STSM GRANTEE: PhD student Anca Ioana Paslaru

HOST: Institute of Parasitology, National Centre for Vector Entomology,University of Zurich, Switzerland (Dr. Eva Veronesi,Prof. Dr. Alexander Mathis)

STSM Topic: Insect vector molecular identification (DNA amplification, mass spectrometry), vector competence (mosquitoes), vector bionomics (invasive mosquitoes, biting midges), vector control.

STSM Type: Regular (from Romania to Switzerland)

COST Action:TD1303

DURATION: 2014-08-25 to 2014-10-13

SHORT SCIENTIFIC REPORT

- **Purpose of the STSM**

The role of mosquitoes as vectors of West Nile virus (WNV)is well known and represents one of the main concerns in the epidemiological and etiological researches of the (re-) emerging disease caused by WNV. In Romania, several epidemics have been reported in the last decade but there is a lack of updated research regarding vector competence of the mosquitoes and the methodology for isolation and identification of WNV in mosquitoes. My research topic is WNV and I am following a PhD program with the thesis entitled "Epidemiological and etiological research regarding West Nile Virus infection in wild and domestic animals". Taking into consideration the above mentioned aspects, the goal of my STSM was to learn new techniques and to gain access to equipment and methods not available in my home institution. The focus of this STSM was to learn the methodology for collection, preservation and identification of mosquitoes putatively involved in the transmission of WNV,to perform vector competence experiments, RNA extraction from mosquito samples (saliva, body parts), reverse transcription real time PCR for the identification of WNV in mosquitoes and learning the methodology for the identification and determination of risk areas for WNV transmission. All this tasks were achieved.

- **Description of the work carried out during the STSM**

My activities were done according to the working plan.

Description of the topics:

Insect vectors identification using morphology and PCR

- Morphological identification of mosquito genera and species.

During my STSM, I have learned about the collection and storage techniques for wild mosquitoes using several trapping approaches (CDC mini-trap with CO_2 and lure, ovitraps, human landing and animal baiting traps). I have developed the skills to identify mosquitoes not only to the genus but also down to species level (*Aedes, Culex, Anopheles, Ochlerotatus*) using morphological traits.

Vector competence experiments, involving different mosquito species.

- Artificial oral infection of mosquitoes and *Culicoides* biting midges.

Preparation of blood meal: I have practiced on washing of red blood cells, followed by spiking with virus (WNV and Sindbis virus).

Feeding device: Three different methods were used 1) hemotek device unit: mosquitoes (*Ae. japonicus*) feeding on a blood meal reservoir (sheep blood) through various types of membranes (chicken skin, parafilm, pig intestine, nescofilm); 2) blood-soaked piece of cotton introduced inside the mosquito cage (*Culex pipiens*); 3) Parafilm on heated glass container (*Culicoides* only).

- Homogenisation of mosquitoes

Adult mosquitoes were homogenized using a motor pestle (Kontas) driven inside an Eppendorf tube containing 100 µl DMEAM medium, for 30 sec. After that, the pestle was easily removed and 900 µl of medium was added. Pestles can be recycled prior to their autoclavation which I was also doing after each homogenization round.

To test all the different stages of vector infection, I was also processing body parts of mosquitoes separately: abdomen (to assess virus infection), thorax & head (to assess virus dissemination) and saliva (to assess virus transmission).

- Saliva collection from mosquitoes

This process is done with live mosquitoes. Individuals were anesthetized on dry ice for few seconds, and then transferred to the stereomicroscope where legs and wings were removed to

avoid their escape. After this step, mosquito's proboscis was introduced into a capillary tube filled with 1µl of mineral oil. The mosquitoes were left to salivate for 30 min.

- Extraction of viral RNA from mosquitoes tissue

This procedure has been done using a standard protocol (Mini Viral-RNA kit; Qiagen)

- Quantification of viral RNA by reverse transcription quantitative RT-qPCR

This procedure was used to determine WNV viral RNA presence in mosquito samples of SE of Romania which were captured in July and brought by me for identification.

Study about methodology of localization, characterization and definition of risk levels and areas for WNV transmission.

- Localisation of mosquitoes breeding sites (larvae and pupae)

The training was carried out in both natural (field) and artificial site (urban) by Dr. Eva Veronesi. Activities performed were: 1) larvae and pupae breeding site localization and characterization for *Aedes* and *Culex* genus; 2) sampling techniques for larvae and pupae of same mosquitoes genera; 3) identification of larvae and pupae artificial breeding sites in urban environment (private gardens).

- Collection of mosquitoes adults with CDC mini traps baited with CO_2 and lure (iGu®). Traps were placed at 9 different places in and around a natural reserve (Thurauen) and run for two consecutive nights every second week. Live mosquitoes were transferred into plastic container and kept in dry ice or at – 20 °C until their identification.

- Oviposition traps for mosquitoes surveillance

The training on this technique was carried out by Dr. Fabrizio Balestrino as part of a European project (EDENext). Ovitraps are aiming to give a confirmation of presence or absence for *Aedes* species based on eggs laid on a substrate provided within the trap. Due to the biology and ecology of this genus, the location of ovitraps is more difficult than for *Culex* species as it needs to take into account not only suitable breeding sites but also areas where adults are circulating.

Ovitraps were placed weekly in different environments (cemetery, forest and urban area). The main purpose was to evaluate the efficiency of ovitraps for the species *Ae. japonicus* using different substrates and infusions. Three type of substrates were compared: 1) germination paper; 2) wooden stick; 3) polystyrene block. In addition, 3 types of infusion were also investigated: 1) deionized water; 2) hay infusion; 3) oak infusion. The infusions were prepared with 5g (hay,

oak)/ l in a container half filled with water. The water used for the experiment was kept at room temperature, uncovered, in order to simulate the rain water (chlorine free water). After the collection, the eggs were counted and stored at +4 °C.

- Animal baited trap

This is an experiment were I participated with PD Dr. Cornelia Silaghi and med vet. Andrea Schönenberger. The purpose of this experiment is to identify host preferences of different species of mosquitoes. Animals used for these experiments were: horses and chicken which were kept inside a coral (horses) or a cage (chicken) covered with a net. Mosquitoes were allowed to approach the animals by lifting up the net for 2 hours, after which animals were clinically examined before dropping down the net and all the insects collected with electric backpack aspirators. Engorged mosquitoes were separated from the rest of the collected insects and stored in petri dishes in dry-ice until further investigation carried out in the lab. After 2 more hours, the same activity was repeated. In addition, human landing technique was also carried out in 4 hour intervals.

This experiment started in May and was performed at two different locations (rural, natural site), on a weekly rotation (1week/location).

- Since I have learned these techniques, I have been also asked by Dr. Veronesi to contribute on an experimental study assessing vector competence for Sindbis virus (SINV) in indigenous population of *Ae. japonicus* (Zürich strain). During this experiment, I therefore made use of all the activities mentioned above (human landing, feeding mosquitoes with infected blood, mosquito salivation, homogenization etc.).

- I also managed to identify some mosquitoes which I brought with me from Romania (350 mosquitoes collected in July in the SE area of the country). The results are: Males – 17 (*An. claviger*), 8 (*An. plumbeus*), 46 (*Cx. pipiens/torrentium*), 1 (*Ae.vexans*) and 3 *Aedes.spp.*

Females – 164 (*Cx. pipiens /torrentium*), 14 (*Oc. caspius*), 13 (*Ae. vexans*), 1 (*An. maculipennis*), 1 (*Cq. richardii*), and 84 *Aedes.spp.*

- Artificial breeding of mosquitoes species (*Culex* and *Aedes*)

Under supervision of PD Dr.Cornelia Silaghi, I contributed in the insectary for the following species: *Ae .japonicus*, *Cx. quinquefasciatus* and *Culicoides nubeculosus*. The steps covered were: 1) egg hatching and larvae breeding in standard plastic tray using validated methods for their feeding; 2) pupae collection and transfer into adult cages (bugdorm) where they were

completing their aquatic stage and emerged as adult imago; 3) collection of *Culicoides* larvae and pupae and transferring into adult cages (pillbox).

- **Description of the main results obtained**

The results of mosquito identification from Thurauen (15.07.14 – 10.09.14) are:

Cx. pipiens/torrentium = 1109, *Ae. vexans* = 4991, *An. plumbeus* = 342, *Ae.cinereus/geminus* = 95, *Ae. geniculatus* = 27, *Oc. Sticticus* = 911, *Cq. Richardii* = 45, *An. claviger* = 27, *An. maculipennis* = 88, *Ae. anulipes* = 3, *Ae.detritus* = 1, *Aedes.spp.*= 278, *Culex.spp.* = 19, *Simulidae* = 397.

After the identification of the mosquito samples from Romania, all the specimens were processed for WNV viral RNA presence: females were separated by genus for a total of 14 pools of ≤25 mosquitoes/each. Viral RNA was extracted following the procedure described above (Qiagen kit). I also did a RT-qPCR for West Nile virus detection in mosquitoes, and all the pools were negative.

The results from animal-baited traps (May-August, the data from September is ongoing):

Total number of collected mosquitoes = 985; nr. of females= 979; nr.of blood-fed females = 678; nr. of males= 6. The mosquito species were:

Aedes spp. = 425 (*Ae. cantans/annulipes* = 221; *Ae. rusticus* = 35; *Ae. japonicus* = 19; *Ae. geniculatus* = 2; *Ae. vexans* = 111; *Ae. cinereus/geminus* = 25; *Oc. Sticticus* = 8; *Ae. cataphylla* = 1);

Anopheles spp. = 361 (*An. claviger* = 280; *An. maculipennis* = 81);

Culiseta spp. = 32 (*Cs. Annulata* = 31; *Cs. Morsitans* = 1);

Coquilletidia spp. =111 (*Cq.richardii* = 111);

Culex spp. = 53 (*Cx. pipiens/torrentium* = 52; *Cx. territans* = 1).

Mosquito in animal baited traps:

1) May: Horses= 244 mosquitoes;

   Chicken= 0 mosquitoes;

2) June/July: Horses = 185 mosquitoes;

   Chicken= 20 mosquitoes;

3) August: Horses = 478 mosquitoes;

Chicken = 38 mosquitoes.

The use of oviposition traps for a mosquito surveillance project is ongoing.

- **Future collaboration with the host institution**

As studies on West Nile virus have high priority at the Swiss laboratory in the framework of a project to assess the risk of future disease transmission in Switzerland, collaborations with research groups from WNV endemic areas are of great value. Since the completion of this full detailed training course, and acquisition of skills and knowledge on entomology for both field and laboratory techniques, Anca would be an excellent contact person in that regard, and at least regular academic exchanges are aimed at (see also paragraph 'Confirmation by the host institution....' Below)

- **Foreseen publication/articles resulting from the STSM**

At least 1 peer reviewed paper will be produced on vector competence studies among Swiss *Ae.japonicus* after oral infection with Sindbis Virus. (1 paper on SINV at least)

- **Confirmation by the host institution of the successful execution of the STSM**

In consideration of the short time that Anca had available during for training here at the University of Zurich (only 7 weeks), she has worked very intensively with a rich detailed programme of activities most of them completely new for her. She went through all the aspects of ecology, surveillance, monitoring and taxonomy of mosquito species in different habitats (urban, forest and rural) together with several laboratory activities. She also took part at several experimental projects both in the field and in the laboratory which gave her a solid understanding of what is implying working as entomologist. After only few weeks that she was training on the identification of mosquitoes, she was already able to independently identify up to 20 species.

Anca is a very motivated and enthusiastic scientist and an excellent learner. She was always willing to do more than needed and working long hours to gain as much experience as possible considering the short visit. She was also using her time on reading relevant papers and books and discussing work-related subjects with all the staff.

It was really a great pleasure to have Anca in our lab and to see how quick she learns and develops scientific thinking. She totally fulfils all the skills and requirements for a successful PhD or a Doctorate in Veterinary Medicine. In fact, we currently clarify whether we could offer Anca the option to prepare a veterinary thesis or at least part of her PhD at our Institute.

- **Other comments**

I especially wish to thank Dr. Eva Veronesi for being an extraordinary supervisor, who coordinated with professionalism, ability and enthusiasm this practical training. Also, I want to thank PD Dr. Cornelia Silaghi, Dr. Fabrizio Balestrino and all members of the Vector Entomology Unit. It was a pleasure to meet and work with them.

Special thanks to Prof. Dr. Alexander Mathis, PhD, Head of the Vector Entomology Unit, for availability and collaboration.

Dr. Prof. Alexander Mathis

Head of Vector Entomology Unit

Dr Eva Veronesi

Senior Scientist