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Foundation
Swiss School of Public Health
Dr. Sandra Nocera
Hirschengraben 82
8001 Zurich

Report - Organisation of the workshop for the project „Virome determination of endemic and invasive mosquitoes in Ticino”

Dear Dr. Nocera,

the project entitled "Virome determination of endemic and invasive mosquitoes in Ticino" has been completed and the foreseen workshop for this project, funded by the SSPH+ (Call for Inter-university Initiatives and Collaborations) could be successfully organised in November 2021.

Below you’ll find the report of the research project and the organisation of the workshop.

Project „Virome determination of endemic and invasive mosquitoes in Ticino"

Vector-borne diseases have become more and more important, representing today 17% of all infectious diseases, and causing more than 700 000 deaths annually[1]. Arthropod vectors play important roles in the transmission of hundreds of different viruses, many of which can severely affect human and animal health. Changing environmental factors and human activities can favor the establishment of invasive arthropod species, affect the vector competence of endemic species, and enhance viral fitness/virulence, thereby potentially contributing to the spread of emerging viral diseases. For example, the Asian tiger mosquito, *Aedes albopictus*, originally endemic in South-East Asia, over the past 40 years has been spreading over almost all continents, including Europe[2]. After its first detection in 2003, tiger mosquito is nowadays established also in Ticino and is spotted occasionally in northern parts of Switzerland, with established colonies also in Basel[3,4,5,6,7,8]. The major concern about the spread of tiger mosquito is related to its ability to carry and transmit mosquitoes borne diseases such as chikungunya, dengue, and Zika[9]. In Europe, several cases of autochthonous transmission of chikungunya and dengue have been reported following cases imported from endemic regions, thus highlighting the real threat that these diseases constitute for public health[10,11]. In Switzerland, autochthonous cases of mosquito-borne viruses transmission have not been reported so far (Federal Office for Public Health, https://www.bag.admin.ch). However, the consolidated presence of the vectors in Ticino, together with the vicinity with countries that experienced autochthonous transmission of mosquitoes borne diseases, increase the risk of indigenous transmissions following imported cases. The development of a methodology for the characterization of the viral population found in endemic and invasive mosquitoes in Ticino is of clear advantage in order to predict and prevent possible mosquitoes borne disease outbreaks.

This project aimed to determine the full, unbiased viral population diversity of *Ae. albopictus* collected in the Canton Ticino based on next-generation sequencing technologies.

The expertise of the Vector Entomology group of the Institute of Microbiology (former Laboratory of Applied Microbiology), SUPSI allowed to obtain field collected specimens of *Ae. albopictus* from Canton Ticino. A total of 538 adult mosquitoes (498 female and 40 male *Ae. albopictus* mosquitoes) were collected from August to September 2019 in five municipalities of the Lugano area, Canton Ticino and subsequently analysed to determine their virome by a metagenomic technique developed by the Institute of Virology of the University of Zurich. From this analysis, a total of 13 viruses from seven different virus families and several unclassified viral taxa were identified. Among the identified viruses there was no genomes of human pathogenic viruses.

Thanks to the present study, we could obtain a first analysis of the virome of established Swiss populations of *Ae. albopictus* and sets the basis for future metagenomic analyses to explore the spatial and temporal dynamics of the virus diversity in these mosquitoes.

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[1] https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases. Accessed 31st January 2022.

[2] Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D (2009) Microbes Infect 11: 1177–1185.

3 Wymann MN, Flacio E, Radczuweit S, Patocchi N, Luthy P (2008). Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 13: pii = 8058.

[4]Flacio E, Engeler L, Tonolla M, Lüthy P, Patocchi N (2015). Parasit Vectors;8:208.

[5]Flacio E, Engeler L, Tonolla M, Müller P (2016). Parasit Vectors. 9:304.

[6]Suter TT, Flacio E, Feijoó Fariña B, Engeler L, Tonolla M, Regis LN, de Melo Santos MA, Müller P (2016). PLoS Negl Trop Dis. 6;10:e0004315.

[7]Ravasi D, Guidi V, Flacio E, Lüthy P, Perron K, Lüdin S, Tonolla M (2018). Parasit Vectors. 11:212.

[8]Biebinger S. Asiatische Tigermücke. Überwachung und Bekämpfung im Kanton Basel-Stadt 2020. Report of Gesundheitsdepartement des Kantons Basel-Stadt, Kantonales Laboratorium. 2020. Available online https://www.kantonslabor.bs.ch/umwelt/neobiota/tigermuecke.html. Accessed 31.01.2022.

[9] Medlock JM, Hansford KM, Schaffner F, Versteirt V, Hendrickx G, et al. (2012). Vector-Borne Zoonotic Dis 12: 435–447.

[10]Rezza G. 2016. Dengue and other Aedes-borne viruses: a threat to Europe? Euro Surveillance. 21(21).

[11]Schaffner F., Medlock J.M. and Van Bortel W. 2013. Public health significance of invasive mosquitoes in Europe. Clinical Microbiology and Infection. 19(8), pp 685-692.

The results of this joint research between the two research Institutes were successfully published in a peer-reviewed journal (Annexe 1. Kubacki J. *et al.* (2020) Metagenomic Analysis of Aedes albopictus Mosquitos from Southern Switzerland. *Viruses* 12, 929; doi:10.3390/v12090929).
The collaboration between our Institutes was very interesting and fruitful, bringing together an interdisciplinary group of scientists with competences in vector entomology, virology, and metagenomics analysis. The collaboration established through this project will certainly lead to further interesting projects in the future in the field of public health.

Organisation of the workshop

In the framework of the SSPH+ Call for Inter-university Initiatives and Collaborations, the organization of a common workshop was envisaged. The workshop was initially intended for the end of summer – beginning of autumn 2020. Because of the SARS-COV-2 pandemic and the impossibility of holding an in-person meeting due to the limitations imposed by the evolution of the pandemic at that time, the workshop was postponed to autumn 2021.
The workshop was organized in the frame of the annual meeting of Swiss Vector Entomology Group (SVEG), which was held on November 25 and 26, 2021 at the Institute of Microbiology (SUPSI) in the new Campus next to the railway station of Mendrisio.
The SVEG was founded in 2009 with the aim to develop vector entomology in Switzerland. The SVEG is organized as an open, informal working group (no membership fee), open to everyone interested in vector entomology and vector-borne diseases (academics, authorities, private sector). The main focus is the organization of an annual scientific meeting (https://www.sstmp.ch/about-us/working-groups/sveg/). The SVEG platform is therefore an ideal place for a workshop on vector-related viruses, because many of its participants either already work directly with viruses and their vectors or are otherwise interested in getting an overview of the activities carried out in Switzerland on arboviruses.
In fall 2021, SUPSI was able to organize a meeting in presence, ensuring all appropriate measures according to the federal guidelines related to the pandemic and the attendance, despite the conjectural situation, was high. In fact, 43 people from the various Swiss regions participated (see Annex 2. List of participants). These included students, researchers, and representatives of cantonal, federal and international institutions.
The program was packed, with a total of 21 presentations divided into 4 sessions, and 5 posters. The program was planned on 2 days: one focused more on the vectors themselves and the other on vector-borne diseases (see Annexes 3. Programm; 4. Abstracts). There was also a very interesting practical demonstration of integrated activities for tiger mosquito control. We could invite also external guests such as Dr. Gioia Capelli, author of the Italian national plan on West Nile virus, and Dr. Florence Fouque from the WHO who gave us an overview on global epidemics related to arboviruses and the measures to be taken. The research project "Virome determination of endemic and invasive mosquitoes in Ticino" was presented by Dr. Kubacki within session no. 4 dedicated to mosquito-associated viruses and their vectors.
During the meeting there was plenty of time for both questions after each presentation and discussions at the end of the sessions. In addition, many aggregative moments were created (coffee breaks, lunches and dinners offered) so that participants could exchange opinions at different times. In fact, the exchanges were quite intense and the scientists got to know each other. The feedback from the participants was very positive, which we are very happy about.

The organization of the workshop involved 10 collaborators from the Institute of Microbiology, SUPSI for a total of 178 working hours spent. All the organizational costs could be covered by the grant obtained for this project. A table with the total costs is presented below. Copies of receipts for third-party costs and working costs are attached to this report.

Table 1. Costs incurred by SUPSI for the organisation of the workshop

Description	Receipt No.	Costs CHF
Working hours IM collaborators (178 hours)	5	9'925.75
Office supplies (block notes, pens, bags, etc.)	6-7	301.50
Social dinner (Antico Grotto Ticino)	8	2'184.00
Coffee breaks – Lunches (SV Ristorante SUPSI)	9-10	2'421.00
Speakers' accommodation expenses (Albergo Milano)	11	108.30
TOTAL		**14'940.50**

We kindly acknowledge for your support that allowed to made it possible to develop and carry out an interesting research work and to exchange our knowledge with other Swiss and international researcher during the meeting organised in November 2021.

Sincerely,

Dr. Eleonora Flacio
Sector Vector Ecology

Dr. Valeria Guidi
Sector Biosafety

Prof. Dr. Mauro Tonolla
IM Director

Bellinzona, 02 February 2022

Attachment:

- 1. Published scientific article "Kubacki J. *et al.* (2020) Metagenomic Analysis of Aedes albopictus Mosquitos from Southern Switzerland. *Viruses* 12, 929; doi:10.3390/v12090929"
- 2. List of participants, SVEG 2021
- 3. Program of the SVEG meeting, 25th-26th November 2021
- 4. Abstracts, SVEG meeting 2021
- 5-11. Copies of receipts for SVEG organizational costs

viruses

MDPI

Article

Viral Metagenomic Analysis of *Aedes albopictus* Mosquitos from Southern Switzerland

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Abstract: A metagenomic study was performed on 498 female and 40 male *Aedes albopictus* mosquitos collected in August and September 2019 in Ticino, a region in southern Switzerland, to address the question regarding the risk of the local transmission of zoonotic viruses. A total of 13 viruses from seven different virus families and several unclassified viral taxa were identified. Reads of insect-specific flaviviruses were present in all pools, and a complete genome of aedes flavivirus was assembled and phylogenetically analysed. The most abundant virus was Wenzhou sobemo-like virus, assembled from 1.3×10^5 to 3.6×10^6 reads in each pool. In a pool of male mosquitos, a complete genome of aedes Iflavi-like virus was detected and phylogenetically analysed. Most importantly, genomes of human pathogenic viruses were not found. This is the first study to determine the virome of *Ae. albopictus* from Switzerland and forms a baseline for future longitudinal investigations concerning the potential role of *Ae. albopictus* as a vector of clinically relevant viruses.

Keywords: metagenomic; *Aedes albopictus*; virome

1. Introduction

The Asian tiger mosquito *Aedes albopictus* plays an important role as a vector of arboviruses, many of which can severely affect human health, including dengue virus (DENV), Zika virus (ZIKV), yellow fever virus (YFV), and chikungunya virus (CHIKV) [1]. Changing environmental factors and progressing human development can support the establishment of invasive arthropod species, affect the vector competence of endemic species, and enhance viral virulence, thereby potentially contributing to the spread of emerging viral diseases. *Ae. albopictus*, originally endemic in South-East Asia, has spread over the past approx. 40 years throughout large parts of the Americas, Africa, Australia, and Southern Europe [2]. In Switzerland, *Ae. albopictus* was spotted for the first time in 2003 in canton Ticino, where since 2007 it has been firmly established; occasionally it is also found in northern parts of Switzerland [3–7]. To date, no autochthonous cases of DENV or CHIKV infections have occurred in Switzerland, while in neighbouring countries, i.e., Italy and France, several cases have been reported. The first transmission of CHIKV by *Ae. albopictus* outside of a tropical area was reported in 2007 in Italy [8]. Several cases of autochthonous DENV and CHIKV virus infections have been linked to *Ae. albopictus* as a vector in France as well [9,10]. Hence, *Ae. albopictus* appears to retain its competence to harbour and transmit zoonotic viruses outside of the tropical regions.

In addition to zoonotic viruses, mosquitos are known to host many different insect-specific viruses (ISVs) which persistently infect mosquitos, but not vertebrates [11], although many ISVs are closely

related to human pathogens [12]. Interestingly, coinfection with some ISVs may modulate or even supress replication of specific zoonotic viruses, such as West Nile virus (WNV), in mosquito cells and therefore inhibit their transmission [13–16]. In addition, coinfection with specific bacteria such as *Wolbachia* sp. may limit the ability of mosquitos to transmit specific zoonotic viruses [17].

As *Ae. albopictus* is endemic in Ticino and progressively invades other regions of Switzerland, and because the presence of the vector facilitates the possibility that zoonotic viruses may be locally transmitted to humans, we addressed the question as to whether these mosquitos indeed harbor such viruses. Specifically, using next-generation sequencing and metagenomic analyses, we determined the full, unbiased virus population diversity of *Ae. albopictus* collected in five municipalities of Lugano in Ticino. A similar study has not previously been performed in Switzerland.

2. Materials and Methods

2.1. Sample Collection

Adult mosquitos were captured in urban and public areas, using electric manual aspirators and entomological nets in August and September 2019 in five municipalities of the Lugano area (Lugaggia, Manno, Porza, Muzzano, and Lugano), canton Ticino, Switzerland (Figure 1). Mosquitos were euthanised by exposure to dry ice [18] and identified to the species level using morphological keys [19–23]. In total, 538 adults (males $n = 40$; females $n = 498$) were collected, divided into 15 pools according to collection date, place, and gender (5–59 mosquitos per pool; see Table 1), and stored at −20 °C until further use.

Table 1. Summary of the mosquito samples used in this study. Male mosquitos were collected at all locations and during the whole sampling period.

Pool Name	Sample Location	Position	Number of Mosquitos	Gender	Sampling Time Point
MO1	Lugaggia	46.062 N 8.969 E	5	female	August
MO2	Manno	46.034 N 8.918 E	16	female	August
MO3	Lugano1	46.028 N 8.964 E	42	female	August
MO4	Porza	46.029 N 8.960 E	21	female	August
MO5	Lugano1	46.028 N 8.964 E	48	female	September
MO6	Lugano1		34	female	September
MO7	Lugano1		35	female	September
MO8	Lugano1		40	female	September
MO9	Muzzano	45.996 N 8.911 E	40	female	September
MO10	Muzzano		40	female	September
MO11	Muzzano		40	female	September
MO12	Muzzano		50	female	September
MO13	Lugano2	45.997 N 8.944 E	59	female	September
MO14	Lugano2		28	female	September
MO15	Mix		40	male	mix

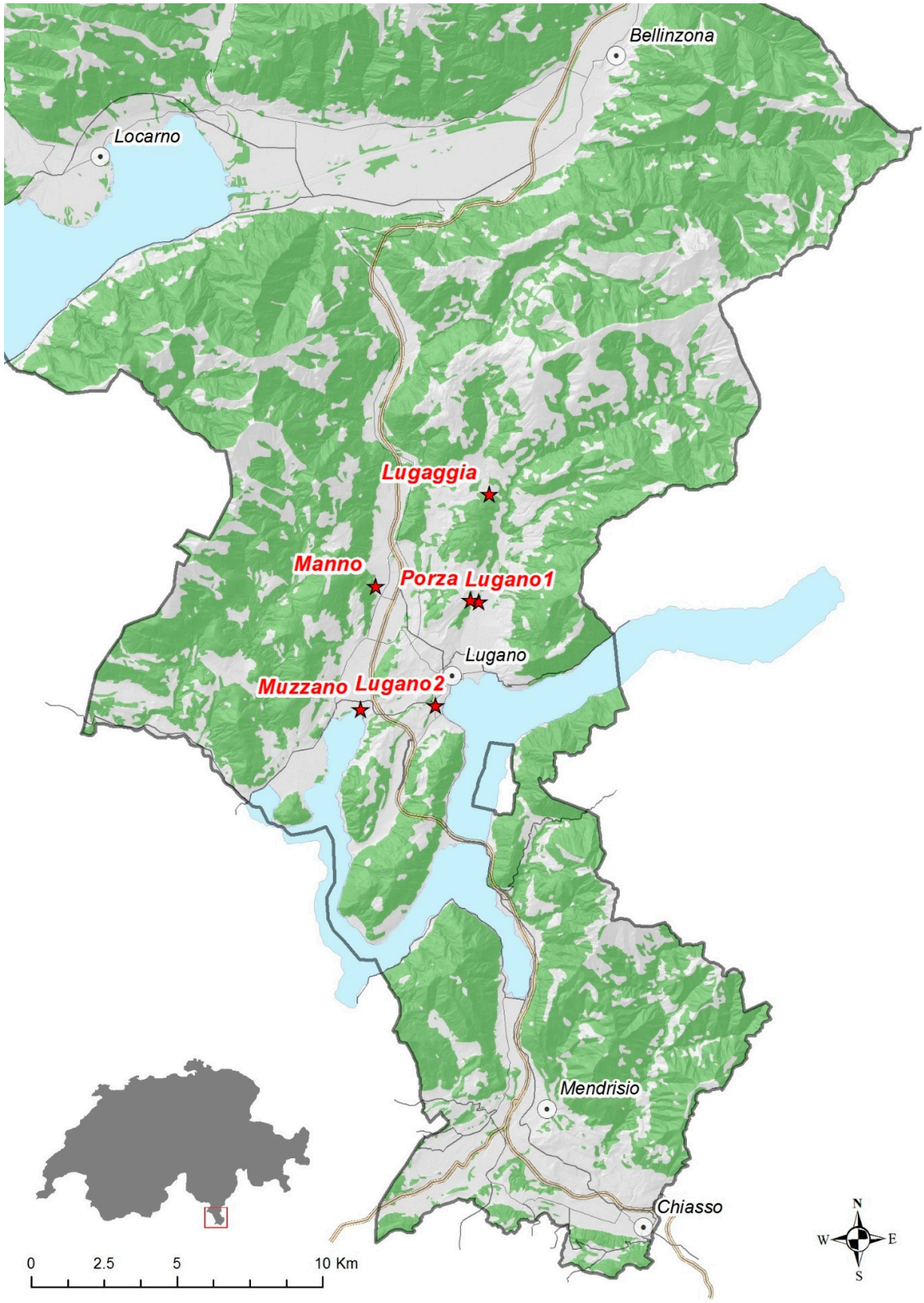

Figure 1. Locations of mosquito collection. Detailed coordinates and sample descriptions are given in Table 1.

2.2. Sample Processing and Sequencing

To each pool, 500 µL of phosphate-buffered saline (PBS) (Merck, Darmstadt, Germany) and a stainless-steel bead (5 mm, Qiagen, Hilde, Germany) were added, and samples were mechanically

homogenised in a TissueLyser II (Qiagen, Hilde, Germany) at 20 Hz for 2 min. Then, homogenates were centrifuged at 16,060× *g* for 5 min, and the supernatants were passed through a 0.45 µm syringe filter (Puradisc, 13 mm, Whatman GE Healthcare, Chicago, IL, USA). To enrich nucleic acids that are protected by a virus capsid, 14 µL of micrococcal nuclease buffer, 1 µL of micrococcal nuclease (both New England Biolabs, Ipswich, MA, USA), and 1 µL of ribonuclease A from bovine pancreas (Merck, Darmstadt, Germany) were added to 134 µL of each filtrate from the previous step and incubated for 15 min at 45 °C and 1 h at 37 °C. Total RNA and DNA were extracted using the QIAmp Viral RNA mini kit (Qiagen, Darmstadt, Germany) without RNA carrier. β-mercaptoethanol (Bio-rad, Cressier, Switzerland) was added at a final concentration of 1% in order to inactivate nucleases. RNA was transcribed using 2.5 µM of random primer with a known 20 nucleotide (nt) tag sequence at the 5′ end (SISPA-N: GTTGGAGCTCTGCAGTCATCNNNNNN) and the RevertAid First Strand H minus cDNA Synthesis Kit (Thermo Fisher Scientific, Basel, Switzerland) following the manufacturer's recommended protocol. Then, 1 µL of RNase H (New England Biolabs, Ipswich, MA, USA) was added to degrade remaining RNA. A premix of 0.8 µM SISPA-N primer, 10× Klenow buffer, and 0.2 mM dNTP was added to 45.5 µL of the first-strand DNA. Following denaturation at 95 °C for 1 min and cooling down on ice, the second strand was synthesized using Klenow polymerase (5 U/20 µL; Thermo Fisher Scientific, Basel, Switzerland) for 15 min at 25 °C followed by 1 h at 37 °C. An additional step of second-strand synthesis using Klenow polymerase was performed at the same conditions, followed by DNA purification using the PureLink® PCR Micro Kit (Invitrogen-ThermoFisher, Waltham, MA, USA). Then, dsDNA was amplified non-specifically by sequence-independent single primer amplification (SISPA). For this, the HotStarTaq DNA polymerase (Qiagen, Darmstadt, Germany) and the SISPA primer (GTT GGA GCT CTG CAG TCA TC) were used under the following conditions: 15 min of activation at 95 °C, 18 cycles of 30 s at 94 °C, 30 s at 58 °C and 1 min at 72 °C, followed by 10 min at 72 °C and cooling down to 4 °C. Finally, the amplified products were purified using the QIAquick PCR purification kit (Qiagen, Darmstadt, Germany). The total DNA was quantified on the Agilent 4200 TapeStation (Santa Clara, CA, USA). Sequencing libraries were made using NEBNext Ultra II DNA library prep kit and NEBNext® Multiplex Oligos for Illumina® (96 Unique Dual Index Primer Pairs) (both New England Biolabs, Ipswich, MA, USA). After quantifying at the TapeStation and pooling at equal molar concentrations, libraries were sequenced using the Illumina NovaSeq system in a single-end 1 × 100 nt run at the Functional Genomics Center Zurich (FGCZ, Zurich, Switzerland).

2.3. Quality Control, Pre-Processing, and Assembly of Metagenomics Reads

Individual metagenomes per sample and the metagenome of all samples combined were assembled and analyzed using the same pipeline. In detail, the technical quality of Illumina single-end (SE) DNA-seq reads was evaluated using FastQC version 0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Raw reads were pre-processed using Trimmomatic (version 0.36) to trim off PCR primers, sequencing adaptors, and low-quality ends (average quality lower than 20 within a 4 nucleotide (nt) window) [24]. Quality-controlled reads (average quality 20 and above, read length 40 and above) were assembled using megahit (version 1.1.3) with multiple k-mers of 21, 29, 39, 59, 79, 99, and 119 [25]. To annotate contigs taxonomically, assembled contigs were compared against the NCBI nt database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/) using BLASTN (version 2.6.0+) [26]. Hits were sorted by bit scores. The top hit was defined as the hit with the maximal bit score. Only hits with e value $\leq 1 \times 10^{-5}$ and bit score ≥ 100 were kept for contig taxonomy annotation. The naïve best-hit method was used to obtain the specific taxa assignment per contig after manual inspection of the alignments. The LCA (lowest common ancestor) algorithm (contig mode) based on multiple BLAST hits was also applied to obtain a more accurate and general taxa assignment [27]. To quantify the abundance of contigs, quality-controlled reads were mapped back to the assembled genomes using BWA-MEM (version 0.7.17) [28]. Mapped reads were quantified using samtools idxstats (version 1.5) [29]. Unmapped reads were extracted again and aligned to an in-house database containing genomes using Bowtie 2 (parameters: -a –very-sensitive –no-mixed –no-discordant -X 1000).

Mapped reads and mapped bases per viral genome were calculated using bedtools. Viral genomes with at least five mapped reads were reported using R markdown (http://rmarkdown.rstudio.com/). Additionally, de novo assembled contigs were further investigated in the metagenomic pipeline of SeqMan Ultra software (Lasergene, DNAStar, USA), viral sequences were analyzed with NCBIs ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/), and phylogenetic trees were built in MEGA X [30]. MUSCLE was used for multiple sequence alignment of the amino acid (aa) sequences, and the maximum likelihood method with 1000 bootstraps replicates was employed to build up phylogenetic trees. Statistical analysis was performed in IBM SPSS software version 24 using Fisher's exact test (p value ≤ 0.05).

The raw sequence reads generated in this study are available at the NCBI sequence read archive (SRA) database under Bioproject accession PRJNA638077. Virus genomes generated have been deposited in GenBank under the accession numbers MT577804, MT577805, and MT591567.

3. Results

In total, 125 million sequencing reads were generated from 15 pools (3.520×10^6 sequencing reads per pool) and applied to reference alignment and de novo analysis to construct contigs. In each mosquito pool, between 1.9% and 36.1% of the total generated sequences were classified as viral reads (Table 2). From a total number of 30,754 contigs, identification of obtained viral contigs using the blastn algorithms resulted in detection of 167 viral contigs belonging to the viral families Flaviviridae, Rhabdoviridae, Iflaviridae, Orthomyxoviridae, Dicistroviridae, Tymoviridae, Genomoviridae, and several unclassified viral taxa. The majority of the detected viral sequences were classified as ISVs. Importantly, no human pathogenic viruses were detected.

Table 2. Viral reads detected shown as a percentage of the total number of reads generated from each pool of mosquitos.

Virus	MO1	MO2	MO3	MO4	MO5	MO6	MO7	MO8	MO9	MO10	MO11	MO12	MO13	MO14	MO15
Aedes albopictus cell fusing agent virus	0.003%	0.0002%	-	-	-	0.0002%	0.0003%	0.0001%	0.0006%	0.0001%	0.0002%	0.0002%	-	-	-
Aedes flavivirus	-	0.5%	0.06%	0.005%	0.03%	0.06%	0.01%	0.02%	0.007%	0.04%	0.02%	0.04%	0.04%	0.002%	0.153%
Aphid lethal paralysis virus	-	-	-	0.001%	-	-	-	-	-	-	-	-	-	-	-
Arboretum virus	-	-	-	-	-	-	-	0.002%	-	0.001%	-	-	-	-	-
Culex Iflavi-like virus 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.6%
Fig fleck-associated virus	-	-	-	-	-	-	-	-	-	0.004%	0.008%	-	-	-	-
Guato virus	-	0.002%	-	0.0004%	-	0.0003%	0.001%	-	-	0.001%	0.002%	0.0003%	-	-	-
Hubei mosquito virus 2	1.3%	0.058%	-	0.42%	0.77%	0.19%	0.3%	0.2%	-	0.037%	-	0.2%	1.2%	-	-
Kaiowa virus	-	0.0002%	-	-	-	0.0002%	-	-	-	-	-	-	-	-	-
Lake Sarah-associated circular virus-48	-	-	-	-	-	-	-	-	-	-	-	0.002%	-	-	-
Plant associated genomovirus 3	-	-	-	-	-	-	-	-	-	-	-	-	0.003%	-	-
Wenzhou sobemo-like virus 4	21.4%	1.3%	33.1%	19.9%	29.0%	13.8%	17.9%	15.7%	20.5%	17.3%	12.6%	15.5%	25.2%	14.4%	31.4%
Whidbey virus	-	0.0002%	-	-	-	-	-	-	-	-	-	-	-	-	-
Total % of viruses	22.7%	1.9%	33.2%	20.3%	29.8%	14.0%	18.2%	16.0%	20.5%	17.4%	12.6%	15.7%	26.5%	14.4%	36.1%
Reads generated in million	4.2	9.4	6.7	7.5	7.1	9.9	12.2	9.5	8.1	20.6	6.7	8.9	6.8	3.5	3.6

3.1. Mosquito-Associated Viruses

In all 15 pools, viral reads of flavivirus or flavivirus-related virus have been detected. In 14 pools, between 0.0020% and 0.5% (Table 2) of the total generated reads were assembled to Aedes flavivirus (AEFV). In the pool MO2, with 42,862 sequencing reads (0.5% of total reads), the complete AEFV of 11,038 nt in length encoding a polyprotein of 3341 amino acids in length was assembled and showed 99.62% similarity to the Aedes flavivirus strain AEFV-SPFLD-MO-2011-MP6 (GenBank accession: AGJ91136.1) (Figure 2) [31]. Additionally, in nine pools contigs with an identity above 99% to Aedes albopictus cell fusing agent virus (CFAV) have been assembled (GenBank acc: AF411835.2) [32].

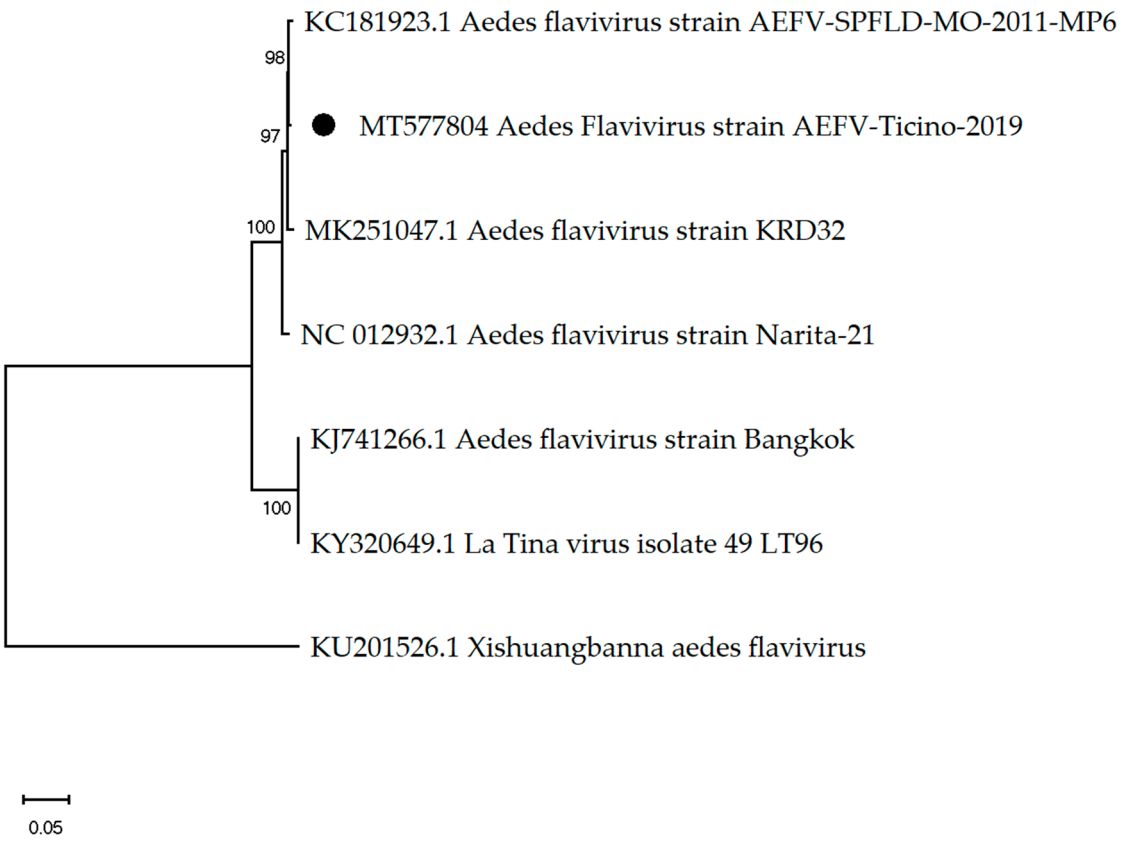

Figure 2. The phylogenetic tree of Aedes flaviviruses constructed using the maximum likelihood method and Kimura 2-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The black dot indicates the genome sequenced in this study.

Interestingly, in pool MO15, the only pool with male mosquitos, a contig from the order Picornavirales with 9666 nt in length has been constructed using 165,192 sequencing reads (4.6% of total reads) (Table 2). Further analysis revealed a 9666 nt genome with 97.47% nt similarity to Culex Iflavi-like virus 4 isolate FTA18 (GenBank acc: MT096522.1) [33] (Figure 3), with a polyprotein starting at nt 298 and ending at nt 9639 (9342 nt and 3113 aa in length). The detection of Aedes iflavi-like virus in a pool of male mosquitos only was statistically significant (p value ≤ 0.05).

Between 1.3×10^5 and 3.6×10^6 sequenced reads (1.333–0.1% of the total reads) (Table 2) have been assembled to Wenzhou sobemo-like virus, which was the most abundant virus detected in all 15 pools. The metagenomic pipeline of SeqMan Ngen revealed in pool MO1 a genome of 2959 nt length and 98% nt identity to Wenzhou sobemo-like virus isolate FTA 15 (GenBank acc: MT096519.1) [33] (Figure 4). A hypothetical protein 1 of 1770 nt/589 aa in length starts at nt 67 and ends at nt 1836, and a hypothetical protein 2 of 1326 nt/441 aa in length starts at nt 1578 and ends at nt 2903. Additionally,

in nine pools, between 0.037% and 1.3% of the total reads generated (Table 2) were assembled to Hubei mosquito virus 2 (GenBank acc: KX882764.1), which has previously been detected in mosquitos collected in China.

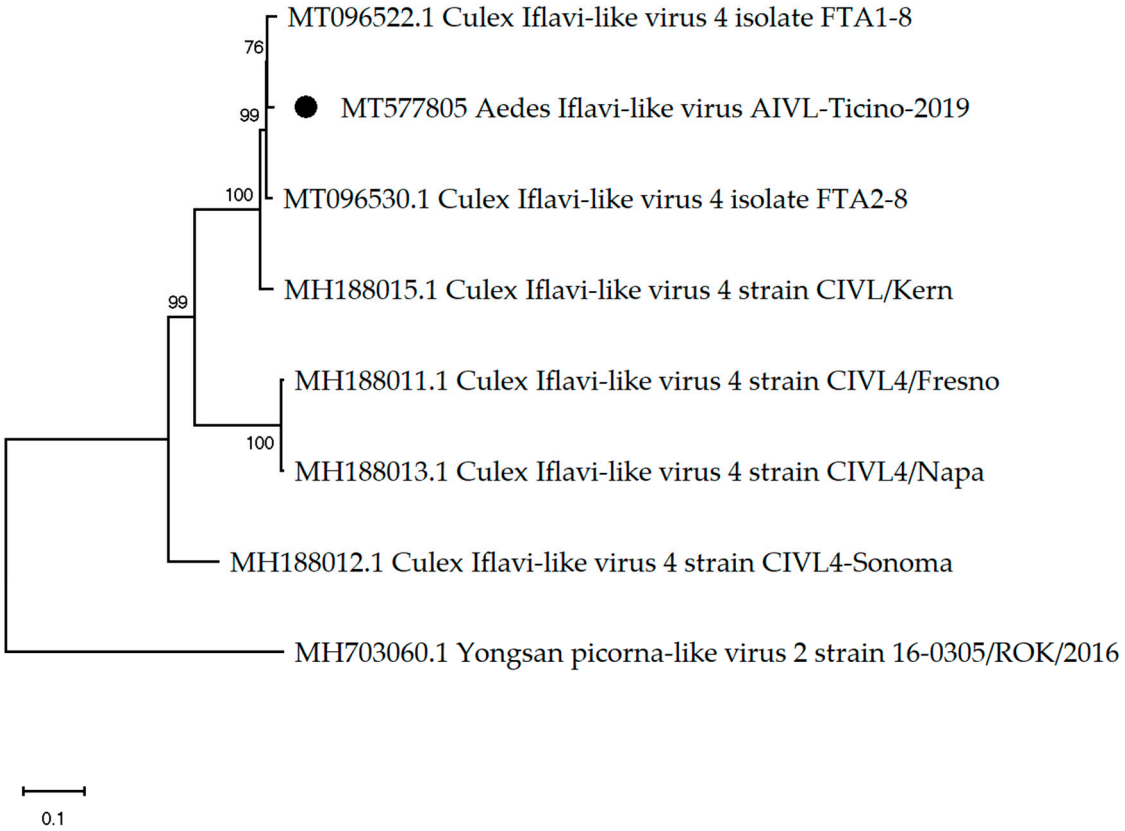

Figure 3. The phylogenetic tree of Aedes iflavi-like virus constructed using the maximum likelihood method and Kimura 2-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The black dot indicates the genome sequenced in this study.

Two contigs belonging to Rebdoviridae, particularly Arboretum virus, were detected in pools MO8 and MO10. Contigs of 1064 nt and 1235 nt in length, generated from 144 and 125 sequenced reads, respectively, showed 79.6% nt identity to novel rhabdoviruses isolated from mosquitos in Peru (GenBank acc: KC994644.1) [34]. One contig of 471 nt generated from 17 sequenced reads belonging to Orthomyoviridae has shown 75.4% nt identity to Whidbey virus strain UW1 pb1 gene (GenBank acc: KX898491.1) identified in *Aedes dorsalis* in Washington. Moreover, in seven pools, sequencing reads of two unclassified viruses were assembled with high nt identity to viruses detected in Brazilian mosquitos. In seven pools, 25,294 reads with 99–100% nt identity to the putative glycoprotein gene of Guato virus (GenBank acc: KT966486.1) have been assembled [35]. In two pools, 23 and 15 reads matched in 99% of the nt to a putative glycoprotein of Kaiowa virus (GenBank acc: KT966481.1 and MF344590.1) [35].

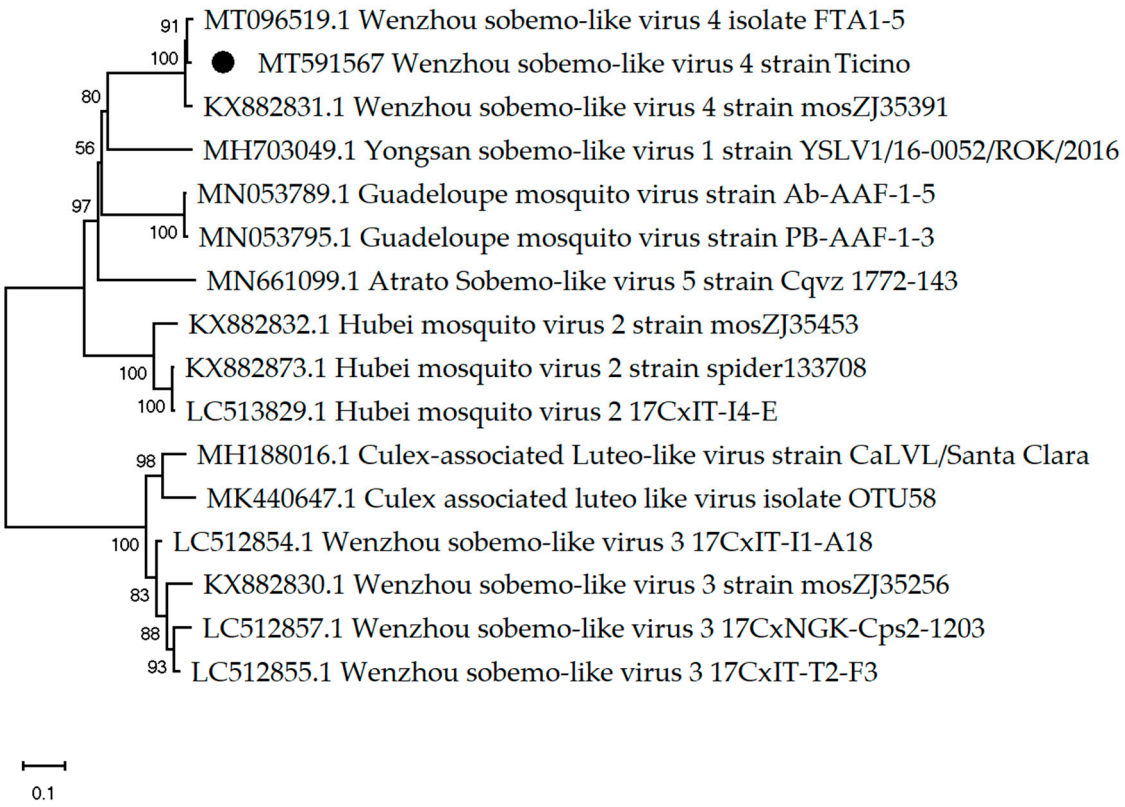

Figure 4. The phylogenetic tree of Wenzhou sobemo-like virus constructed using Table 2. parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The black dot indicates the genome sequenced in this study.

3.2. Other Insect-Associated Viruses

In pool MO4, three contigs with lengths ranging from 307 to 794 nt showed 93.3%–99% nt similarity to members of the family Dicistroviridae, in particular to Aphid lethal paralysis virus isolate ALPV-CE (GenBank acc: MK704471.1), and Aphid lethal paralysis virus isolate ALPV-An (GenBank acc: JX480861.1) identified in *Aphis nerii* and several other insects species [36]. That was the only pool in which sequences assembled to Aphid lethal paralysis virus have been detected.

3.3. Other Viruses

Two contigs of 526 and 839 nt length showing 81% and 90% nt identity, respectively, to Lake Sarah–associated circular virus-48 were generated in pool MO12. This virus belongs to the CRESS DNA viruses identified from Lake Sarah in New Zealand (GenBank acc: KP153505.1) [37]. Viral reads assembled to a plant virus, specifically Fig fleck-associated virus from the family Tymovirdae, were detected in pools MO10 and MO11 (824 and 518 respectively) (GenBank acc: NC_015229). In pool MO13, 0.003% of the total reads have been assembled to plant associated genomovirus 3, a virus from the Genomoviridae family (GenBank acc: MH939439.1).

3.4. Wolbachia Sp. Detection

Finally, since only a fraction of the reads was found to have a viral origin (1.9% to 36.1%), we also investigated the presence of other infectious agents, Wolbachia in particular. Indeed, between 290 and 14,763 reads were assembled to Wolbachia wAlbB in each pool, thereby confirming that *Ae. albopictus* is naturally infected with Wolbachia strains.

4. Discussion

It is well known that arthropods constitute a major reservoir for many different viruses and therefore play an important role in virus spread and evolution [38,39]. High species diversity, worldwide distribution, and dense population support viral transmission and increase the risk of emerging and remerging viral diseases. Some mosquitos, including *Ae. albopictus* and *Ae. Aegypti,* are vectors for several medically important viruses such as ZIKAV, DENV, CHIKV, and WNV [40–45]. RNA viruses are particularly predisposed to causing new emerging diseases due to their inherently high mutation rate, which facilitates adaptation to new hosts [46,47]. Therefore, surveying the virome of invading mosquitos, in particular of species that are known vectors of human viruses, is of utmost importance in order to (i) detect potential spots of disease outbreaks and (ii) understand the role of mosquitos in virus evolution and spread.

In the current study, we used viral metagenomic sequencing to determine the virome of *Ae. albopictus* collected in Southern Switzerland. Importantly, genomes of medically relevant viruses were not detected. The most abundant virus genomes detected were Wenzhou sobemo-like virus and AEFV. Wenzhou sobemo-like virus has previously been found in mosquitos [48], but plants can also serve as natural host of sobemoviruses [49]. AEFV is an ISFV which replicates in mosquito cells but is unable to replicate in mammalian cells [50–52]. While to our knowledge this is the first report of fully sequenced AEFV in Switzerland, the virus has previously been detected in *Ae. albopictus* collected in Northern Italy, with a prevalence of 77.5% in 2008 and 16.8% in 2012 [53,54].

It would be interesting to investigate whether a high prevalence of ISFVs such as AEFV in Southern Switzerland (this study) and Northern Italy [53,54] correlates with a low prevalence of other medically important viruses. Indeed, cell fusing agent virus (CFAV) and *Culex* flavivirus, two other members of the ISFVs, are known to inhibit WNV, ZIKV, and DENV [15,55,56]. On the other hand, medically important arthropod-borne flaviviruses may have evolved from ISFVs [57,58].

Not only viruses have been shown to inhibit the replication of other viruses. *Wolbachia* sp. is an intracellular endosymbiont that reduces the ability of *Ae. aegypti* to transmit ZIKV and DENV by restricting their replication [59]. Indeed, *Wolbachia*-infected *Ae. aegypti* mosquitos have been released into the environment in Australia with the aim to supress the spread of DENV [17]. *Wolbachia* reads have been detected in all our mosquito pools. This was expected, as previous studies revealed a prevalence of *Wolbachia* in *Ae. albopictus* of >95% [60–62].

An interesting observation of this study was that Aedes iflavi-like virus genomes were found exclusively in a pool of male mosquitos. The most closely related viruses have been detected in mosquitos collected in Spain and California [33,63]. Considering that most of the studies have been performed with female mosquitos, and that the iflavi-like virus has been found in a pool of female *Culex* spp. in California, there is no explanation why in the present study iflavi-like virus has been detected only in a pool of male *Ae. albopictus*. However, the number of mosquitos collected at different locations varies greatly, and the presence of the virus may be location-specific. While it is rather unlikely that only a single male mosquito in the pool hosted the virus when considering the large number of assembled reads, this possibility cannot be excluded. The transmission of ISVs is not fully understood yet, and the transmission occurs mainly vertically from the female to progeny or on the breeding sites [33,64]. Further investigations would be necessary to determine whether this virus is sex- or location-specific.

In conclusion, this study presents a snapshot of the virome of established Swiss populations of *Ae. albopictus* and sets the basis for future metagenomic analyses to explore the spatial and temporal dynamics of the virus diversity in these mosquitos. This kind of study may also contribute to a better understanding of virus–virus interactions and thereby support novel strategies to prevent arbovirus diseases. The world of the ISFVs and ISVs remains largely unexplored but, if better known, may yield important insights into viral evolution and the role of these viruses in the emergence and transmission of pathogenic viruses.

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References

1. Medlock, J.M.; Hansford, K.M.; Schaffner, F.; Versteirt, V.; Hendrickx, G.; Zeller, H.; Van Bortel, W. A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. *Vector Borne Zoonotic Dis.* **2012**, *12*, 435–447. [CrossRef] [PubMed]
2. Paupy, C.; Delatte, H.; Bagny, L.; Corbel, V.; Fontenille, D. *Aedes albopictus*, an arbovirus vector: From the darkness to the light. *Microbes Infect.* **2009**, *11*, 1177–1185. [CrossRef] [PubMed]
3. Wymann, M.N.; Flacio, E.; Radczuweit, S.; Patocchi, N.; Luthy, P. Asian tiger mosquito (*Aedes albopictus*)—A threat for Switzerland? *Eurosurveillance* **2008**, *13*, 3–4. [CrossRef] [PubMed]
4. Flacio, E.; Engeler, L.; Tonolla, M.; Lüthy, P.; Patocchi, N. Strategies of a thirteen year surveillance programme on *Aedes albopictus* (*Stegomyia albopicta*) in southern Switzerland. *Parasites Vectors* **2015**, *8*, 208. [CrossRef]
5. Flacio, E.; Engeler, L.; Tonolla, M.; Müller, P. Spread and establishment of *Aedes albopictus* in southern Switzerland between 2003 and 2014: An analysis of oviposition data and weather conditions. *Parasites Vectors* **2016**, *9*, 304. [CrossRef]
6. Suter, T.T.; Flacio, E.; Feijoó Fariña, B.; Engeler, L.; Tonolla, M.; Regis, L.N.; de Melo Santos, M.A.; Müller, P. Surveillance and Control of *Aedes albopictus* in the Swiss-Italian Border Region: Differences in Egg Densities between Intervention and Non-intervention Areas. *PLoS Negl. Trop. Dis.* **2016**, *10*, e0004315. [CrossRef]
7. Ravasi, D.; Guidi, V.; Flacio, E.; Lüthy, P.; Perron, K.; Lüdin, S.; Tonolla, M. Investigation of temperature conditions in Swiss urban and suburban microhabitats for the overwintering suitability of diapausing *Aedes albopictus* eggs. *Parasites Vectors* **2018**, *11*, 212. [CrossRef]
8. Sambri, V.; Cavrini, F.; Rossini, G.; Pierro, A.; Landini, M.P. The 2007 epidemic outbreak of Chikungunya virus infection in the Romagna region of Italy: A new perspective for the possible diffusion of tropical diseases in temperate areas? *New Microbiol.* **2008**, *31*, 303–304.
9. Delisle, E.; Rousseau, C.; Broche, B.; Leparc-Goffart, I.; L'Ambert, G.; Cochet, A.; Prat, C.; Foulongne, V.; Ferre, J.B.; Catelinois, O.; et al. Chikungunya outbreak in Montpellier, France, September to October 2014. *Eurosurveillance* **2015**, *20*, 21108. [CrossRef]
10. La Ruche, G.; Souarès, Y.; Armengaud, A.; Peloux-Petiot, F.; Delaunay, P.; Desprès, P.; Lenglet, A.; Jourdain, F.; Leparc-Goffart, I.; Charlet, F.; et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. *Eurosurveillance* **2010**, *15*, 19676.
11. Hall, R.A.; Bielefeldt-Ohmann, H.; McLean, B.J.; O'Brien, C.A.; Colmant, A.M.; Piyasena, T.B.; Harrison, J.J.; Newton, N.D.; Barnard, R.T.; Prow, N.A.; et al. Commensal Viruses of Mosquitoes: Host Restriction, Transmission, and Interaction with Arboviral Pathogens. *Evol. Bioinform. Online* **2016**, *12* (Suppl. 2), 35–44. [CrossRef] [PubMed]
12. Vasilakis, N.; Tesh, R.B. Insect-specific viruses and their potential impact on arbovirus transmission. *Curr. Opin. Virol.* **2015**, *15*, 69–74. [CrossRef] [PubMed]
13. Hall-Mendelin, S.; McLean, B.J.; Bielefeldt-Ohmann, H.; Hobson-Peters, J.; Hall, R.A.; van den Hurk, A.F. The insect-specific Palm Creek virus modulates West Nile virus infection in and transmission by Australian mosquitoes. *Parasites Vectors* **2016**, *9*, 414. [CrossRef] [PubMed]
14. Goenaga, S.; Kenney, J.L.; Duggal, N.K.; Delorey, M.; Ebel, G.D.; Zhang, B.; Levis, S.C.; Enria, D.A.; Brault, A.C. Potential for Co-Infection of a Mosquito-Specific Flavivirus, Nhumirim Virus, to Block West Nile Virus Transmission in Mosquitoes. *Viruses* **2015**, *7*, 5801–5812. [CrossRef] [PubMed]

15. Bolling, B.G.; Olea-Popelka, F.J.; Eisen, L.; Moore, C.G.; Blair, C.D. Transmission dynamics of an insect-specific flavivirus in a naturally infected Culex pipiens laboratory colony and effects of co-infection on vector competence for West Nile virus. *Virology* **2012**, *427*, 90–97. [CrossRef] [PubMed]
16. Hobson-Peters, J.; Yam, A.W.; Lu, J.W.; Setoh, Y.X.; May, F.J.; Kurucz, N.; Walsh, S.; Prow, N.A.; Davis, S.S.; Weir, R.; et al. A new insect-specific flavivirus from northern Australia suppresses replication of West Nile virus and Murray Valley encephalitis virus in co-infected mosquito cells. *PLoS ONE* **2013**, *8*, e56534. [CrossRef]
17. Hoffmann, A.A.; Montgomery, B.L.; Popovici, J.; Iturbe-Ormaetxe, I.; Johnson, P.H.; Muzzi, F.; Greenfield, M.; Durkan, M.; Leong, Y.S.; Dong, Y.; et al. Successful establishment of Wolbachia in *Aedes* populations to suppress dengue transmission. *Nature* **2011**, *476*, 454–457. [CrossRef]
18. Flacio, E.; Bricalli-Rossi-Pedruzzi, A.; Bernasconi-Casati, E.; Patocchi, N. *Culicidae fauna* from Canton Ticino and report of three new species for Switzerland. *Mitt. Schweiz. Entomol. Gesell.* **2014**, *87*, 163–182.
19. Becker, N.; Petric, D.; Zgomba, M.; Boase, C.; Madon, M.B.; Dahl, C.; Kaiser, A. *Mosquitoes and Their Control*, 2nd ed.; Springer: Berlin/Heidelberg, Germany, 2010; p. 577.
20. Romi, R.; Pontuale, G.; Sabatinelli, G. Le zanzare italiane: Generalità e identificazione degli stadi preimaginali (Diptera: Culicidae). *Fragm. Entomol.* **1997**, *29*, 1–141.
21. Schaffner, F.; Angel, G.; Geoffroy, B.; Hervy, J.-P.; Rhaiem, A.; Brunhes, J. *The Mosquitoes of Europe. An Identification and Training Programme*; IRD Editions: Montpellier, France, 2001.
22. Severini, F.; Toma, L.; Di Luca, M.; Romi, R. Italian Mosquitoes: General Information and Identification of Adults (Diptera, Culicidae). *Fragm. Entomol.* **2009**, *41*, 213–372. [CrossRef]
23. Stojanovich, C.J.; Scott, H.G. *Llustrated Key to the Adult Male Mosquitoes of America (North of Mexico)*; P. Stojanovich and H. G. Scott: Madison, WI, USA, 1997.
24. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef] [PubMed]
25. Li, D.; Liu, C.M.; Luo, R.; Sadakane, K.; Lam, T.W. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* **2015**, *31*, 1674–1676. [CrossRef] [PubMed]
26. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
27. Huson, D.H.; Albrecht, B.; Bağcı, C.; Bessarab, I.; Górska, A.; Jolic, D.; Williams, R.B.H. MEGAN-LR: New algorithms allow accurate binning and easy interactive exploration of metagenomic long reads and contigs. *Biol. Direct* **2018**, *13*, 6. [CrossRef]
28. Li, H.; Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **2009**, *25*, 1754–1760. [CrossRef]
29. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef]
30. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
31. Haddow, A.D.; Guzman, H.; Popov, V.L.; Wood, T.G.; Widen, S.G.; Haddow, A.D.; Tesh, R.B.; Weaver, S.C. First isolation of *Aedes* flavivirus in the Western Hemisphere and evidence of vertical transmission in the mosquito *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae). *Virology* **2013**, *440*, 134–139. [CrossRef]
32. Crochu, S.; Cook, S.; Attoui, H.; Charrel, R.N.; De Chesse, R.; Belhouchet, M.; Lemasson, J.J.; de Micco, P.; de Lamballerie, X. Sequences of flavivirus-related RNA viruses persist in DNA form integrated in the genome of *Aedes* spp. mosquitoes. *J. Gen. Virol.* **2004**, *85 Pt 7*, 1971–1980. [CrossRef]
33. Birnberg, L.; Temmam, S.; Aranda, C.; Correa-Fiz, F.; Talavera, S.; Bigot, T.; Eloit, M.; Busquets, N. Viromics on Honey-Baited FTA Cards as a New Tool for the Detection of Circulating Viruses in Mosquitoes. *Viruses* **2020**, *12*, 274. [CrossRef]
34. Vasilakis, N.; Castro-Llanos, F.; Widen, S.G.; Aguilar, P.V.; Guzman, H.; Guevara, C.; Fernandez, R.; Auguste, A.J.; Wood, T.G.; Popov, V.; et al. Arboretum and Puerto Almendras viruses: Two novel rhabdoviruses isolated from mosquitoes in Peru. *J. Gen. Virol.* **2014**, *95 Pt 4*, 787–792. [CrossRef] [PubMed]
35. Pauvolid-Corrêa, A.; Solberg, O.; Couto-Lima, D.; Nogueira, R.M.; Langevin, S.; Komar, N. Novel Viruses Isolated from Mosquitoes in Pantanal, Brazil. *Genome Announc.* **2016**, *4*, e01195-16.

36. Dombrovsky, A.; Luria, N. The Nerium oleander aphid *Aphis nerii* is tolerant to a local isolate of Aphid lethal paralysis virus (ALPV). *Virus Genes* **2013**, *46*, 354–361. [CrossRef] [PubMed]
37. Dayaram, A.; Galatowitsch, M.L.; Argüello-Astorga, G.R.; van Bysterveldt, K.; Kraberger, S.; Stainton, D.; Harding, J.S.; Roumagnac, P.; Martin, D.P.; Lefeuvre, P.; et al. Diverse circular replication-associated protein encoding viruses circulating in invertebrates within a lake ecosystem. *Infect. Genet. Evol.* **2016**, *39*, 304–316. [CrossRef]
38. Shi, M.; Lin, X.D.; Tian, J.H.; Chen, L.J.; Chen, X.; Li, C.X.; Qin, X.C.; Li, J.; Cao, J.P.; Eden, J.S.; et al. Redefining the invertebrate RNA virosphere. *Nature* **2016**, *540*, 539–543. [CrossRef]
39. Li, C.X.; Shi, M.; Tian, J.H.; Lin, X.D.; Kang, Y.J.; Chen, L.J.; Qin, X.C.; Xu, J.; Holmes, E.C.; Zhang, Y.Z. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. *eLife* **2015**, *4*, e05378. [CrossRef]
40. Olson, J.G.; Ksiazek, T.G.; Suhandiman; Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. *Trans. R. Soc. Trop. Med. Hyg.* **1981**, *75*, 389–393. [CrossRef]
41. Delatte, H.; Paupy, C.; Dehecq, J.S.; Thiria, J.; Failloux, A.B.; Fontenille, D. Aedes albopictus, vector of chikungunya and dengue viruses in Reunion Island: Biology and control. *Parasite* **2008**, *15*, 3–13. [CrossRef]
42. Delatte, H.; Gimonneau, G.; Triboire, A.; Fontenille, D. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. *J. Med. Entomol.* **2009**, *46*, 33–41. [CrossRef]
43. Chang, C.; Ortiz, K.; Ansari, A.; Gershwin, M.E. The Zika outbreak of the 21st century. *J. Autoimmun.* **2016**, *68*, 1–13. [CrossRef]
44. Yang, C.F.; Hou, J.N.; Chen, T.H.; Chen, W.J. Discriminable roles of Aedes aegypti and *Aedes albopictus* in establishment of dengue outbreaks in Taiwan. *Acta Trop.* **2014**, *130*, 17–23. [CrossRef] [PubMed]
45. Rezza, G. Dengue and chikungunya: Long-distance spread and outbreaks in naïve areas. *Pathog. Glob. Health* **2014**, *108*, 349–355. [CrossRef] [PubMed]
46. Taylor, L.H.; Latham, S.M.; Woolhouse, M.E. Risk factors for human disease emergence. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **2001**, *356*, 983–989. [CrossRef] [PubMed]
47. Woolhouse, M.E.; Gowtage-Sequeria, S. Host range and emerging and reemerging pathogens. *Emerg. Infect. Dis.* **2005**, *11*, 1842–1847. [CrossRef] [PubMed]
48. Sanborn, M.A.; Klein, T.A.; Kim, H.C.; Fung, C.K.; Figueroa, K.L.; Yang, Y.; Asafo-Adjei, E.A.; Jarman, R.G.; Hang, J. Metagenomic Analysis Reveals Three Novel and Prevalent Mosquito Viruses from a Single Pool of *Aedes vexans nipponii* Collected in the Republic of Korea. *Viruses* **2019**, *11*, 222. [CrossRef]
49. Sõmera, M.; Sarmiento, C.; Truve, E. Overview on Sobemoviruses and a Proposal for the Creation of the Family Sobemoviridae. *Viruses* **2015**, *7*, 3076–3115. [CrossRef]
50. Hoshino, K.; Isawa, H.; Tsuda, Y.; Sawabe, K.; Kobayashi, M. Isolation and characterization of a new insect flavivirus from *Aedes albopictus* and *Aedes flavopictus* mosquitoes in Japan. *Virology* **2009**, *391*, 119–129. [CrossRef]
51. Cook, S.; Moureau, G.; Kitchen, A.; Gould, E.A.; de Lamballerie, X.; Holmes, E.C.; Harbach, R.E. Molecular evolution of the insect-specific flaviviruses. *J. Gen. Virol.* **2012**, *93 Pt 2*, 223–234. [CrossRef]
52. Calzolari, M.; Zé-Zé, L.; Vázquez, A.; Sánchez Seco, M.P.; Amaro, F.; Dottori, M. Insect-specific flaviviruses, a worldwide widespread group of viruses only detected in insects. *Infect. Genet. Evol.* **2016**, *40*, 381–388. [CrossRef]
53. Roiz, D.; Vázquez, A.; Rosso, F.; Arnoldi, D.; Girardi, M.; Cuevas, L.; Perez-Pastrana, E.; Sánchez-Seco, M.P.; Tenorio, A.; Rizzoli, A. Detection of a new insect flavivirus and isolation of *Aedes flavivirus* in Northern Italy. *Parasites Vectors* **2012**, *5*, 223. [CrossRef]
54. Grisenti, M.; Vázquez, A.; Herrero, L.; Cuevas, L.; Perez-Pastrana, E.; Arnoldi, D.; Rosà, R.; Capelli, G.; Tenorio, A.; Sánchez-Seco, M.P.; et al. Wide detection of *Aedes flavivirus* in north-eastern Italy—A European hotspot of emerging mosquito-borne diseases. *J. Gen. Virol.* **2015**, *96 Pt 2*, 420–430. [CrossRef]
55. Schultz, M.J.; Frydman, H.M.; Connor, J.H. Dual Insect specific virus infection limits Arbovirus replication in *Aedes mosquito* cells. *Virology* **2018**, *518*, 406–413. [CrossRef]
56. Baidaliuk, A.; Miot, E.F.; Lequime, S.; Moltini-Conclois, I.; Delaigue, F.; Dabo, S.; Dickson, L.B.; Aubry, F.; Merkling, S.H.; Cao-Lormeau, V.M.; et al. Cell-Fusing Agent Virus Reduces Arbovirus Dissemination in *Aedes aegypti* Mosquitoes In Vivo. *J. Virol.* **2019**, *93*, e00705-19. [CrossRef]

57. Cammisa-Parks, H.; Cisar, L.A.; Kane, A.; Stollar, V. The complete nucleotide sequence of cell fusing agent (CFA): Homology between the nonstructural proteins encoded by CFA and the nonstructural proteins encoded by arthropod-borne flaviviruses. *Virology* **1992**, *189*, 511–524. [CrossRef]
58. Kuno, G.; Chang, G.J.; Tsuchiya, K.R.; Karabatsos, N.; Cropp, C.B. Phylogeny of the genus Flavivirus. *J. Virol.* **1998**, *72*, 73–83. [CrossRef]
59. Chouin-Carneiro, T.; Ant, T.H.; Herd, C.; Louis, F.; Failloux, A.B.; Sinkins, S.P. Wolbachia strain wAlbA blocks Zika virus transmission in *Aedes aegypti*. *Med. Vet. Entomol.* **2020**, *34*, 116–119. [CrossRef]
60. Ahantarig, A.; Trinachartvanit, W.; Kittayapong, P. Relative Wolbachia density of field-collected *Aedes albopictus* mosquitoes in Thailand. *J. Vector Ecol.* **2008**, *33*, 173–177. [CrossRef]
61. Dutton, T.J.; Sinkins, S.P. Strain-specific quantification of Wolbachia density in *Aedes albopictus* and effects of larval rearing conditions. *Insect Mol. Biol.* **2004**, *13*, 317–322. [CrossRef]
62. Kittayapong, P.; Baisley, K.J.; Sharpe, R.G.; Baimai, V.; O'Neill, S.L. Maternal transmission efficiency of Wolbachia superinfections in *Aedes albopictus* populations in Thailand. *Am. J. Trop. Med. Hyg.* **2002**, *66*, 103–107. [CrossRef]
63. Sadeghi, M.; Altan, E.; Deng, X.; Barker, C.M.; Fang, Y.; Coffey, L.L.; Delwart, E. Virome of >12 thousand Culex mosquitoes from throughout California. *Virology* **2018**, *523*, 74–88. [CrossRef]
64. Saiyasombat, R.; Bolling, B.G.; Brault, A.C.; Bartholomay, L.C.; Blitvich, B.J. Evidence of efficient transovarial transmission of Culex flavivirus by *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.* **2011**, *48*, 1031–1038. [CrossRef] [PubMed]

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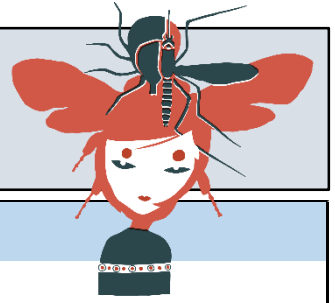
Swiss Vector Entomology Group - meeting 2021

SUPSI, via Flora Ruchat-Roncati 15, 6850 Mendrisio



Thursday 25 November 2021

Vectors

09.30-10.30	Arrival and small breakfast - sala polivalente (A0.08)
10.30-10.40	M. Tonolla - Welcome and introduction of the Institute of Microbiology/DACD/SUPSI
10.40-12.40	**Session 1 - Surveillance and control of Invasive Mosquito in Switzerland** Chaired by E. Flacio & T. Suter
10.40-11.10	Keynote lecture: History of mosquitoes activities in Canton Ticino E. Flacio
11.10-11.30	*Aedes albopictus* management in the canton of Basel-City S. Biebinger
11.30-11.50	Measures taken to eradicate a population of *Aedes albopictus* in a residential area in the City of Zurich G. Müller
11.50-12.10	Risk-based mapping tools for surveillance and control of invasive mosquito *Aedes albopictus* in Switzerland D. Ravasi
12.10-12.30	Swiss Mosquito Network: a national coordination network for the activities on invasive mosquitoes L. Engeler
12.30-12.40	Discussion
12.40-14.00	Lunch - sv ristorante (campus canteen)
14.00-14.50	**Practical demostration of integrated control activities on invasive mosquitoes -** in front of the main entrance
14.50-17.00	**Session 2 - Vector ecology** Chaired by F. Schaffner & M. Bernasconi
14.50-15.10	Temperature preferences of mosquitoes and biting midges D. Hug
15.10-15.30	Thermal preference of Culicoides biting midges under laboratory and semi-field conditions A. Hochstrasser
15.30-15.50	Mathematical modelling of mosquito movement N. Chitnis
15.50-16.10	3D video tracking of mosquitoes exposed to topical repellents in the arm-in-cage test reveal excito-repellency of mosquitoes upon contact rather than spatial repellency M. Fatou
16.10-16.30	Anopheles gambiae mosquitos' microbiota and resistance to insecticide S. Le Bissonnais
16.30-16.50	Multi-insecticide resistant malaria vectors in the field remain susceptible to malathion, despite the presence of Ace1 point mutations P. Müller
16.50-17.00	Discussion
17.00-17.20	Coffee break - sala polivalente (A0.08)
17.20-17.40	Poster session - sala polivalente (A0.08)
from 19.00	Social dinner - Antico Grotto Ticino (viale alle Cantine 20, 6850-Mendrisio) - by SUPSI bus (10 min) or on foot through the town of Mendrisio (30 min) for both meet in front of the main entrance at 18:45

Friday 26 November 2021

Vector-borne diseases

08.30-09.30	Arrival and small breakfast - sala polivalente (A0.08)
09.30-11.20	**Session 3 - Management of vector-borne diseases** Chaired by E. Veronesi & F. Fouque
09.30-10.10	Keynote lecture: WNV and other arboviroses in the Italian National Plan: when One Health falls into practice G. Capelli
10.10-10.30	Challenges for preparing against arboviral diseases epidemics and the role of vector control F. Fouque
10.30-10.50	Citizen action for sustainable dengue control in sub-Saharan Africa P. Müller
10.50-11.10	Swiss app "Tick Prevention / Zecke": seven years of experience with crowdbased research and prevention. W. Tischhauser
11.10-11.30	Skin microbial probiotics to reduce pathogen transmission to humans and animals D. Lucas-Barbosa
11.30-11.40	Discussion
11.40-12.00	Coffee break - sala polivalente (A0.08)
12.00-12.30	**Visit of the Istitute of Microbiology and DACD/SUPSI -** departure from A0.08
12.30-14.00	Lunch - sv ristorante (campus canteen)
14.00-16.00	**Session 4 - Mosquito-associated viruses and their vectors** Chaired by V. Guidi & J. Kubacki
14.00-14.20	Handling infected arthropods under biosafety containment level 3 (BSL-3) E. Veronesi
14.20-14.40	Japanese encephalitis virus: vector competence of Swiss mosquito species, and viral in vitro evolution J. Ettlin
14.40-15.00	Evaluation of the vector competence for dengue and chikungunya viruses of *Aedes albopictus* from southern Switzerland V. Guidi
15.00-15.20	Viral metagenomic analysis of endemic and invasive arthropod vectors J. Kubacki
15.20-15.40	Surveillance of flaviviruses in southern Switzerland, 2018-2021 S. Cazzin
15.40-15.50	Discussion
15.50-16.00	Meeting closure

All meals (breakfast, coffee breaks, lunches and dinner) will be provided by the organisation thanks to the **support of SSPH+**

Attention: to enter the SUPSI building you must have a valid COVID certificate.

SVEG meeting 2021: List of abstracts

Session 1.

Surveillance and control of Invasive Mosquito in Switzerland

Keynote lecture: **History of mosquitoes activities in Canton Ticino**

Eleonora Flacio

Institute of Microbiology, University of applied sciences and arts of Southern Switzerland, Via Flora Ruchat-Roncati 15, 6850 Mendrisio

Until 2000 in Canton Ticino, a Swiss region located South of the Alps, the knowledge on mosquito distribution was scarce. Some faunistic investigations related to malaria occurrence in the area were lead until the beginning of last century and others started at the end of that century to support control measures against wetland mosquitoes causing nuisance in residence areas close to lake Maggiore. A general evaluation on mosquito species was needed to assess the biodiversity in all main natural areas and on the origin of nuisance to improve the control measures on mosquitoes and the risk of diseases transmission. Not only European native species needed investigations but also the exotic species *Aedes albopictus* (the tiger mosquito), which was rapidly spreading in the nearby Italian urban settlements since the 90's, which was of concern due to its potential ability to carry numerous arboviruses and its extreme nuisance.

Over the years, several faunistic censuses of wetland mosquitoes have been carried out, which have demonstrated a good biodiversity in these environments and have allowed the identification of new species for the Canton and some for Switzerland. Furthermore, it has been shown that control actions carried out in natural areas are effective both in limiting nuisance in neighboring urban areas and in maintaining an extremely low risk of disease transmission.

At the same time, a system of surveillance and control of the tiger mosquito has been built up over the years since 2000, which has made it possible to manage the presence of this exotic species on the territory, containing its density and the risk of disease transmission. This system also acts on the other two exotic species *Ae. japonicus* and *Ae. koreicus*. The surveillance system adopted in Canton Ticino is also the basis for other systems adopted in Switzerland against this exotic species. Furthermore, around the basic surveillance system many researches have been developed in order to improve the whole system.

Today it can be said that the view on mosquitoes in Canton Ticino has improved and that the management of these insects has become routine throughout the territory.

Aedes albopictus management in the canton of Basel-City

Susanne Biebinger

Chemie- und Biosicherheitsinspektorin, Koordination Tigermücke Gesundheitsdepartement Basel-Stadt Kantonales Laboratorium Kontrollstelle für Chemie- und Biosicherheit (KCB) Kannenfeldstrasse 2, 4056 Basel

Since the first detection of *Ae. albopictus* in the canton Basel-Stadt in 2015 the number and size of areas with populations are continuously increasing. In a pilot project lasting from 2020 – 2024 an organization and a project group has been set up including different stakeholders involved in the monitoring and control of *Ae. albopictus*. Monitoring based on trapping, larval sampling and reports by citizens is carried out by the SwissTPH. Control actions are taken within the perimeter of repeated detections of *Ae. albopictus*. These include the regular treatment of catch basins with larvicide and public participation achieved by the distribution of flyers as well as house visits with personal instructions.
2021 an awareness campaign has been run adressing the general public as well as specific interest groups using many different channels of information. A major challenge are garden associations in which *Ae. albopictus* is rapidly spreading. The geographical position of the canton Basel-Stadt requires a regular exchange of information and coordination between neighbouring countries and cantons.

Measures taken to eradicate a population of _Aedes albopictus_ in a residential area in the City of Zurich

Gabi Müller

City of Zurich, Department of Public Health and Environment, Urban Pest Advisory Service, Eggbühlstrasse 23, 8050 Zurich

The Urban Pest Advisory Service (UPAS) is responsible for urban pests in the city of Zurich, Switzerland. We provide approximately 2,000 consultations per year concerning pests and their control, survey the pest situation in the city and control rodents in public areas.

Since the first and expected finding of the Asian tiger mosquito, *Aedes albopictus* on the long-distance bus station in the center of Zurich in 2016, the monitoring and control of this invasive mosquito species has become an additional task for the UPAS. To date there is no established tiger mosquito population within the perimeter of this bus station despite several repeated introductions by the more than 200 long-distance coaches arriving per day.

In September 2018, six adult tiger mosquitoes from Wollishofen, a suburban residential area approximately 5 km away from the bus station, were sent to the UPAS for identification. For 2019, the Canton of Zurich had laid-out a monitoring scheme for *Ae. albopictus* in Wollishofen including an area of about 11 ha. This plan covered the period from May to October. The City of Zurich, UPAS, was responsible for the information of the residents, the reduction of breeding sites by door-to-door visits and larval control in the gullies with Bti. At the end of the season, the area where adults and eggs were still found was reduced to hot spots in two gardens. During 2020, monitoring, door-to-door visits and prophylactic control of mosquito larvae in the gullies continued. This approach seemed to be very successful. No tiger mosquitoes or eggs were found during the monitoring seasons 2020 and 2021. There is a high likelihood that an established population of *Ae. albopictus* was successfully eradicated.

Risk-based mapping tools for surveillance and control of invasive mosquito *Aedes albopictus* in Switzerland

Damiana Ravasi[1], Francesca. Mangili[2], David Huber[2], Lukas Engeler[1], Mauro Tonolla[1], Eleonora Flacio[1]

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[2] Dalle Molle Institute for Artificial Intelligence Studies, University of Applied Sciences and Arts of Southern Switzerland, via la Santa 1, 6962 Lugano-Viganello

The presence of the Asian tiger mosquito*, Ae. albopictus*, in Switzerland was recorded for the first time in 2003 in Canton Ticino, south of the Alps. Today it is considered well established in most urban areas of the canton and also in part of Canton Graubünden. Here, surveillance and control to contain its spread are regularly implemented. Climate change, along with passive transportation, is driving the spread of *Ae. albopictus* in the rest of Switzerland. A national-scale surveillance programme has been set up in 2013 to monitor the expansion of *Ae. albopictus* and other invasive mosquitoes along the main communication routes (highways, airports and river ports). Since then, *Ae. albopictus* has been observed in Swiss cities north of the Alps (e.g., Basel, Zurich and Geneva) with possible establishment of small populations. Therefore, decision-making tools are urgently needed by the local authorities in order to prioritize and optimize vector surveillance and control actions. We developed an empirical machine-learning model for spatio-temporal distribution of *Ae. albopictus* based on historical mosquito monitoring data from Canton Ticino and socio-environmental factors considered important for the establishment of the vector. This allows producing maps of risk scenarios for diffusion of *Ae. albopictus* in Swiss cities, which should provide local authorities with critical information to promptly react through intensification of surveillance and treatments in the areas where there is higher risk of introduction and establishment of mosquito populations.

Swiss Mosquito Network: a national coordination network for the activities on invasive mosquitoes

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[5] City of Zurich, Department of Public Health and Environment, Urban Pest Advisory Service (UPAS), Eggbühlstrasse 23, 8050 Zürich

The Asian tiger mosquito (*Aedes albopictus*) has been detected for the first time in Switzerland in Ticino in 2003 and north of the Alps in 2013. Two additional invasive species *Ae. japonicus* and *Ae. koreikus* have been found for the first time in Cantons of Aargau and Ticino in 2008 and 2013 respectively. To coordinate their surveillance in 2017 the Federal Office for the Environment commissioned SUPSI, in collaboration with SwissTPH, UNIL and UPAS, to set up the Swiss Mosquito Network (SMN), which became operational in 2020. The aim of the SMN is to coordinate the activities with respect to the surveillance of invasive mosquitoes and to centralise the collected data at national level. Its structure consists of a coordination centre and four reporting offices, where citizens can report suspect mosquitoes on a dedicated web page. In case of reports of *Ae. albopictus* in a new area, the SMN carries out on-site inspections to assess the situation. In addition,

the SMN operates a national surveillance programme at potential points of entry, primarily along motorways, and supports the cantons in setting up their own surveillance programmes. In 2021, 19 of the 26 Swiss Cantons initiated a monitoring programme. Here, we will present the structure of the SMN, its activities over the past two years, the results of the various monitoring programmes and give an overview of the current distribution of invasive Aedes mosquitoes Switzerland.

Session 2.

Vector ecology

Temperature preferences of mosquitoes and biting midges

David Hug[1], Raphaela Ziegler[1], Alec Hochstrasser[1], Alexander Mathis[1], Wolf U. Blanckenhorn[2], Niels Verhulst[1]

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[2]Department of Evolutionary Biology and Environmental Studies, University of Zürich, Switzerland

The mathematical equation of the basic reproduction number R0 for pathogens that are biologically transmitted by insect vectors contains eight factors. All but two of these factors are related to the insects and/or the pathogens (e.g., vector daily survival rate, extrinsic incubation period of the pathogen). These characteristics are directly or indirectly dependent on climate (mainly temperature). 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 (mean ambient air temperatures) but did not consider if vectors have thermal preferences (behavioural thermoregulation) 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 (a large outdoor cage) and field conditions.

Experiments with the thermal gradient setup with laboratory-reared *Aedes aegypti* and *Ae. japonicus* showed that these insects preferred cooler temperatures than their physiological optimum proposed in the literature1,2. This was true for the two feeding states examined (blood-fed and sugar-fed). These results were supported by semi-field outdoor cage experiments, in which a controlled population size was used. Adult mosquitoes were found eight (blood-fed) or four (sugar-fed) times more often in the cooler resting boxes than warm ones, and the mosquitoes also preferred to lay their eggs in cooler water bodies. Two field locations were selected at low altitude (Zürich, Thurauen) and high altitude (Braunwald, Hasliberg). At each location, five testing sites were chosen, ten meters apart from each other and in a row, to incorporate microhabitat differences. Every testing site consisted of mosquito traps for collecting both host seeking and resting mosquitoes, and also oviposition opportunities were provided. In addition, UV light traps to collect biting midges were included. Temperature and humidity as well as air pressure were measured using data loggers. The field data that need to be analysed in detail revealed clear differences in mosquito abundances and species composition across altitude and individual trapping sites.

Future experiments with additional species and infection states will further indicate how mosquitoes behaviourally thermoregulate. By including preferred temperatures and temperatures on microclimatic scales, models for vector abundance and disease outbreaks can be improved.

Thermal preference of Culicoides biting midges under laboratory and semi-field conditions

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Hematophagous biting midges of the genus Culicoides (Diptera: Ceratopogonidae) are vectors of numerous animal and human pathogens. In Europe, they recently attracted attention as vectors of the bluetongue virus, causing disease in sheep and cattle, which was controlled by compulsory vaccination. Temperature is a strong driver of vector development and pathogen transmission, e.g., by determining the extrinsic incubation period.

We investigated the thermoregulatory behaviour of field-collected Culicoides biting midges via laboratory and semi-field experiments. Female insects were released in the laboratory in a Plexiglas enclosure1, where they were exposed to a temperature gradient (15-25 °C) under controlled humidity. Their movement was recorded for 15 minutes and analysed with the EthoVision software2. In the semi-field experiment, biting midges were released in a large cage (90 x 45 x 45 cm) situated under a canopy in proximity of vegetation in summer conditions. A LED mini trap was placed at each end of the cage, where cooling elements or heating mats generated microclimates with temperature differences of 2 °C on average. During each trial, the traps were concurrently operated 6 times for 30 seconds every 30 minutes to capture locally aggregated insects. Results indicate that Culicoides avoid high temperatures and prefer cooler sites ($F_{1,2506} = 137.7$, $p < 0.001$, in laboratory conditions; $F_{1,26} = 30.0$, $p < 0.001$, in semi-field conditions). This preference did not appear to differ significantly between individuals which were fed on sugar only and those that additionally received a blood meal ($F_{1,2500} = 0.12$, n.s.). These results are relevant for fine-tuning the modelling of the role of biting midges as vectors, currently as well as under climate change scenarios.

Mathematical modelling of mosquito movement

Nakul Chitnis

Department of Epidemiology and Public Health Swiss Tropical and Public Health Institute Socinstrasse 57, Postfach, 4002 Basel

Mosquito strategies for foraging for blood meals are poorly understood when compared to larger animals that can be tracked individually with GPS sensors. We review the ecological literature on foraging behaviour and the mathematical analysis of animal movement. We present some simple models for analysing mark-release recapture data on mosquitoes from Tanzania and Switzerland.

3D video tracking of mosquitoes exposed to topical repellents in the arm-in-cage test reveal excito-repellency of mosquitoes upon contact rather than spatial repellency

Mathurin Fatou, Steven N Fry and Pie Müller

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The efficacy of topical repellents is usually assessed in the arm-in-cage test in which study participants expose their repellent-treated forearm at regular intervals into a cage containing host-seeking female mosquitoes until the repellent's protection effect fails. However, this test does not measure how mosquitoes actually interact with a repellent-treated arm other than counting landings and bites. In addition, the small size of the cage casts doubts on how representative the assay is for the real use scenario given mosquitoes rely on sequentially integrating context-dependent stimuli up to several meters away from the human host. To understand how mosquitoes actually interact with the topical repellent in the arm-in-cage test, we measured the behaviour of host seeking mosquitoes using a real-time 3D infrared video camera system. We tracked the flight paths of *Aedes aegypti* and *Anopheles stephensi* as they interact with a repellent-treated or an untreated forearm in the arm-in-cage test. The tested repellents included 20% ethanolic solutions of N,N-diethyl-meta-toluamide, p-menthane-3,8-diol, icaridin and ethyl butylacetylaminopropionate. Interestingly, we found that mosquitoes do not seem to be repelled at a distance by volatile chemicals but rather they are excito-repelled upon contact with the treated skin, suggesting that these repellents are either no true repellents or the arm-in-cage assay might not be a suitable proxy for measuring the efficacy of topical repellents.

Anopheles gambiae mosquitos' microbiota and resistance to insecticide

Sandra Le Bissonnais, Jacob Koella

Laboratory of Ecology and Epidemiology of Parasites, Institute of Biology, University of Neuchâtel

Recent studies show that the mosquito interactions with his microbiota could potentially be a target to fight vector-borne diseases. Despite an enthusiasm for the subject, the acquisition process and the precise role of the mosquito microbiota in the immune system or in the resistance to insecticide remains relatively unknown. Here we want to investigate, as a first step, if removing a part of the bacterial communities using antibiotics could impact the mosquito's resistance to insecticide, with or without the infection with Plasmodium. In a second time, the composition of the microbiota populations, both sensitive and resistant, will be analyses using NGS (MiSeq metabarcoding 16S pipeline). Those results could give new insights on what to look for in the mosquito-microbiota interaction and lead to new tools for vector control.

Multi-insecticide resistant malaria vectors in the field remain susceptible to malathion, despite the presence of Ace1 point mutations

Nadja C Wipf, Wandrille Duchemin, France-Paraudie A Kouadio, Behi K Fodjo, Christabelle G Sadia, Mouhamadou S Chouaïbou, Pascal Mäser, Konstantinos Mavridis, John Vontas and Pie Müller

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Insecticide-based mosquito control has saved millions of lives from malaria and other vector-borne diseases. However, the emergence and increase of insecticide resistant Anopheles populations seriously threatens to derail malaria control programmes. Surveillance of insecticide resistance and understanding the underlying molecular mechanisms are key for choosing effective vector control strategies. The aim of our study was to characterise the degree and mechanisms of resistance in three *Anopheles coluzzii* field populations from southern Côte d'Ivoire, specifically Agboville, Dabou and Tiassalé. All three populations were resistant to bendiocarb, deltamethrin and DDT, but not or only very weakly resistant to malathion. The absence of malathion resistance is an unexpected result because we found the acetylcholinesterase mutation Ace1-G280S at high frequencies, which would typically confer cross-resistance to carbamates and organophosphates, including malathion. Notably, Tiassalé was the most susceptible population to malathion while being the most resistant one to the pyrethroid deltamethrin. The resistance ratio to deltamethrin between Tiassalé and the laboratory reference colony was 1,800 fold. By sequencing the transcriptome of individual mosquitoes, we found numerous cytochrome P450-dependent monooxygenases – including CYP6M2, CYP6P2, CYP6P3, CYP6P4 and CYP6P5 – overexpressed in all three field populations. This could be an indication for negative cross-resistance caused by overexpression of pyrethroid-detoxifying P450s that may activate pro-insecticides, thereby increasing malathion susceptibility. In addition to the P450s, we found several overexpressed carboxylesterases, glutathione S-transferases and other candidates putatively involved in insecticide resistance. At the SVEG Meeting, we will present phenotypic and genotypic data and discuss how negative cross-resistance may be exploited in the fight against multi-resistant malaria vectors

Session 3.

Management of vector-borne diseases

Keynote lecture: WNV and other arboviroses in the Italian National Plan: when One Health falls into practice

Gioia Capelli

Istituto Zooprofilattico Sperimentale delle Venezie, viale dell'Università, 10 - 35020 Legnaro – Padova, Italy

Among vector borne infections, West Nile virus (WNV) showed an increasingly wider spread in temperate regions of Europe, including Italy. During the last decade, WNV outbreaks have been recurrently reported in mosquitoes, horses, wild birds, and humans, showing great variability in the temporal and spatial distribution pattern. Other arboviroses are currently authoctonous in Italy, such as Usutu virus, Toscana virus (transmitted by sandflies) and tick-borne encephalitis. Alongside the circulation of authocthonous viruses, Italy, as many other european countries, is at risk of importation of other viruses such as dengue, chikungunya and zika. These introductions may led to local transmission due to the presence of competent mosquitoes, as witnessed by the chikungunya and dengue foci notified in the past in Italy.

For many years the responce to these infections has been organized separatly in specific Plans of surveillance and control targeted to the medical, veterinary and entomological parts. The long experience of WNV monitoring and the evaluation of the results has shown that control activities are much more effective and cheaper if organized in an integrated way. We have been able to demonstrate, for example, that entomological and veterinary surveillance can act as a trigger for human monitoring activities. Therefore in 2020 the National Plan for the prevention, surveillance and response to arbovirosis (2020-2025) was enacted. This Plan is an integrated Plan at different levels: i) first because it reunites all the viruses of interest transmitted by different vectors; ii) second because it contains all the actions to be implemented in the medical, veterinary and entomological fields, iii) and third because it provides for the integration of the different actions in relation to the results of the individual surveillance, which can act as a trigger for subsequent actions, in a system based on mutual trust. In this report we present the path that led to the birth of the Integrated Plan and the first evaluation of the successes and limits of this approach using as example the WNV virus hystory in Italy.

Challenges for preparing against arboviral diseases epidemics and the role of vector control

Florence Fouque

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The vector-borne diseases are accounting for about 17% of the communicable diseases worldwide and are claiming more than 700,000 lives every year. The distribution of some of them is now global, putting three quarters of the world population at risks of emergence of epidemics. Although malaria remains the most deadly VBD, the arboviral diseases are more disruptive for societies because epidemics can develop quickly in non-immune and unprepared populations, due to the main vectors domestic behavior and adaptability. The prevention against the VBDs is a great concern for a majority of the countries worldwide and action plans and frameworks are recommended by the WHO. To respond to these concerns a Global Vector Control

Response (GVCR) was developed in 2017 and approved by the countries with plan of action for 2017 to 2030. And a new Global Arbovirus Initiative (GAI) is currently under development. In the first part of this presentation, the main elements of the GVCR and GAI will be presented. In the second part of the presentation, a specific country example will be discussed with the Preparedness Plan for Surveillance and Interventions on Emerging Vector-Borne Diseases (VBDs) in Southern Switzerland. The objective of the plan is to provide Public Health Authorities with a framework of preventive and control measures according to the situation and level of epidemic risks. The plan includes strategies for preventing and managing potential outbreaks, as well as the surveillance and control activities with a specific focus on Aedes-borne diseases transmitted by *Aedes albopictus* mosquitoes. The plan is divided into various phases representing the different steps for all potential situations, ranging from no vectors and no transmission risk to epidemic levels with multiple autochthonous/local cases of hospitalization (and deaths) until the end of the epidemic. An algorithm presents how decisions are taken to move from one phase of the plan to another, with detailed activities for different partners and strategies at each specific phase. The activities are planned for disease surveillance and clinical case management, vector surveillance and control, communication, and coordination. This preparedness plan will be evaluated for a better understanding of the strengths and weaknesses of the system and where adjustments are needed, into a dynamic process, with the final objective to allow a flexible and adapted response to outbreaks events.

Citizen action for sustainable dengue control in sub-Saharan Africa

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Transmitted by day biting, highly anthropophilic invasive Aedes mosquitoes, dengue is a life-threatening disease that continues to be neglected, although it has increased in incidence over six folds since 2000. Particularly in sub-Saharan Africa, Aedes control programmes are either inexistent or mainly follow a top down approach with sporadic insecticide spray campaigns targeting adult mosquitoes. However, these interventions have only a very limited, short-term impact and are environmentally not sustainable since these Aedes mosquitoes breed in small water containers that are ubiquitous in urban areas. Therefore, targeting the juvenile stages is more promising but requires the active support of the affected communities. The current project aims at mobilising and engaging local residents in the sustainable control of Aedes mosquitoes by removal of potential larval breeding sites and mass trapping of egg-laying females. We are collecting baseline data to assess the association between housing, human behaviour and mosquito habitat suitability through surveys and mosquito collections in Abidjan, the largest city of Côte d'Ivoire, which has recently experienced multiple dengue outbreaks. In consultation with local residents, we are designing a community based intervention to reduce Aedes breeding sites. To test whether the intervention in this African context reduces mosquito densities, we are implementing a cluster randomised trial over the course of a whole year. In the trial, we are also testing whether mass trapping with simple traps targeting egg-laying females adds to the effectiveness of the larval source management. The key outcome of this project will be clear policy recommendations for the control of Aedes-borne arboviruses with community mobilisation in urban sub-Saharan Africa.

Swiss app "Tick Prevention / Zecke": seven years of experience with crowdbased research and prevention.

Werner Tischhauser [1,2,3]

[1] Stadt Zürich, Umwelt- und Gesundheitsschutz, Schädlingsprävention und -beratung

[2] A&K Strategy GmbH

[3] ZHAW, Dep.N, Life Sciences and Facility Management: "Fighting bites with bytes – promoting public health with crowdbased tick prevention"

As a byproduct of a research project, the app "Tick Prevention" was developed at the Zurich University of Applied Sciences (ZHAW). The prevention tool explains the correct behavior in the case of a tick bite. The warning function shows the current tick risk in the field and raises awareness of the seasonally varying tick risk potential. The information section explains the common protective measures to avoid contact with ticks. After a tick bite, the user enters the tick bite in the app diary, whereupon the app periodically reminds the user to check for symptoms. This helps to detect and treat Lyme disease in an early stage.

Since March 2015, around 45,000 "crowdbased" tick bite data have been submitted, which were used to validate the spatial tick model (map.geo.admin.ch - tick bite model). With 1 million app calls per year, the awareness-raising benefit on the topic of "Tick-borne diseases" has been proofed.

Skin microbial probiotics to reduce pathogen transmission to humans and animals

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Biting insects, such as mosquitoes and biting midges, exploit skin odours to locate their hosts, and they are vectors of numerous pathogens Most of the odours emitted by the skin of humans and mammals are produced by skin bacteria. It is, therefore, the individual characteristic skin microbiota that make us and animals more of less attractive to mosquitoes1. A novel solution for protection against insect vectors could be achieved by manipulating the skin bacterial commensals2. Manipulation of the human skin microbiome is already being used to treat several skin conditions.

In our project, we collected volatiles from the arms of 119 volunteers, and their attraction to four mosquito species (*Aedes aegypti*, *Ae. albopictus*, *Anopheles gambiae*, *Culex quinquefasciatus*) is being tested using a uniport olfactometer. We aim at correlating the mosquito attractiveness with the human odour signature to identify the key volatile compounds mediating insect attraction. In addition, we created in-vitro human skin microbiota community models with 4 bacterial species at different ratios, reflecting the skin microbiota composition of a highly attractive and that of a poorly attractive individual. Also, models in which skin microbiota varies in terms of species biodiversity are investigated. Bacteria are grown in a so-called 'sweat medium'. Mosquitoes of *Aedes aegypti* were differently attracted to the in-vitro models. For instance, increasing the number of bacterial species resulted in unexpected synergistic effects, with some complex models being up to 3x less attractive to female mosquitoes than the simpler models. We are testing mosquito attraction to the odours emitted by these bacteria communities in landing assays and will collect volatiles emitted by these microbial communities using a dynamic headspace collection set up. Skin bacteria are also being bioengineered to produce less of odours attractive to mosquitoes, and changes in attractiveness will

be tested. With respect to animals, we are currently identifying skin bacteria characteristic of different sheep breeds and testing their attractiveness to biting midges (see poster by Jost et al.).

The current option for protection of humans against biting insects is the topical application of chemical repellents that evaporate within hours, whereas a microbial-based repellent could offer longer protection to humans as well as animals.

Session 4.

Mosquito-associated viruses and their vectors

Handling infected arthropods under biosafety containment level 3 (BSL-3)

Eva Veronesi

Eva Veronesi Consultancy, Zurich

The design and set-up of laboratories intended for the use of pathogens affecting animal and human health and handling of infected live arthropods (e.g. mosquitoes), requires various biocontainment precautions to protect the workers from infection, the environment from pathogen escape, and the quality of our data from cross-contamination as well as data standardization and reproducibility.

Courses on biocontainment design, waste management, risk assessment, accident response, and handling of infected animals, including flying insects, are also very demanded due to the lack of qualified trained people able to run these types of courses. Moreover, there aren't many laboratories designed and equipped for these kinds of procedures and experiments.

Large classrooms of "in-person trainees" attending a course on biocontainment for risk group 3 (RG3) pathogens and infected arthropods handling is not feasible due to the minimum number of people able to access the biocontainment lab and safe measurements.

The course here proposed is based on a new innovative approach to virtual reality training, where the trainee can follow the course online with interactive tools that guide step-by-step to its completion. A final test at the trainee's site will follow to verify the level of knowledge acquired and the adequacy of their infrastructure, providing tailored technical assistance on biocontainment and laboratory set-up.

Japanese encephalitis virus: vector competence of Swiss mosquito species, and viral in vitro evolution

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Japanese encephalitis virus (JEV) is a pathogen relying on mosquitoes, mainly of the genus Culex, for the transmission between the amplifying hosts including wild birds and pigs. Horses and humans are regarded as dead-end hosts occasionally developing severe neurological symptoms after infection. The virus is currently present in tropical and temperate regions of Asia and Oceania but has repeatedly been detected outside these regions. Therefore, the introduction of JEV to Europe and its spread by local mosquitoes may only be a question of time. For Culex pipiens, the most abundant mosquito species in Europe, JEV vector competence has been shown in the laboratory. Furthermore, direct transmission of JEV in pigs via oronasal shedding has recently been demonstrated under experimental conditions1, representing a potential thread in areas with

intensive pig farming. As a basis for risk assessment, we are investigating the vector competence for JEV of abundant mosquito species in Switzerland (*Aedes albopictus* from Ticino, *Ae. japonicus* and *Ae. vexans* from the Zürich area). For this purpose, the mosquitoes are fed on blood spiked with JEV (two strains, 'Laos', 'Cambodia') and incubated under fluctuating temperature regimes realistic for Switzerland (summer day: 16°C-28°C; hot spell: 20°C-32°C). Subsequently mosquito body parts and saliva are analysed by RT-qPCR and cell isolation to determine infection, dissemination and transmission rates of the virus2. First data showed that at temperatures between 16°C to 28°C JEV 'Laos' can be transmitted by *Cx. quinquefasciatus*, whereas in *Ae. albopictus* only dissemination of the virus has been shown. Moreover, we explore whether series of single-host cycles result in virus adaptions leading to altered virus fitness. To this end, we have generated a collection of alternatingly and directly passaged (10 passages) viruses in vitro in porcine cells (monocyte-derived macrophages (pMDM), endothelial cells (PEDSV.15) and mosquito cells (C6/36). These viruses will be genetically analysed by next-generation RNA sequencing (NGS), and their transmissibility will be assessed by *Cx. quinquefasciatus*.

Evaluation of the vector competence for dengue and chikungunya viruses of *Aedes albopictus* from southern Switzerland

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Arboviruses have become increasingly of concern in several European countries. In Switzerland, no transmissions of mosquito-borne human diseases have been reported. However, the presence of *Ae. albopictus*, especially in densely populated urban areas of southern Switzerland, together with a high number of travellers returning with arboviral infections rise the risk for local transmissions. To better understand the risk of arbovirus transmission in Canton Ticino, Switzerland, we investigated the vector competence of local *Ae. albopictus* for dengue (DENV) and chikungunya (CHIKV) viruses. Females of *Ae. albopictus* were reared in the laboratory from field-collected eggs (Muzzano) and F1-F3 generations were orally exposed to DENV-2 (dengue serotype 2, Bangkok) or CHIKV (strain 06.21, E1-226V mutated). Half of the fully engorged females (n= 2854) were exposed to an additional non-infectious blood meal 4 days post-exposure (second group). The mosquitoes were kept under either a fluctuating temperature regime (16-28°C) or at constant temperature (27°C; CHIKV only) for 4, 5, 7 or 10 days post oral feeding.

The results indicate that *Ae. albopictus* from Canton Ticino can efficiently transmit CHIKV. The infection rates (IR) and transmission efficiencies (TE) ranged between 45-93% (IR) and 3–40% (TE) (constant temperature), and 60-97% (IR) and 3-32% (TE) at the fluctuating temperature regime at all the tested time points. Infection, dissemination and transmission rates were significantly higher for mosquitoes exposed to the infectious blood meal only compared to the second group. No significant differences were observed for the saliva viral load according to the experimental group, incubation time or temperature.

The IRs of mosquitoes exposed to DENV-2 ranged between 4-14%. Dissemination or transmission of the virus was not confirmed in any of the mosquitoes exposed to DENV-2, indicating that the *Ae albopictus* strain from the Muzzano area is not suitable for DENV-2 transmission.

Viral metagenomic analysis of endemic and invasive arthropod vectors

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Climate-driven changes and subsequent establishment of new vectors and reservoir species raise the risk of spillovers of viral diseases. Since several years, the Asian tiger mosquito, *Aedes albopictus*, which is capable of transmitting several different dangerous viruses to humans, including chikungunya virus, dengue virus, and zika virus, is established in Switzerland, although autochthonous incidences of virus transmissions have not yet been reported. Ticks also play a major role in the transmission of vector-borne diseases such as tick-borne encephalitis virus and Lyme borreliosis both in Switzerland and abroad.

To investigate viral abundance in various vectors, a sequencing protocol that includes a step for enrichment of virus particles has been established. A metagenomic study was performed on 498 female and 40 male *Aedes albopictus* mosquitos collected in August and September 2019 in Ticino, southern Switzerland, and on 4200 ticks collected in May 2021 in 10 Swiss cantons.

In mosquitos, 13 viruses from seven different virus families and several unclassified viral taxa were identified. Analysis of tick samples revealed two places with ticks hosting tick-borne encephalitis virus.

Those are the first studies to determine the virome of *Ae. albopictus* and ticks from Switzerland. Next Generation Sequencing supports the detection and surveillance of viruses that have not previously been reported in Switzerland but may become more important in the future, e.g., due to climate change.

Surveillance of flaviviruses in southern Switzerland, 2018-2021

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In the Mediterranean basin and in the eastern part of Europe several outbreaks caused by the West Nile flavivirus (WNV) have occurred in the last decades. Although this virus was notoriously present in warmer areas, since 2018 it seems to show a geographical expansion towards the north with outbreaks in birds, horses and human cases, firstly in Germany and then in 2020 in Netherland. Furthermore, in 2020 a human case and some samples of birds and mosquitoes were found positive for WNV and Usutu virus (USUV) in two Italian areas very close to the Swiss border. In recent years, an active surveillance has been launched in the southern part of Switzerland in order to detect the presence of WNV and USUV in mosquitoes.

Here we present data from the surveillance for flaviviruses that has been carried out from 2018 to 2021 in several locations of the Canton Ticino. Box-Gravid Mosquito (BOX) traps coupled with honey-baited FTA filter papers have been placed and monitored every two weeks from July to September. In 2020, CDC traps baited with dry ice were also used in parallel at all sampling sites. Viral RNA has been extracted from pools of up to 50 *Culex pipiens* or *Culex modestus* specimens, as well as from the FTA cards and analysed by semi-nested endpoint PCR specific for flaviviruses. Any positive results has been further confirmed by sequencing and species specific real-time PCR.

So far, only USUV has been found to circulate in mosquitoes from Canton Ticino. In 2018, no flaviviruses were detectable in the selected locations during the surveillance period. In 2019, a total of 2'480 mosquitoes were sampled using BOX traps and one FTA card resulted positive for USUV in a site of the Mendrisiotto district. The analysis of mosquitoes recovered from this trap confirmed the positivity for USUV. In 2020, 2'413 mosquitoes were collected and one mosquito pool resulted positive for USUV from a CDC trap located in a site of the Locarnese district, while no FTA cards resulted positive for flaviviruses. For the current mosquito season, the surveillance is ongoing.

This type of surveillance is very important in Canton Ticino where urban areas are intrinsically connected with natural and forest areas. Usutu virus has already found the conditions for its spread and these same conditions could allow the cycle of WNV in the coming years.

Posters

Inventory of Culicidae in and around the nature reserve "Grande Cariçaie" (Lake Neuchâtel) 2019

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For the first time an extensive study on Culicidae was carried out in the biggest wetland area of Switzerland. The "Grande Cariçaie" is a nature reserve located at the shore of Lake Neuchâtel in Western Switzerland. We found a rich diversity of 17 different native species, with monthly sampling of female adult mosquitoes and larvae both in in natural environments and in peri-urban zones. The species composition is representative for a nature reserve, while in comparison to other wetlands, the proportion of flood-water mosquitoes is rather low. The results also show a strong connection and exchange between nature and non-nature zones for mosquitoes.

Update on wetland mosquito fauna in southern Switzerland (Ticino)

Sylvie Flämig and Eleonora Flacio

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With the goal to update knowledge about wetland mosquito fauna in the canton of Ticino (southern Switzerland), mosquitoes were sampled monthly between June and September 2018. To also check for nuisance for nearby residents, sampling sites were located in 2 nature reserves as well as in adjacent urban areas. Centers for Disease Control (CDC) miniature light traps were used to collect adults. Larval stages were sampled using a standard pint dipper. A total of 20'311 mosquitoes (both juvenile and adult stages) were collected at 26 sampling sites.

The majority of mosquitoes were caught at the "Bolle di Magadino" (1500 ha), a protected meadow landscape of international importance. A total of 17 different species were recorded. All species identified in earlier studies (2003 – 2011) were confirmed in 2018. The share of *Aedes sticticus* has decreased relatively to *Aedes vexans*. Also *Ae. cantans* has been found far less frequently. The species complex *Anopheles maculipennis* s.l. has become more abundant compared to earlier studies. Still its density currently does not pose a risk of malaria disease transmission. Generally the sites in the surrounding settlements did not produce high catch numbers.

The second protected area, "lake Muzzano" (22 ha) shows an equally rich diversity of species with 13 different mosquito species caught. However, this reserve and the nearby urban areas are largely dominated by Coquillettidia species (both *Cq. richiardii* and *Cq. buxtoni*) and *Cx. pipiens*. Larval treatments against the former are difficult to implement and could have a considerable ecological impact. New approaches to control should be discussed.

Skin microbials to reduce pathogen transmission to animals by biting midges

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Biting midges (Ceratopogonidae, genus Culicoides, 'no-see-ums') are of huge veterinary importance, mainly as biological vectors of disease agents, such as e.g. bluetongue virus (sheep, cattle) and African horse sickness virus. In addition, biting midges cause nuisance and insect bite hypersensitivity, mainly in horses. Currently, there are no effective methods to control biting midges since synthetic insecticides have limited and/or short-lived efficacy against biting midges.

Like mosquitoes, biting midges are attracted to hosts by carbon dioxide released in their breath and by their body odours which are mainly produced by skin bacteria1. It has been shown that differences in attractiveness between human individuals to mosquitoes is mediated by these volatiles (attractive and repellent compounds) released from the skin (see presentation by Dani Lucas-Barbosa). We hypothesise that sheep also differ in their attractiveness to biting midges. The aim of the study is to identify sheep skin bacteria that attract or repel biting midges.

First, sheep odour and skin bacteria samples were collected from animals of the three most prevalent breeds in Switzerland (white alpine sheep, black-brown mountain sheep, brown headed sheep). From each breed, 30 animals from three different farms were sampled, and samples were taken from the left ear, the belly and the left leg (total 270 bacteria and odour samples). The attractiveness of the odour samples will be assessed in a newly designed and optimized dual-choice Y-tube olfactometer with laboratory reared Culicoides nubeculosus and field collected biting midges. The bacteria on the sheep skin are identified by metabarcoding at the V1-V2 region of the 16S rRNA gene by Illumina sequencing. First results revealed 181 different bacteria genera present on sheep skin of which 48 could be identified at species level, such as Staphylococcus equorum and Rhodococcus fascians. Simultaneously, the skin bacteria are cultivated on various agar media (sweat, sheep blood) and genetically identified. In a following step, the culture-grown bacteria of the most and least attractive sheep will be evaluated for attractiveness with Culicoides in the Y-Tube olfactometer.

Ultimately, repellent bacteria could be applied to sheep and bacteria producing attractive compounds to traps, to establish a 'push-pull system' (lure the biting midges away from the sheep and into the traps) for an effective and long-lasting control.

Monitoring of invasive Aedes mosquitoes along Swiss traffic routes

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While the spread and distribution of *Aedes albopictus* has been well documented in southern Europe, its dispersal patterns across the Alps remain less consistently assessed, limiting a projection of future scenarios beyond its current range and impeding a more targeted mosquito surveillance. Therefore, we set out to monitor the presence and frequency of invasive Aedes mosquitoes across and beyond the Alps over consecutive years deploying a sentinel trapping network. We placed oviposition and BG-Sentinel traps at potential points of entry with a focus on motorway service areas across Switzerland and controlled them for the presence of mosquitoes every other week from June to September between 2013 and 2020. Over the eight years of monitoring, we identified three invasive Aedes species, including *Ae. albopictus*, *Ae. japonicus* and *Ae. koreicus*. Based on the frequency and distribution patterns we conclude that *Ae. albopictus* and *Ae. koreicus* are being passively spread primarily along the European route E35 from Italy to Germany, crossing the Alps. In contrast, our data indicate that *Ae. japonicus* has been steadily expanding its range from northern Switzerland across almost the entire country through active dispersal.

Behavioural temperature preferences of *Aedes aegypti* and *Ae. japonicus* under semi-natural conditions

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Mosquito-borne diseases impose a high burden on human and animal health. Temperature strongly influences basic life parameters of mosquitoes, such as egg, larval and adult development, but also the propagation/development of the pathogens in the insect (extrinsic incubation period, EIP). Therefore, the vector capacity of mosquitoes is strongly temperature-dependent and affected if mosquitoes behaviourally thermoregulate through microhabitat selection.

Recently, the locomotory behaviour of adult *Aedes aegypti* and Ae. japonicus mosquitoes under different temperature regimes was investigated in a laboratory setup1,2. These experiments were complemented by studying the thermal preference of female *Ae. aegypti* for egg laying (oviposition) and of sugar-fed and blood-fed female *Ae. japonicus* for resting behaviour under semi-natural conditions. The experiments were conducted in large, netted cages which were located either indoors (cage: 3 x 2.2 x 2 m) for the oviposition or outdoors (semi-field cage: 4.9 x 2.8 x 2.2 m) for the resting behaviour experiments. The experimental cages contained three oviposition cups or resting boxes, respectively, representing three randomized temperature treatments by placing cooling elements or electric heating mats under the oviposition cups and resting boxes, with an additional untreated ('ambient') control treatment. These temperature treatments generated microclimates with temperature differences of 1-3 °C between the oviposition cups (measured just above the water surface with dataloggers), and 8-10 °C between the resting boxes (average temperature in the

lower half of the box measured with a thermal imaging camera). Mosquitoes were released into the cages in groups (30-50 per oviposition experiment, or approx. 150 individuals per resting behaviour experiment; 9-12 replicates). The number of eggs in the oviposition cups or the number of mosquitoes in the resting boxes were counted at certain time points during the experiments.

Consistent with our previous laboratory results, the mosquitoes preferred cooler than ambient temperatures to lay their eggs and to rest. For instance, eight times more blood-fed *Ae. japonicus* rested in the cool box than the warm box across all trials (GLMM, $p<0.001$). Our findings confirm that mosquitoes are able to behaviourally thermoregulate by selecting microhabitats for different activities such as oviposition and resting. This highlights the urgent need to account for this ability in models for a realistic prediction of mosquito distribution and mosquito-borne disease outbreaks.

Amount in CHF corresponding to the recorded hours (rilevamento attività):

		2021			
		Q4			
		November		December	
Giustificativo	Descrizione	Costi	Ricavi	Costi	Ricavi
75001120	Rilevamento attività	15,621.58			
75001121	Rilevamento attività			571.20	
75001223	Buchung, Sachkonto 30.11.2021 75001223	-6,267.03			
Totale		9,354.55		571.20	

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Matita	0,60		
USB 16GB	8.00		
Felpa/Giacchetto	36.00		
Maglietta Polo	22.00		
Vino Merlot	13.00		
Vino Bianco	13.00		
Confezione vino	1,20		
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Power Bank	27,50		
Borsa di carta	3.00		
Borsa per PC nera (14")	23.00		
Post-it	2,50		
Post-it segnapagina	1.00		
Moleskine	18,50		
Borsa di tela	2.00	50	100,00
Righello	0,50		
Schiscetta Food for brain	5,70		

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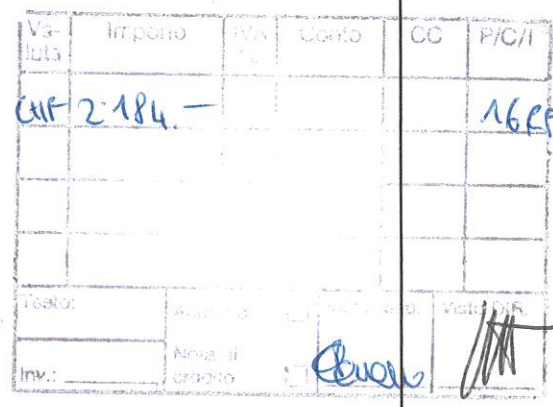
Indirizzo
CAP Città
Telefono

Spettabile
Istituto microbiologia
C.Att. Sig.ra Eleonora Flacio PhD
Via flora Ruchat-Roncati 15

6850 Mendrisio

DESCRIZIONE	IMPORTO
la vostra visita del 25.11.2021 presso l'Antico Grotto Ticino	CHF 2'184.00
ci permettiamo di inviarvi la fattura	
TOTALE	CHF 2'184.00



7.7% Iva inclusa N.IVA CHE-114.122.489 Fr. 156.14
N.Rif. Nr. 676 645
Vi chiediamo gentilmente di saldare l'allegata fattura entro 30 giorni

GRAZIE PER LA PREFERENZA ACCORDATACI

Odette Raith

Mendrisio, 23.12.21
Eleonora Flacio
COD: 16RAZVIROME

VEG

CO GROTTO TICINO

a Alle Cantine 20
CH-6850 MENDRISIO
TEL. 091-6467797
% inclusa CHE-114.122.489
N.Rif. 676 645

Cassa 1 25-11-2021

A 13

TAVOLO 21

ostrano	*1100.00
egetariano	*350.00
tto bibite	*609.00
Ticinese	*41.00
lico	*4.00
er weissbier	*45.00
ld	*35.00
	*2184.00

*2184.00

EURO) *2080.00
*156.14

www.grottoticino.com
zie per la vostra visita
rvito: C2

00000092 22:44

ANTICO GROTTO TICINO

Via Alle Cantine 20
CH-6850 MENDRISIO
TEL. 091-6467797
IVA 8% inclusa CHE-114.122.489
N.Rif. 676 645

#0001 Cassa 1 25-11-2021

FATTURA 12

TAVOLO 21

22 Menu nostrano	*1100.00
7 Menù vegetariano	*350.00
29 Pacchetto bibite	*609.00
1 Rosso Ticinese	*41.00
1 Analcolico	*4.00
6 Erdinger weissbier	*45.00
7 Braugold	*35.00
Subtotale	*2184.00

SU FATTURA *2184.00
CONTANTI(EURO) *2080.00
IVA 7.7% *156.14

Firma Cliente: Eleonora Flores

Vi ha servito: C2

C2 00000089 22:44

Einzahlung Giro | Versement Virement | Versamento Girata

nzahlung für / Versement pour / Versamento per
REDIT SUISSE (CH) AG

Zahlungszweck / Motif versement / Motivo versamento

Flacio Eleonora

Da: Flacio Eleonora
Inviato: giovedì, 25 novembre 2021 17:14
A: 'Raith'
Oggetto: R: proposta menu per cena SVEG giovedì 25 novembre

Gentile Signora Raith, sembra che saremo in 28.

A presto
Eleonora

University of applied sciences of Southern Switzerland - SUPSI
Department of Enviroment Constructions and Design
Institute of microbiology
Vector ecology

Eleonora Flacio, PhD
Senior Researcher (resp.)

via Flora Ruchat-Roncati 15
CH-6850 Mendrisio

T +41 58 666 62 47
M +41 76 580 58 23
Skype: pinkmosquito
eleonora.flacio@supsi.ch
www.supsi.ch/im

Da: Flacio Eleonora
Inviato: lunedì, 22 novembre 2021 11:34
A: 'Raith' <info@grottoticino.ch>
Oggetto: R: proposta menu per cena SVEG giovedì 25 novembre

Gentile Signora Raith,
allora:
saremo 33 persone.
Facciamo il fortait delle bevande da lei proposto
Di base rimaniamo 50% menu nostrano e 50% menu vegetariano

Grazie mille per i parcheggi ☺

Cordiali saluti
Eleonora Flacio

University of applied sciences of Southern Switzerland - SUPSI
Department of Enviroment Constructions and Design
Institute of microbiology
Vector ecology

Eleonora Flacio, PhD
Senior Researcher (resp.)

via Flora Ruchat-Roncati 15
CH-6850 Mendrisio

T +41 58 666 62 47
M +41 76 580 58 23
Skype: pinkmosquito
eleonora.flacio@supsi.ch
www.supsi.ch/im

Da: Raith <info@grottoticino.ch>
Inviato: lunedì, 22 novembre 2021 10:23
A: Flacio Eleonora <Eleonora.Flacio@supsi.ch>
Oggetto: Re: proposta menu per cena SVEG giovedì 25 novembre

Gentile Signora Flacio,
purtroppo la sala balcone non ha abbastanza posti per tutti
cosi abbiamo pensato che prepariamo la sala boccalino e la saletta in mezzo

per il parcheggio mettero le sedie, cosi che avete 4 posteggi

Vi alllego la carta del vino cosi magari fatte la scelta prima e noi possiamo servirlo direttamente
Al vostro arrivo
Potrei consigliavi anche la magnum Piccolo Ronco di Valsangiacomo vini Mendrisio
Se avete piacere di un grande formato

Cordiali saluti
Pdette Raith

ANTICO GROTTO TICINO

Viale Alle Cantine 20

6850 Mendrisio

Tel. 091 646 77 97

Cel. 076 434 08 56

Da: Flacio Eleonora <Eleonora.Flacio@supsi.ch>
Data: martedì, 16 novembre 2021 14:50
A: Raith <info@grottoticino.ch>
Oggetto: R: proposta menu per cena SVEG giovedì 25 novembre

Gentile Signora Raith, per me va bene ☺
Allora confermo la cena presso di voi giovedì 25 novembre verso le 19.00-19.30 (poi le farò sapere con più precisione).
Altre domande:

- Potremmo avere la sala balcone?
- Posso avere 4 posti macchina nei pressi del grotto?

Cordiali saluti
Eleonora

University of applied sciences of Southern Switzerland - SUPSI
Department of Enviroment Constructions and Design
Institute of microbiology
Vector ecology

Eleonora Flacio, PhD
Senior Researcher (resp.)

via Flora Ruchat-Roncati 15

CH-6850 Mendrisio

T +41 58 666 62 47
M +41 76 580 58 23
Skype: pinkmosquito
eleonora.flacio@supsi.ch
www.supsi.ch/im

Da: Raith <info@grottoticino.ch>
Inviato: martedì, 16 novembre 2021 11:26
A: Flacio Eleonora <Eleonora.Flacio@supsi.ch>
Oggetto: Re: proposta menu per cena SVEG giovedì 25 novembre

Gentile signora Flacio,

come da richiesta le invio la nostra proposta di menu nostrano e vegetariano.
Mi sono permessa di aggiungere anche un forfait per le bevande.

Resto a sua disposizione per eventuali domande.

Cordiali saluti
Odette Raith

ANTICO GROTTO TICINO

Viale Alle Cantine 20
6850 Mendrisio
Tel. 091 646 77 97
Cel. 076 434 08 56

Da: Flacio Eleonora <Eleonora.Flacio@supsi.ch>
Data: lunedì, 15 novembre 2021 22:08
A: "info@grottoticino.ch" <info@grottoticino.ch>
Oggetto: proposta menu per cena SVEG giovedì 25 novembre

Gentile Signora Right,
ci siamo sentite appena adesso al telefono.

- Giovedì prossimo 25 novembre, organizzo una cena per un convegno.
- Saremo 39 persone
- La cena dovrebbe iniziare 19.00-19.30
- Mi serve un'offerta per un menu nostrano. Consideri che qualcuno preferirà mangiare vegetariano. La lista di chi mangerà vegetariano o no, la potrò però avere solo giovedì stesso. Quindi le propongo di fare metà e metà.

In attesa di una sua risposta
Cordiali saluti
Eleonora Flacio

University of applied sciences of Southern Switzerland - SUPSI
Department of Enviroment Constructions and Design
Institute of microbiology
Vector ecology

Eleonora Flacio, PhD
Senior Researcher (resp.)

via Flora Ruchat-Roncati 15
CH-6850 Mendrisio

T +41 58 666 62 47
M +41 76 580 58 23
Skype: pinkmosquito
eleonora.flacio@supsi.ch
www.supsi.ch/im

Proposte Menu

Menu Nostrano

Tagliere di salumi vari
insalatina di nervetti e borlotti
sott'aceto
***
Polenta servita con:
brasato di manzo al vino rosso
coniglio nostrano al forno
luganighetta in umido con cipolle
funghi porcini
gorgonzola
***
Sorbetto all'uva coretto con grappa

50.--

Menu vegetarino

Insalatina di soncino con crostini
all'vinaigrette di lampone
***
Risotto alla zucca mantecato
al Zincarlin
***
Polenta e funghi porcini
***
Sorbetto all'uva coretto con grappa

Fr. 50.--

FORFAIT BEVANDE

Merlot Riserva
Enrico Trapletti, Coldrerio
1 bott ogni 3 persone
Acqua minerale naturale e frizzante
Caffè
FR. 21.-- p.p.

SUPSI
Istituto Microbiologia
Via Flora Ruchat-Roncati 15
6850 Mendrisio

T: 058 666 62 47
M: 076 580 58 23

Numero cliente: 16RA2VIROME

Rif: Eleonora Flacio
eleonora.flacio@supsi.ch

Antico Grotto Ticino
viale alle Cantine 20

6850 Mendrisio

T: +41 91 646 77 97

info@grottoticino.ch

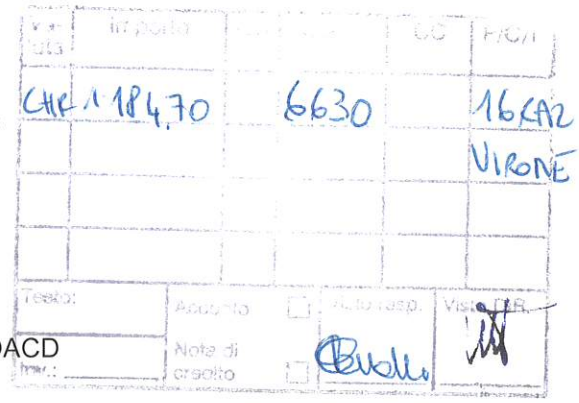
Mendrisio, 25 novembre 2021

ORDINE D'AQUISTO
BESTELLUNG
COMMANDE

DESCRIZIONE ARTICOLO	N°ARTICOLO	PREZZO UNITARIO	QUANTITÀ	Persona referente (interno IM)
menu		50	28	Eleonora Flacio
bevande		21	10	Eleonora Flacio

Tonolla Mauro
Responsabile IM

SV (Svizzera) SA
SV Ristorante SUPSI
Campus Mendrisio
Via Flora Ruchat-Roncati 15
6850 Mendrisio
CHE-110.062.405 MWST



svrestaurant

SUPSI
Istituto Microbiologia/DACD
A.c.a. Dr Valeria Guidi
Via Mirasole 22A
6500 Bellinzona

Rechnung

16RA2 VIRONE

Seite 1 von 1

Evento del 26.11.2021

Rechnungs-Nr.	Kunden-Nr.	Kostenstelle / Auftrag	Bestell-Nr / Lieferanten-Nr	Rechnungs-Datum
90307900	**30000**	**27534100**		**26.11.2021**

Menge	Artikelnr. / Beschreibung	MwSt.%	Einzelpreis	Gesamtpreis
1.00	Fattura N. 218 del 26.11.2021	2.5	30.00 CHF	30.00 CHF
1.00	Fattura N. 218 del 26.11.2021	7.7	1,154.70 CHF	1,154.70 CHF
	Bruttobetrag			**1,184.70 CHF**

inkl. 2.5 % MwSt. von 30.00 CHF: 0.73 CHF
inkl. 7.7 % MwSt. von 1,154.70 CHF: 82.56 CHF
Nettobetrag: 1,101.41 CHF

Zahlbar innerhalb von 30 Tagen ohne Abzug

SV (Schweiz) AG
Bank: UBS Switzerland AG, 8098 Zürich IBAN: CH7600230230346999308 SWIFT/BIC: UBSWCHZH80A

▼▼▼ Vor der Einzahlung abzutrennen / A détacher avant le versement / Da staccare prima del versamento ▼▼▼

Empfangsschein / Récépissé / Ricevuta

Einzahlung für / Versement pour / Versamento per
UBS Switzerland AG
8098 Zürich
Zugunsten von / En faveur de / A favore di
SV (Schweiz) AG
Memphispark
Wallisellenstrasse 57
8600 Dübendorf
Konto / Compte / Conto **01-145-6**
CHF
1184.70
Einbezahlt von / Versé par / Versato da
SUPSI
Istituto Microbiologia/DACD
Via Mirasole 22A
6500 Bellinzona

Einzahlung Giro

Einzahlung für / Versement pour / Versamento per
UBS Switzerland AG
8098 Zürich
Zugunsten von / En faveur de / A favore di
SV (Schweiz) AG
Memphispark
Wallisellenstrasse 57
8600 Dübendorf
Konto / Compte / Conto **01-145-6**
CHF
1184.70

609

Versement Virement

Keine Mitteilungen anbringen
Pas de communications
Non aggiungete comunicazioni

Referenz-Nr. / N° de référence / N° di riferimento
25 68510 00000 03000 09030 79000

Einbezahlt von / Versé par / Versato da
SUPSI
Istituto Microbiologia/DACD
Via Mirasole 22A
6500 Bellinzona

Versamento Girata

2481X
609.601
442.06

25685100000003000090307900O

Die Annahmestelle
L'office de dépôt
L'ufficio d'accettazione

010001184707>256851000000300009030790000+ 010001456>

SV (Schweiz) AG

Ristorante Subsi Campus Mendrisio
Via Francesco Cantenazzi 23
6850 Mendrisio
CHE-110.062.405 MWST

	CHF
42 X 11.50 CHF	
Colazione	483.00
Trasporto	15.00
15 X 11.50 CHF	
Pasta	172.50
20 X 15.00 CHF	
Autentico	300.00
6 X 0.60 CHF	
Ketchup, Mayo, Senf	3.60
Insalata comp.	9.00
Contorni caldi	12.50
2 X 5.00 CHF	
Küche warm	10.00
4 X 2.90 CHF	
Coca Cola 5dl	11.60
2 X 2.90 CHF	
Nestea Peach 5dl	5.80
2 X 2.90 CHF	
Apfelsaft 5dl	5.80
Elmer Citro 5dl	2.90
Michel Cranberry 3.3	2.90
Rivella rot 5dl	2.90
Henniez blau/grün 5d	2.40
22 X 3.50 CHF	
Minerale mista	77.00
22 X 2.40 CHF	
Henniez blau/grün 5d	52.80
Trasporto	15.00

Totale CHF 1,184.70

Commento: evento del 26.11.2021

6 - Debitor 1,184.70

Unterschrift:

.....
Profit Center: 1 Restaurant

Percentuale IVA	Netto	Lordo	IVA
(2) 2.50	29.27	30.00	0.73
Reduzierter Satz			
(1) 7.70	1,072.14	1,154.70	82.56
Standard Satz			

26.11.2021 16:55 #:218 Op:2753402 C:27534
02 S:27534
Vi ha servito : Ihr SV Team Mendrisio

Mille grazie e arrivederci!

Offerta

Committente

Nome e Cognome	Eleonora Flacio
Numero telefono	058 666 62 47
Indirizzo di fatturazione	da comunicare

Evento

Data	**giovedì, 25. novembre 2021**
Ora	**tutto il giorno**
Tipo di Evento	Colazione-Pranzo-Pausa caffé
Luogo	**Campus Mendrisio - Aula polivalente (A0.08)**
Numero partecipanti	38

Offerta culinaria

Offerta	Quantità	Prezzo	Totale
Colazione - ore 9:30 - 10:30	35	Fr. 11.50	Fr. 402.50
Macchina del caffé + The			
Succo d'arancia			
Acqua gasata e naturale			
Brioche miste			
Bichermüesli in monoporzione			
Cesto frutta fresca			
Pranzo - ore 12:30 - 14:00	38		
Tavolo riservato in mensa			
i partecipanti consegnano un buono alla cassa per la fatturazione finale			
Pausa Caffé - ore 17:00 - 17:20	38	Fr. 8.00	Fr. 304.00
Macchina del caffé + The			
Succo d'arancia			
Acqua gasata e naturale			
Pasticceria Mignon			
Bevande - ore 18:00			
Acqua naturale e gasata (0.5L)	22	Fr. 2.40	Fr. 52.80
Succhi di frutta misti (2.5dl)	22	Fr. 3.50	Fr. 77.00
Total		**Fr.**	**836.30**

Costi aggiuntivi

Tipologia servizio	Descrizione	Quantita	Costo	Totale
Trasporto	consegna della merce	3	Fr. 15.00	Fr. 45.00
Total			**Fr.**	**45.00**

Riassuntivo costi

Totale offerta culinaria	Fr.	836.30
Totale costi aggiuntivi	Fr.	45.00
Costi exta	Fr.	-
Totale costo Evento	**Fr.**	**881.30**

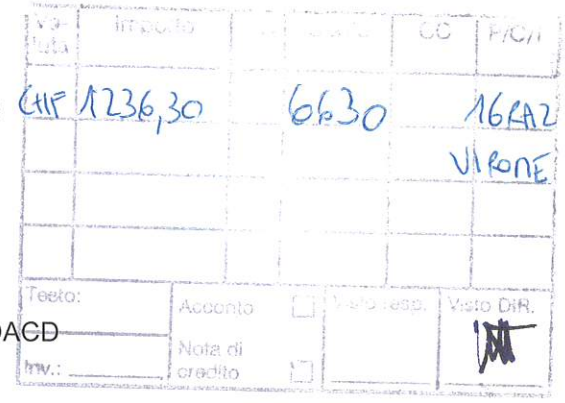
Conferma numero partecipanti

Il **numero definitivo dei partecipanti** va comunicato **entro 4 giorni lavorativi prima dell'evento**. Il numero confermato dei partecipanti sarà utilizzzato come base per la fatturazione.

Costo del personale

Il servizio del personale ammonta a CHF 45.00/ora a collaboratore. Le ore di fatturazione iniziano 30 minuti prima dell'ora di inizio stabilita e terminano 30 minuti dopo la partenza dell'utlimo ospite.

SV (Svizzera) SA
SV Ristorante SUPSI
Campus Mendrisio
Via Flora Ruchat-Roncati 15
6850 Mendrisio
CHE-110.062.405 MWST



svrestaurant

SUPSI
Istituto Microbiologia/DACD
A.c.a. Dr Valeria Guidi
Via Mirasole 22A
6500 Bellinzona

16RA2VIROME

Rechnung

Evento del 25.11.2021

Rechnungs-Nr.	Kunden-Nr.	Kostenstelle / Auftrag	Bestell-Nr / Lieferanten-Nr	Rechnungs-Datum
90307899	**30000**	**27534100**		**26.11.2021**

Menge	Artikelnr. / Beschreibung	MwSt.%	Einzelpreis	Gesamtpreis
1.00	Fattura N. 217 del 25.11.2021	2.5	30.00 CHF	30.00 CHF
1.00	Fattura N. 217 del 25.11.2021	7.7	1,206.30 CHF	1,206.30 CHF
	Bruttobetrag			**1,236.30 CHF**

inkl. 2.5 % MwSt. von 30.00 CHF: 0.73 CHF
inkl. 7.7 % MwSt. von 1,206.30 CHF: 86.24 CHF
Nettobetrag: 1,149.33 CHF

Zahlbar innerhalb von 30 Tagen ohne Abzug

SV (Schweiz) AG
Bank: UBS Switzerland AG, 8098 Zürich IBAN: CH7600230230346999308 SWIFT/BIC: UBSWCHZH80A

▼▼▼ Vor der Einzahlung abzutrennen / A détacher avant le versement / Da staccare prima del versamento ▼▼▼

npfangsschein / Récépissé / Ricevuta

zahlung für/Versement pour/Versamento per

UBS Switzerland AG
8098 Zürich

gunsten von / En faveur de / A favore di

SV (Schweiz) AG
Memphispark
Wallisellenstrasse 57
8600 Dübendorf

nto / Compte / Conto **01-145-6**
HF

1236.30

bezahlt von / Versé par / Versato da

25685100000003000090307899

SUPSI
Istituto Microbiologia/DACD
Via Mirasole 22A
6500 Bellinzona

Einzahlung Giro **Versement Virement** **Versamento Girata**

Einzahlung für/Versement pour/Versamento per

UBS Switzerland AG
8098 Zürich

Zugunsten von / En faveur de / A favore di

SV (Schweiz) AG
Memphispark
Wallisellenstrasse 57
8600 Dübendorf

Konto / Compte / Conto **01-145-6**
CHF

1236.30

609

Keine Mitteilungen anbringen
Pas de communications
Non aggiungete comunicazioni

2481X

Referenz-Nr./N° de référence/N° di riferimento

25 68510 00000 03000 09030 78999

Einbezahlt von / Versé par / Versato da

SUPSI
Istituto Microbiologia/DACD
Via Mirasole 22A
6500 Bellinzona

609.601

442.06

Die Annahmestelle
L'office de dépôt
L'ufficio d'accettazione

0100001236305>256851000000030000903078999+ 010001456>

SV (Schweiz) AG

Ristorante Subsi Campus Mendrisio
Via Francesco Cantenazzi 23
6850 Mendrisio
CHE-110.062.405 MWST

	CHF
35 X 11.50 CHF	
Colazione	402.50
Trasporto	15.00
23 X 13.00 CHF	
Autentico	299.00
17 X 9.50 CHF	
Pasta	161.50
Apfelsaft 5dl	2.90
Apfelsaft 5dl	2.90
2 X 2.50 CHF	
Patisserie	5.00
Coca Cola Zero 5dl	2.90
Coca Cola Zero 5dl	2.90
Henniez blau/grün 5d	2.40
4 X 2.90 CHF	
Insalata Piccola	11.60
Nestea Peach 5dl	2.90
Rivella blu 5dl	2.90
Pepita 5dl	2.90
38 X 8.00 CHF	
Caffè pausa	304.00
Trasporto	15.00

Totale CHF 1,236.30

Commento: evento del 25.11.2021

6 - Debitor 1,236.30

Unterschrift:

.....

Profit Center: 1 Restaurant

Percentuale IVA	Netto	Lordo	IVA
(2) 2.50	29.27	30.00	0.73
Reduzierter Satz			
(1) 7.70	1,120.06	1,206.30	86.24
Standard Satz			

25.11.2021 17:50 #:217 Op:2753402 C:27534
02 S:27534
Vi ha servito : Ihr SV Team Mendrisio

Mille grazie e arrivederci!

Offerta

Committente

Nome e Cognome	Eleonora Flacio
Numero telefono	058 666 62 47
Indirizzo di fatturazione	da comunicare

Evento

Data	**venerdì, 26. novembre 2021**
Ora	**tutto il giorno**
Tipo di Evento	Colazione-Pranzo-Pausa caffé
Luogo	**Campus Mendrisio - Aula polivalente (A0.08)**
Numero partecipanti	42

Offerta culinaria

Offerta	Quantità	Prezzo	Totale
Colazione - ore 8:30 - 9:30	42	Fr. 11.50	Fr. 483.00
Macchina del caffé + The			
Succo d'arancia			
Acqua gasata e naturale			
Gipfel al burro			
Bichermüesli in monoporzione			
Cesto frutta fresca			
Pausa Caffé - ore 11:20 - 11:40	42	Fr. 7.00	Fr. 294.00
Macchina del caffé + The			
Succo d'arancia			
Acqua gasata e naturale			
Cioccolatini			
Pranzo - ore 12:20 - 13:50	42		
Tavolo riservato in mensa			
i partecipanti consegnano un buono alla cassa per la fatturazione finale			
Bevande - ore 16:00			
Acqua naturale e gasata (0.5L)	22	Fr. 2.40	Fr. 52.80
Succhi di frutta misti (2.5dl)	22	Fr. 3.50	Fr. 77.00
Total		**Fr.**	**906.80**

Costi aggiuntivi

Tipologia servizio	Descrizione	Quantita	Costo	Totale
Trasporto	consegna della merce	3	Fr. 15.00	Fr. 45.00
Total			**Fr.**	**45.00**

Riassuntivo costi

Totale offerta culinaria	Fr.	906.80
Totale costi aggiuntivi	Fr.	45.00
Costi exta	Fr.	-
Totale costo Evento	**Fr.**	**951.80**

Conferma numero partecipanti

Il **numero definitivo dei partecipanti** va comunicato **entro 4 giorni lavorativi prima dell'evento**. Il numero confermato dei partecipanti sarà utilizzzato come base per la fatturazione.

Costo del personale

Il servizio del personale ammonta a CHF 45.00/ora a collaboratore. Le ore di fatturazione iniziano 30 minuti prima dell'ora di inizio stabilita e terminano 30 minuti dopo la partenza dell'utlimo ospite.

ALBERGO MILANO ★★★

Mendrisio, 26.11.2021

Spettabile
SUPSI
Att. Signora Eleonora Flacio, PhD
Via Flora Ruchat-Roncati 15
6850 Mendrisio

Fattura Nr.°15214.612

Egregi Signori,

Per il soggiorno presso l'Albergo Milano le fatturiamo:

25.11.2021-26.11.2021 - 612 - Spettabile CAPELLI GIOIA

Dettagli riservazioni	Pers-Qta	Prezzo	Giorni		Totale
Camera	1	105.00	1	CHF	105.00
Consumazioni (3.7%)	103.75	3.70			
Tassa di promovimento (3.7%)	1.25	0.04			
Tassa di soggiorno	1	3.30	1	CHF	3.30
Tassa di soggiorno (0%)	3.30	0.00			
				CHF	**108.30**

IVA No.CHE - 102.452.402

Consumazioni (3.7%)	103.75	3.70
Tassa di soggiorno (0%)	3.30	0.00
Tassa di promovimento (3.7%)	1.25	0.04

Vi ringraziamo per la fiducia accordataci
Nella speranza di poterla di nuovo accogliere, la preghiamo di accettare i nostri più cordiali saluti.

Hotel Milano
Tatjana Vukobrat

21.12.21, Mendrisio

COD. 16RA2VIROME

Eleonora Flacio

CH-6850 Mendrisio - Via Franscini 6 (Piazzale Stazione) - Tel. +41 (0)91 646 57 41 - Fax +41 (0)91 646 17 64
Internet: http://www.hotel-milano.ch - E-mail: info@hotel-milano.ch N. IVA CHE-102.452.402

ALBERGO MILANO ★★★

Spettabile
SUPSI
Att. Signora Eleonora Flacio, PhD
Via Flora Ruchat-Roncati 15
6850 Mendrisio

Mendrisio, 19.12.2021

Gentile Signora Flacio,

In allegatole inviamo la nostra fattura:

15214 Pernottamento della Sig.ra Capelli Fr. 108.30

Ringraziando per la fiducia accordataci la preghiamo gentilmente di voler effettuare il pagamento tramite la polizza allegata entro 30 giorni dal ricevimento della stessa.

In attesa cogliamo l'occasione per porgerle i nostri migliori saluti.

Hotel Milano

T. Vukobrat

CH-6850 Mendrisio - Via Franscini 6 (Piazzale Stazione) - Tel. +41 (0)91 646 57 41 - Fax +41 (0)91 646 17 64
Internet: http://www.hotel-milano.ch - E-mail: info@hotel-milano.ch N. IVA CHE-102.452.402

Ricevuta

Conto / Pagabile a
CH94 8080 8001 8187 7266 2
Maria Teresa Gerosa Albergo Milano
Via Motta 2b
CH-6850 Mendrisio

Pagabile da (nome/indirizzo)

Valuta CHF

Importo

Punto di accettazione

Sezione pagamento

Conto / Pagabile a
CH94 8080 8001 8187 7266 2
Maria Teresa Gerosa Albergo Milano
Via Motta 2b
CH-6850 Mendrisio

Pagabile da (nome/indirizzo)

Valuta CHF

Importo

Info Hotel Milano

Da: Info Hotel Milano <info@hotel-milano.ch>
Inviato: domenica, 21 novembre 2021 12:53
A: 'Flacio Eleonora'
Oggetto: R: prenotazione singola Gioia Capelli

Gentile Signora Flacio,
con la presente confermo la prenotazione sottoscritta.

Grazie e cordiali saluti

Tatjana

ALBERGO MILANO
VIA FRANSCINI 6
6850 MENDRISIO
Tel. +4191/646 57 41
www.hotel-milano.ch

Check-in 14:00/23:00
Check-out 6:30/11:00

Da: Flacio Eleonora <Eleonora.Flacio@supsi.ch>
Inviato: sabato, 20 novembre 2021 18:26
A: info@hotel-milano.ch
Oggetto: prenotazione singola Gioia Capelli

Gentile Signora Tatiana, ci siamo appena sentite al telefono,
mi serve una singola per giovedì 25 sera a nome di Gioia Capelli (eventualmente anche parcheggio, ma non lo so ancora).
La Dr Capelli sarà ospite della SUPSI, quindi mandi pure la fattura a me.

Cordiali saluti
Eleonora Flacio

University of applied sciences of Southern Switzerland - SUPSI
Department of Enviroment Constructions and Design
Institute of microbiology
Vector ecology

Eleonora Flacio, PhD
Senior Researcher (resp.)

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