E-SOVE

The 22nd Conference
Sofia, Bulgaria

2022

PROCEEDINGS OF

THE 22ND EUROPEAN SOCIETY FOR VECTOR ECOLOGY CONFERENCE

Meeting following a pandemic;
bringing clarity to vector ecology in days of uncertainty

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Special Thanks To:

- Kivanc Sevim, Mert Okbay, Selin İnsel

WELCOME ADDRESS

Dear Friends and Colleagues,

As globalization, the Covid-19 pandemic and the Russia-Ukraine war continue to impact every aspect of research and generate new problems regarding vector-borne diseases, many scientists have continued their work with no less enthusiasm than before. Vector-borne infections continue to persist, the latest examples being the reports of deaths related to the WNV from Greece and increasing dengue cases from France. Obviously, this is quite the time for us to unite once again.

During the Conference on October 11-14th, we will listen to some intriguing research on "Vector Phylogeny, Taxonomy and Biogeography," "The Role of Citizen Science and Outreach for the Surveillance of Vector Species," "Host-Pathogen-Vector Interactions," while most of our oral presentations will cover "Vector Ecology and Behaviour" and "Surveillance and Management of Vector-Borne Diseases." Brilliant poster presentations await the participants on these topics.

ESOVE Conferences have always been the primary platform for exchanging ideas and creating long-lasting collaborations on vector ecology research. This year, I'm eager to present you the session "New Projects & Updates from Different Networks" as much time has passed since we last met to discuss key developments from where we've left. We will listen to the most recent findings regarding new projects such as CLIMOS, which strives towards a better understanding of climate and environmental drivers of sand fly borne diseases in Europe, as well as RIVOC, a regional collaborative research project and network for enhancing innovative research on emerging and main vector-borne pathogens in plants and vertebrates through a One Health approach. We will find out more about the latest on INOVEC, which is a research and innovation partnership for boosting the surveillance and control of mosquito vectors of emerging arboviruses. Among them, RIVOC has lent great support to the conference this year by sending outstanding keynote speakers and students. Finally, we will be receiving key updates from VectorNet and some impressive AIM surveillance and control records out of 22 years from Switzerland.

Incidentally, right before the conference kicks off on October 10th, the AIM-COST Action (www.aedescost.eu) will have a face-to-face training on "Finding, using and interpreting maps and models of invasive mosquitoes" at the Sofia Balkan Hotel.

I would like to express my deep appreciation to Major Dhillon and Michelle Brown for their constant support in making this Conference happen. We've put together a great program with the help of the ESOVE Scientific Committee, consisting of Alexandra Chaskopolou, Andrei Mihalca, Bruno Mathieu, Claire Garros, Gregory L'Ambert, Ozge Erisoz Kasap, Vit Dvorak. Many thanks to them and all the participants who have submitted their outstanding abstracts.

Lastly, I would like to briefly convey the thoughts and feelings of our Scientific Committee regarding our expectations from the conference. The Society has been a welcoming scientific environment for those of us who have been attending ESOVE Conferences since we were doctoral students. Today, the efforts of our students who have been working towards generating new knowledge on key research areas remain our top priority. Therefore, we could not be prouder to create a collaborative atmosphere where students get to meet junior and senior scientists across different disciplines and cultures. We look forward to inspiring and challenging each other and sharing our laughter again.

I sincerely wish that everybody will benefit from the upcoming Conference. Stay healthy...

Filiz Gunay
ESOVE Director

PROGRAM AT A GLANCE

	Tuesday 11th October
09.00-09.15	**Conference opening address and Welcome**
09.15-09.45	Keynote Lecture: Thierry Lefevre
09.45-11.00	**Symposium 1: Vector Phylogeny, Taxonomy and Biogeography** Chairs: Bülent Alten, Alexandra Chaskopoulou
11.00-11.30	Coffee Break
11.30-13.00	**Symposium 2: Vector Ecology and Behavior - I** Chairs: Bruno Mathieu, Gizem Oguz
13.00-14.30	Lunch
14.30-16.00	**Symposium 3: Vector Ecology and Behavior - II** Chairs: Tatiana Sulesco, Ognyan Mikov
16.00-16.30	Coffee Break
16.30-18.00	**Symposium 4: Vector Ecology and Behavior - III** Chairs: Thierry Lefevre, Francis Schaffner
	Wednesday 12th October
	Excursion to Plovdiv
	Thursday 13th October
09.00-09.30	Keynote Lecture: Richard Allan
09.30-11.00	**Symposium 5: Surveillance and Management of Vector Borne Diseases - I** Chairs: Eleonora Flacio, Filiz Günay
11.00-11.30	Coffee Break
11.30-13.00	**Symposium 6: Surveillance and Management of Vector Borne Diseases - II** Chairs: Helge Kampen, Nazl Ayhan
13.00-14.30	Lunch
14.30-16.00	**Symposium 7: Surveillance and Management of Vector Borne Diseases - III** Chairs: Mihaela Kavran, Grégory L'Ambert
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10.30-11.00	**Poster Sesssion**
11.30-13.00	**Symposium 9: New Projects & Updates from Different Networks** Chairs: Vít Dvořák , Didier Fontenille
13.00-14.30	Lunch
14.30-16.00	**Symposium 10: Host Pathogen Vector Interactions - I** Chairs: Renke Lühken, Lison Laroshe
16.00-16.30	Coffee Break
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SCIENTIFIC PROGRAM

Tuesday 11th October

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	Symposium 1: Vector Phylogeny, Taxonomy and Biogeography **Chairs:** Bülent Alten, Alexandra Chaskopoulou	
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10.00-10.15	**Shirly Elbaz**	Environmental constraints on the distribution of the vector and non-vector sand fly species of Israel
10.15-10.30	**Bülent Alten**	Sand flies (Diptera: Psychodidae) and pathogen circulation in Balkan countries: region-wide entomological survey
10.30-10.45	**Francis Schaffner**	AIMSurv protocol and mosquito diversity survey to complement each other: the example of Luxembourg
10.45-11.00	**Tobias Lilja**	Genetic relationship between Aedes cinereus and Aedes geminus
11.00-11.30	**Coffee Break**	
	Symposium 2: Vector Ecology and Behavior - I **Chairs:** Bruno Mathieu, Gizem Oguz	
11.30-11.45	**David López-Peña**	Altitudinal distribution pattern of *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) in Castellón province, Eastern Spain
11.45-12.00	**Kıvanç Sevim**	Determining the effects of low temperatures on the life cycle of *Aedes* (*Stegomyia*) *aegypti* (Linneus,1762) (Diptera: Culicidae)
12.00-12.15	**Ayda Yılmaz**	Effects of Different Temperatures on The Biology of *Phlebotomus tobbi*
12.15-12.30	**Gizem Oğuz**	Gene expression changes of three *Phlebotomus tobbi* Adler, Theodor & Lourie, 1930 (Diptera: Psychodidae) transcripts under different environmental conditions
12.30-12.45	**Sarah Groschupp**	*Culicoides* biting midges (Diptera: Ceratopogonidae) in Germany in winter: 'vector-free period' or not?
12.45-13.00	**Maia Tsikolia**	Stored sheep wool as source of mosquito attractant volatiles
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	Symposium 3: Vector Ecology and Behavior - II **Chairs:** Tatiana Sulesco, Ognyan Mikov	
14.30-14.45	**Tatiana Şuleşco**	Host-feeding patterns and West Nile virus infection in mosquitoes from the Republic of Moldova
14.45-15.00	**Alexander Mathis**	Towards exploiting skin bacteria to protect animals against insect vectors
15.00-15.15	**Felix Sauer**	Analysing the efficiency of CO2-traps as barrier against Culexmosquitoes
15.15-15.30	**Carmen Villacañas de Castro**	Breeding site selection and carry-over effects of *Culex* species along a land-use gradient.
15.30-15.45	**Pie Müller**	3D video tracking unravels how mosquitoes interact with topical repellents in the arm-in-cage test

15.45-16.00	**Paul Taconet**	Insecticide resistance and feeding behavior of malaria vectors in two areas of rural West-Africa : spatiotemporal distribution, drivers, and predictability
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	Symposium 4: Vector Ecology and Behavior - III **Chairs:** Thierry Lefevre, Francis Schaffner	
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16.45-17.00	**Eleonora Flacio**	Risk-based mapping tools for surveillance and control of the invasive Asian tiger mosquito in Switzerland
17.00-17.15	**Nikos Perros**	An update on short-term forecasting for larvae and adult mosquito abundances and epidemiological risk in wide-area mosquito control programs
17.15-17.30	**David Hug**	Temperature preferences of mosquitoes and biting midges
17.30-17.45	**Laor Orshan**	The emergence and re-emergence of zoonotic cutaneous leishmaniasis in northwest Negev Israel, through the lenses of the sand fly monitoring findings
17.45-18.00		

Wednesday 12th October

Excursion to Plovdiv

Thursday 13th October

09.00-09.30	**Keynote Lecture:** Richard Allan	The Social Determinants, Surveillance and Control of Leishmaniasis across Northern Syria – through a Decade of Conflict
	Symposium 5: Surveillance and Management of Vector Borne Diseases - I **Chairs:** Eleonora Flacio, Filiz Gunay	
09.30-09.45	**Marina Bisia**	Entomological surveillance in the Attica region (Greece) as part of integrated mosquito management.
09.45-10.00	**Cedric Marsboom**	Vector-borne disease risk estimation based on commercial veterinary laboratory tests in Germany
10.00-10.15	**Miguel Ángel Miranda**	AIMSurv-AIMCOST: Why to harmonize the surveillance of Aedes invasive mosquito species in Europe?
10.15-10.30	**William Wint**	Invasive Mosquitoes: A Roadmap from Sampling to Modelling and Dissemination
10.30-10.45	**Vladimir Ivović**	Detection, identification and genotyping of Borrelia spp. in ticks of Coastal-Karst and Littoral-Inner Carniola regions in Slovenia
10.45-11.00	**Nazlı Ayhan**	Detection and Isolation of Sindbis virus from Field Collected Mosquitoes in Timimoun, Algeria
11.00-11.30		**Coffee Break**
	Symposium 6: Surveillance and Management of Vector Borne Diseases - II	

	Chairs: Helge Kampen, Nazli Ayhan	
11.30-11.45	**Ognyan Mikov**	Sandflies and human visceral leishmaniasis in Bulgaria
11.45-12.00	**David López-Peña**	Zoonotic vector diseases situation in Valencian Autonomous Region (Eastern Spain) along the period 2010-2020
12.00-12.15	**Miguel Angel Miranda**	"I'll be back": a new bluetongue outbreak in the Balearic Islands after 18 years of silence.
12.15-12.30	**Petr Halada**	MALDI-TOF mass spectrometry: A powerful method for blood meal identification in insect vectors
12.30-12.45	**Manel Silva**	Monitoring *Aedes aegypti* in Portugal with a novel optical sensor system for the automatic classification of mosquitoes
12.45-13.00	**Yvon Perrin**	VECTRAP: Applicability of a mass trapping strategy against *Aedes albopictus* and *Aedes aegypti*
13.00-14.30		**Lunch**
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14.45-15.00	**Isra Deblauwe**	Finding the balance between efficiency and budget: preventive invasive mosquito species (IMS) surveillance.
15.00-15.15	**Wadaka Mamai**	New developments on mosquito mass-rearing for the sterile insect technique application
15.15-15.30	**Nanwintoum Séverin Bimbile Somda**	Adult mosquito predation and potential impact on the sterile insect technique
15.30-15.45	**Grégory L'Ambert**	Autodissemination method against *Aedes albopictus* in a field experiment in Southern France
15.45-16.00	**Nazli Ayhan**	Update of the Vector-borne Viruses circulating in Europe in the Shadow of Sars-CoV-2
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16.30-18.00		**POSTER SESSION**

GALA DINNER

Friday 14th October

09.00-09.30	**Keynote Lecture:** Didier Fontenille	Greening cities and vectorial risks for human, animal and plants
	Symposium 8: The Role of Citizen Science and Outreach for the Surveillance of Vector Species **Chairs:** Claire Garros, Alessandra della Torre	
09.30-9.45	**Javiera Rebolledo**	Monitoring of Exotic Mosquitoes (MEMO+) project in Belgium: The passive surveillance component
09.45-10.00	**Alessandra della Torre**	AIM-COST expands mosquito monitoring by Citizen Science across Europe: first results from Mosquito Alert Italia network.
10.00-10.15	**Sandra Gewehr**	Mosquito Vision: An interactive application for the prediction of mosquito nuisance for citizens
10.15-10.30	**Rody Blom**	Unwanted guests: mosquitoes in the domestic environment during winter
10.30-11.00		**POSTER SESSION**
11.00-11.30		**Coffee Break**

	Symposium 9: New Projects & Updates from Different Networks **Chairs:** Vit Dvorak, Didier Fontenille	
11.30-11.45	**Vít Dvořák**	CLIMOS - towards better understanding of climate and environmental drivers of sand fly borne diseases in Europe
11.45-12.00	**Claire Garros**	RIVOC (2021-2025): a regional collaborative research project and network to enhance innovative research on emerging and main vector-borne pathogens in plants and vertebrates through an One Health approach
12.00-12.15	**Pie Müller**	INOVEC: a research and innovation partnership for enhancing the surveillance and control of mosquito vectors of emerging arboviruses
12.15-12.30	**Zran Innocent Tia**	Efficacy of a ‘lethal house lure’ against Culex quinquefasciatus from Bouaké, central Côte d’Ivoire.
12.30-12.45	**Marieta Braks**	VectorNet: Putting vectors on the map
12.45-13.00	**Lukas Engeler**	Evolution of invasive mosquito surveillance and control in Switzerland in the last 22 years
13.00-14.30	**Lunch**	
	Symposium 10: Host Pathogen Vector Interactions - I **Chairs:** Renke Luhken, Lison Laroshe	
14.30-14.45	**Charlotte Linhout**	The potential role of Aedes japonicus mosquitoes as spillover vector for West Nile virus in the Netherlands
14.45-15.00	**Gioia Bongiorno**	Species abundance, seasonal dynamics and natural infection prevalences of sand flies from Sardinia Italian island
15.00-15.15	**Lison Laroche**	Sand fly and Toscana virus: impact on vector life-history traits using experimental infection and potential effects on transmission
15.15-15.30	**Renke Lühken**	Host-feeding patterns of European mosquitoes in relation to land use
15.30-16.00	**Coffee Break**	
	Symposium 11: Host Pathogen Vector Interactions - II **Chairs:** Carlos Barceló, Maria Bourquia	
16.00-16.15	**Maria Bourquia**	Combining biodiversity conservation and vector ecology: characterization of Culicoides and mosquito fauna at the National Zoological Garden of Rabat, Morocco
16.15-16.30	**Magdalena Laura Wehmeyer**	A global meta-analysis on mosquito host-feeding patterns
16.30-16.45	**Anastasia Angelou**	Bioclimatic factors associated with *Culex* population abundance and WNV cases in Greece
16.45-17.00	**Romain GIROD**	Entomological investigation of a Rift Valley Fever epizootic/epidemic reveals new aspects of the vectorial transmission of the virus in Madagascar
17.00-17.15	**Carlos Barceló**	Pathogen detection of mosquitoes collected in different environments of Mallorca, Spain

ORAL PRESENTATION ABSTRACTS

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1

Keynote Abstract

The ecological roles of mosquitoes, with special reference to their contribution to pollination

Thierry Lefevre

MIVEGEC (CNRS-IRD-Montpellier University), Montpellier, France

Abstract

Vector control is a cornerstone in the fight against mosquito-borne pathogens. However, the impact on ecosystem functioning of reducing or eliminating mosquito vector populations remains poorly understood. Recent studies highlight that, although the ecological roles of most mosquito species might be redundant with other non-vector species, changes in mosquito abundance can alter food web dynamics (bottom-up and top-down trophic cascades), as well as inter- and intraspecific competition. In addition, the importance of mosquitoes to plant reproduction is currently controversial. While some studies show that mosquitoes can effectively transferred pollen between plants, resulting in seed sets, others suggest that their role to global pollination is minor. Here, I will briefly review the possible ecological roles of mosquitoes, and present new findings on the contribution of the mosquitoes *Culex pipiens molestus* and *Aedes albopictus* to pollination of two flowering plants.

2

Vector-borne pathogens as climate refugees

Kamil Erguler[1], Dušan Veljko Petrić[2], Sirri Kar[3], Yiannis Proestos[1], George Zittis[1], Mina Petrić[4], Filiz Gunay[5], Bulent Alten[5], Jos Lelieveld[6]

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Abstract

Persistent global warming, due to human-made greenhouse gas emissions, is expected to substantially affect biodiversity, species phenology and ranges, and ecosystem processes. Since mosquitoes and the pathogens they transmit are exposed to these global trends, serious consequences on human and environmental health are expected. While Europe is predicted to become increasingly habitable to many vector species, its neighbouring southern territories will be increasingly inhabitable. Here, we investigate the extent to which climate change will shift vector-borne disease risk by examining the common house mosquito, *Culex pipiens*. We focus on the extended geospatial domain of Europe, the Middle East, and North Africa and employ temperature-driven time to adult emergence as a proxy for environmental support on high vector abundance and pathogen transmission risk. Using climate models and different shared socio-economic pathways, we project mosquito development and survival until the end of the century, reveal the regions of pronounced impact, and identify the expected time intervals when such changes become discernible. While highly invasive species, such as *Aedes albopictus*, benefit from human travel and transport to disperse risk over Europe, residential mosquito fauna will most directly be affected by climate change, exacerbating existing and setting off new vulnerabilities against circulating and/or invading pathogens.

3

Environmental constraints on the distribution of the vector and non-vector sand fly species of Israel

Shirly Elbaz[1,2]*, Laor Orshan*[1]*, Maya Davidovich-Cohen*[1]*, Gal Zagron*[3]*, Takuya Iwamura*[4]

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Abstract

The spatial distribution and habitat preferences of vectors are recognized as major factors that limit the geographical range of vector-borne diseases. For ground-dwelling arthropod vector species such as sand flies, specific edaphic factors can be most important, since they develop and sustain in soil. In Israel, there are fourteen known species of *Phlebotomus* (Diptera: Psychodidae). Most of these species are proven or suspected vectors of three *Leishmania* pathogens (*Leishmania major, L. tropica* and *L. infantum).* In order to examine the potential distribution and habitat suitability of *Phlebotomus* sand fly species, ensemble species distribution model approach was applied. For modeling, robust complied national database of Phlebotomus species recorded in Israel over the past 19 years was combined with environmental factors of five categories: climate, soil, topography, vegetation, and rock. Based on model results the distribution binary maps of vector and non- vector species were compared for ecological differences. In addition, vectors predicted distributions were regressed with the presence of *Leishmania*-infected females to confirm the potential risk.

Model results show high variation between the different sand fly species regarding the predicted distributions. Climate factors contribute the most to the distribution, mostly precipitation and average annual temperature. Lithography and slope were found significant for species related to mountain areas. Soil properties had little effect on the distribution. Proven or suspected vector species were found in more sites than non-vector species, and their geographic ranges were wider than those of non-vector species. Regression between vector distributions and sites with specific *Leishmania* infected females showed high vector –parasite geographic compatibly for only few vectors. Predicted distribution maps for these species better reflect the potential risk of endemic transmission of *Leishmania*. Understanding the distribution and habitat preferences of sand flies can help predict current and future disease transmission risks and help decision makers assess future threats and plan prevention and intervention actions.

4

Sand flies (Diptera: Psychodidae) and pathogen circulation in Balkan countries: region-wide entomological survey

Bulent Alten[1], Vit Dvorak[2], Ozge Erisoz Kasap[1], Petr Volf[2], Yusuf Ozbel[3], Dusan Petric[4], Vladimir Ivovic[5], Ognyan Mikov[6], Jovana Stefanovska[7], Franjo Martinkovic[8], Jasmin Omeragic[9], Igor Pajovic[10], Nazli Ayhan[11], Ankelejda Velo[12]

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Abstract

Sand flies (Diptera: Psychodidae) are medically important vectors of human and veterinary disease-causing agents. Among these, the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), and phleboviruses are of utmost importance. Despite such significance, updated information about sand fly fauna is missing for Balkan countries where both sand flies and pathogens are historically present and recently re-emerging. Therefore, a review of historical data on sand fly species composition and distribution in the region was followed by a large-scale entomological survey in eight Balkan countries to provide a recent update on local sand fly fauna.

The entomological survey was conducted at 1189 sampling stations in eight countries (Bulgaria, Bosnia and Herzegovina, Croatia, Kosovo, Montenegro, North Macedonia, Serbia and Slovenia), covering 49 settlements and 358 sampling sites between June and October in the years 2014 and 2017, accumulating 130 sampling days. We performed a total of 1189 trapping nights at these stations using two types of traps (light and CO2 attraction traps) in each location. Sampling was performed with a minimal duration of 6 (Montenegro) and a maximal of 47 days (Serbia) between 0–1000 m.a.s.l. Collected sand flies were morphologically identified.

In total, 8490 sand fly specimens were collected. Morphological identification showed presence of 14 species belonging to genera *Phlebotomus* and *Sergentomyia*. Historical data were critically reviewed and updated with our recent findings. Six species were identified in Bosnia and Herzegovina (2 new records), 5 in Montenegro (2 new records), 5 in Croatia (2 new records), 9 in Bulgaria (5 new records), 11 in North Macedonia (1 new record), 10 in Serbia (no new records), 9 in Kosovo (3 new records) and 4 in Slovenia (no new records).

This study presents results of the first integrated sand fly fauna survey of such scale for the Balkan region, providing first data on sand fly populations for four countries in the

study area and presenting new species records (proven and suspected vectors) for six countries and updated species lists for all surveyed countries. Our findings also demonstrate presence of virus including new virus species and leishmania circulation by sand flies.

5

AIMSurv protocol and mosquito diversity survey to complement each other: the example of Luxembourg

Francis Schaffner[1], Alexander Weigand[2], Christian Ries[2]

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Abstract

The effectiveness of mosquito-borne diseases prevention and control plans is directly related to the knowledge and understanding of local mosquito fauna, in terms of species presence (both native and alien), abundance and seasonality. Therefore, and since the mosquito fauna of Luxembourg was only partially known, we did construct the first comprehensive mosquito diversity and distribution database for the Grand Duchy as a base for an atlas. To achieve this, we did collect and synthesise existing mosquito occurrence data, and implement field surveys to acquire original field data. We also implemented the AIMSurv2020 protocol (within AIM-COST Action project) to survey invasive species at Points of Entry. We assembled, on one side, 642 mosquito taxa occurrences from published or unpublished sources from the period 1997 to 2018, and on the other side 22 citizen data and 735 original mosquito occurrences obtained from 560 sites distributed across the Grand Duchy and both collected over the period 2019-2021. The 3,592 AIMSurv ovitrap/nights did remain negative. Updated mosquito distribution maps are available for the 28 species (31 taxa) reported to occur in Luxembourg to date (journal Ferrantia, 2022). The assembled data reveal the taxon *Culex pipiens/torrentium* to be the most widespread and frequently encountered, and probably the most abundant taxon in Luxembourg. The other taxa that show a wide-ranging distribution are *Anopheles claviger s.s.*, *An. maculipennis* s.l., *Ae. geniculatus*, *Culiseta annulata*, *Ae. annulipes/cantans*, *Cs. morsitans*, *Cs. longiareolata*, *An. maculipennis* s.s., *Cx. territans*, and *Ae. rusticus*. Taxa that have a very limited distribution range are *Ae. cinereux/geminus*, *Ae. sticticus*, *Ae. vexans*, *Coquillettidia richiardii*, *Ae. refiki*, *An. petragnani*, *Cs. glaphyroptera*, *Cx. modestus* and *Uranotaenia unguiculata*. The territory has been colonised in recent years by two new species: *Cs. longiareolata* and the alien *Ae. japonicus*. Further possible changes may occur, with the introduction of additional invasive *Aedes* container-inhabiting species. There is no environmental or climatic reason that *Ae. albopictus* will not establish populations. A primary risk assessment performed for the mosquito species reported to occur in Luxembourg plus the invasive species *Ae. albopictus* shows that at least 15 species could be involved in a pathogen or parasite transmission.

6

Genetic relationship between *Aedes cinereus* and *Aedes geminus*

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Abstract

Identification of the sibling mosquito species *Aedes cinereus* and *Ae. geminus* can only be confirmed by examination of the male gonostylus. Since females and immature stages are morphologically indistinguishable, the two species are often reported together, but one or both are vectors of West Nile virus, *Francisella tularensis* and *Setaria* parasitic worms. To facilitate molecular identification of the two species, males were collected in Finland, France and Sweden, identified, and used to sequence several DNA marker loci, as well as genome wide ddRAD sequencing. Of the genetic markers examined (COI, ND4, ND5, ITS2, Actin and ACE2) none showed any distinction between specimens morphologically identified as *Ae. cinereus* or *Ae. geminus*. The genetic differences that were found did not provide sufficient stable phylogenetic differences to indicate that the specimens belonged to different taxa. Similarly, the genome wide ddRAD based SNP analysis did not reveal any genetic distinction between the specimens that were identified based on morphological features of the males. Our results did not, therefore, indicate any reproductive isolation between the two morphological forms but instead that the diagnostic character represents morphological variation within the same population.

7

Altitudinal distribution pattern of *Aedes* (Stegomyia) *albopictus* (Skuse, 1894) in Castellón province, Eastern Spain

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Abstract

Aedes (Stegomyia) *albopictus* (Skuse, 1894), an invasive and allochthonous mosquito native from Southeast Asia, is expanding its geographical distribution area worldwide. First, taking advantage of anthropic means of transport used in both the movement of human beings and the transport of goods, and second, due to the effects of climate change and global warming, which are favouring the presence and establishment of this species in many regions of the world.

Stable populations of tiger mosquito were detected for the first time in Spain during 2004 in Cataluña, since then this insect has been increasing its distribution mainly throughout the Mediterranean coast line of the country. In the province of Castellón, southern border with Catalonia, its presence was detected in 2010 in Benicàssim, a coastal town located at 16 meters above sea level (m a.s.l.). From then on Ae. albopictus has continued colonizing new localities, firstly those coastal ones with suitable conditions for the completion of its life cycle, and later the inland ones with unfavourable characteristics.

The results of 6 years of fortnightly surveillance and monitoring of each and every one of the 135 municipalities that make up the province reveals that 87 of them are currently affected by this mosquito. Likewise, the altitudinal distribution pattern is provided ranging from 6 to 985 metres so far. Nevertheless, the altitudinal profile of the province reaches 1,245 metres, fact that, together with the associated climatic conditions at that elevation might be limiting its spread and distribution pattern.

The study of this culicid is of great importance for human health since it is able to act as a vector of arboviruses, and even more after being reported seven imported cases of dengue, and one of zika in the province since 2016 to 2021, making possible the autochthonous infection of emerging and/or re-emerging diseases.

8

Determining the effects of low temperatures on the life cycle of *Aedes* (Stegomyia) *aegypti* (Linneus,1762) (Diptera: Culicidae)

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Abstract

One of the invasive mosquitoes, *Aedes aegypti* is able to transmit important disease pathogens such as Dengue, Yellow Fever, Chikungunya, and ZIKA. In the Western Palearctic Region, Madera Island, Southern Russia, Georgia, and Turkey has an established population of *Ae. aegypti*. In this study, the effects of low constant temperatures (5°C, 10°C, 15°C, 20°C, and 26°C) on *Ae. aegypti* life stages were investigated for the colony rearing under laboratory conditions.

Eggs of *Ae. aegypti* from laboratory conditions (26°C, %60 rH, 12:12 L:D) were left to hatch at constant temperatures in the climate chambers. Development from egg to adult were monitored. Cohorts consisting of 30 female and 30 male (F_0) individuals were placed under temperatures where adults emergence was observed. Effects on two different food sources (Fish food and Brewer's Yeast) were also investigated at these low temperatures. Egg hatching was observed at all temperature conditions and it has been observed that there is a direct correlation between the temperatures and the egg hatching rates. Development was completed up to the L_4 larval stage at 15°C. In this study, adult emergence was only observed at 20°C and 26°C temperature conditions. Accordingly, adults survived as 26,06±13,4 days for females, 24,83±15,52 days for males at 20°C, 28,83±16,36 days for females and 16,03±5,8 days for males at 26°C. At 26°C, adult life table parameters were rate of natural increase (r_m)=0.24, generation time (T_c)=21.46 and net productivity (R_o) = 186.50. Life table parameters could not be calculated at 20°C because the new generation eggs (F_1) could not hatch.

This research is a preliminary study to understand the effects of low temperatures on the all-development stages of Ae. aegypti. These results could help to estimate the adaptations for cold habitats and the cold tolerance ranges. The data obtained from this study will contribute to a better control program and the creation of distribution models for this species and the other invasive species.

9

Effects of Different Temperatures on The Biology of *Phlebotomus tobbi*

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Abstract

Phlebotomus tobbi, the proven vector of Leishmania infantum, is a widely distributed species across the Mediterranean Basin. Knowledge regarding the biology of *P. tobbi* is restricted to the field data, and the effects of environmental conditions on its life cycle have been poorly understood. In this study, we evaluated the effects of different temperature conditions on the developmental rate, adult survival, and fecundity of laboratory reared *P. tobbi* for the first time. Horizontal life tables were constructed using the cohorts of adults maintained at 18°C, 23°C, and 27°C with a day length of 14 h and relative humidity of 55-60%. Survival of adult *P. tobbi* significantly reduced as the temperature increased, while the fecundity increased with the increase in temperature. The fastest development was observed at 27 °C for all immature stages as well as from egg to adult emergence. Any of the larvae hatched from the eggs laid by females maintained at 18°C pupated. Increased temperature shortened the life span of *P. tobbi* as expected, and the data would be useful when predicting the future distribution and phenology of this medically important sand fly species.

10

Gene Expression Changes of Three *Phlebotomus tobbi* Adler, Theodor & Lourie, 1930 (Diptera: Psychodidae) Transcripts Under Different Environmental Conditions

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Abstract

Phlebotomus tobbi is an important vector sand fly species, widely distributed in many Middle East and Eastern Mediterranean countries including Turkey. It is the proven vector of both cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) agent, *Leishmania infantum* and also has a role on transmitting of several Phleboviruses. The main goal of the present study to reveal the differential gene expressions of this species under different constant temperature conditions and also to compare gene expression levels between natural populations dwelling in distinct geographical region in Turkey.

Cacophony (PtCac), which is known to be directly affected by temperature and two salivary gland proteins, PtSP32 and PtSP38, which have possible roles on the leishmania protection of vertebrate hosts, were used for gene expression experiments. To determine the thermal effect on gene expression, two *P. tobbi* laboratory colonies were reared under 23°C and 27°C constant temperature conditions. Sand fly sampling was also held in three distinct areas in Turkey (Adana, Datca and Yozgat), to establish the regional differences on the gene expression levels of natural *P. tobbi* populations. The relationship between gene expression levels of natural populations and climatic variables was analyzed with climatic data obtained from meteorology stations.

A positive correlation was observed between PtCac expression of laboratory colony and temperature, while negative correlations were observed in field samples with both temperature and relative humidity. For PtSP32, no relationship with temperature was detected but a negative correlation was observed with relative humidity in natural populations. Although no differences were observed between PtSP38 expression levels among regions, positive correlation was detected in laboratory colony. These findings indicated that changes in the environmental conditions can drive the expression levels of *P. tobbi* genes, which can influence population dynamics and transmission risk of the diseases.

11

Culicoides biting midges (Diptera: Ceratopogonidae) in Germany in winter: 'vector-free period' or not?

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Abstract

The emergence of bluetongue and Schmallenberg diseases in Central Europe in 2006 and 2011, respectively, demonstrated that biting midge-borne diseases are not necessarily linked to tropical and subtropical regions. Instead, they might show up wherever competent vectors occur, provided introduction of the causative viruses and climatic conditions appropriate for vector activity and virus replication.

It has been assumed that bluetongue and Schmallenberg viruses are transmitted only during the warm season and *Culicoides* vector species are not or hardly active in the cold season. However, this is questioned by recent reports of vector activity and virus transmission in temperate climatic zones during winter time and before the background of climate warming.

To verify if *Culicoides* vectors are active and feed on blood in winter in Germany, we trapped biting midges throughout the country during various periods from October to April in 2019 to 2021, using UV-light traps operated on farms with animal husbandry once a week for 24 hours. The analysis of collections focused on the Obsoletus Group and Pulicaris Complex which are known to contain vector species.

Activity of the species was observed any time at more than half of the trapping sites, with large differences between weekly catches and trapping sites. Most samples contained a few individuals, but some over 200, with higher numbers being collected indoors than outdoors. Similarly, samples contained none to well over 100 blood-fed specimens and considerable numbers of collections from November to March exceeded the threshold number of five parous *Culicoides* per night and trap, as defined by European Commission regulation 1266/2007 for a vector-free period.

The results over the three study years demonstrate that Obsoletus Group and Pulicaris Complex biting midges are active in winter, although regional and temporal fluctuations occur. They suggest that collections be made continuously and as widespread as possible to obtain reliable up-to-date information on vector activity and allow substantiated risk assessments for biting midge-borne diseases. The concept of a ‚vector-free period‘ fixed at one point of time for a complete country needs to be reconsidered.

12

Stored sheep wool as source of mosquito attractant volatiles

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Abstract

Investigating new tools for protection of humans and livestock against biting arthropods still remains an important task. The aim of this work was to study new natural means for mosquito control and surveillance. The literature has shown that sheep and other animal odor inclusion increases light trap catch of Rift Valley fever virus (RVFV) mosquito vectors and this odor may be the primary factor of mosquito attraction. We investigated commercially available stored sheep wool as attractant against adult female Aedes aegypti mosquitoes. The volatiles from sheep wool were collected by various techniques of headspace (HS) extractions and hydrodistillation. The extracts were analyzed using gas chromatography–mass spectrometry (GC-MS) and gas chromatography – the flame ionization detector (GC-FID) with GC-MS. A total of 52 volatile compounds were detected, many of them known for their mosquito attractant activity. Seven compounds were not previously reported in sheep products. The volatile compositions of the extracts varied significantly across the collections depending on the extraction techniques or types of fibers applied. Bioassays revealed that the dynamic (vibrating) bait setup with sheep wool significantly improved mosquito catches compared to the static setups or the setups without wool. Sheep wool is a readily available, affordable, and environment-friendly material and has the potential to be used as a mosquito management and surveillance component in dynamic bait setups.

13

Host-feeding patterns and West Nile virus infection in mosquitoes from the Republic of Moldova

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Abstract

West Nile virus (WNV) is considered one of the most important zoonotic diseases in Europe causing increased concern to public and veterinary health. The mosquito host-feeding patterns play a crucial role in understanding the transmission ecology of vector-borne pathogens. WNV is widely distributed in Eastern Europe and maintained by a complex transmission cycle involving different species of mosquitoes and birds. WNV had been neglected for a long time and at present is recognized as an emerging threat across several countries in Eastern Europe, including the Republic of Moldova. The last screening of mosquitoes and ticks for WNV was conducted between 1970s and 1990s, resulting in isolation of WNV from Ixodidae ticks. The focus of the here presented study was the evaluation of the mosquito host-feeding pattern and WNV circulation in the Republic of Moldova. A total of twenty-four mosquito taxa were collected by CDC light traps and CO2 baited CDC traps between 2016 and 2019 from 50 sampling sites in the Republic of Moldova. Overall, 581 DNA sequences of the cytochrome b gene have been obtained from the mosquito blood meal content. Cattle, pig and goat were the most commonly found, while Culex pipiens s.s./C. torrentium were found to have the highest host diversity including a high number of bird species. The species' role as enzootic vector was supported by the detection of WNV in four pools of C. pipiens s.s./C. torrentium via Pan-Flavivirus-PCR. Two positive WNV pools of the lineage 2 were detected for Dubăsari and Cahul regions with sampling sites located in Jagorlic Reserve and Slobzia Mare in 2019. Our data provide updated information on the vector capacity of different native mosquito species and indicate active circulation of WNV in Moldova.

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Towards exploiting skin bacteria to protect animals against insect vectors

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Abstract

Mosquitoes (Culicidae) and biting midges (Ceratopogonidae, genus *Culicoides*) use carbon dioxide and body odours to find their blood meal host. Skin bacteria play an important role in the production of these body odours. Differences in skin microbiota of individual humans were shown to correlate with difference in attractiveness to mosquitoes. In-vitro bacterial community models representing the skin composition of individuals highly attractive to *Anopheles gambiae* mosquitoes were also more attractive to *Aedes aegypti* mosquitoes than the models of poorly attractive individuals. Similarly, the preference of mosquitoes for different host species, including humans, cows, and great apes, was correlated with their skin bacterial profiles and the odours emitted. This opens the possibility to protect animals from biting insects by supplementing the skin microbiome with probiotics. We are investigating this approach in a sheep model. The skin bacterial composition of three body parts (belly, left ear, left leg) of 89 adult female sheep from three different breeds from nine farms was determined by metabarcoding. The main phyla identified were Actinobacteriota, Firmicutes, Proteobacteria and Bacteriodota. Principal coordinate analysis showed clustering of the bacterial taxa by farm and body site, whereas breed had only a marginal influence. A subset of bacteria was cultured, and Y-tube olfactometer experiments revealed bacterial isolates that were either deterring or attracting laboratory-reared *Culicoides nubeculosus* and field collected biting midges. In further studies, a repellent bacterial strain will be selected for application on sheep and tested whether this confers protection against biting midges. Applying skin probiotics could be a novel solution for long-lasting protection, in contrast to the topical application of repellents that evaporate within hours.

Further reading: Lucas-Barbosa, D., M. DeGennaro, A. Mathis, and N.O. Verhulst (2021): Skin bacterial volatiles: propelling the future of vector control. Trends in Parasitology: 2183.

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15

Analysing the efficiency of CO_2-traps as barrier against *Culex* mosquitoes

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Abstract

The use of CO2-traps is the most common method to collect mosquitoes for monitoring or surveillance purposes. Moreover, it was demonstrated that the mass trapping of host-seeking females by means of CO2-traps can significantly reduce the abundance of *Anopheles* or *Aedes* mosquitoes, thus reducing the local risk of mosquito-borne pathogen transmission. However, the potential of CO_2-traps to control *Culex* mosquitoes, the main vectors of West Nile virus, is unclear. This study aims to analyse the efficiency of CO_2-traps as barrier against Culex mosquitoes. In addition, we analysed different factors, potentially affecting the sampling efficiency of CO_2-traps, including the use of BG-Lure (Biogents, Regensburg, Germany) as additional attractant and the effect of the position of the trap (e.g. its surrounding vegetation). In 2021, the study was conducted at eight sites located between Leipzig and Wittenberg in Eastern Germany, which is considered the main circulating area of West Nile virus in Germany. At each site, 11 to 15 BG-Sentinel 2 traps (Biogents, Regensburg, Germany) were placed in a ring around an area of approximately 1 ha. Every second CO_2-trap was equipped with BG-Lure. The traps were run for four days and emptied after one and four days, respectively. In addition, six garden pop-up bags each were installed as artificial resting sites inside and outside of the ring of BG-traps and sampled with a hand-made aspirator before and after the collection periods. Overall, four collection periods between May and September were conducted. A total of 45,000 mosquitoes (expected number of specimens: ~65,000) from 27 taxa have been identified, so far. *Culex pipiens s.l./Cx. torrentium* (50%) and *Aedes vexans* (40%) were the dominant taxa. Preliminary analyses demonstrated that the CO_2-traps may lead to a short-term reduction of *Culex* mosquitoes, but cannot completely prevent mosquito activity inside the controlled area. Further analyses will evaluate the effect of BG-Lure and the position of the traps on the number of collected mosquitoes and mosquito species. The results shall provide insight into the potential of CO_2-traps for the control of Culex mosquitoes and can give detailed information for the sampling design in mosquito monitoring programmes.

16

Breeding site selection and carry-over effects of *Culex* species along a land-use gradient.

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Abstract

In the current scenario of climate warming, the outbreak risk of mosquito-borne diseases such as West Nile virus (WNV) will increase in central Europe. To properly assess the vectorial capacity of Culex species and effectively design control and monitoring plans, it is essential to understand the underlying factors which determine population development. Therefore, field experiments were carried out between 2020-2022 to analyse ecological factors influencing the breeding site selection of *Culex* spp. in different urban and suburban habitats in Germany, and how the environmental conditions of the breeding habitats effect the fitness of the emerging adults (so called carry-over effects). Firstly, sampling sites were selected along a land-use gradient from vegetation-dominated areas to urban settlements, where ovitraps were exposed and equipped with a temperature logger recording hourly. Egg lays in the ovitraps were regularly collected and later identified to species level by molecular biological analysis using PCR assays. Females preferred breeding habitats with intermediate urbanisation levels and laid significantly more egg rafts as mean temperatures increased. In a second field study, insect cages with artificial breeding habitats containing first instar larvae of *Culex torrentium* were installed along the same land-use gradient and regularly checked for adult emergence. Adults were taken to the lab and wings were dissected and measured. Results showed that larval developmental time decreased with higher temperatures in urban sites, while mortality increased. Simultaneously, adults from urban sites also had a decreased wing length and wing centroid size, indicating decreased fecundity. Wing asymmetry increased with higher temperatures, which might be indicative of environmental stress. These results provide new insights into the microhabitat-dependent ecology of *Culex* species and can refine our understanding of the spatio-temporal development of *Culex* populations.

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3D video tracking unravels how mosquitoes interact with topical repellents in the arm-in-cage test

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Abstract

Topical repellents provide protection against mosquito bites, while their behavioural mode of action is still largely unknown. Nevertheless, the arm-in-cage test is the standard behavioural bioassay recommended to assess the efficacy of topical repellents and constitutes the basis for product label claims. In the arm-in-cage test, study participants expose their repellent-treated forearm for a few minutes into a cage containing host-seeking mosquitoes. The procedure is repeated at regular time intervals until participants receive a mosquito bite that concludes the test and provides an estimate for the repellent's complete protection time. However, the arm-in-cage test as such does not reveal the repellent's behavioural mode of action. To understand how mosquitoes interact with topical repellents in the arm-in-cage test, we measured the behaviour of host-seeking mosquitoes using a 3D infrared video camera system. We tracked mosquito flight paths of individual *Aedes aegypti* and *Anopheles stephensi* females during the exposure of either a repellent-treated or an untreated forearm. The applied repellents included 20% (m/m) ethanolic solutions of N,N-diethyl-meta-toluamide (DEET), p-menthane-3,8-diol (PMD), icaridin and ethyl butylacetylaminopropionate (EBAAP). Compared to the untreated forearm, all four repellents reduced the number of bites, while the flight trajectories revealed the mosquitoes making multiple, directed contacts with the arm and are primarily irritated upon contact. Since previous studies have shown the same repellents exhibiting an odour-based repellency or odour masking effect, we conclude that repellents may have different context-dependent mode of actions, while the arm-in-cage test is biased towards measuring contact-irritancy; hence the arm-in-cage test may erroneously underestimate repellent efficacy or even fail repellents that would actually be effective under a real use scenario.

18

Insecticide resistance and feeding behavior of malaria vectors in two areas of rural West-Africa : spatiotemporal distribution, drivers, and predictability

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Abstract

Insecticide resistance and behavioural adaptation of malaria mosquitoes impact the efficacy of long-lasting insecticide nets – currently the main malaria vector control tool. To develop and deploy complementary, efficient and cost-effective control interventions, a good understanding of the drivers of these physiological and behavioural traits is needed. In this data-mining work, we modelled a set of indicators of physiological resistances to insecticide (prevalence of three target-site mutations) and biting behaviour (early- and late- biting, exophagy) of anopheles mosquitoes in two rural areas of West-Africa, located in Burkina Faso and Côte d'Ivoire, using mosquito field collections and multi-source environmental data. The objectives were to assess the small-scale spatial and temporal heterogeneity of the indicators, and to better understand their drivers, at scales that are consistent with operational action. The explanatory variables covered a wide range of potential environmental determinants of vector resistance to insecticide or feeding behaviour: vector control, human availability and nocturnal behaviour, macro and micro-climatic conditions, landscape, etc. The resulting models revealed many statistically significant associations, although their predictive powers were overall weak. We interpreted and discussed these associations in light of several topics of interest, such as respective contribution of public health and agriculture in the development of physiological resistances, biological costs associated with phycological resistances, biological mechanisms underlying biting behaviour, and impact of micro-climatic conditions on time or place of biting. To our knowledge, our work is the first studying insecticide resistance and feeding behaviour of malaria vectors at such fine spatial scale with such a large dataset of both mosquito and environmental data.

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Impact of Irradiation on vector competence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) for dengue and chikungunya Viruses.

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Abstract

Effective control strategies against arthropod disease vectors are amongst the most powerful tools to prevent the spread of vector-borne diseases.

The sterile insect technique (SIT) is an effective and sustainable autocidal control method that has recently shown effective population suppression against different *Aedes* vector species worldwide. The SIT approach for mosquito vectors requires the release of radio-sterilized male mosquitoes only, but currently available sex separation techniques cannot ensure the complete elimination of females resulting in short-term risk of increased biting rate and arboviral disease transmission.

In this study, we compared for the first time the transmission of dengue and chikungunya viruses in *Aedes aegypti* and *Aedes albopictus* females exposed as pupae to an irradiation dose of 40 Gy. Females of both species were fed on blood spiked with either dengue or chikungunya viruses, and body parts were tested for virus presence by real-time RT-PCR at different time points.

No differences were observed in the dissemination efficiency of the dengue virus in irradiated and unirradiated *Ae. albopictus* and *Ae. aegypti* mosquitoes. The dissemination of the chikungunya virus was higher in Ae. albopictus than in *Ae. aegypti*, and irradiation increased the virus load in both species. However, we did not observe differences in the transmission efficiency for chikungunya (100%) and dengue (8-27%) between mosquito species, and irradiation did not impact transmissibility. Further implications of these results on the epidemiology of vector-borne diseases in the field are discussed.

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Risk-based mapping tools for surveillance and control of the invasive Asian tiger mosquito in Switzerland

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Abstract

The Asian tiger mosquito *Aedes albopictus* is spreading globally due to human trade and travel, climate change and lack of control. In Switzerland, it is largely established in urban areas south of the Alps. Cities north of the Alps can be considered at risk of establishment. Our aim is to provide local authorities north of the Alps with a tool to focus surveillance and control of the mosquito in the areas where the risk of establishment is higher. To achieve this, we develop a risk model that learns from the historical data on *Ae. albopictus* occurrence in the Canton of Ticino, south of the Alps, and links it to socio environmental predictors, computing a probability of establishment. The model is then extrapolated to Switzerland and more finely to the cities of Basel, Lausanne and Zurich. In addition, we investigate if we can obtain more precise and realistic risk scenarios for the spread of *Ae. albopictus* when considering the winter microclimatic conditions of catch basins, one of the major sites of oviposition and egg overwintering in temperate urban areas. For this part we develop a microclimate model to predict the average microclimatic winter temperatures of catch basins, based on available local meteorological parameters. We then use the microclimate model to correct the predictions of the risk model for Basel, Lausanne and Zurich. The results indicate that the risk of establishment for *Ae. albopictus* in temperate urban areas increases when microhabitat temperatures are considered.

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An update on short-term forecasting for larvae and adult mosquito abundances and epidemiological risk in wide-area mosquito control programs

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Abstract

Since 2018, many wide-area mosquito control programs in Greece have field data collection and epidemiological awareness embedded as prerequisites, hence reflecting the maturity of such methodologies as a result of scientific research and the industries' initiative. The most important challenge in such programs remains the development, testing, optimization and operational establishment of the optimum workflow in compliance with the basic need of all mosquito control programs: that of a better Decision Support System (DSS).

In this communication six major digital operational tools of Ecodevelopment are briefly presented which are based on systematic data collection (raw field data, processed and open-source data, from 2010 and onwards in 5/13 regions of Greece), developed in collaboration with renowned Universities and Research Institutes and implemented gradually from 2015 and onwards:

- eBite, an online platform for real-time recording, monitoring, visualizing and analysing of field monitoring data and related control actions
- eFACTORY, a data engineering pipeline for real-time error-proofing and pre-processing of more than 300 variables derived from multiple sources (field data, remote sensing, epidemiological data, etc.)
- BoL, a weekly predictive model for the presence-absence of mosquito larvae on a breeding site-specific scale (operational in the Region of Central Macedonia since May 2022)
- BAd, a daily-run predictive model for the abundance of adult Culex spp., Aedes spp., and Anopheles spp. mosquitoes on a settlement level (operational in 2,415 villages in four regions of Greece since 2020)
- BAR, a weekly predictive risk model for West Nile Virus on a settlement level (operational in Central Macedonia from 2020 for 1,000 villages)
- Mosquito Vision, a mobile application based on the predictions of BAd which provides daily forecasting for mosquito nuisance at a settlement level in four regions.

Parts of these operational tools are components of the EYWA system (EarlY WArning System for Mosquito-borne Diseases), which has been awarded with the EIC Horizon Prize for Epidemics. This research was co-financed by the European Regional Development Fund of the European Union and Greek national funds under the call RESEARCH–CREATE–INNOVATE (project code: T2EDK-02070) and under the

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Temperature preferences of mosquitoes and biting midges

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Abstract

Mosquitoes (Culicidae) and biting midges (Ceratopogonidae, genus *Culicoides*) are two vector taxa that spread numerous pathogens of human or veterinary significance such as West Nile virus and bluetongue virus. The impact of temperature on vector-borne disease dynamics and distributions has been studied intensively, especially to model the effect of climate change. However, these studies used macroclimatic data and did not consider if vectors have thermal preferences and select specific microhabitats. This project focuses on preferred temperatures of mosquitoes and biting midges of different life stages, feeding stages and infection states. Investigations are done under laboratory (thermal gradient setup with video tracking), semi-field (large outdoor cage) and field conditions. Thermal gradient setup experiments showed that laboratory reared *Aedes aegypti* and *Ae. japonicus* behaviourally thermoregulate, preferring cooler resting temperatures than their physiological optimum proposed in the literature[1]. These results were supported by semi-field experiments: Adult mosquitoes were found eight (blood-fed) or four (sugar-fed) times more often in the cooler resting boxes than in the warm ones. Field studies at two altitudes revealed a thermal preference in host seeking mosquitoes (CO2 traps) and in biting midges (UV light trap), but the orientation is species dependent.

Reference

[1]Ziegler, R., W.U. Blanckenhorn, A. Mathis, and N.O. Verhulst (2022). Video analysis of the locomotory behaviour of Aedes aegypti and Ae. japonicus mosquitoes under different temperature regimes in a laboratory setting. Journal of Thermal Biology 105: 103205.

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The emergence and re-emergence of zoonotic cutaneous leishmaniasis in northwest Negev Israel, through the lenses of the sand fly monitoring

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Abstract

The transmission of zoonotic cutaneous leishmaniasis as a result of Leishmania major has long been known in Israel. About fifteen years ago, a geographical spread of the disease to areas where endemic transmission was previously unknown was observed, leading in 2010 to the emergence of an outbreak in several localities in the Northwest of the Negev. Morbidity rates peaked in 2011 and 2012, and gradually decreased in 2013-2015. In the following years, only sporadic morbidity was reported. The situation changed at the end of 2021, with a re-emergence of the disease in the region.

Monitoring of sand flies in the area began in 2011 and has continued since then with varying extent and frequency. Sand flies were collected by modified CDC light traps operated without light, placed in an updraft position with openings about 10cm above the ground, and baited with ~1 kg of dry ice. The catch was brought to the lab counted, identified and subsamples of the females were analyzed for the detection of Leishmania DNA. Phlebotomus papatasi, the vector of *L. major* was the only species in most of the catches. Its densities were very high in 2013, gradually declined, and remained relatively low until 2020. *Leishmania major* infection rates assessed in females were high in 2011, declined until 2015, and were very low between 2016 and 2020 peaking again in the second half of 2021.

The results demonstrate a positive correlation between the infection rates of sand flies with Leishmania and morbidity. This observation indicates that the re-emergence of the disease in the area is probably a result of changes in the natural transmission cycle of the parasite and not a result of changes related to the human population.

We hope that the multi-year data, together with the current collection effort, will enable us to point out the environmental factor(s) that caused or enabled the observed change, and formulate hypotheses about their modes of action. Such insights can be used for data-based predictions and hopefully also for effective intervention actions.

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The Social Determinants, Surveillance and Control of Leishmaniasis across Northern Syria – through a Decade of Conflict

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By the end of 2021, the number displaced by war, violence, persecution and human rights abuses stood at 89.3 million, of which 53.2 million were displaced within their home countries. Disease, conflict, urban destruction and mass population displacement are inextricably linked. Transmission of vector, water and sanitation borne diseases ramps up very quickly under the conditions associated with conflict and displacement. The same context changes which drive up disease transmission, directly increase human vulnerability to disease infection and death, until access to effective shelter, disease control, health and other vital services, are put into place. Malaria, dengue fever and, or Leishmaniasis are predominant causes of suffering and death as in many of the conflicts that have characterised our world over recent decades. However, studies on the interlinked nature of the multiple factors associated with conflict and epidemiological changes in vector borne diseases, and their effective control, remain few. Cutaneous leishmaniasis (CL) has historically been present in Syria, mainly in the western part of the country. However, the overall epidemiology of leishmaniasis has been heavily reshaped by the war in Syria (2011- present) that is responsible for 27% (6.8 million) of the world's refugees, and for 6.7 million people living in internal displacement (at the end of 2021). Protecting people displaced, isolated, or returning to damaged homes, when at their most vulnerable, is vital.

I will review a decade of key study data that helps to elucidate how and why Leishmaniasis epidemiology has changed during the war, and explore the feasibility, scale and effectiveness of old and new prevention tools and strategies designed to protect the most vulnerable from infection.

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Entomological surveillance in the Attica region (Greece) as part of integrated mosquito management.

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Abstract

A plethora of different mosquito species plays an important role as vectors of pathogens. Especially invasive mosquito species (*Aedes albopictus* in particular for Greece) pose a threat to the expansion of vector-borne diseases in new geographic areas. Therefore, entomological surveillance and population data collection are needed for mapping the risk of disease transmission in an area, and also for taking actions to reduce the risks. In the present study, 57 adult mosquito traps (BG-Sentinel 2) are operating continuously since February 2021, alongside the 114 ovitraps for egg collection, in the Attica region of Greece. The trapping locations were chosen based on the geostatistical method of stratified random sampling. The BG- Sentinel traps, except for the lure that is provided by the manufacturer, are connected with a gas cylinder that, under specific pressure, releases CO2 that act as an attractant for adult mosquitoes. Briefly, the samples (adult mosquitoes and eggs) are collected and transferred to the lab where the morphological identification of adult mosquitoes takes place along with the counting of eggs. The results for adult mosquitoes and eggs are presented with static and online thematic maps to the relevant stakeholders of the Region. Weekly, based on the obtained results, the Benaki Phytopathological Institute in collaboration with Local Authorities (Regional Units and Municipalities) jointly decide the appropriate measures that need to take place for mosquito management.

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Vector-borne disease risk estimation based on commercial veterinary laboratory tests in Germany

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Abstract

Commercial veterinary laboratories conduct large numbers of tests on several tick-borne diseases in domestic animals. This data is a treasure trophy of information both to map the seasonal and regional distribution miof these diseases and to analyse changes in time in a spatial and time series sense.

For this analysis data was used from tests performed by the laboratory LABOKLIN (Bad Kissingen, Germany) upon request by German veterinarians. As an example, we used the occurrence of Babesia spp., parasites transmitted by ticks to dogs. In Germany, such data are available at the five-digit post code level.

In this presentation we analyse how a series of issues related to such data bases may affect the outcome of spatial distribution models using babesia dogs as example. More specifically we discuss:

- How data clusters, i.e. areas with a higher density of reports, may affect the outcome of spatial distribution models and how this can be dealt with.
- How polygon input data (postcode areas) can be converted into finer resolution raster output data and whether this improves outputs.
- How the diagnostic method used, i.e. antigen versus antibody detection, may affect the output of distribution models.
- Whether such databases allow to compare distribution patterns in time.

In the discussion we highlight which pitfalls to avoid and how these outcomes may contribute to improve disease management. The high-level risk maps are routinely made freely available through a public website.

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AIMSurv-AIMCOST: Why to harmonize the surveillance of Aedes invasive mosquito species in Europe?

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Abstract

In recent decades several Aedes invasive mosquito species have invaded Europe. These species have important implications because of aggressive biting behaviour and potential transmission of vector-borne diseases. In 2018, the COST action Aedes Invasive Mosquito (AIM) (https://www.aedescost.eu) established a transboundary network of partners and institutions across Europe to improve surveillance, control and communication about AIM species. Harmonization of sampling methods and procedures was soon recognised as one key issue for improving mosquito surveillance. To meet this goal, AIM-COST organized a training school in early 2020 that resulted in the development of a harmonized protocol for AIM species in Europe, defining standardised sampling methods, frequency and length of the sampling period, as well as the reporting (AIMSurv protocol; https://www.aedescost.eu/aimsurv). The AIM-Surv protocol was implemented by volunteer teams from 24 European and near-neighbouring countries in 2020, 2021 and 2022 resulting in the first pan-European AIM surveillance dataset. The core data file of AIMSurv2020 contains 19,130 samples with nearly 450,000 eggs and 82,000 adults. The 2020 data is available as open-access file in GBIF (https://doi.org/10.15470/vs3677) which will be updated with 2021 and 2022 data, providing an unprecedented resource for accurate comparison of AIM surveillance, abundance, and seasonality between countries and/or regions at the continental level. These data also have the potential to be compared with other AIM sampling strategies, such as citizen science photographic records sent via the Mosquito Alert app, also implemented in collaboration with AIM-COST.

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Invasive Mosquitoes: A Roadmap from Sampling to Modelling and Dissemination

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Abstract

Aedes Invasive Mosquitoes (AIM-COST) is a COST project aiming to recommend best practices in Aedes invasive mosquito surveillance, control, modelling, and mapping. One of the key outputs, currently in the final stages of preparation, is the AIM-COST Roadmap. This aims to provide public health professionals with recommendations of the most effective ways to make integrated assessments of invasive mosquito distributions, while ensuring that the technical outputs are presented in a way that is comprehensible to a range of public health users.

The Roadmap sets out the basic types of sampling designs, provides guidance on determining the number and locations of collection sites in relation to the anticipated aims of the sampling programme, and summarises the sampling protocols used in the recent continent wide AIMsurv invasive mosquito sampling initiative that has been implemented each year since 2020.

Well-specified data cleaning and curation procedures ensures that the data used to map the results or passed on to modellers for spatial or mathematical analysis is reliable and error free. The importance of accumulating information on both the presence and absence of a population in a region by performing consistent and standardised data collection is emphasized.

The Roadmap summarises recommended methodologies for modelling invasive mosquitos and some common issues related to them. Two broad categories are covered: (i) spatial distribution modelling, which provides detailed maps from the sampled data, and (ii) mathematical modelling, which uses measured or simulated development processes or life history parameters to estimate population abundance and dynamics.

Finally, the Roadmap recognises that the technical outputs of the sampling and modelling are often not readily comprehensible for users that are not intimately familiar with the techniques used. It therefore examines how the technical results are most appropriately visualised as maps, charts or presentations so that the data produced by the technicians can be effectively interpreted and acted upon by those responsible for planning vector mitigation strategies, fundraising or public facing risk communication.

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Detection, identification and genotyping of *Borrelia spp.* in ticks of Coastal-Karst and Littoral-Inner Carniola regions in Slovenia

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Abstract

The density and spread of tick vector species have increased throughout Europe in the last 30 years, leading to an increase of *Lyme borreliosis* cases, including in Slovenia. The aim of this study was to isolate Borrelia strains and to determine the prevalence of *B. burgdorferi s.l.* and *B. miyamotoi* in *I. ricinus* in the two regions of the country (Coastal-Karst and Littoral-Inner Carniola) using cultivation and PCR.

In 2019, we isolated *B. burgdorferi s.l.* by culture method in 28/559 (5%) ticks from both regions. Culture negative samples (531 ticks) were additionally tested by real-time PCR. In 155 PCR positive samples, a fragment of flaB or glpQ was amplified and further sequenced to identify Borrelia species. Using both methods, the Borrelia prevalence was 32.7% in Coastal-Karst and 33.0% in Littoral-Inner Carniola region respectively. Genotyping of Borrelia isolates revealed that most belonged to the *B. garinii*, 17/28 (60,1 %) were identified as *B. garinii* subtype Mlg2.

Of all tick samples tested in 2019, 8/398 (2 %) were PCR positive for *B. miyamotoi.*

The presence of *B. burgdorferi s.l.* in ticks from the Littoral-Inner Carniola region was slightly more frequent than in ticks from Coastal-Karst region. Based on previous studies in this region, we had expected more ticks to be infected with *B. afzelii*, but genotyping revealed that *B. garinii* was most abundant.

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Detection and Isolation of Sindbis virus from Field Collected Mosquitoes in Timimoun, Algeria

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Abstract

Sindbis virus (SINV) is a zoonotic alphavirus (family Togaviridae, genus Alphavirus) that causes human diseases in Africa, Europe, Asia and Australia. Occasionally, SINV outbreaks were reported in South Africa and northern Europe. Birds are the main amplifying hosts while mosquitoes play the role of primary vector. Culex mosquitoes were collected in Algeria and subsequently tested for SINV. SINV RNA was detected in 10/40 pools (922 mosquitoes). A strain of SINV was isolated from a pool displaying high viral load. Whole genome sequencing and phylogenetic analysis showed that the SINV Algeria isolate is most closely related to a Kenyan strain. This is the first record of SINV in Algeria and more broadly in northwestern Africa which can be a potential risk for human in circulating area. Further studies are needed to measure the impact in public health through seroprevalence studies and inclusion of SINV a differential diagnosis of other arboviral diseases.

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Sandflies and human visceral leishmaniasis in Bulgaria

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Abstract

Phlebotomine sandflies are known to transmit parasites, bacteria and viruses that affect humans and animals in many countries worldwide. During the 20th century, they were considered to transmit human visceral leishmaniasis (VL) and sandfly fever in Bulgaria. Early studies on species composition in the first half of the century revealed the presence of five species of genera *Phlebotomus* and *Sergentomyia* in the country. After a 60-year gap in the sandfly research with no data between 1950 and 2010, the studies on sand fly fauna were renewed in 2011.

Fourteen different regions of Bulgaria were sampled for sandflies between 2011 and 2014 using different light traps and CO_2 traps with 991 specimens collected. Moreover, thirteen regions were sampled between 2015 and 2016 during the VectorNet field campaign in Bulgaria with 1268 specimens collected. Most of them were identified using morphological keys and confirmed by PCR and MALDI-TOF assays. The contemporary species list includes *Ph. alexandri*, *Ph. balcanicus*, *Ph. kandelakii*, *Ph. neglectus*, *Ph. papatasi*, *Ph. perfiliewi s.l.*, *Ph. sergenti*, *Ph. tobbi*, *S. dentata*, and *S. minuta*.

The first imported human VL case was reported in 1921 and the first domestic case occurred in 1937. Until 1953, 57 autochthonous cases were reported in the country, 50 of which were children. During the following 35 years, only sporadic cases were recorded. Since 1978, VL is a mandatorily notifiable disease in Bulgaria.

Between 1988 and 2021, 166 confirmed VL cases (both autochthonous and imported) were registered in Bulgaria. Of them, 155 were primary autochthonous cases, four cases of relapse, and seven imported cases of disease from other European countries with endemic VL distribution. Predominantly affected was the male sex (n = 121) compared to the female (n = 45), with a ratio of 2.7:1, while the number of infected children (n = 82) and adults (n = 84) was almost identical.

The predominant sandfly species found in Bulgaria are *Ph. neglectus*, *Ph. perfiliewi* and *Ph. tobbi*. All these are proven vectors of Leishmania infantum causing zoonotic VL and human VL, and should be considered the suspected vectors of VL in the country.

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Zoonotic vector diseases situation in Valencian Autonomous Region (Eastern Spain) along the period 2010-2020

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Abstract

The animal diseases incidentally transmitted to humans are call zoonoses. The zoonotic agents are pathogens such as bacteria, viruses, parasites fungi and other biological elements. Their transmission can be both in a direct or indirect way. Regarding the second way, stand out those transmitted by the so-called vectors.

The recent year evidences indicate that an increase on the number of cases of vector-borne zoonoses have taken place. Climate change; globalization with an increase in international traffic of goods and people associated; as well as the transfer of pathogens from wild to domestic reservoirs and, from these, to man have been the main causes.

In Spain, the Epidemiological Surveillance Services of each autonomous region are the public entities in charge of registering and reporting the cases of zoonotic diseases occurred. This information is notified to European organizations such as European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC).

This communication provides data about the situation of vector-borne zoonotic diseases that affect human beings in Valencian Autonomous Region (Eastern Spain). Information concerning to notifiable diseases along the 2010-2020 period has been collected. It has been verified that the vector agents belong to two groups: Diptera of the Psychodidae Phlebotominae and Culicidae families, and also to ticks of the Ixodidae family. A compulsory epidemiological surveillance on the following seven diseases is carried out in Valencia: Leishmaniasis, Malaria, Chikungunya, Dengue, Zika, Mediterranean spotted fever and Relapsing fever due to ticks. Some of them are endemic while others are imported. Finally, the evolution of the registered cases has been analysed; actions carried out by health authorities for its prevention and/or control are evaluated and the level of affectation of human vector arthropods is discussed since it is a tool to assess the thought increase happened in the arthropod vector populations associated with zoonoses.

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"I'll be back": a new bluetongue outbreak in the Balearic Islands after 18 years of silence.

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Abstract

Bluetongue (BT) is a viral disease that affects ruminants and is transmitted by blood-feeding biting midges (*Culicoides spp.*). The last time BT was detected in the Balearic Islands (Spain) was in 2003. Since then, no further outbreaks were detected until June 2021, when a new outbreak of bluetongue affected the island of Mallorca. The main known vector species in Spain are *Culicoides imicola* and the species of the Obsoletus complex. Samples collected from animals showed that the circulating serotype was BTV-4. In order to obtain further information about the epidemiology of the recent outbreak, adult *Culicoides spp.* were collected using UV traps from farms and natural areas in Mallorca in September and October 2020, as well as from May to October 2021. Collections were grouped by pools according to species, date and place of sampling and were kept in cold conditions to allow virus detection by molecular techniques at the Spanish reference laboratory for BT. A total of 6976 females of biting midges grouped in 242 pools were submitted for BTV molecular detection. 16 pools collected from farms in 2021 resulted positive to BTV-4, and from these, 15 pools included *C. imicola* and one pool included *C. newsteadi*, while all pools collected in 2020 resulted negative to BTV. The obtained results indicate that *C. imicola* is still the major BTV vector in Majorca, while more importantly, this is the first time that *C. newsteadi* is incriminated in the transmission of BTV in Spain.

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MALDI-TOF mass spectrometry: A powerful method for blood meal identification in insect vectors

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Abstract

Determination of blood meal sources of hematophagous arthropods is crucial for understanding transmission cycles of vector-borne diseases in endemic areas. Most of currently used methods are nevertheless laborious, expensive and challenged by tiny volumes of rapidly degraded host blood. A promising approach towards blood meal source identification was recently developed for sand flies employing peptide mass mapping-based MALDI-TOF mass spectrometry (MS) analysis of host-specific haemoglobin peptides generated by trypsin digestion of the engorged blood [1].

The method was first tested on lab-reared sand flies, allowing correct host identification of 100% experimentally fed females until 36h post blood meal (PBM) and for 80% of samples even 48h PBM and thus providing longer reliable blood source determination than other nowadays used methods. The blind study using field-caught sand flies yielded unambiguous host identification for 96% of females collected during a field survey in Greece. Moreover, the method successfully determined blood meals of engorged females collected in Bosnia & Herzegovina and Croatia and stored frozen in ethanol for several years prior to the analysis. The approach also works on blood meals spotted on a filter paper that represents a simple and low-cost alternative of sample storage enabling easy shipment at ambient temperatures from regions of collection to MS facilities for the analysis. Furthermore, it allows correct identification of mixed blood meals as was demonstrated on both experimentally fed and field-collected sand flies and reliable differentiation of closely related host species within the same genus.

MALDI-TOF mass spectrometry was shown as an accurate and fast method for blood meal identification with a minimal sample input. Beside sand flies, it was applied also on Culex mosquitoes and may be universally applicable to various hematophagous arthropods.

Reference:

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Monitoring *Aedes aegypti* in Portugal with a novel optical sensor system for the automatic classification of mosquitoes

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Abstract

The *Aedes aegypti* mosquito was first identified on Madeira Island (Portugal) in 2005. Since then, its distribution has been expanding throughout the most densely populated areas on the southern edge of the island.

Seven years after its identification, the first dengue outbreak occurred on this island, the first in Europe since 1928. The outbreak began on September 26th of 2012 and ended on March 3rd of 2013, with 1080 confirmed infections and over 2000 probable cases. To help reduce the risk of future outbreaks, local health authorities have been monitoring species distribution across the island, particularly in the most densely populated areas. However, the invasion process is still ongoing, thus increasing the risk of future outbreaks in the island and the spread of the species to other regions.

Understanding the dynamics of this species is critical for targeted local control strategies, thus new surveillance methods need to be employed and tested. With this aim in mind, the VECTRACK project, led by IRIDEON, developed a "smart" trap, utilizing a bioacustic sensor. This sensor, which was calibrated through machine learning methods, will be able to identify multiple mosquito species, including species such as *Aedes albopictus* and *Aedes aegypti*, and send information about the amount of captures and their place, in real time, to the team in charge of the surveillance. After a testing period in Barcelona, Algarve and Águas de Moura, this new sensor was implemented in Madeira Island to access its viability as a surveillance tool.

In this work, we will present the results of the first months of captures in Madeira and accessing what extra information this new type of "smart trap" can give us, compared to the usual surveillance methods. Hopefully, with the advance of these methods and technology, it will be possible to monitor a countries points-of-entry for Aedes invasive species in real time, greatly improving the time of response in order to prevent these carriers of arboviruses from expanding.

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VECTRAP: Applicability of a mass trapping strategy against *Aedes albopictus* and *Aedes aegypti*

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Abstract

Aedes aegypti and *Ae. albopictus* are among the most important public health issues due to their ability to transmit human arboviruses, as well as a major source of nuisance. As the systematic use of insecticide against adults or larvae is an inappropriate strategy, the only accepted method of reducing the abundance of these vectors but also harming mosquitoes is through social mobilisation. Research and evaluation of new complementary methods without environmental and health effects remain a priority. For a few years, mass trapping is studied as a complementary or alternative control strategy. The objective of Vectrap project (PNR-EST-ANSES n°2020/1/114, 2020-2024) is to demonstrate the effectiveness, feasibility and acceptability of a new control strategy based on massive trapping against both species using 2 types of traps - catching gravid and host-seeking females - and on citizen mobilisation. The project is conducted in 3 different regions (2 in metropolitan France, 1 in Martinique Island), with 8 sites for each location. Effectiveness is assessed in terms of reducing the number of mosquitoes, with the help of weekly or bi-monthly captures, and swap in behaviour and perceptions of the population based on psychometric studies (questionnaire surveys). The ultimate goal of the project, planned for 3 years, is to demonstrate the effectiveness of this mass trapping method in order to provide municipalities wishing to control these species with an acceptable and cost-effective strategy. During the first campaign in 2021, A difference in abundance between control and treated sites after treatment was observed in the Rhône-Alpes area, but not for the Montpellier area, while the campaign had to be suspended in Martinique due to the social context. For the second campaign, trapping is complemented by larval control to increase the effectiveness of the strategy.

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Integrating SIT in *Aedes albopictus* control in Serbia: MRR trials

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Abstract

Asian Tiger Mosquito, *Aedes* (Stegomyia) *albopictus* (Skuse, 1894) was for the first time recorded in Serbia in 2009 on the border crossing to Croatia. Afterwards species has continued its invasion along the highway towards the neighboring towns and villages. In 2019 species was established in Novi Sad (second largest city in Serbia) and has been spreading successfully to the all parts of city. Conventional mosquito control measures did not provide significant reduction of the Asian Tiger Mosquito. Therefore, we decided to start integrating SIT in control of this invasive species. To do this, four mark-release-recapture (MRR) experiments were performed to estimate size of the local population, longevity and flight range of sterile males. Two areas were selected: area where the sterile male mosquitoes will be released and the control area where only conventional measures are conducted by private mosquito control company. In both control (20 ovitraps and 12 HLC in 20 ha) and release (20 ovitraps and 40 HLC in 20 ha) areas monitoring started two weeks before the first release and finished one week after the fourth release. There were four weekly releases of males for MRR studies. Ovitraps were employed to register possible induced sterility. Control area and release area are both urban types of habitats, but for sampling stations we selected mainly areas rich in vegetation. Local strain of *Ae. albopictus* was reared and amplified, male mosquitoes sterilized and delivered from Centro Agricoltura Ambiente, Crevalcore, Italy. Males were released from two points, 50m distanced and recaptured on 1st,2nd,4th, and 6th day after release by four collectors sampling at 10 stations (1:30h) each h sampling day. At control area two collectors conducted HLC sampling once a week. Results of four MRR trials will be presented.

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Finding the balance between efficiency and budget: preventive invasive mosquito species (IMS) surveillance.

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Abstract

Early detection and control of invasive mosquito species (IMS) at points of entry (PoEs) are of paramount importance to slow down any possible establishment. Preventive actions are however more difficult to sell to the authorities than reactive actions. Since 2007, IMS monitoring in Belgium is project-based and short-term without a future guarantee. Based on available literature and experiences from Belgium and neighbouring countries, a recommendation for a cost-efficient long-term IMS monitoring programme was made. We reviewed the important factors to take into account: the IMS and PoEs to be surveyed, the collection methods to be used, and the options to decrease the workload and the costs. The following recommendations were proposed to the Belgian authorities in 2020:

- The targeted IMS should be *Ae. albopictus*, as it is posing the highest risk for human Aedes-borne disease transmission in the near future. To cover the full *Ae. albopictus* introduction or activity period, active sampling should be carried out between mid-April and end-October.
- From a practical and feasibility perspective, active monitoring should focus on the 10 most important high-risk PoEs. Yet, a yearly evaluation of these and potential new high-risk PoEs with new data on import origin, frequency and volume is important. To follow the spread along the highway, it is also recommended to actively monitor the next main parking lot following an *Ae. albopictus* positive one.
- Active monitoring should be supplemented by passive monitoring. Citizen science should cover entire Belgium, including camping places and cities, with PoE-targeted citizen science at less important high-risk PoEs (e.g. ports, airports).
- Sampling design should involve BG-Sentinel traps, oviposition traps and larval sampling. Yet, some alternatives are possible to make it even more cost-efficient.
- Collaboration with local partners at the PoE or colonised area is essential.
- Operational research is recommended to be able to incorporate innovations into the monitoring.

Finally, a sustainable, structured and long-term IMS management programme in Belgium should not only integrate active and passive entomological surveillance, but

also vector control and Public Health surveillance. Anno 2022, a large part of this recommendation was implemented, but there is still room for improvement.

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New developments on mosquito mass-rearing for the sterile insect technique application

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Abstract

Over 80% of the world's population lives in areas at risk of transmission of one or more mosquito borne diseases. While the effectiveness of chemical vector control is decreasing as mosquitoes develop insecticide resistance, there is an urgent need for innovative mosquito control methods, and one such is the sterile insect technique (SIT). The SIT is an insect birth control method using area-wide inundative releases of sterile insects to reduce reproduction in a field population of the same species. The Joint Food and Agriculture Organization of the United Nations / International Atomic Energy Agency (FAO/IAEA) Centre of Nuclear Techniques in Food and Agriculture is supporting the integration of the sterile insect technique (SIT) with existing national vector control strategies, to help delay, curtail and contain the spread of pathogen-carrying mosquitoes in Europe and worldwide. Since 2004, the Insect Pest Control Laboratory (IPCL) has been developing the SIT package against mosquitoes and mass-rearing is one of the essential steps in the process. Substantial efforts have been made in the development of equipment and protocols for mass-rearing, sex-separation, irradiation, handling, packing, transport, release, and quality control. Mass-rearing racks and cages, automatic larval counter, larval diet ingredients were developed and successfully tested. Rearing methods including larval rearing water quality for optimal pupae production and quality of the resulted adults were determined. A phased conditional approach for mosquito SIT application was also proposed. The WHO and IAEA produced a joint guidance framework for testing SIT as a vector control tool against Aedes-borne diseases.

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Adult mosquito predation and potential impact on the sterile insect technique

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Abstract

The sterile insect technique is a promising environmentally friendly method for mosquito control. This technique involves releasing laboratory-produced sterile males into a target field site, and its effectiveness may be affected by the extent of adult mosquito predation. Sterile males undergo several treatments. Therefore, it is vital to understand which treatments are essential in minimizing the risks of predation once released. The present study investigates the predation propensity of four mantis species (Phyllocrania paradoxa, Hymenopus coronatus, Blepharopsis mendica, Deroplatys desiccata) and two gecko species (Phelsuma standingi, P. laticauda) on adult *Aedes aegypti*, *Ae. albopictus* and *Anopheles arabiensis* mosquitoes in a laboratory setting. First, any inherent predation preferences regarding mosquito species and sex were evaluated. Subsequently, the effects of chilling, marking, and irradiation, on predation rates were assessed. The selected predators effectively preyed on all mosquito species regardless of the treatment. Predation propensity varied over days for the same individuals and between predator individuals. Overall, there was no impact of laboratory treatments of sterile males on the relative risk of predation by the test predators, unless purposely exposed to double the required sterilizing irradiation dose. Further investigations on standardized predation trials may lead to additional quality control tools for irradiated mosquitoes.

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Autodissemination method against *Aedes albopictus* in a field experiment in Southern France

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Abstract

Aedes albopictus, after its installation in metropolitan France in 2004 represents now an important source of nuisance in sectors where marsh mosquitoes have been controlled for more than 60 years. It is also, and as episodes of autochthonous transmissions prove it every year, a good vector of arboviruses of major health importance. Even if an emergency response plan exist with biocides against adult mosquitoes, sprayed around travelers when they are virus-carriers, preventive actions have to be implemented to reduce the vector densities in urbanized areas which remains high despite effort on community involvement -the most used strategy- whereas it's results are often disappointing.

A method of self-dissemination (autodissemination AD) of larvicide made upon a commercial trap was developed in our lab, studied in the field and finally tested in a configuration representative and compatible with reasonable operational conditions near Montpellier during summer 2021. With more than 0.25 AD station per hectare (around one house over 4), a significative decrease in adult densities was observed (up to 40% depending on the area), as well as an effective contamination of the breeding sites. However, this decrease is not considered as sufficient regarding the population's expectations. However, integrated strategies using different synergized methods could improve the efficacy of autodissemination.

These results are discussed from an operational control perspective of *Aedes albopictus*.

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Update of the Vector-borne Viruses circulating in Europe in the Shadow of Sars-Cov-2

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Abstract

The last 5 years witness to the importance of the Public Health burden of emerging infectious pathologies in the world and particularly in Europe. A significant proportion was caused by pathogens vectored by biting arthropods in relation to the phenomenon of global warming and the "tropicalization" of southern Europe and the Mediterranean basin.

In Europe, the distribution of arthropod vectors such as ticks, mosquitos, sand flies and biting midges of viral diseases was effected by environmental modifications, climate change and urbanization and widened. Vector-borne viral diseases have a growing importance for human and animal health, with prominent outbreaks reported in humans (e.g. West Nile, Tick-borne encephalitis, Sindbis, Toscana virus) and animals (e.g. Bluetongue, Usutu, West Nile virus) in recent years in Europe. West Nile fever is caused by the West Nile virus has been recorded in numerous European countries include Italy, France and Romania with increase numbers. Tick-borne encephalitis is endemic in 27 European countries today with considerable increase of the incidence. Toscana virus show distribution in south Europe and cause human cases in both residence and tourists which was started recording more in patient with central nervous system diseases.

In this presentation, our aim to have an update on epidemiology of vector-borne viruses in Europe in the shadow of Sars-Cov-2 pandemic.

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Keynote Abstract

Greening cities and vectorial risks for human, animal and plants

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Abstract

Global growth occurs mainly in cities where 70% of the world's population is expected by 2050 (United Nations 2018). Urbanization is a process whereby some habitats are lost; others are reduced and new ones are created. Urban areas are mosaics of residential, commercial and industrial built-up areas intertwined with green spaces that can provide habitat for animals, plants, and their potential pathogens and vectors. Many studies have shown the positive impact of urban greening on biodiversity, human health and well-being. Nevertheless, few studies have addressed the possible negative impact of greening cities on human, animal and plant health, including the emergence of diseases related to pathogens transmitted by arthropods such as mosquitoes, ticks or leafhoppers.

Urban vegetation can provide a suitable ecological niche for several arthropod species, including mosquitoes, hemiptera and ticks. Some of these species are already adapted to the urban environment, such as *Aedes albopictus* and *Culex pipiens*, and will therefore develop almost 'naturally' in the city allowing the circulation of diseases, such as dengue or West Nile fevers for example. For some other species more adapted to rural areas, the development of green screens can create a continuum from the rural to the urban environment, leading to the spread of vectors into cities. According to plant vector borne diseases, knowledge is further limited. But, with urban ornamental plants that can be potential hosts, for example of *Xylella fastidiosa*, there is a need to consider the potential spread of this pathogen to peri-urban crops, such as vineyards or olive trees, which would have a significant economic impact.

Therefore, well-documented data is still needed to fully assess the impact of urban greening on vertebrate and plant vector-borne diseases. An interdisciplinary and cross-sectoral One Health approach, bringing together all partners and components interested in making city healthier, is the most relevant way to be able to propose prevention, management and control actions that are shared by all stakeholders.

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Monitoring of Exotic Mosquitoes (MEMO+) project in Belgium: The passive surveillance component

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Abstract

Introduction of exotic mosquito species (EMS) especially the tiger mosquito (Aedes albopictus) is a potential threat for human health in Belgium and across Europe. Therefore, surveillance of this specific EMS is of paramount importance to early detect its introduction and postpone its establishment and as such prevent local transmission of viruses such as chikungunya, dengue or Zika. In this context, the MEMO project, funded by Belgian health and environment authorities, performed active surveillance at potential points of entry between 2017 and 2020 with the aim to detect EMS. The project was successful and several introductions of Ae. albopictus were reported. To scale up the surveillance across the whole Belgian territory the MEMO+ project started in 2021 adding a passive surveillance component based on citizen science to the existing active surveillance.

A citizen science platform, MuggenSurveillance/SurveillanceMoustiques was launched end of May, 2022. The platform (a website) has two main purposes: the notification portal of tiger mosquitoes in Belgium and a repository of information for the general population on mosquitoes (eg. life cycle, breeding sites,..), EMS and mosquito-borne-diseases. Citizens can upload pictures of a potential *Ae. albopictus* on the website, after answering some filtering questions about size, color and stripes in legs. Pictures are then analysed to determine whether or not it is a tiger mosquito and feedback is provided to the notifiers.

Since the launch of the website, 214 pictures have been uploaded. So far, three notifications were positive, i.e. identified as a tiger mosquito. All the others were mostly the common-house mosquito (22,5%), other mosquito species (16,8%) or other insects (60,7%). Of the positive notifications, one came from southern-France, another from Kenia and one from Belgium. This last one triggered a field visit to further investigate on site the presence of *Ae. albopictus* with larval collection, ovitraps and adult-trap. After three field visits its presence could not be confirmed. However, larvae of another EMS, Aedes japonicus, were found.

Based on these preliminary results, passive surveillance relying on citizens appear to be an effective tool for surveillance of EMS in Belgium and seems to have a potential also for tiger mosquitoes.

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AIM-COST expands mosquito monitoring by Citizen Science across Europe: first results from Mosquito Alert Italia network.

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Abstract

Mosquito Alert Italia is a network coordinated by the Medical Entomology (MedEnt) group of Sapienza University aiming to introduce citizen science (CS) into mosquito monitoring. We here describe the results of two parallel studies conducted in Italy either exploiting Mosquito Alert (MA) app for mobile phones (originally developed in Spain and later made available in 20 languages within the AIM-COST Action framework) (1), and involving a selected group of undergraduate students (2).

1- Mosquito Alert app was launched in Italy in late 2020 and promoted by press releases and social media and in partner's websites. From Oct 2020 to end of 2021, 4,487 mosquito reports were recorded in MA, mostly from Rome (753) and other large cities in northern-eastern regions. A total of 2,653 reports associated to photographic records were examined by MA' Digital EntoLab experts: i) 651 were unambiguously identified as *Cx. pipiens* (24.5%), 865 as *Ae. albopictus* (32.6%), 665 as Culicidae (25%) and 467 were discarded; ii) 3 *Ae. koreicus*, 1 *Ae. japonicus*, and 1 *Ae. japonicus/koreicus* were recorded; iii) despite peak of mosquito densities in Italy is in Aug-Oct, most reports (733, of which 479 with photos) were recorded in Jun-July, reflecting need of more effective MA promotion activities in the late part of the mosquito season; iv) most *Cx. pipiens* were recorded in May-July and in Oct-Nov 2021, while most *Ae. albopictus* in Jun-Sept, reflecting the actual relative frequencies of the two species in Italy.

2- Students at Sapienza and Tor Vergata Universities of Rome were asked to send photos of mosquito by MA and to provide the recorded specimens to MedEnt. Twenty-nine students participating the project collected 460 specimens, which were identified as *Cx. pipiens* (43.3%), *Ae. albopictus* (34.6%). One *An. maculipennis*, 1 *Ae. detritus* and 10 *Cs. longearolata* and 2 *Cs. annulata* were also identified.

Despite the low-profile communication campaign, results show the potential of CS approaches in complementing conventional entomological mosquito monitoring. More extensive, effective and regular promotion of the app during the entire mosquito season is necessary to increase collections of records/specimens.

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Mosquito Vision: An interactive application for the prediction of mosquito nuisance for citizens

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Abstract

Throughout the last decade Citizen science applications are mostly used for the passive surveillance of mosquitoes and for the mapping of breeding sites, offering the advantage of a potentially very high geographical resolution of field data which is critical for predictive modeling.

The Mosquito Vision application was developed in 2020 for informing citizens about nuisance from mosquitoes, using a large time-series of field data from the monitoring networks operated in the frame of mosquito control projects implemented by Ecodevelopment. Five-day predictions for the nuisance at evening hours, mainly produced by *Aedes caspius*, and during the night, predominantly provoked by *Culex pipiens*, are uploaded daily for 2,415 settlements in four regions in Greece. 288 evaluations from 47 peers showed an average declination of felt to predicted nuisance level for evening hours of 0.7 class out of 5 nuisance classes, and for nuisance at nighttime of only 0.1 class.

Progress in and preliminary results of the new functionalities of Mosquito Vision in response to user demands are presented and concern

- Strengthening of the existing network of weather stations with 10 private stations in poorly covered areas of Central Macedonia to optimize short-term weather forecasts
- Development of a predictive model for urban areas through the installation of a canvas of 76 GAT-traps and ovitraps per 1 km^2 in Thessaloniki
- Development of a predictive model for daytime nuisance produced by Aedes albopictus through the installation of 60 GAT traps and the comparison of Human Landing Catches with ovitraps (AIMApse protocol)
- Integration of interactive citizen science functionalities for the evaluation of nuisance predictions and the information about new or cryptic breeding sites

Aspiration of this project is an increase in the active involvement of Greek citizens in the problematic of mosquitoes which represent not only a nuisance issue but also an increasing risk for public health.

Mosquito Vision is a component of the EYWA system (EarlY WArning System for Mosquito-borne Diseases), which has been awarded with EIC Horizon Prize for Epidemics. The presented research is funded under the Greek Research and

Innovation Strategies for Smart Specialisation call (RIS 3, project code: KMP6_077890).

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Unwanted guests: mosquitoes in the domestic environment during winter

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Abstract

The omnipresent mosquito species Culex pipiens is the primary vector of Usutu virus (USUV) and West Nile virus (WNV) in North-Western Europe. *Culex pipiens* consists of two behaviourally distinct, yet morphologically identical biotypes, namely *Cx. pipiens pipiens* and *Cx. pipiens molestus*. Interestingly, *Cx. pipiens pipiens* enters diapause in winter, whereas *Cx. pipiens molestus* remains active and continues blood-feeding in the domestic environment. It currently remains unclear 1) what the spatial distribution is of *Cx. pipiens* biotypes throughout winter in the Netherlands, 2) how urbanization may affect this distribution, 3) on which vertebrate species winter-active *Cx. pipiens* feed throughout winter, and 4) whether, in the Netherlands, *Cx. pipiens* can maintain arboviruses throughout winter. To this end, we used a citizen science-approach, using the web-based application 'Muggenradar', to receive mosquitoes collected indoors by the Dutch general public in February 2021. Our call resulted in the submission of more than 7,000 insects in ten days, of which 5,744 were identified as mosquitoes. From these 5,744 mosquitoes, we identified 2,544 (44.3%) as *Cx. pipiens*. From the mosquitoes identified as *Cx. pipiens*, subsets were selected to assess the effects of urbanization and the presence of (flooded) crawl spaces on biotype distribution, which were identified via molecular diagnostics (qPCR). Higher levels of urbanization (expressed as no. of households per km2) and presence of flooded crawl spaces were significantly associated with higher proportions of *Cx. pipiens molestus*. Furthermore, preliminary analyses on blood-engorged specimens indicated that the vast majority (82.3%) were *Cx. pipiens molestus*. Analyses to determine blood-host origin via PCR and sequencing are ongoing. Furthermore, collected mosquitoes will be pooled in monospecific pools per region and tested for the presence of USUV and WNV. We conclude that there is a strong effect of both small-scale and large-scale environmental conditions on the relative distribution of Cx. pipiens biotypes. Our citizen science approach thus provides valuable insights into vector- and arbovirus distributions, especially during the winter season.

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CLIMOS - towards better understanding of climate and environmental drivers of sand fly borne diseases in Europe

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Abstract

Among vector-borne diseases, those caused by sand fly-borne pathogens such as parasitic protozoans of the genus Leishmania or various phleboviruses constitute an important public health and veterinary concern in many regions including Europe. The ongoing climatic and environmental changes are expected to expand the distribution of sand fly vectors into new regions, emphasizing the need for establishment of field data-driven, predictive early warning system (EWS) accessible and applicable at local scale to generate predictions, to assist policy recommendations and contribute to effective risk management.

CLIMOS - Climate Monitoring and Decision Support Framework for Sand Fly-borne Diseases Detection and Mitigation with Cost-benefit and Climate-policy Measures – includes 29 partners across 16 countries and aims to complement and build on previous EU-funded projects (EDEN, EDENext, Vectornet) that accumulated vast knowledge on vector-borne diseases, their causative agents and vectors in Europe and adjacent regions. By coordinating efforts of data, climate and environmental scientists, health-care and veterinary practitioners, technology platform designers and at-risk communities, CLIMOS will conduct innovative and applied research seeking to better prepare for current and future impacts of climate and environmental changes on

human and animal health, using sand flies and the diseases they transmit as a model system.

CLIMOS aims to empirically characterize the microclimatic, environmental, demographic and epidemiologic aspects associated with longitudinal datasets on country-specific sand fly abundance and animal infection rates at different geographical scales. These data, acquired by surveillance networks in 10 European and neighboring countries (Austria, Croatia, Czech Republic, France, Germany, Italy, Israel, Portugal, Spain, Turkey), will feed into epidemiological-climatic and environmental predictive mathematical models of realistic human-induced climatic changes scenarios to help develop an early warning system of infection and disease designed with the input of partner public health ministries for public use. In addition, a foresight exercise will be conducted, leading to different plausible scenarios for health and other impacts of effects of climate and environmental changes to SFBDs. Novel technologies will be developed to monitor and mitigate human-sand fly contact.

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RIVOC (2021-2025): a regional collaborative research project and network to enhance innovative research on emerging and main vector-borne pathogens in plants and vertebrates through an One Health approach

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Abstract

Southern France and the Occitanie region, like the rest of the world, is facing the emergence of diseases affecting plants, animals and humans, of which causative agents (viruses, parasites, bacteria) are transmitted by arthropod vectors. Global changes are accelerating the emergence and spread of these diseases. RIVOC is a regional collaborative research project and network to enhance innovative research on emerging vector-borne pathogens in plants and vertebrates through a One Health approach in the Occitanie region.

Through several funding and training actions, RIVOC aims to (i) mobilize and structure the academic community and the regional partners and stakeholders on the issue of infectious vector-borne risks in a "one health" approach, (ii) fund collaborative research to develop sustainable socio-economic solutions for control and surveillance, and (iii) support and increase the visibility of the training offer for the academic world and socio-economic partners in terms of vectors and vector-borne diseases in human, animal and plant health.

We will review some examples of innovative and collaborative projects and trainings that were developed and granted in the framework of RIVOC. Achieving these objectives, RIVOC will lead to the identification and reduction of vector-borne risks and their impacts on agricultural production, biodiversity and tourism on the economy, major issues of the Region.

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INOVEC: a research and innovation partnership for enhancing the surveillance and control of mosquito vectors of emerging arboviruses

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Abstract

Over the past 10 years, arboviral diseases, such as dengue, Zika, chikungunya and yellow fever, have (re)emerged with increasing prevalence and severity. Although these arboviral diseases are more prevalent in tropical countries, increasing numbers of autochthonous cases are being reported from European countries; hence concerns about the potential for the establishment of these pathogens in temperate regions are raising. New, affordable, scalable and community-based vector control measures are urgently needed to prevent the introduction, spread and establishment of Aedes-borne diseases in Europe and beyond. The INOVEC consortium funded by the Horizon Europe-MCSA-SE will gather a large pan-European, cross-sectoral and multidisciplinary network to develop, optimise and promote integrated approaches and innovative tools for the surveillance and control of mosquito vectors of emerging arboviruses. INOVEC gathers 21 academic and non-academic institutions specialised in vector biology, social sciences and product development to stimulate basic and applied research, strengthen capacities, promote career development, and to facilitate knowledge and technology transfer to countries at increasing risk of arboviral diseases. The final aim is to contribute to international efforts to improve global health and human well-being by reducing the burden of vector-borne diseases.

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Efficacy of a 'lethal house lure' against *Culex quinquefasciatus* from Bouaké, central Côte d'Ivoire.

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Abstract

Background: Eave tubes technology is a novel method of insecticide application that uses electrostatic coating system to boost insecticide efficacy against resistant mosquitoes. A series of previous experiments have shown encouraging insecticidal impact against malaria vectors. This study was undertaken to assess the effects of the eave tube approach on other Culicidea, in particular *Culex quinquefasciatus* under laboratory and semi field conditions.
Methods: Larvae of local *Cx. quinquefasciatus* from Bouaké were collected and reared to adult stage in the laboratory and cylinder tests performed to determine resistance status. WHO standard 3 min cone bioassays were conducted on pyrethroid treated netting versus various exposure period using cone on eave tubes treated inserts. Eave tubes bioassay method utilizing smelly socks as attractant was then performed at increasing exposure periods (1 min up to 60 min) on 10% beta-cyfluthrin and residual activity monitored over several months. Release recapture experiment within enclosure around experimental huts fitted with windows, eave tubes untreated or treated with insecticide (10% beta-cyfluthrin) was conducted to determine house entry preference of *Cx. quinquefasciatus* and impact of tubes on survival of this species.
Results: Bouaké *Cx. quinquefasciatus* displayed high intensity of resistance to three out of four classes of insecticides currently used in public health. With 3 min exposure in cone tests, 10% beta-cyfluthrin treated insert induced 100% mortality *of Cx. quinquefasciatus* whereas LLINs only killed 5%. With reduced exposure to the eave tube inserts, mortality was still 100% after 2 min, 88.7% after 1 min and 44% for a transient exposure of 30 s. Mortality with 10% beta-cyfluthrin treated inserts was > 80% up to 7 months post-treatment. Data suggest that Cx. quinquefasciatus have strong preference to enter house through the eaves than windows. Beta-cyfluthrin treated inserts were able to kill 52% of resistant Cx. quinquefasciatus released in within the enclosure.
Conclusion: Eave tubes technology is a novel delivery method of insecticide to the house. It attracts nuisance host seeking *Cx. quinquefasciatus* mosquitoes and control them effectively despite high level of resistance they have developed.

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VectorNet: Putting vectors on the map

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Abstract

Public and animal health authorities face many challenges in surveillance and control of vector-borne diseases (VBDs). Those challenges are principally due to the multitude of interactions between vertebrate hosts, pathogens and vectors in continuously changing environments. VectorNet, a joint project of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) facilitates risk assessments of VBD threats through the collection, mapping and sharing of distribution data for ticks, mosquitoes, sand flies and biting midges that are vectors of pathogens of importance to animal and/or human health in Europe. Here we describe the development of the network, that celebrated its 10th anniversary in 2020, and the value of its most tangible outputs, the vector distribution maps, that are freely available online.

Interestingly, since 2019, this community has been supplemented with a structured entomological network (one person per country) that is harmonized with other networks within the European public and animal health agencies. This VectorNet Entomological Network (VEN) encompasses 51 countries: EU/EEA (26+3), EU Enlargement policy (7) and European Neighbourhood Policy partner (15) countries. A strong professional VEN is intended to encourage national governments to ensure sustainable vector surveillance.

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Evolution of invasive mosquito surveillance and control in Switzerland in the last 22 years

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Abstract

The Asian tiger mosquito (*Aedes albopictus*) was first detected in Ticino, Switzerland's southernmost Canton, in 2003 and north of the Alps in 2013 and has been spreading steadily ever since. The Asian bush mosquito (*Ae. japonicus*) was first found in 2008 in the Canton of Aargau, spread very quickly and is now present in almost all Cantons. *Ae. koreicus* was found in Ticino for the first time in 2013, but is not spreading to the same extent as the other two species mentioned above. Due to the continuous spread of *Ae. albopictus* since its first detection, activities to monitor and control the invasive mosquito in Switzerland have been gradually expanded. In the Canton of Ticino, the surveillance system has been running since 2000 and was continuously expanded since then. In 2013, the national surveillance programme along motorways, in international airports and harbours began, and since 2016, surveillance programmes have been gradually introduced in numerous Cantons. In the same year, the national guidelines for surveillance and control of invasive mosquitoes were published, and in 2017 the establishment of a national mosquito network began. The aim of the Swiss Mosquito Network (SMN) is to coordinate invasive mosquito surveillance activities at national level and centralise the data collected; it is fully operative since 2020. Collaborations with neighbouring France and Germany have also been established. Here we provide an overview of this development and show the current spread of the three invasive mosquito species present in Switzerland.

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The potential role of *Aedes japonicus* mosquitoes as spillover vector for West Nile virus in the Netherlands

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Abstract

In recent years *Aedes japonicus* has invaded Europe and since 2013 a population of this species has been found near Lelystad, the Netherlands. This mosquito species is a known vector for a range of arboviruses, possibly including West Nile virus (WNV). As WNV emerged in the Netherlands in 2020, it is important to investigate the vectorial capacity of Dutch mosquito species to estimate the risk of future outbreaks and further spread of the virus. Therefore, this study aimed to evaluate the potential role of Aedes japonicus mosquitoes in WNV transmission and possibly spillover from birds to dead-end hosts in the Netherlands. We conducted human landing catches (HLC) in Lelystad in June, August and September 2021 to study the diurnal and seasonal host seeking behavior of *Aedes japonicus*. Furthermore, we looked into their host preference in relation to birds. In a lab study we investigated the vector competence of field-caught *Aedes japonicus* mosquitoes for two isolates of WNV at two different temperatures. Based on the data generated in these studies, we developed a Susceptible-Exposed-Infectious-Recovered (SEIR) model that specifically estimates the risk of WNV spillover from birds to humans via *Aedes japonicus* under the condition that the virus is introduced and circulates in an enzootic cycle in a given area. Our HLC results show that *Aedes japonicus* mosquitoes are active throughout the day, with peaks in activity in the morning and evening. In comparison with June and September, the highest abundance was recorded in August. For the host preference experiment we documented a small number of mosquitoes feeding on birds; only six blood fed females were caught over four full days of sampling. Finally, our lab-based vector competence experiments with *Aedes japonicus* show a higher transmission rate of WNV at 28 °C (7%), compared to the transmission rates at more realistic summer temperatures (22 °C) in the Netherlands (3%). Combining the field and laboratory derived data, our model predicts that *Aedes japonicus*, even though it prefers to bite on dead-end hosts, can still be responsible for a high initial WNV invasion risk when present at high numbers and when temperatures are high.

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Species abundance, seasonal dynamics and natural infection prevalences of sand flies from Sardinia Italian Island

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Abstract

Phlebotomine sand flies are considered to be expanding their boundaries into territories where they were not previously established and lengthening their seasonal activity period in those where they were endemic. As ectothermic insects, their presence are climate constrained, particularly minimum winter temperatures affecting larval survival and cold and rainy summers that reduce adult biting period. In Italy they are vectors of zoonotic visceral and cutaneous leishmaniasis caused by Leishmania infantum as well as Toscana Virus and other phleboviruses endemic in our country. The aim of this study is to report sand fly species distribution, seasonal dynamics and natural infection prevalence in Sardinia one of the biggest Italian island. This study was performed from 2020 to 2021 using CDC and BG-sentinel traps. All collected specimens were morphologically identified and molecularly tested by RFLP and RT-PCR for pathogen detection, Leishmania spp. and Toscana Virus respectively. While sand flies from Olmedo (SS) was hand captured and dissected for parasite isolation and colture.

A total of 1.673, specimens were identified (sex ratio of 0.9 M) as follow *Ph. perniciosus* 60.0%, *Ph. perfiliewi* 17.4% and *Se. minuta* 22.5%. Seasonal population dynamics shows a bimodal peaks distribution in all provinces for *Ph. perniciosus* and *Se. minuta* showing earlier activity peaks in July and September and delayed peaks in August and October respectively. While *Ph. perfiliewi* highlighted a trimodal peaks distribution (May, July and September).

Altogether 145 pool (N=1445) were molecularly analyzed, of which 20 (1.4%) tested positive for Leishmania natural infection in 5 of the 8 tested provinces, the highest positive prevalence has been registered in Sassari (0.8%), with higher positive vector species prevalence for *Se. minuta* (0.5%) followed by *Ph. perniciosus* (0.3%) and *Ph. perfiliewi* (0.07%). Dissection analysis, isolation and culture highlighted presence of 3 *L. tarentolae* (10.0%) and 1 *Trypanosoma platydactyli* (3.3%) strain obtained only from Se. minuta. Analyses are pending with regard to Toscana Virus detection.

The putative Leishmania and Phlebovirus vectors were differently represented in the investigated sites with different densities. These preliminary analyses highlighted the presence of the main Italian vectors and improve knowledge of presence and distribution of phlebotomine-borne diseases.

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Sand fly and Toscana virus: impact on vector life-history traits using experimental infection and potential effects on transmission

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Abstract

Sand flies are hematophagous insects belonging to the family Psychodidae and the subfamily Phlebotominae. During blood meal taken by the female sand flies, several pathogens such as Leishmania and Phleboviruses, including the Toscana virus (TOSV) can be transmitted to the host. Toscana virus infects humans and can cause neuroinvasive infection in the Mediterranean region during the warm season. Currently, there is little information on the natural cycle of Phleboviruses in their sand fly vector. Thus, this study aims to determine, the TOSV infection dynamics in its major vector *Phlebotomus perniciosus*, and the infection impact on vector life-history traits. Female sand flies were infected with TOSV via blood feeding under experimental conditions. We observed systemic dissemination around four days post-infection, which could lead to a shorter extrinsic incubation period. Moreover, we showed an impact of infection on hatching time, which is longer for the eggs of infected females. Based on this result and the fact that TOSV can be transmitted transovarially in *Ph. perniciosus*, we hypothesize that this could have an impact on vectorial capacity and lead to an increase in the transmission risk period. These experimental results help to better understand the virus maintenance in sand fly populations and its natural cycle.

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Host-feeding patterns of European mosquitoes in relation to land use

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Abstract

Mosquitoes (Diptera: Culicidae) are the most important arthropod vectors of pathogens. Understanding their blood-feeding behavior is an essential element to assess the vectorial capacity of a mosquito population. Therefore, this study aims to analyze mosquito spatial-temporal host-feeding patterns by barcoding blood-fed female mosquitoes. Between 2012 and 2019, mosquitoes were sampled at different sites in Germany, Iran, Moldavia and Romania. Blood-fed specimens were analyzed by PCR with vertebrate specific primers and subsequently processed with Sanger sequencing. The sequences were compared to sequences from GenBank to identify the host species. The host specificity of the mosquitoes was tested by calculating the checkerboard score (C-score). In addition, land use information based on the Copernicus global land cover layers were extracted from each sampling site to analyze its influence on the mosquito-host interaction. In total, we collected 4,827 blood-fed specimens of 48 different mosquito taxa. The molecular biological analysis revealed 82 host taxa, including 3 amphibia, 42 bird, 36 mammal and 1 reptile species. The five dominant host species were domestic cattle (34%), human (18%), pig (13%), horse (9%) and roe deer (6%). C-scores indicated a random structure in the host-feeding patterns, i.e. mosquitoes use a broad range of different host species. Nevertheless, most mosquito species preferred certain host groups. Species from the genus *Aedes* predominantly fed on mammals, while we detected a balanced proportion of mammal and bird blood in *Culex* specimens. The analyses showed that the mosquito-host interaction is significantly affected by land use factors, e.g. at forest sites, human blood was predominantly detected in Aedes mosquitoes, while at unforested sites, particularly in urban areas or in shrublands, humans are more likely to be bitten by Culex mosquitoes. Land use changes can influence the abundance and species composition of mosquitoes and vertebrates affecting vector-host dynamics. This large data with a standardized molecular screening method gives a unique insight in the spatial-temporal host-feeding patterns of mosquitoes and the relevance for mosquito-host interaction on the epidemiology of mosquito-borne diseases.

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Combining biodiversity conservation and vector ecology: characterization of Culicoides and mosquito fauna at the National Zoological Garden of Rabat, Morocco

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Abstract

Zoos are considering to be essential places for the conservation of wild animal species. It is essential to prevent their infection especially for those belonging to threatened or extinct species. Indeed, zoo captive ruminants are susceptible to Culicoides and mosquito borne-viruses. In order to further evaluate the risk of pathogen transmission by those vectors in zoos, it is essential to identify the potential vector species, as well as the communities in which they evolve.

For this purpose, Culicoides and mosquito species composition was investigated in ten sites at the National Zoological Garden of Rabat, Morocco, from March to June 2021 for Culicoides and in four sites from April to June 2022 for Mosquito at the National Zoological Garden of Rabat, Morocco.

Culicoides were collected using the OVI (Onderstepoort Veterinary Institute) trap from dusk until dawn every two weeks. Mosquitoes were collected using the CDC light trap combined with a CO2 source as an attractant, operated for 24 hours, on two consecutive days every two weeks.

In total, 1584 individuals belonging to the genus *Culicoides* were collected (88% females and 12% males) belonging to at least ten different species. Among the species collected *C. newsteadi* -33%-, *C. imicola* -24%-, *C. circumsciptus* -19%- and *C. obsoletus*/*C. scoticus* -8%-, constituted the majority of the total catches. These species are proven or suspected vector species of bluetongue and African hose sickness viruses in the Mediterranean basin.

For mosquito, 455 individuals belonging to 4 species of 3 genera were collected (91.9% females and 2.41% males), respectively *Culex pipiens* -94%-(vector species of West Nile and Rift Valley fever viruses), *Culiseta longiareolata* -5%- (vector of avian malaria parasite), *Aedes caspius* and *Ae. Detritus* -representing together less than 1%-.

To our knowledge this is the first study investigating Culicoides and mosquito fauna on wild zoo animals in Morocco. Indeed, zoo animals are susceptible to Culicoides and Mosquito borne-viruses. In addition to the zoo's unique structure, the environment in which it is embedded must be considered when it comes to risk analysis, vector control, and vector-borne disease management.

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A global meta-analysis on mosquito host-feeding patterns

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Abstract

Mosquito host-feeding patterns are an important factor shaping the mosquito's vector capacity. As the interaction between vectors and hosts determine transmission cycles and risk of pathogen spill over, the understanding of host selection is important to assess the risk for human and animal health. Host selection can depend on intrinsic (e.g. mosquito genetics) as well as extrinsic factors (e.g. host availability). For example, anthropophagic mosquitoes are potential vectors for pathogens transmitted between humans (e.g. chikungunya virus), while opportunistically feeding mosquitoes can serve as bridge vectors for zoonotic viruses (e.g. West Nile virus).

In order to investigate mosquito host-feeding patterns, we collected the data from 339 scientific publications, covering a timeframe of nearly eight decades (1942-2019). We included studies, which sampled engorged mosquito females and screened the bloodmeal for hosts using any serological or molecular biological method. The collected and standardized parameters comprised mosquito species, blood meal hosts, collection method, method for blood meal analysis, time and date, and, if provided, land use and landscape information per study. These data on 544.425 identified mosquito blood meals allow wide range of in-depth analysis of the host-feeding ecology of mosquitoes. For example, 277 of the taxa (57.67%) fed on humans, making them potential vectors of pathogens relevant for public health. Furthermore, the data indicate different host-feeding patterns: while some mosquito species like Culex quinquefasciatus show a broad host range, clear preferences for non-human mammalian species are evident for *Culex tritaeniorhynchus*. This comprehensive meta-analysis helps to understand the interaction between mosquito and host species to understand global transmission patterns of mosquito-borne pathogens.

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Bioclimatic factors associated with *Culex* population abundance and WNV cases in Greece

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Abstract

The transmission of West Nile Virus is known to be affected by multiple heterogenous factors related to the behavior and interactions between reservoir (birds), vector (*Culex*-mosquitos), and hosts (humans). The aim of the present study was to determine the association of various climatic factors and Earth Observation (EO) parameters with the Culex mosquito abundance and the occurrence of WNV cases in Greece, from 2010-2020. Climate data were acquired from ERA5 (European Centre for Medium-Range Weather Forecasts) and EO parameters from satellite image processing (Sentinel 2, Landsat 7 και Landsat 8). Culex abundance data were obtained through the mosquito surveillance network of ECODEVELOPMENT S.A and epidemiological data from the Hellenic National Public Health Organization. The association between mosquito abundance and climate factors was examined by univariable and multivariable linear and polynomial regressions. Associations with reported WNV cases were assessed by logistic regression and generalized linear models. Correlation heatmaps were used to visualize the dependence of vector abundance with EO parameters and climate variables of winter and spring. *Culex* abundance was highly correlated with temperature, but also with composite indicators derived from temperature, relative humidity, wind speed and total precipitation. Regarding EO parameters, the mean surface temperature during the month of April was the most informative predictors of mosquito populations followed by Normalized Difference Vegetation Index (NDVI) and Normalized Difference Water Index (NDWI). Concerning the variability of climate and EO parameters against the occurrence of human WNV cases a series of associations were observed, not only from the analysis of cross-sectional data but also from the examination of the impact of winter and spring conditions. The stronger determinants were temperature, dew-point temperature and NDWI. Environmental parameters can play a critical role in understanding WNV epidemiology. In future research, more focus should be given to the temporal and spatial consistency of the identified associations.

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Entomological investigation of a Rift Valley Fever epizootic/epidemic reveals new aspects of the vectorial transmission of the virus in Madagascar

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Abstract

After twelve years of silence, a new epizootic of Rift Valley fever (RVF) has affected cattle in several regions of Madagascar. The first human cases were confirmed in April 2021 in the Southeast region as part of a multidisciplinary investigation which included an entomological survey aiming at identifying the mosquito species present, describing some aspects of their bio-ecology and unravelling their potential role as Rift Valley fever virus (RVFV) vectors.

Adult mosquitoes were collected night and day using different methods during April / May 2021 in three villages where suspected human/cattle or confirmed human RVF cases were observed. Mosquitoes were then morphologically identified, bloodmeal sources of engorged females were determined by PCR and the presence of RVFV RNA was sought in males and both engorged and unfed females by qRT-PCR.

A total of 2,806 mosquitoes was collected. At least 26 species were identified. *Anopheles* (74.3%) and *Culex* (16.6%) were the predominant genera and *Anopheles mascarensis* the predominant species. RNA of RVFV was detected in 7.2% of the 512 monospecific pools tested i.e. in ten distinct mosquito species. Most (71.6%) of the 450 engorged females tested had taken a mixed bloodmeal (human/zebu) while 21.3% fed on zebu only and 7.1% on human only. At last, 74.6% of engorged females from RVFV-positive pools had taken a mixed bloodmeal.

Beyond the recognized RVFV major Malagasy vectors (*Cx. antennatus*, *Cx. tritaeniorhynchus*), other species such as *An. coustani*, *An. squamosus*, collected in large numbers, were positive for RVFV. Of note, it is the first time that *An. mascarensis*, an endemic species of the Malagasy sub-region, locally involved in the transmission of malaria parasites, was found naturally infected with RVFV. Moreover, zoo-anthropophilic females naturally infected with RVFV were collected close to confirmed human cases. This strongly supports the hypothesis of RVFV circulation between domestic animals, mosquitoes and humans. As most of the mosquito species here incriminated in RVFV transmission exhibits nocturnal activities, the use of

impregnated mosquito nets distributed by the National Malaria Control Program should be reinforced to prevent and control RVFV transmission.

62

Pathogen detection of mosquitoes collected in different environments of Mallorca, Spain

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Abstract

Vector-borne diseases represent a public health problem worldwide. Mosquitoes can transmit arboviruses and other pathogens that cause diseases to human, domestic animals, and wildlife. Surveillance of these agents of diseases and the identification of potential vectors and hosts provide valuable data to assess the local transmission risk. A total of 18,207 mosquitoes (432 blood-fed) of ten different species were collected between July 2020 and October 2021 by using adult traps in 22 sample sites covering urban, peri-urban, rural, natural conservation areas and dog pounds. DNA, RNA extraction and pathogen screening were performed with different qRT-PCRs and gel PCRs with subsequent sequencing. Pathogens detected included avian malaria parasites Plasmodium spp. and *Haemoproteus* spp. in *Aedes albopictus* and *Culex pipiens s.l.* The filarial nematodes *Dirofilaria* repens and *Dirofilaria immitis* were detected in *Aedes caspius* and *Cx. pipiens s.l.* During the screening of flaviviruses and alphaviruses, West Nile Virus (WNV) was identified in *Ae. albopictus* collected in a peri-urban area. The detection of pathogens in mosquitoes collected in surveillance programs for invasive species as *Ae. albopictus* and other mosquito species in different environments could improve control intervention strategies against vector-borne diseases. Part of this project has been funded by the AIM COST Action CA17108 is funded by COST (European Cooperation in Science and Technology).

POSTER PRESENTATION ABSTRACTS

Vector Phylogeny, Taxonomy and Biogeography

Vector Ecology and Behaviour

Surveillance and Management of Vector Borne Diseases

The Role of Citizen Science and Outreach for the Surveillance of Vector Species

New Projects & Updates from Different Networks

Host Pathogen Vector Interactions

P1

Analysis of the role of the microbiota in the physiology of *Culex* larvae

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Abstract

Wolbachia is a genus of intracellular bacteria infecting many invertebrate species, which receives a particular interest as it affects its hosts in diverse ways. While its interaction with nematodes is clearly mutualistic via participation to worm nutrition, its success in arthropod species is generally explained by its ability to manipulate host reproduction. Recently some *Wolbachia* strains have however been identified as nutritional endosymbionts in bedbugs and planthoppers. *Culex* mosquitoes are known to host maternally-inherited *Wolbachia* with a high prevalence ratio in diverse populations with no known impact on the mosquito fitness except reproductive manipulation, hence appear as an interesting model to further investigate whether such nutritional benefit may also happen in mosquitoes.

Larvae of mosquito species that do not harbor *Wolbachia* have been found to require microbial colonization for normal development. In this study, we aim to test if *Wolbachia* has a nutritional role during larval development of *Culex quinquefasciatus*. We successfully produced larvae carrying *Wolbachia* but no culturable microbiota, and found that *Wolbachia* by itself is not able to sustain larval development with a standard sterile diet. To produce germ-free adults, we used a transient colonization system that was recently developed for *Aedes* mosquitoes in our laboratory. Larvae are colonized with a bacterial strain which supports larval development yet can be eliminated by a simple change of diet, allowing to obtain germ-free adults. In this project, we adapted this protocol and produced *Culex* mosquitoes harbouring *Wolbachia* but no culturable bacteria with a conventional timeline. This indicates that our bacterial strain is able to support development until adulthood, even though *Culex* larvae generally develop in "dirty" water. In parallel, we treated mosquitoes with antibiotics to remove the intracellular bacteria in non-sterile conditions. In sum, we have produced critical tools to investigate whether *Wolbachia* affects larval development in otherwise sterile conditions.

P2

BopCo, a barcoding facility for organisms and tissues of policy concern, and its role in the identification of vector species

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Abstract

The Barcoding facility for organisms and tissues of policy concern (BopCo: https://bopco.myspecies.info/) is a Belgian federal focal point for identifying biological materials upon request. It provides access to the expertise and infrastructure necessary to identify organisms of policy concern and their derived products. The species identifications are based on classical morphological characters and/or DNA analyses, such as Sanger sequencing (e.g. DNA-barcoding), RFLP and microsatellites. If necessary, BopCo also relies on, for example, environmental DNA and/or Next Generation DNA Sequencing technologies. Species of policy concern include, but are not limited to, vector species of diseases and parasites, invasive alien species, and agricultural pest species. BopCo's identification service is available to all stakeholders (both governmental and private) who deal with biological material of policy concern and who require species identifications.

This contribution provides an overview of BopCo's identification activities with respect to host and vector species of diseases and parasites that are potentially harmful to humans and/or (domestic) animals. In this context, BopCo is currently involved in four projects: (1) monitoring (exotic) disease vector mosquito species (Culicidae) in Belgium and the Netherlands, (2) identifying intermediate host snails of Trichobilharzia trematodes responsible for recent outbreaks of cercarial dermatitis (swimmer's itch) in Belgium, (3) identifying snails and slugs in Greece that act as intermediate hosts of metastrongyloid nematodes provoking cardiopulmonary parasitoses in domestic and wild cats, and (4) screening the trematode vector capacity of assassin snails (Genus *Anentome*) in Thailand and the international aquarium trade. The former two projects will be presented in more detail, while the latter two projects are dealt with in separate contributions by respectively Vanderheyden *et al.* and Chomchoei *et al.*

A list of processed requests by BopCo is available on our website (https://bopco.myspecies.info/projects).

P3

The role of Infravec2 in ISIDORe, the newborn Horizon Europe project on integrated services for infectious diseases outbreak research

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Abstract

ISIDORe is an EU-funded project under the Horizon Europe program HORIZON-INFRA-2021-EMERGENCY-02 that unites a multidisciplinary consortium of 17 European research infrastructures to offer integrated services for facing current and future infectious disease outbreaks. It is coordinated by ERINHA, the European Research Infrastructure of Highly Pathogenic Agents. This interdisciplinary project brings together 154 European service providers from 32 countries for a total funding of €21 million. The project provides access to key services to support projects for the study of SARS-CoV-2, its variants and other potential pathogens and it is part of the EU-Commission´s biodefense preparedness plan, the Health Emergency Preparedness and Response Authority (HERA) Incubator.

The purpose is to assemble the largest and most diverse research and service-providing instrument to study infectious diseases in Europe, from structural biology to clinical trials. Giving scientists access to the whole extent of our state-of-the-art facilities, cutting-edge services, advanced equipment and expertise, in an integrated way and with a common goal, will enable or accelerate the generation of new knowledge, intervention tools, and epidemic-prone pathogens in general.

Infravec is one of the 17 umbrella partners, providing services and infrastructure access at no cost. We will here introduce the opportunities available in the field of vector entomology and vector-borne disease research.

P4

The use of new molecular techniques for evaluating the effects of mosquito control on biodiversity

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Abstract

EID Méditerranée is a public operator in charge of mosquito control in temporary flooded wetlands along the coast of five French Mediterranean departments since the 60's. Among the 61 species found in Metropolitan France[1], mainly two native species are targeted because of their nuisance: *Aedes (Ochlerotatus) caspius and Ae. (Oc.) detritus*. Nowadays, only *Bacillus thuringiensis var. israelensis* (Bti) is duly authorised for controlling the breeding sites of both species along the French Mediterranean coast. Despite its specificity, Bti treatments may cause some side effects in other non-target dipteran species such as chironomids[2]. The indirect impact on aquatic and terrestrial food chains should therefore be evaluated.

A study[3] on the diet of four paludicole passerine species was recently carried out in the frame of the Occitania Coastal Reedbeds Project[4]. The results of this study based on new molecular techniques highlighted a high consumption of chironomids by the four bird species throughout the year, and the presence of several mosquito species in three of the four birds studied. Among the rural species targeted by the EID-Med operations, *Ae. detritus* was only detected on the diet of the *Acrocephalus melanopogon*. Based on these initial results, the proposed study uses the methodology of environmental DNA as a tool to assess the potential unintentional effect of mosquito control on the invertebrates populating the reedbeds.

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P5

A web-based platform for the integration and dissemination of entomological and epidemiological data and provision of predictive analytic tools related to West Nile Virus in Greece.

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Abstract

The repeated West Nile Virus (WNV) outbreaks in an increasing number of countries during the past twelve years, witness for the existence of an increasing risk of mosquito-borne diseases to human population in Europe. The plethora of available monitoring data and the need for supportive tools for decision making gave rise to the development of an early warning system for WNV in Greece.

We present the EMPROS platform that has been developed to this end and aggregates, consolidates and enriches the entomological and ornithological field data, providing as well mosquito abundance predictions and risk predictions for WNV on a municipality down to a settlement level with the ultimate goal of representing a tool that serves the needs of all stakeholders involved in the control of WNV in Greece.

The platform visualizes entomological and sentinel chickens field data collected within the monitoring networks of Ecodevelopment S.A., enriched with environmental proxies (NDVI, NDMI, NDWI, NDBI) geomorphological and meteorological data (for the sampling date and predictions for the upcoming 10 days) at the sampling sites, as well as information about pathogen circulation in mosquitoes and sentinel chickens.

Additionally, the platform, depicts predictions from a full suite of predictive models which provide predictions about mosquito abundance at the trapping sites and on a settlement level, and risk predictions for human cases of West Nile Virus on a settlement and on municipality level. The models are both deterministic and data-driven in nature and based on the environmental, meteorological and geomorphological data as inputs to generate their outputs.

The platform implements a role-based access to provide per-region data to specific users, giving full access to the national public health authorities.

In summary the EMPROS platform represents a practical tool which stores an enormous amount of data and provides supportive services for decision-making to the relevant stakeholders.

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P6

Crimean-Congo Hemorrhagic Fever Virus and Rift Valley fever Virus, are they circulating in Tunisia?

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) and Rift valley fever (RVF) are arthropod-borne zoonotic diseases with clinical relevance worldwide, caused by Crimean-Congo haemorrhagic fever virus (CCHFV, Nairoviridae family) and Rift valley fever virus (RVFV, Phenuiviridae family) respectively. In order to investigate the prevalence of these viruses in ruminants in Tunisia we conducted a retrospective serological investigation and identified the potential risk factors for seropositivity among cattle, sheep, and goats in Tunisia. Serum samples were randomly chosen from twenty-six localities in Tunisia belonging to four different bioclimatic zones, and analysed for anti-CCHFV and anti-RVFV IgG and IgM antibodies using enzyme-linked immunosorbent assays (ELISAs) and indirect immunofluorescence assays (IIFA). We found the overall seroprevalence of IgG antibodies in ruminants to be 8.6% (76/879) and 2.3% (16/699) against CCHFV and RVFV, respectively. Risk factor analysis revealed that breed (P-value=0.005), age (P-value=0.007; OR=0.259; [CI%: 0.090.743]), season (P-value=0.001), bioclimatic zone (P-value=0.001), and tick infestation (P-value=0.001; OR=17.489; [CI%: 4.929-62.051]) were significantly associated with CCHFV seropositivity in cattle.
For sheep only breed (P-value=0.001), season (P-value=0.008) and bioclimatic zone (P value=0.001) were shown to have a significant association with CCHFV seropositivity. On the other hand, breed (P-value=0.023), bioclimatic zone (P-value=0.001) and tick infestation (P-value=0.001; OR=35; [CI%: 7.173-170.773]) were significantly associated with CCHFV seropositivity in goats. In contrast, there was no significant association between any of the different analysed risk factors in cattle or goats with RVFV seropositivity; while only age and season (P-value=0.001), were significantly associated with RVFV seropositivity in sheep. The CCHFV and RVFV seroprevalences reported in ruminants in this study highlight an important public health threat that needs to be further explored, by conducting fine-scale eco epidemiological studies to investigate the dynamics of the vector-host-environment interactions of these viruses in Tunisia using a One Health based approach. For example, humans at high risk of exposure to CCHFV and RVFV (e.g. slaughterhouse workers) should be considered together with the CCHFV infection rates in *Hyalomma* ticks and their distribution, to better predict and respond to eventual human cases of CCHF in Tunisia.

P7

Evaluation of the effectiveness of the mechanical UNFO-PLS system in Swiss drains as a mechanical control method to contain invasive mosquitoes in urban breeding sites.

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Abstract

Background: The container-breeding invasive exotic mosquito *Aedes albopictus* has spread rapidly over the last few decades across Europe. Integrated vector management (IVM) has been implemented to control its numbers. Here, we evaluated the efficacy of one mechanical systems available on the market that aim to prevent mosquitoes from gaining access to manhole water, thus preventing reproduction in these receptors. SUPSI collaborated in the development and subsequently tested one of these systems proposed by the Italian company UNFO PLS s.r.l. (https://www.unfo-pls.com).

Methods: The experiment was done in the municipality of Balerna, Switzerland, in order to limit differences due to variable conditions. Three study zones were selected, each containing four experimental and four control manholes. The UNFO Pest Lock System was placed in the experimental manholes and compared to the control manholes without the device during the 2020 e 2021. This allows us to compare the differences in the presence and abundance of mosquito breeding container larval stages to determine the effectiveness of the mechanical system. All developmental stages were modeled separately with generalized mixed-effect models using negative binomial distributions.

Results: Mosquito counts at all developmental stages were significantly lower at manholes fitted with UNFO-PLS devices compared to control manholes. The percent reduction in mosquito counts at the L_1 stage was 95.2%, at the L_2 stages was 96.3%, at the L3 stages was 97.2%, at the L_4 stages was 95.0% and at the pupal stage was 92.6%. Additionally, models which included all possible interactions between manhole type, year, and day of year did not produce substantially better fits than the main model assessed for any developmental stage.

Conclusions: UNFO-PLS devices are highly effective at reducing the presence of L1-L4 stage and pupal stage mosquitos in sewer system drainage holes present in Balerna (southern Switzerland). The use of this tool is interesting in an integrated *Ae. albopictus* control system. In fact, for manholes, which represent main breeding sites that cannot be removed, it can replace the application of larvicides. However, the cost-benefit ratio against the use of the larvicides must be evaluated.

P8

On-Site Real-Time West Nile Virus Surveillance To Improve The Reaction Time Of Adulticide Mosquito Control Management

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Abstract

Arthropods are playing an increasing role in the success of pathogen transmission on a global scale. One of the important players in this process are mosquitoes and the mosquito-borne diseases, which often culminates in outbreaks, sometimes affecting millions of people and animals. One of the major groups of mosquito-borne diseases is Flaviviruses with prominent human pathogens, such as West Nile virus (WNV), dengue, Zika viruses. Nowadays, WNV is appearing more and more in several countries, thanks in part to climate change and other factors. WNV is endemic in parts of Europe, the Middle East, West Asia, Australia, Africa, and across the American continent. The primary vector for WNV is *Culex pipiens*, and some *Aedes* and *Anopheles* species may be competent vectors as well. Proper, accurate, and comprehensive vector control, surveillance, and laboratory diagnostics are important not only in WNV endemic countries but other places as well. Currently, most data on the presence of the virus comes from event-based surveillance. Here we demonstrate a protocol that can be used to detect the presence of certain viruses on-site within a short timeframe, permitting mosquito control activities for outbreak prevention. With the mobile laboratory we present, it is possible to detect the virus directly from mosquitoes within 3-4 hours, on the spot. This method provides an opportunity to process freshly caught mosquitoes in the trunk of a car under variable field conditions. This process contributes greatly to the increasing use of targeted, environmentally friendly, biological mosquito control but most importantly it facilitates the fine-tuning of outbreak prevention mosquito control activities.

P9

Accuracy of the automated mosquito counting device BG-Counter 2

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Abstract

The transmission of pathogens is an important capability of mosquitoes. Prevention methods such as the application of insecticides or the removal of potential breeding sites are common practice. However, the timing of their application requires exact knowledge on the spatial-temporal occurrence of the vector species. Mosquitoes are usually collected with CO2-baited traps and subsequently identified by morphological and molecular biological methods. This procedure is very time consuming, which prevents a short-term assessment of the risk of local mosquito-borne pathogen transmission. Automatic counting traps allow a timely decision-making on the prevention measures against pathogen transmission. The BG-Counter 2 (Biogents) counts insects and discriminates by size directly in the field. Placed on CO2-baited traps, captured insects are categorized as “mosquito sized”, “too small to be a mosquito” and “too large to be a mosquito”. All information is transmitted to a cloud server at 15-minute intervals. In order to assess the accuracy of this counting device, traps were placed all over Germany which were run in daily and bimonthly intervals. The BG Counter 2 was attached to a CO2-trap (BG-Trap-Station) and a modified version for gravid mosquitoes. All captured mosquitoes were identified morphologically. The number of mosquitoes where compared to the output of the counter to assess their accuracy.

P10

Results of a two-year Slovenian nationwide mosquito surveillance program

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Abstract

Invasive mosquito species (IMS) are of great public health importance due to the risk of transmission of various zoonoses. As knowledge about their distribution, seasonality and abundance is crucial for the control of mosquito-borne diseases, a Slovenian mosquito surveillance program was established in 2020. We sampled adult mosquitoes once per month at 226 sites using one of 3 types of traps: BG Sentinel trap, CDC light trap, CO2 baited trap or CDC Gravid trap. The captured adult mosquitoes were stored on dry ice immediately after collecting the traps. Mosquitoes were identified to species level by morphology or DNA barcoding. When setting the traps, we paid attention to the presence of mosquito larvae in aquatic habitats. If larvae were observed, a sample was taken and stored in 75% ethanol until further analysis in the laboratory. Larvae were also regularly taken from local cemeteries, which have proven to be ideal habitats for IMS in the past. Ovitraps were placed in selected communities to monitor the abundance of IMS. A total of 63,346 adult mosquitoes and 7,884 mosquito larvae were caught in 2 years of sampling. Twenty-five mosquito species were identified - 22 native species and all 3 IMS previously known in the country (*Ae. albopictus*, *Aedes koreicus*, *Ae. j. japonicus*), with *Ae. albopictus* dominating over the latter two species. In ovitraps, only *Ae. albopictus* and *Ae. j. japonicus* were present. Most of the native mosquito fauna was provided by 3 species, *Ae. vexans*, *Ae. sticticus* and *Culex pipiens* s. l. 5 rare species were caught: *Anopheles hyrcanus*, *Orthopodomyia pulcripalpis*, *Ochlerotatus detritus*, *Ochlerotatus pulcritarsis* and *Uranotaenia unguiculata*. Through monitoring we are gaining important new data on the distribution of IMS in the country, new data on the distribution of all three species and important missing information on the native mosquito fauna. Monitoring should continue in the future as it is an important part of the country's preparedness plan for possible future disease outbreaks.

The work was carried out as part of Action CA17108 of AIM-COST and the project "Establishment of monitoring of vectors and vector-borne diseases in Slovenia" (V3-1903).

P11

Field efficacy of VectoMax® FG biological larvicide against *Aedes albopictus* and *Culex pipiens* in urban catch basins

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Abstract

The exotic invasive tiger mosquito, *Aedes albopictus*, appeared in southern Switzerland in 2003. The spread of the mosquito has been surveyed constantly since then, and an integrated vector management has been implemented to control its numbers. In public areas, the control measures focus on the aquatic phase of the mosquito with removal of breeding sites and applications of larvicides, mainly in catch basins. VectoMax® FG (Valent Biosciences) is a combined larvicidal formulation of *Bacillus thuringiensis var. israelensis* (strain AM65-52) and *Bacillus sphaericus* (strain ABTS-1743). This biological mosquito larvicide presents the advantage of having a longer efficacy (i.e., one month), compared to other similar products, reducing therefore the application efforts both in terms of quantity of product used and personnel employed. Here, in 2021, we evaluated the efficacy of VectoMax® FG against *Aedes albopictus* and *Culex pipiens* under real conditions in urban catch basins in southern Switzerland with the aim of giving the best guidelines to the local authorities in charge of the application.

P12

Agriculture and public health promote diflubenzuron resistance in the West Nile vector *Culex pipiens*

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Abstract

The control of arthropod vectors using chemical insecticides is still a fundamental strategy to prevent or reduce the diseases they transmit, but the onset of insecticide resistance is hampering the success of control programs. Frequently, insecticide resistance in vector species is considered an evolutionary effect due to applications of vector control measures (for public health purpose). Nevertheless, agricultural practices can also play a major role in promoting vector resistance. Indeed, the same insecticidal compounds are often used against both agricultural pests and disease vectors, therefore vectors that may breed in rural areas can be exposed to insecticides used in agriculture. A connection could therefore arise between the fields of agriculture and public health in promoting insecticide resistance in vector species. However, assessing the relative contribution of selective pressure derived from agricultural and public health in resistance evolution is often difficult due to lack of historical data series.

Here, we showed a role of agricultural applications in maintaining alleles that confer resistance to diflubenzuron (DFB) in the mosquito *Culex pipiens* in the Emilia-Romagna region. In this area, DFB has been largely employed against orchard pests during the 1980s-1990s, while its use against disease vectors, including *Cx. pipiens*, started in the 2000s. By analysing DNA sequences of current (i.e. 2018-2020) and historical samples (i.e. 1986-1993) collected in agricultural and rural sites, we showed that: *i*) DFB resistant alleles were already present in historical individuals, indicating that such resistance in *Cx. pipiens* goes back to before the use of DFB in mosquito control; *ii*) resistant alleles were present only in samples collected in agricultural sites, showing a spatial correlation between insecticide resistance and agricultural insecticide use; *iii*) applications of DFB against disease vectors from 2000 onwards enhanced the frequency and distribution of resistant alleles. On the whole, such evidence bring out important implications for control and resistance management.

P13

Pyrethroid Resistance in the Two Mosquito Vector Species, *Aedes albopictus* and *Culex pipiens*: a Wake-up Call for Europe

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Abstract

The indigenous *Culex pipiens* and the invasive *Aedes albopictus* are among the most abundant mosquito species in Europe and represent an increasing public health concern due to them transmitting arboviruses including West-Nile, chikungunya and dengue. The usage of pyrethroids to control mosquitoes as well as agricultural pests is widespread but can favour the selection of pyrethroid-resistance (PR) phenotypes and the rise in frequency of mutations in the voltage-sensitive-sodium-channel (VSSC) gene, associated with PR. Despite its importance, this issue has rarely been studied so far.

Herein we present WHO-bioassay data obtained for *Ae. albopictus* and *Cx. pipiens* from Italy together with results of PCR-genotyping of *kdr*-mutations, i.e. V1016G mutations in *Ae. albopictus* populations across Europe and of L1014/F/C/S mutations in *Cx. pipiens* populations across Italy. Further data on genotypic and phenotypic PR in European populations of both species were obtained from a bibliographic research.

In *Ae. albopictus*, literature data and WHO-bioassay results reveal reduced pyrethroid susceptibility in 7 out of 11 populations from Italy, as well as in Albania, Greece and Spain. Genotyping of over 3,400 specimens from 19 European countries shows widespread presence of the V1016G mutation in Italy (reaching frequencies >50%) and in 8 other countries (Bulgaria, France, Georgia, Malta, Romania, Spain, Switzerland and Turkey) at frequencies ranging between 1 to 7% per site.

In *Cx. pipiens*, bioassay results reveal reduced susceptibility in all Italian populations analysed (reaching mortalities <20%), as well as in Spain and Greece. Presence of mutations L1014F/C/S was investigated in Italy and Greece, where they reach frequencies >90%.

The present data reveal widespread PR in Italian *Ae. albopictus* populations and highlight the risk of PR spreading also to other European populations. An even more worrisome situation is observed for *Cx. pipiens*: despite the low number of studies available, PR appears to be widespread, reaching levels high enough to surely compromise the efficacy of pyrethroid-treatments, which represent at the moment the only available weapon in case of arboviral disease transmission. These evidences

represent a wake-up call for mosquito-surveillance programs across Europe and prompts extensive monitoring as well as implementation of PR management plans.

P14

Detection of the invasive mosquito *Aedes aegypti* on the island of La Palma, Canary Islands (Spain).

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Abstract

Due to the risk of introduction of invasive mosquitoes belonging to the genus *Aedes*, the Canary Islands were included in the National Programme of Entomological Surveillance in Ports and Airports in 2013, with the main objective of early detection of the entry of invasive alien species vectors of arboviruses. The Medical Entomology Laboratory of the University Institute of Tropical Diseases and Public Health of the Canary Islands (IUETSPC), as the main responsible for the implementation of this project in the Canary Islands, confirmed in December 2017 the detection of *Aedes aegypti* in Fuerteventura, which had been eradicated from the islands in the mid 50's. After the control work carried out and the rapid action of the responsible authorities, it could be declared eradicated again in June 2019. At the end of February 2022, the IUETSPC received two culicid larvae from an ovitrap near the Port of Santa Cruz de La Palma. The morphological characteristics of the specimens were consistent with the species *Aedes aegypti*. After molecular confirmation and reporting the finding to the competent authorities, the Government of the Canary Islands confirms the new introduction of Aedes aegypti in La Palma. The work has started by intensifying the number of traps, ovitraps and Bg-Sentinel, together with the revision of artificial breeding sites in the initially established dispersion radius around the detection point. These breeding sites have been treated preventively due to the detection of mosquito larvae and pupae of species native to the islands. In addition, the General Directorate of Public Health has activated epidemiological surveillance throughout the island in order to receive notification of bites. Although the work is still ongoing, so far, no new specimens of the invasive mosquito have been detected.

P15

Age-Grading of *Anopheles gambiae* s.s. mosquitoes using MALDI-TOF MS protein profiling

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Abstract

Background & Objective: Malaria is transmitted by the bite of an infected female *Anopheles* mosquito. Transmission can only occur if a mosquito survives the extrinsic incubation period of 10 to 14 days. Assessing the age structure of mosquito populations could help in evaluating the impact of control methods. Traditional techniques are cumbersome and subjective. Malaria vectors have been shown to undergo proteomic changes as they age. MALDI-TOF is a mass spectrometry technique that uses laser technology for protein profiling and identification. This study aimed to determine if MALDI-TOF MS is capable of distinguishing different age groups of Anopheles gambiae s.s. mosquitoes based on their protein profiles.

Materials & Method:
Anopheles gambiae sensu stricto mosquitoes (Kilifi strain) were reared in a laboratory controlled environment at KEMRI-Wellcome Trust insectary. Mosquito were reared to different physiological and chronological ages. The individual life history of each mosquito was recorded including mating, blood-feeding and oviposition. Approximately 100 mosquitoes per group of distinct physiological and chronological age were processed using MALDI TOF MS.

Results & Discussion: Principal components analysis was used to explore differences in each group’s spectrum profiles. Age-grading databases were created using ClinProTools and validated with spectra of unknown age. We expect to present the protein profiles of different ages of mosquitoes and database accuracy. We also aim discussing whether the method is worth exploring more widely in the field.

Conclusion & Recommendation: The information will be used to validate the importance of MALDI-TOF MS as a new tool for entomological surveillance

P16

Blood meal inhibition by the essential oil of *Lippia alba* (Verbenaceae) against *Anopheles gambiae* and *Aedes aegypti*

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Abstract

Background: The management of chemical insecticide resistance in mosquito vectors and the desire to develop alternative and/or complementary vector control methods have encouraged evaluations of the biological effects of plant extracts against mosquitoes. *Lippia alba* is an aromatic plant whose essential oil has insecticidal and repellent effects against Culicidae. The aim of this study was to evaluate the blood meal inhibiting effect of the essential oil extracted from the leaves of *L. alba* harvested in Abidjan on *Anopheles gambiae* and *Aedes aegypti*, which transmit the pathogens causing malaria and dengue respectively.

Methods: The essential oil of *Lippia alba* was obtained by hydrodistillation of the leaves using a Clevenger. The susceptible strains Kisumu (*An. gambiae*) and SBE (*Ae. aegypti*) are maintained in rearing at the Vectopôle insectarium. The blood meal inhibition effect was evaluated using a water bath- feeders- water bath circuit connected by pipes, pig membranes and rabbit blood. The essential oil was evaluated at 1%, 2.5%, 5% and 10%.

Results: *L. alba* essential oil caused 35%, 39.67%, and 49.67% blood meal inhibition at concentrations of 1%, 2.5%, and 5% respectively with *An. gambiae*. At the 10% dose, the inhibition rate in *An. gambiae* decreased (15.5%). The essential oil at 1%, 2.5%, 5% and 10% resulted in 30.33%, 36%, 57.67% and 98.43% blood meal inhibition against *Ae. aegypti* respectively.

Conclusion: The essential oil of *L. alba* causes significant blood meal inhibition in *Ae. aegypti*. The low inhibition observed in *An. gambiae* with the 10% concentration invites further investigations.

P17

Surveillance of the invasive tiger mosquito *Aedes albopictus* (Diptera:Culicidae) in Skopje from 2017-2021

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Abstract

The Asian tiger mosquito, *Aedes albopictus*, is an invasive mosquito species that is considered a potential vector of about 22 arboviruses, among which dengue, chikungunya and Zika. The monitoring and surveillance of native and invasive species of mosquitoes (Diptera:Culicidae) was established in Skopje in 2013, and in the RN Macedonia in 2016. In 2016 for the first time, the presence of *Aedes albopictus* (Skuse, 1894) was established in RN Macedonia, in the southeastern part, and in 2017 in the Capital Skopje has been recorded. Surveillance in the city of Skopje for period 2017-2021 was carried out by trapping adult mosquitoes with at three BiogentMosquitaireTM PLUS trap made in Germany, and liquid CO2 was used as an attractant. The trapping of adult mosquitoes was in the period from May to the end of October each year. The dynamics of changing the nets was every two weeks. The caught material was taken for laboratory analysis in the Center for Public Health -Skopje, where the determination and quantitative analysis was performed. The determination of mosquitoes was performed through taxonomic-morphological characteristics of the units according to the key of Becker, N., Petric, D., Zgomba, M., Boase, C., Minoo, M., Dahl, C., Kaiser, A., - Mosquito and their control, Second edition, Springer, 2010. From a five-year monitoring and surveillance, a total of 87,158 adult mosquitoes were caught, of which 984 were *Ae.albopictus,* that is, 1.13% of the population of the fam.Culicidae is the Asian tiger mosquito. Of them, 28 were males, and the remaining 956 were females. For a period of five years the surveillance, we have information that the Asian tiger mosquito is spreading very quickly in new settlements and its numbers are increase in the territory of the Skopje. Global warming, climate characteristics, rural-urban population migration and faster urbanization of Skopje enabled *Ae.albopictus* to be established in about 80% of populated areas in Skopje in a short period of time. Of the invasive mosquito species, only *Aedes albopictus* is present in Skopje.

P18

Investigation on key aspects of *Aedes koreicus* mating biology

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Abstract

Aedes koreicus is an alien mosquito firstly detected in Europe in 2008. While in some colonised areas the species showed a low tendency to spread, remaining confined to the sites of introduction, in other regions such as Northern Italy, the so-called Korean bush mosquito expanded its distribution over the years. Despite being discovered more than a decade ago in the European territory, the mating biology of *Ae. koreicus* is largely unknown. Our research investigated aspects that may influence the reproductive success of this mosquito, such as autogeny, mating behaviour, *Aedes albopictus* competitive mating, and the presence of the endosymbiont *Wolbachia pipientis*.

Ae. koreicus autogeny was tested in three different groups of male and female mosquitoes housed in BugDorm® cages (cage 1, N=123 males, 134 females; cage 2, N=116 males, 146 females; cage 3, N=103 males, 138 females). Our laboratory mosquitoes, deriving from the initial established Italian population, showed no evidence of autogeny up to three weeks from the initial co-caging.

We observed the species mating behaviour in a modified BugDorm® cage where virgin males (N=190) and virgin females (N=240) ready for copula were caged together, dissecting the female spermathecae at twelve to thirteen hours intervals for sperm visualisation. Mating activity was observed after 25.5 hours of co-caging, and evidence of motile sperm in *Ae. koreicus* female spermathecae was found in 28% of females (N=25) sampled after 31 hours of co-caging with males.

Laboratory reared *Ae. albopictus* males (N=27) ready for copula introduced to virgin *Ae. koreicus* females (N=22) displayed an aggressive mating behaviour. After five days, all female spermathecae were dissected to evaluate whether a successful sperm transfer had occurred. Despite repeated interactions between *Ae. albopictus* males and *Ae. koreicus* females, no sperm was detected in the 66 female spermathecae.

Wolbachia was not detected in *Ae. koreicus* from field collected during the early stages of colonisation in Italy (N=21).

Reproductive success plays a fundamental role in mosquito establishment and population growth. Our preliminary results could inform further studies and assist in

determining the invasive potential of *Ae. koreicus* and the public health risk posed in newly colonised areas.

P19

Landscape structure sequence influences culicid species composition: mosquito community analysis in six transects, East to West Germany

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Abstract

Like any animal and insect, mosquitoes have special requirements to their habitat, which is determined, among others, by biotope availability and landscape structure. To relate species occurrence, frequency and distribution to landscape and assess ecological dependencies, mosquito collections were carried out in different regions of Germany in a transect approach along the rivers Oder, Spree, Elbe, Weser and Rhine (2x). Each transect included the landscape structures 'floodplain', 'agricultural land', 'deciduous forest', 'coniferous forest', 'urban area' and 'cemetery'. To analyse the mosquito communities in the various transects and landscape structures, adult mosquitoes were collected by EVS traps equipped with dry ice to attract host-seeking females. In a three-week-rhythm, one EVS trap per landscape structure and transect was operated over night for sixteen hours each, from mid-April to late October 2020. The collection data obtained were compared regarding trapping success between the different landscape structures of the same transect and between the same landscape structures of different transects.

The species spectrum produced by the EVS traps consisted of five out of six mosquito genera occurring in Germany (*Aedes, Anopheles, Coquillettidia, Culex, Culiseta*), with considerable differences between transects. Over all transects, 20 mosquito species were trapped, with the highest number (n=16) in the Weser transect (location Höxter) and the lowest number (n=9) in one of the two Rhine transects (location Troisdorf). By contrast, the highest number of individuals (n=9,418) was trapped in the Oder transect (location Frankfurt) and the lowest number (n=51) in the Troisdorf Rhine transect. With regard to landscape structures, most species were trapped in floodplains (n=10) and deciduous forests (n=7) whereas in agricultural lands (n=4), coniferous forests (n=3) and urban areas (n=4) relatively few species were caught.

In conclusion, the availability of biotopes and breeding sites (both natural and artificial) determines the composition and abundance of mosquitoes. However, the sequence of landscape structures has a major influence on the species composition.

P20

Characterization of Wolbachia endosymbiont in mosquitoes of Ticino, Switzerland

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Abstract

Wolbachia is the most widespread group of intracellular bacteria belonging to the alpha-proteobacteria. Estimated to be present in 66% of insect species, this endosymbiont is maternally transmitted and has evolved several strategies to manipulate host reproduction. In mosquitoes, Wolbachia can interfere with host reproduction by inducing cytoplasmic incompatibility, thereby having an interesting role in biological control. The present study aim to characterize *Wolbachia* strains in four mosquito species present in Ticino, southern Switzerland: *Culex pipiens*, *Aedes albopictus*, *Aedes japonicus* and *Aedes koreicus*.

Mosquitoes has been collected in several locations of Ticino during summers 2020, 2021 and 2022 using different traps: CDC traps baited with dry ice, BOX gravid mosquito traps, BG-sentinel mosquito traps and manual aspirators. For this preliminary study 50 *Cx. pipiens*, 50 *Ae. albopictus*, 113 *Ae. japonicus* and 255 *Ae. koreicus* were collected and analysed. As *Cx. pipiens* and *Ae. albopictus* are known to be naturally infected, they were analysed as single specimens, while all Ae. koreicus and *Ae. japonicus* were analysed in pools of maximum 20 specimens to assess the presence or absence of *Wolbachia*. Each species was analysed with a specific set of primers in order to characterize the *Wolbachia* strains.

Among the 50 *Ae. albopictus* analysed, 92% were positive for the two strains WAlb A and WAlb B, 6% only for the strain WAlb A, and one mosquito was negative. All *Cx. pipiens* were positive for Wolbachia and the Wpip groups have been identified in 39 mosquitoes (61,5% Wpip group IV, 28,2% group II and 10,2% group III) . All *Ae. japonicus* tested negative for *Wolbachia*, while one pool of *Ae. koreicus* tested positive and has been characterized with a MLST approach. Phylogenetic analysis showed that this strain has the greatest similarity with an Italian strain recently found in *Ae. koreicus*. Further analysis are necessary to investigate and better characterize this new Wolbachia strain of *Ae. koreicus* from southern Switzerland.

P21

Survey of parasitic larval trematodes in the assassin snails *Anentome helena* and *A. wykoffi* from Thailand

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Abstract

The assassin snail genus *Anentome* Cossmann, 1901 is widely distributed in freshwater habitats throughout Southeast Asia, especially Thailand. One of its species, *viz. A. helena* (von dem Busch, 1847), is an intermediate host of various parasitic trematodes. As such, this study investigates the taxonomic diversity of larval trematodes infecting *Anentome* species in Thailand. To this end, 1107 specimens of two *Anentome* species, *viz. A. helena* and *A. wykoffi* (Brandt, 1974) were sampled from 25 localities in Thailand and screened for the presence of larval trematodes. Morphological and DNA sequence analysis based on the nuclear ribosomal internal transcribed spacer 2 (ITS2) and the mitochondrial cytochrome oxidase subunit I (COI) genes were combined to identify the larval trematodes. Species delimitation analyses were applied to explore species boundaries among the larval trematodes. In total 62 specimens of *Anentome* were infected by larval trematodes, *i.e. A. helena* with n = 33 and *A. wykoffi* with n = 29, yielding an overall prevalence of 5.6%. The larval trematodes included zoogonid cercariae, heterophyid metacercariae, and echinostome metacercariae. The DNA analyses confirmed their assignment to the families Zoogonidae, Heterophyidae and Echinostomatidae. Thus, this is the first report of (1) zoogonid cercariae infecting *A. helena*, (2) heterophyid metacercariae infecting *A. helena*, and (3) echinostome metacercariae infecting *A. wykoffi*. Moreover, species delimitation analyses provided evidence of tentative species-level differentiation between *Echinostoma* sp. from Thailand and *E. mekongi* from Cambodia, as well as within *E. caproni, E. revolutum* and *E. trivolvis*.

P22

Hot and cold spots in *Culex pipiens/torrentium* vector-borne disease transmission

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Abstract

Background: Observational studies show that the spatial pattern of mosquito-borne disease outbreaks is far from random, which suggests that that occurrence follows a pattern that is predictable. This would imply that some parts of the landscape are more vulnerable than others. Based on these observations we tested the hypothesis that, within the rural landscape, small patches of forest might be potential hotspots for mosquito-borne disease occurrence. These are characterised by, high densities of the vector species *Culex pipiens/torrentium*, high densities of local breeding birds, the presence of intermediate and long distance migratory birds, and favourable microclimatic conditions for mosquito survival and pathogen transmission. Coldspots are sites where at least one of these elements is missing.

Method: To test if there are hot- and coldspots in the landscape, we conducted a field study at five paired sites, one in a grassland (coldspot) and one in a patch of forest (hotspot). We sampled the sites for twelve consecutive weeks from 28th June to 9th of September 2021. At each site we sampled, the adult mosquito communities, using BG-Pro traps, local bird communities, using point-transect counts, the microclimate using weather stations and the presence of WNV, USUV and Plasmodium spp., with quantitative PCR. To distinguish differences between hot- and coldspots, parametric and non-parametric analyses of variance were executed.

Results: In line with expectations, hotspots foster denser bird communities, host higher abundances of adult *Culex pipiens/torrentium* and accommodate more beneficial microclimatic conditions for vector competence when compared to coldspots. Against expectations, several coldspots were found to host considerable numbers of *Culex pipiens/torrentium*, whereas two hotspots were found to host surprisingly low numbers.

Conclusion: Overall, the results suggest that the hypothesized hotspots contain all critical elements needed for mosquito-borne disease occurrence, whereas grasslands in general lack one or more elements. Together with observations in the field, the findings of this study highlight the importance of recognizing landscape heterogeneity

as an explanatory factor in the mapping of mosquito borne diseases. Moreover, the results give incentive to reconsider the scale at which effects of landscape characteristics are studied and modelled.

P23

In the heat of the moment: temperature fluctuations promote mosquito development

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Abstract

Temperature is commonly acknowledged as one of the primary forces driving mosquito populations, most notably by influencing metabolic rates and survival. Changes in temperature may be cyclic in nature, as with seasonal and diurnal cycles. However, they may also be directional as a result of global processes such as climate change and local effects such as urban-heat islands. The consequences of temperature fluctuations are generally understudied but may severely affect development as has been described for other insects. Additionally, each developmental stage might be impacted in different amounts. To gain insight in the effects of temperature fluctuations on mosquito development, survival and sex-ratio for the cosmopolitan species *Culex pipiens s.l.*, we developed an inexpensive, easily reproducible and open-source Arduino-based temperature control system, to emulate natural sinusoidal fluctuations. We compared commonly used constant (mean) temperature and block schemes with natural sinusoidal fluctuations as well as an extreme variant with twice its amplitude, whilst keeping the number of degree-days over all treatments the same. While no effect was detected on survival and sex-ratio, development sped up considerably with increasing temperature fluctuation, especially during the pupal stage. These results highlight the need for including (natural) oscillations in experiments that use temperature as a variable.

P24

How mosquitoes are distributed across the urban fabric

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Abstract

Human alterations of the environment are a major driver of mosquito abundance and mosquito species composition. The resulting changes have led to a heterogeneous landscape where some areas have very high abundance of mosquitoes. However, which factors contribute to generating this heterogeneity in mosquito abundance is poorly understood. Here, we set out to investigate how different life stages of mosquitoes (eggs, larvae and adults) are distributed and what environmental driver generate these distributions. We carried out this research in the urban fabric, with its highly heterogeneous ecosystem. We used a setup of six paired locations that were selected based on differences in population density and vegetation composition. Our results indicate that while eggs and larval stages were far more abundant in highly populated areas, adult mosquitoes were more abundant in lowly populated sites. These results coincide with differences in abundance of larval habitats (higher in more populated areas) and differences in the composition of blood feeding hosts and major differences in microclimatic conditions (more amenable in the less populated areas). These results suggest that, depending on the life stage, different habitat characteristics are governing mosquito distributions. While adult life is mostly spent in the greener spaces (with higher host availability and more suitable microclimate), larval life stages are spend in the densely populated areas (with its lack of aquatic predation?). Overall, these results highlight the importance of landscape heterogeneity to understanding mosquito distributions, and suggest that mosquito dispersal may be considerably further than previously thought.

P25

Blood meal analysis of *Aedes* invasive mosquito species (Diptera, Culicidae) from urban environments

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Abstract

Mosquitoes play a key role as vectors of several pathogens of public health relevance. The emergence and invasion of exotic vector species increase the likelihood that additional vector-borne diseases may appear in regions that have not encountered them previously. Since blood feeding is a fundamental mosquito behaviour with consequences for pathogen transmission, identification of host organisms can reveal pathogen – mosquito – host associations influenced by environmental and biological parameters. The blood feeding patterns of mosquitoes refer to their vectorial capacity and allow to estimate contact rates that represent an essential component in epidemiological studies of mosquito-borne pathogens. Such information helps to understand disease ecology and plays a key role in vector control as epidemic prevention. Along with many European countries, Hungary also faced the rapid spread of alien *Aedes* species, i.e. all *Ae. albopictus*, *Ae. japonicus* and *Ae. koreicus* have been established in our country. This has allowed us to set the objective of profiling the feeding preferences of *Aedes* invasive mosquito species that occur in the region. As a pilot study, blood-fed mosquitoes collected from urban habitats (using Gravid Aedes Traps, Biogents) were subjected to PCR-based blood meal identification analysis. Altogether, 50 „invasive” and 50 „native” mosquito specimens were included in the analyses, mostly *Ae. albopictus* and *Culex pipiens*, respectively, but all the investigated species showed a preference for human blood, unsurprisingly. In the present work, we show, that for appropriate preventive measures, we need to pay close attention to mosquito vector – human interactions, particularly in urban environments, from monitoring activities to the investigation of feeding habits. Since our study demonstrates the pattern of *Aedes* invasive mosquitoes' host feeding at limited points in time and place, as well as variability exists among populations, our results are not generalizable to other locations or times. Still, our findings highlight the importance of blood meal analyses of mosquitoes and address these research questions repeatedly over time and space.

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Using wooden sticks as oviposition support – what do we miss?

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Abstract

Ovitraps are easy-to-use and inexpensive traps for detecting potentially invasive container-breeding *Aedes* mosquitoes, particularly the Asian tiger mosquito (*Aedes albopictus*). These traps consist only of a black container filled with water, and an oviposition support. This oviposition support has, in contrast to the smooth surface of the container, a rough surface, which several *Aedes* mosquitoes prefer for laying their eggs. Commonly used oviposition supports are filter papers, wooden sticks (mansonite or wooden mouth spatula) or polystyrene. With the exception of filter paper, which covers the entire inner surface of the container, wooden sticks or polystyrene leave the inner surface completely or largely uncovered, which could be used for egg laying as well. The aim of this study is to investigate whether observtions of container-breeding *Aedes* species can be missed if only eggs laid on the oviposition support are examined. In 2022, five ovitraps (1 L capacity) were set up at six locations in the province of Salzburg in Austria. Wooden mouth spatulas roughened with sandpaper were used as oviposition support. From May until October the traps are checked at weekly intervals. At each check the water and the oviposition support was changed and the inner surface of the container was wiped with a paper tissue. The eggs on the stick and tissue were counted and the species of the eggs determined (using morphological characteristics and multiplex PCR). We present results from our analysis on differences in the number of eggs from the stick and the tissue, and discuss whether occurrences could be overlooked by examining only the oviposition support.

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Mosquito and avian-based West Nile virus (WNV) surveillance systems as an integral component of integrated vector control (IVM) programs with a focus on Peloponnese, Greece

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Abstract

West Nile virus (WNV) is a mosquito-borne virus circulating in a sylvatic / rural cycle between birds and mosquitoes of the genus Culex, and under certain environmental conditions it spills over to human settlements where it infects humans and equines causing large outbreaks. The unpredictable occurrence of WNV outbreaks dictates the establishment and maintenance of surveillance systems for quantifying WNV activity levels, assessing public health risk and guiding vector control interventions. Mosquito and avian-based surveillance is considered an important tool for gauging WNV circulation and is commonly used in contemporary Integrated Vector Management (IVM) programs. Here we provide information retrieved from a multi-year WNV surveillance system conducted in Peloponnese, Greece which included monitoring of avian hosts and mosquito vectors. Information relating to a) virus circulation in mosquitoes and wild-bird species, b) impact of environmental parameters on virus circulation, and c) vector control response will be presented. The role of WNV surveillance systems, such as this one, in mitigating the risk of disease transmission and enhancing our understanding on the ecology of WNV seems to be crucial.

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List of mosquito species (Diptera, Culicidae) in the city of Gyumri in Armenia

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Abstract

Background: Mosquitoes of the Culicidae family are of great practical importance as carriers of pathogens of protozoa, bacterial and viral diseases in humans and animals. The presented results of entomological studies were conducted in the city of Gyumri in the spring-autumn season of 2021. A list of species of mosquitoes of the family Culicidae caught in the territory of the city is given. Gyumri is the second-largest city in Armenia, with a population of 120.000. The city is located at an altitude of 1550 meters above sea level in the northwestern part of the republic. Landscape: Middle mountain steppe. Climate: Moderate, relatively dry warm summers and cold winters.

Methods: The CDC Miniature Light Trap was used to collect mosquitoes. Traps were set in the basements of apartment buildings, in park areas and recreation areas, and also in private houses where farm animals are kept. Identification of mosquito species was carried out according to morphological keys (A.Gutschevich, A.Stachelberg). An interactive key was also used to identify mosquito species MosKeyTool and online resource (http://wrbu.si.edu/vectorspecies/keys).

Results: As a result of entomological studies, 1137 adults of mosquitoes of the Culicidae family were collected, and after identification, 8 species from 4 genera were registered. Dominant species: *Culex pipiens* Linnaeus, 1758, (37%) it is the principal vector of West Nile virus, *Culex modestus* Ficalbi, 1890, (24%), *Culex torrentium* Martini, 1925, (15%), *Culex theileri* Theobald, 1903, (11%). Infrequent species: *Culiseta annulata* Schrank, 1776, (7%), *Culiseta longiareolata* Macquart, 1838, (5%). Rare species: *Anopheles sacharovi* Favre, 1903, (0,7%), (Malaria, Dirofilariasis), *Aedes caspius* Pallas, 1771, (0,3%), (Rift Valley fever).

Conclusions: For the first time, a database was created on the species composition and spatial distribution of mosquitoes in different conditions of the urban landscape, with cartographic data. Further improved morphological and molecular identification methods will allow this list to be refined in the upcoming years, with annual updates of monitoring databases. This database is an important source of information for the control of disease vectors and, consequently, the diseases they cause, as well as for assessing the diversity of the Culicidae family in the city of Gyumri.

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DNA identification and diversity of the vector mosquitoes *Culex pipiens* s.s. and *Culex torrentium* in Belgium

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Abstract

Culex pipiens s.s. is one of the most common mosquito found breeding in human habitats throughout the temperate northern hemisphere. The species is easily confused with *Culex torrentium* as they only differ by a few subtle morphological characteristics. Further, *Culex pipiens* biotypes, which are morphologically indistinguishable, are known vectors of West Nile virus (WNV) and several other arboviruses. We used DNA barcoding methods to assess the occurrence and diversity of *Cx. torrentium* and *Cx. pipiens* s.s., as well as *Cx. pipiens* biotypes molestus and *pipiens* and their hybrids, in Belgium.

During a nationwide monitoring program (MEMO, Monitoring of Exotic Mosquitoes), 31 selected sites in Belgium were monitored over three consecutive years (2017-2019) for the introduction of exotic mosquitoes (Diptera: Culicidae). 34,401 collected mosquito samples (adults, larvae) were morphologically identified as *Culex pipiens/Cx. torrentium*, of which 1689 specimens were selected at random for species identification using DNA barcoding. In the present study, a total of 401 *Culex torrentium* and 1248 *Culex pipiens* s.s. were DNA barcoded (956 biotype pipiens, 227 biotype *molestus*, and 29 hybrids). Based on the alignments of the generated sequences (COI) analyses were run in Arlequin to assess the genetic diversity. The results confirm that both species are widespread in Belgium. *Pipiens* biotype hybrids were found in 13 sites where both biotypes occurred in sympatry. While *Cx. torrentium* sequences revealed many COI haplotypes, *Cx. pipiens* s.s. showed only one abundant haplotype. This latter observation may either reflect a recent population-wide demographic or range expansion, or a recent bottleneck, possibly linked to a *Wolbachia* infection. Additionally, new evidence is provided for the asymmetric but limited introgression of the *molestus* biotype into the *pipiens* biotype.

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High-resolution optical identification of *Aedes albopictus* and *Aedes aegypti* eggs

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Abstract

Invasive mosquito species of the genus *Aedes* pose a risk to public health due to their ability to transmit viral diseases (e.g. Dengue, Chickungunya, Zika), which is why there is a need to monitor and control these species. The objective of this project was to develop a high-resolution optical instrument to identify differences in the chorion structure of eggs of the two main vector species (Aedes albopictus and Aedes aegypti). This system would allow a rapid analysis of the samples collected in the field and a quantification of the abundance of the two species when both are present, in order to improve arboviral disease surveillance. In the first part of the project, researchers from the Centro Regional de Investigación en Salud Pública/Instituto Nacional de Salud Pública (CRISP/INSP) collected Aedes eggs in Mexico using ovitraps, while the Vector Ecology Unit (SUPSI-DACD-Institute of Microbiology) performed the optical analysis of Aedes eggs with a high-resolution microscope. The results obtained were confirmed by molecular analysis.

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DNA barcoding and identification of intermediate terrestrial host gastropods of metastrongyloid cat parasites in Greece

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Abstract

Metastrongyloid nematodes are cardiopulmonary parasites affecting domestic cat (*Felis catus*) and wildcat (*Felis silvestris*) populations throughout Europe. Terrestrial snails and slugs may act as intermediate hosts of these parasites. The present study reports on the identity of the intermediate hosts in areas in Greece where domestic cats and wildcats occur in sympatry. In order to survey the prevalence and expansion of the nematode parasites, an accurate identification of their intermediate snail and slug hosts is important. Yet, detecting the presence of parasites in the intermediate hosts requires a destructive approach by which a morphological identification of the intermediate hosts becomes difficult. Moreover, juvenile gastropods often lack their species-specific characteristics. Therefore, DNA barcoding is used in addition to the morphological identification. DNA sequences used for species identification include the universal COI gene fragment (cytochrome c oxidase subunit I), the 16S ribosomal RNA and the ITS2 gene fragment (internal transcribed spacer 2). Hitherto, infected snails and slugs included *Eobania vermiculata, Helix lucorum, Cornu aspersum*, *Ambigolimax valentianus, Limax* cf. *conemenosi* and *Tandonia* cf. *sowerbyi*. These species are efficient colonizers of new areas, and have been introduced in several parts of Europe. Therefore, there are concerns about the possible further spread of the nematode parasites they can carry, the more so as in its first stage the survey provided new occurrence data on three metastrongyloid species, viz. *Aelurostrongylus abstrusus, Troglostrongylus brevior*, and *Angiostrongylus chabaudi.*

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Population genetic structure of the exotic Asian bush mosquito, *Aedes japonicus*, in Belgium suggests multiple introduction pathways

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Abstract

Aedes japonicus has expanded beyond its native range and has established populations in several European countries, including Belgium where the species was first reported in 2002 at Natoye. During a recent nationwide monitoring programme (MEMO, Monitoring of Exotic Mosquitoes, 2017-2020), Natoye was monitored to check for the efficiency of the extensive elimination campaign undertaken between 2012-2015. Also, the species was surveyed along the Belgian border with Germany where *Ae. japonicus* has established and has been expanding. The objectives were: (1) to verify the origin of specimens that were collected each year at Natoye during MEMO and (2) to investigate the origin of new introductions into eastern Belgium, assuming these derive from established populations in western Germany. To this end, genotypic variation at seven microsatellite loci was determined in specimens collected before and after the eradication at Natoye and from specimens collected along the shared Belgo-German border. German reference samples were included. The microsatellite data shows a difference between specimens collected before and after the eradication campaign, suggesting new introduction(s). The genotype results also indicate that some specimens survived the eradication. At the border, clustering results confirm a link between the specimens collected in Belgium and the western German populations. Whether these introductions from Germany occur via passive human-mediated ground transport or by natural spread cannot be determined at this moment. Our results indicate the complexity of controlling invasive species, since four years of intense control measures were insufficient to eradicate *Ae. japonicus* which are entering Belgium via Germany.

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Longitudinal study of *Phortica* spp. in Central Italy: oakwood association and infection prevalence of the oriental eyeworm *Thelazia callipaeda*

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Abstract

The genus *Phortica* includes around 130 species, but in Europe five species have been recorded, with *P. variegata* and *P. semivirgo* largely distributed in the continent with common ecological requirements and population dynamics, *P. erinacea* and *P. goetzi* respectively detected in Bulgary and Turkey, and *P. oldenbergi* in Germany. The last species is originated from Africa and was lately introduced in Europe in the 20th century and since then no other reports on its distribution in the continent have been published. According to literature, only *P. variegata* has been identified as natural vector of *T. callipaeda* in Europe, but recently it was also demonstrated the competence of *P. oldenbergi* in the transmission of these oriental eyeworm. In this contest, we herein present results about the abundance of *Phortica* spp. in Central Italy and the prevalence of natural infection in lachryphagous males. Samplings were conducted during the past five years in wooded areas of Lazio region: Fabrica di Roma (VT), Foglino (RM), Fonte Nuova (RM), Manziana (RM), Rocca Priora (RM), Sabaudia (LT), Torrimpietra (RM) and Turona (VT) using decaying fruits and entomological nets as baits. Identification analysis describes presence of *P. variegata* in all sites, in particular in Manziana where the abundance of species is 75%, followed by *P. oldenbergi* (23%) and *P. semivirgo* (1,8%). A total of 23 out of 1316 lachryphagous of *P. variegata* (1.7%; 95% CI: 0.6% – 2.8%) were tested positive for *T. callipaeda*, particularly in Manziana, were the eyeworm was stably diagnosed from 2017 to 2020, followed by Fonte Nuova, Foglino Park and Turona (1%). These results indicate the stable presence in oakwood forests of *P. variegata* in sympatry with *P. oldenbergi* and *P. semivirgo*, focusing attention of thelaziosis spread in Central Italy which seems to be a hot-spot of this zoonosis. Additionally, the finding for the first time in Italy of African *Phortica* spp. draws attention to the possible entry routes of other alien species in our country, possibly through fruit trading. This also highlights the need to conduct surveys to identify potential pests or disease vectors in ecological contexts not commonly monitored for these purposes.

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Interplay between vector biting rhythms and human habits accounts for high residual malaria transmission in Burkina Faso despite the extensive coverage of insecticide treated nets (LLINs)

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Abstract

Burkina Faso is among the 10 high sub-Saharan countries where a stalling in the fight against malaria has been registered and the incidence remains still very high despite the periodical LLIN distribution campaigns recommended by WHO since 2010. Our overarching goal is to understand how vector behaviours in response to LLINs (i.e. increased zoophagy, outdoor biting and/or altered biting rhythms) and their interactions with human activities and net usage can contribute to this epidemiological scenario. In previous studies, conducted within 5 years after LLIN introduction in Goden village in Burkina Faso, we defined a scenario of marked zoophily in the dominant vector species (A. coluzzii and A. arabiensis) despite sporozoite (SR=6.1%) and entomological inoculation (EIR=1.4 infective bites/person/hour, ibph) rates in the range observed in pre-intervention settings. We here present results of Human Landing Catches (HLC) conducted in 2020 in Goden during the whole mosquito biting period (4pm-8am) alongside a survey on human habits and net usage. This integrated approach of study offered interesting epidemiological insights. Vector population had a SR of 2.7%; modelling analysis indicated an intense biting pressure (mean 30.3 mosq/pers/hour) during human sleeping hours from 8pm to 6am and a non-negligible biting activity (2.2 mosq/pers/hour) before and after this time window. Overall, no difference in mosquito biting pressure has been observed between indoors and outdoors. Under an unrealistic scenario of full human exposure, the mean EIR during the 16h-HLC is 9.1 ibp. However, adjusting human exposure according to LLIN usage as estimated by human survey, the mean EIR drops to 1.7 ibp. Interestingly, 0.4 ibp occurs before 8pm and after 6am when 100% of the inhabitants are awake and thus fully exposed to bites. This shows how interaction between human activities and vector host-seeking pressure contributes to a non-negligible gap in LLIN protection. We expect that this interplay may represent one of the main factor accounting for residual malaria transmission in other epidemiologically similar settings characterized by high vector density and insecticide resistance.

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