We observed that starting time of locomotor activity of hybrids was different than this of parental species. Hybrids had the same behavior as their male parents. However, the duration of activity in parental species was different and shorter than hybrids, where it was similar and long.
Conclusion: Our findings suggest that locomotor activity would be under the control of circadian rhythm. The duration of hybrids activity is long, suggesting the possibility of an introgression of gene flow in both directions.

P81 Inaccuracies of morphological keys to distinguish *Culiseta annulata* and *Culiseta subochrea* in Spain

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Mosquitoes (Diptera: Culicidae) are a key threat in public and animal health since they can transmit some of the most devastating diseases in the world. Their correct identification is important for epidemiological purposes, as mosquito borne diseases can be transmitted by some particular species. Nowadays, DNA sequencing has become a useful method in mosquito taxonomy. The DNA barcoding approach, establishes that species can be identified using a short fragment (650bp) of the Cytochrome Oxidase I gene region. This approach has been widely used in several countries, but it is still not widespread in the surveillance programs of Spain. Here we present the results of our attempt to identify two closely related species, *Culiseta subochrea* and *Culiseta annulata*, by both morphological and molecular techniques. We have found some discrepancies between the identification methods, with some specimens showing mixed characters of both species. We emphasize the urgent need to revise morphological keys available to distinguish these species.

Delıneatıon of sand fly specıes of Turkey usıng DNA Barcodes

P82

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DNA barcoding has been widely used as a complementary tool for sand fly taxonomy as well as a rapid technique for the blood meal identification and the pathogen detection in wild caught specimens. In this work we created cytochrome c oxidase 1 (CO1) DNA Barcodes for the sand fly species collected in 30 provinces covering the three biogeographical regions of Turkey. A total of 740 DNA sequences were analysed both for morphologically distinct species and specimens with cryptic identitiy. DNA barcodes were found to be successful to identify all the morphologically distinct specimens correctly. The mean Kimura 2-parameter sequence divergence within species was 0.60 %, whereas the mean interspesific genetic distance was 16. 30 %. The CO1 barcodes were also found to be helpful in discriminating morphologically similar females belonging to *Adlerius* subgenus. The results obtained from species delimitation methods indicated that the cryptic species diversity level among Turkish sand flies is higher than previously reported. The species accumulation curve obtained for the 32 species, including the provisional species showed that our sampling effort was adequate (Chao1= 34.49, 95 % C.I. 32.37 - 48.94) for the total area investigated. The Mediterranean biogeographic region was found to have the richest sand fly fauna with 22 species (Chao1=22.00, 95 % C.I. 22.00 - 23.31), followed by the Anatolian and the Black Sea regions that comprised 19 (Chao1=19.00, 95 % C.I. 19.00 - 21.64) and 11 (Chao1=14.93, 95 % C.I. 11.55 - 39.28) species, respectively. In conclusion, this study provides the whole CO1 barcode database for the sand fly species distributed in Turkey, for the first time and in concordance with the previous reports, indicates the effectivenes of barcoding technique in solving taxonomic problems and revealing the biological diversity.

Funding: Hacettepe University Scientific Researches Unit (01001601001), The Scientific And Technological Research Council of Turkey (SBAG: 114S999), Identification and incrimination of mosquito and sand fly vectors of viral diseases and pathogens present in refugee zones of the Turkish and Jordanian borders with Syria (GEIS Research Project Award).

P83 First molecular identification and phylogeny of Moroccan *Anopheles sergentii* (Diptera: Culicidae) based on ITS2 and COI sequences

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Anopheles sergentii known as the oasis vector or the desert malaria vector is considered the main vector of malaria in the southern parts of Morocco. Its presence in Morocco is confirmed for the first time through sequencing of mitochondrial DNA (mDNA) cytochrome c oxidase I (COI) barcodes and nuclear ribosomal DNA (rDNA) second internal transcribed spacer (ITS2) sequences and direct comparison with specimens of *An. sergentii* of other countries. The DNA barcodes (n=39) obtained from *An. sergentii* collected in 2015 and 2016 showed more diversity with ten haplotypes, compared to three haplotypes obtained from ITS2 sequences (n=59). Moreover, the comparison using the ITS2 sequences showed closer evolutionary relationship between the Moroccan and Egyptian strain than the Iranian strain. Nevertheless, genetic differences due to geographical segregation were also observed. This study provides the first report on the sequence of rDNA-ITS2 and mtDNA COI, which could be used to better understand the biodiversity of *An. sergentii*.

Vector Taxonomy, Systematics & Phylogeny category

Croatian mosquito fauna Barcoding

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Determination of mosquitoes, together with geographic distribution, are key steps in developing vector control strategies. The mosquito identification mainly relies on morphological identification of specimens using dichotomous keys. The biggest disadvantage of this type of identification is that mosquito scales can be easily rubbed off or are often damaged when they are collected or carelessly stored. It can also be problematic when distinguishing morphologically similar species. DNA barcoding is a worldwide method of biodiversity recognition and the world's base is continually being supplemented. Mitochondrial genes have become the primary molecular tool for analyzing genetic diversity in mosquitoes. Currently, the most commonly used barcode region is a 50-segment of the mitochondrial gene Cytochrome Oxidase I (COI). DNA based identification makes it possible to use sequence data in order to determine the species and their sub-species. Fifty-three species of mosquitoes have been recorded in Croatia so far. Determining the genetic status of this insect group will be of great taxonomic, phylogenetic and phylogeographic importance since Croatia stands out for its diversity of habitats that affect genetic diversity. During the project "DNA Barcoding of the Croatian Fauna" mosquitoes were sampled from the continental, alpine and Mediterranean regions of Croatia in mosquito seasons in 2017. and 2018. Within the sampled mosquitoes twenty-three species have been determined and molecularly identified, including two invasive mosquito species *Aedes japonicus* in the continental and alpine regions and *Aedes albopictus* in the continental and Mediterranean region of Croatia. We expect that by the end of this project we will have barcoded as much as possible of the known species in Croatia, established their systematic membership and complemented the world base (BOLD and GenBank).

P85 Loop-mediated isothermal amplification (LAMP) based assays for mosquito identification

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Surveillance of mosquitoes is a time-consuming task as specimens collected in the field need to be identified in the laboratory, by morphological or by molecular means (PCR/sequencing, MALDI-TOF mass spectrometry). Loop-mediated isothermal amplification (LAMP) is a robust and easy method to amplify DNA with high specificity and efficiency under isothermal conditions, and the method can be applied under field conditions, especially advantageous for remote areas. We have developed specific LAMP assays for the rapid identification of indigenous and invasive container-breeding aedine species, including *Aedes aegypti, Ae. albopictus, Ae. cretinus, Ae. geniculatus, Ae. japonicus*, and *Ae. koreicus*. These assays rely on easily assessable visible color changes after successful amplification. Additionally, these assays can be run in duplex reactions and assessed with lateral flow dipsticks. Further LAMP-based field tests are currently being developed in the frame of an international project (MosqDyn: Optimised methods for precision pest surveillance and control of vectors of disease ; project lead AviaGis, Belgium), in order to identify different *Culex* and *Anopheles* vectors in the tropics.

Vector Taxonomy, Systematics & Phylogeny category

Phylogenetic analysis of two sympatric malaria mosquito species in Sweden: *Anopheles daciae* and *An. messeae*

P86

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The species *Anopheles messeae* and *An. daciae* are close relatives. Two nucleotide differences in the second intergenic translated region (ITS2) of the ribosomal RNA separate them as different species. Both species are present in several European countries. We here report the presence of *An.daciae* in southern and central Sweden. Twenty mosquitoes, morphologically determined as *An.maculipennis* s. l., were collected at two locations in province of Småland, south Sweden. The ITS2 fragment was sequenced and the specimens could be classified as either *An. messeae* or *An. daciae*. Fragments of the mitochondrial genes ND4 and ND5 and a fragment of the nuclear gene Hb were sequenced and for all three loci, there were sequence differences between individuals. However, as these differences did not correlate with those of the ITS2 sequences, they could not help separating the specimens into *An. messeae* or *An. daciae*. The specimens grouped in different patterns for all sequenced genes. For the 20 specimens and five additional specimens collected in north of the province Uppland, the amplified ITS2 fragment was sequenced using Illumina Miseq sequencing. Resulting reads were compared using BLAST to a database of ITS2 sequences with variation only on the five nucleotides that vary between *An. messeae* and *An. daciae*. For the two nucleotides that are considered diagnostic for the species no intra-individual variation could be detected, but each *An. daciae* specimen carried several ITS2 sequence variants for the three other nucleotides, which varied between the species. In the *An. messeae* specimens there was no intra-individual variation. Among the specimens that we analysed, no specimen revealed intermediate levels of the two diagnostic nucleotides, which is expected for a hybrid *An. messeae/daciae* specimen. Admittedly, the results are based on a small sample, but they suggest that the hybrids are disadvantaged and that the two species are well separated.

P87 Finding *Aedes cretinus*: current state in Greece

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After the first description of *Aedes cretinus* from specimens from Crete in 1921, little research reported on the presence of the species in Greece, mostly during last 10 years. As a result of *Aedes albopictus* occurrence in Greece and under the framework of LIFE CONOPS project (www.conops.gr), from 2014 several entomological surveillance activities are implemented for the presence of invasive mosquito species (IMS) in Greece. The presence of Ae. cretinus was confirmed from collected adults and/or adults that emerged in the laboratory from our network with oviposition traps in Greece. Our surveillance network provides us information about the presence of *Ae. cretinus* and its possible displacement by *Ae. albopictus.* Before the invasion of *Ae. albopictus* this species was the only day-biting mosquito that has been recorded in several areas in Greece. Now, based on our IMS collections, this species is located only in two areas in Greece: in Rethymno (regional unit of Crete) and in Leros island.

Unveil the use of Cytochrome Oxidase c Subunit 1 (COI) as a molecular taxonomic tool to assess the phylogeny and diversity of mosquitoes from Nelliampathi, a part of Western Ghats, Kerala, India

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Monitoring mosquito vector population is a fundamental part of vector control surveillance programmes. Recent epidemics of mosquito-borne diseases in India, specifically in Kerala have extended the need for detailed understanding of the distribution of vector mosquitoes in this region. The COI gene-based molecular taxonomic approaches improve the precision and accuracy of vector mosquitoes identification since conventional taxonomic methods showed subtle morphological variations between organisms. Methodology: Cytochrome c oxidase subunit I gene of field-collected mosquitoes was used for the identification of mosquito species. Neighbour-joining tree of *Aedes, Anopheles, Culex* and *Armigeres* species was constructed using the MEGA7 software. The genetic divergence was assessed using the NJ-K2P method. The diversity of mosquito specimens in the study region was estimated using the Shannon index. Here we used SPSS, Venn diagram plotter for statistical analysis. Results: The COI- based mitochondrial DNA Analysis revealed the distinct clustering of individual mosquito species within every genus together with strong bootstrap support. In total, our investigation productively identified three species of *Aedes*, three species of *Anopheles*, three species of *Culex*, followed by one species of *Armigeres* mosquito. *Aedes aegypti, Aedes albopictus*, and *Culex quinquefasciatus* were noted as the most widespread, observed at all the three study sites. Conclusion: This study employed for the first time COI- gene-based approach to identify the mosquito vectors collected from Nelliampathi, Kerala, India. The COI- gene-based method is a convenient tool for the identification of vector mosquitoes since it possesses reliability, and accuracy than conventional methods.

P89 Landscape genetics of *Culicoides* (Diptera: Ceratopogonidae) vector species: evaluation of cryptic diversity and gene flow in the Palearctic region

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Landscape genetics is an approach for understanding how geographical and environmental features structure genetic variation. Whether landscape patterns, in particular those created by human land uses, influence gene flow and its intensity to understand the spread of vector-borne diseases. *Culicoides* dispersal capacities are described as 'dispersive stratified' which results from the combination of processes occurring at a short distance combined with jumps over long distances. Although winds and local air movements have an undeniable role in the dispersal of *Culicoides*, anthropogenic factors also play a predominant role, particularly at the local level (host distribution, landscape patterns). Our overall objective is to characterize the dispersal abilities of two main vector species in Europe, *C. obsoletus* and *C. chiopterus*, showing different host-vector behaviors. However, *Culicoides obsoletus* is reported in sympatry with one morphologically indistinguishable species, C. scoticus, and other morphologically related species, *C. chiopterus*, *C. dewulfi* and *C. montanus*. Moreover, recently, several authors have reported the existence of cryptic diversity within the species commonly called *C. obsoletus*. Sequencing a portion of the *Cox1* mitochondrial gene of 3,200 *C. obsoletus/C. scoticus* individuals from 17 European countries revealed two clades within *C. scoticus* and confirmed the presence of at least three undescribed phylogenetic clades (*C. obsoletus* clade O2, *C. obsoletus* clade Dark and one not yet named) close to *C. obsoletus*. These results are reinforced by rDNA16S mitochondrial gene sequences and a gene coding for ribosomal rDNA18S, over the entire haplotypic diversity resulting from *Cox1* barcoding. Then, we investigated how dispersion shapes the spatial arrangement of *C. obsoletus* genetic diversity in Europe using 13 microsatellite markers by observing patterns of allelic frequency distributions. This work is the first step to a more comprehensive study on the landscape genetics of two main vector species in the Palearctic region, *C. obsoletus* and *C. chiopterus*.

P90

Identification of Italian malaria vectors by MALDI-TOF MS method: preliminary data

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Background: Mosquito species are normally identified by morphological keys whereas PCR-based methods are used to discriminate sibling species belonging to ~~a~~ species complex. However, the morphological approach requires availability of well-preserved specimens and entomological expertise; molecular methods are expensive and time-consuming. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid technique that was recently applied for the identification of arthropods of medical interest. In order to assess the capability of this approach to identify Italian mosquito species, in particular anopheline malaria vectors, a MALDI-TOF MS analysis was implemented.

Methods: Over the time adult mosquitoes were collected by CDC and battery-powered aspirators during entomological surveillance activities and within national/international projects (cryptic malaria-cases 2017 monitoring, WND-monitoring, RC/IZSPB05/2010, EDEN, INFRAVEC). Mosquitoes species belonging to *Anopheles, Culex, Aedes, Ochlerotatus, Culiseta* and *Coquillettidia* genera were previously identified at morphological level; in presence of complex of species molecular approach (PCR and sequencing) was also applied. Moreover, one leg from each specimen was subjected to MALDI-TOF MS analysis and the signature peak spectra were selected for creation of a reference database. Lastly 30 blind-coded samples from different mosquitoes species were tested by comparing them with such spectra database and displayed as dendrogram.

Results: MALDI-TOF MS correctly identified 100% of Italian Culicidae to genera level. Preliminary results show an exact identification of 12 mosquitoes specimens so far analyzed at species level including *Anopheles(An.) labranchiae, An. superpictus* and *An. claviger*.

Conclusion: The MALDI-TOF MS procedure show a rapid and correct species identification of adult mosquitoes, including *An. labranchiae,* morphologically indistinguishable from other members of the *An. maculipennis* complex. Due to its specificity and rapidity, MALDI-TOF MS is a promising tool for entomological surveys particularly useful when local indigenous cases occur in malaria-free countries, including Italy. Further implementations of this method on mosquito early stages is currently ongoing.

P91 Ticks on migratory birds as a proved spreading way for Crimean-Congo hemorrhagic fever virus: one-year acarological data from three Italian stop-over sites

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Migratory birds are considered vehicle for arthropods infected by pathogens as the Crimean-Congo haemorrhagic fever (CCHF) virus, an agent responsible for an endemic zoonosis in sub-Saharan Africa, Asia and East Europe, transmitted mainly by *Hyalomma* ticks and causing serious illness in humans. Within the project funded by the Italian Ministry of Health aimed to study the role of migratory birds as tick-carriers in spreading CCHFV into Europe, 975 ticks were examined in spring 2017: 717 on birds ringed while staging on Mediterranean islands used as major stop-over sites on return migration from Africa and 258 collected by ground dragging. Collection sites were Ventotene Island (Latium) with 620 ticks on birds, Ustica Island (Sicily) with 71 ticks on birds and 91 from the ground, and Asinara Island (Sardinia) with 26 ticks on birds and 126 from the ground. Tick specimens were identified by morphology and molecular techniques to determine the immature stages, the latter particularly for *Hyalomma* sp. specimens and doubtful cases, and then screened for CCHFV. A Real Time RT-PCR assay allowed to detect CCHF virus in a nymph of *Hyalomma marginatum rufipes* from a Whinchat (*Saxicola rubetra*) on Ventotene. This finding is the first case for Western Europe and confirms the risk of CCHFV introduction via migratory birds. The majority of ticks collected on birds was represented by early stages of *Hyalomma* spp. (65%), *Ixodes* spp. (23%) and other species (12%). Such virological survey is a useful approach to record the presence of allochthonous *Hyalomma* spp. and *Amblyomma* spp. ticks and to improve, by molecular tools, the knowledge of little known species like some belonging to the subgenus *Ixodes* (*Ixodes*). Here we report the acarological results on tick species identified during the first year of this ongoing project, planned to continue also during next year.

Vector Taxonomy, Systematics & Phylogeny category

Constructing a morphological reference collection of native and exotic mosquito species (Diptera: Culicidae) in Belgium

P92

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Morphological reference collections are essential to acquire solid background knowledge on target species. This knowledge is directly applicable to public health, by supporting research through reliable identifications and by having an overview of medically important species for monitoring and surveillance projects. Until recently, information on mosquito species and their distribution in Belgium was scarce. The nationwide survey of mosquito biodiversity performed within the MODIRISK project between 2007 and 2010 changed this. Additionally, in July 2017 the three-year active monitoring project MEMO started with focus on monitoring exotic mosquitoes at 23 locations in Belgium which were selected based on their high potential of being points of entry of exotic mosquito species. For both projects, morphological reference collections are established and curated at the Royal Belgian Institute of Natural Sciences (RBINS). These collections contain specimens representing all life stages in order to provide a complete morphological overview of the mosquitoes present in Belgium. Adults are stored at -20 °C after field collecting and subsequently dry pinned. Larvae and eggs are stored in 80% ethanol and slide-mounted using DMHF. Additionally, a digital photo database is created containing images of all available life stages and sexes for each species. The results of the MODIRISK project, together with the inventory of Belgian mosquito species in existing collections of the RBINS led to an updated checklist representing 31 mosquito species. Two more species, *Culiseta longiareolata* and *Anopheles pharoensis*, were found for the first time during the MEMO project in 2017. In total 1491 specimens from 33 species and 100 locations are included in the collection. The morphological reference collections support the monitoring of introduced exotic mosquito species which are intercepted in Belgium as a result of globalization and global warming.

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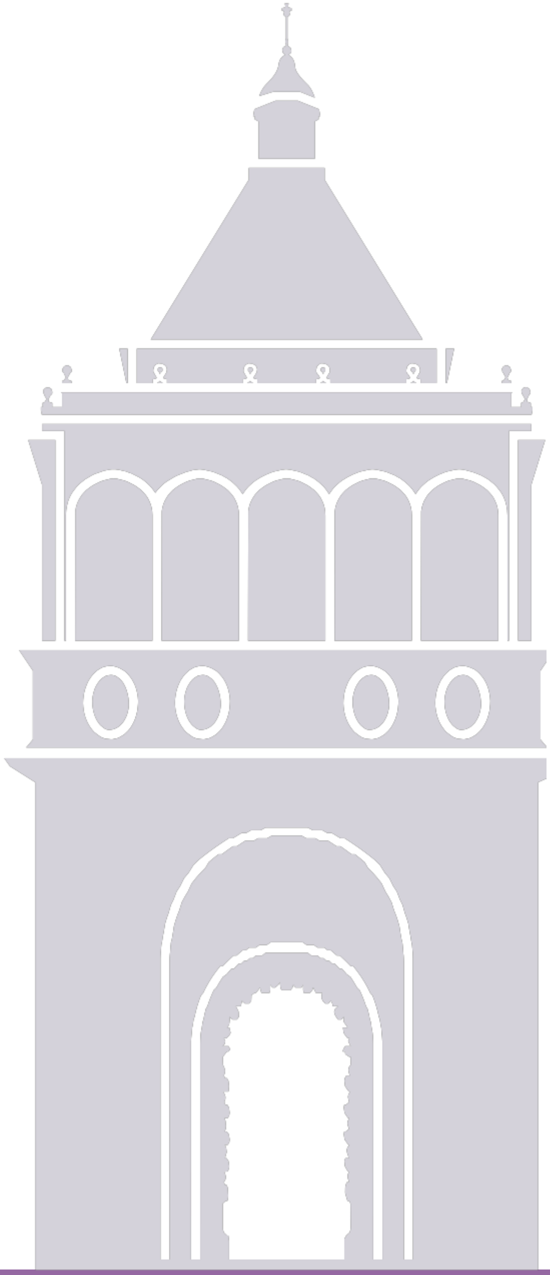

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