

COST TD1303 EURNEGVEC

STSM Report "Evaluation and Harmonization of adult sand fly and mosquito sampling methods"

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1. Purpose of the STSM

Short Term Scientific Missions (STSM) "**Evaluation and Harmonization of adult sand fly and mosquito sampling methods**" (Reference code: COST-STSM-ECOST-STSM-TD1303-220614-044123; Host institution: University of Novi Sad; Period: 22/06/2014 to 29/06/2014; Approved amount: EUR 800) in collaboration with Prof.Dr. Dusan Petric and according to the Work Plan previously provided.

Recently, in two sibling projects ongoing in Europe, EdenNext and Vbornet, the researchers working in both projects revealed that even though useful data had been gathered on vectors and vector-borne diseases in Europe, there is an important gap of knowledge on distribution of sand fly and mosquito species related vector borne pathogens in the Balkan area together with some other part of west Palearctic. According to the results of last risk analysis performed by Vbornet team, risk maps show these gaps in particularly Balkans with some differences between the countries. For instance, there is almost no information on sand fly species distribution/taxonomic list in some parts while in others are not precise and/or dated. To understand exact distribution pattern and routes of these vectors and pathogens and describe vector's dispersion in transition areas like Balkans is very important subject for estimating possible future public health risks and threats to Europe.

Therefore, Short Term Scientific Missions was aimed (1) to exchange the knowledge and tools related to the collection methods between applicants and host researcher, (2) to evaluate state-of-the-art and new techniques of adult sampling; (3) to collect sand fly and mosquitoes in the locations where no information is available in Serbia and Kosovo; (4) to clarify and standardize storage techniques which are necessary for future parasitic or viral studies and identification methods for sand fly and mosquitoes; (5) to strength the existing networks and fostering collaborations by allowing scientists from other participating COST countries to visit University of Novi Sad that provided opportunities to achieve the aims.

Research done contributed to the TD1303 COST Action objectives of "One Health" concept in the ecology of vector-borne diseases (WG1). It also allowed participants to

exchange experience in the field work methods and learn new techniques and gain access to specific sampling methods, not available in their own countries. In addition, I fully believe that this STSM was one of the most important opportunities which contributed to the COST action objectives (WG1) of investigating a neglected vectors and diseases from the "One Health" concept perspective, using standardized techniques. During the fieldwork, the applicants exchanged their experience in the field of insect vectors. In particular, three PhD students from Turkey, France and Serbia, and one local undergraduate student and one local MSc student from Kosovo were trained during the study of vector sampling techniques and identification of sand fly and mosquito species.

2. The work carried out during the STSM

a. Sampling in the field

The field samplings of adult sand flies and mosquitoes were done mainly from rural environment using CDC Light Traps, NS2 type dry-ice baited traps, CDC Type Light Traps baited with dry-ice, BG sentinel traps, sticky paper traps coated with castor oil at specific sampling points in different microhabitats such as wall hall, poultry, barn wall, vegetation, sheep barn, goat barn, sylvatic area etc. , and conventional mouth aspirators according to the insects' behavior. Traps were set up before sunset, around 07:00 PM, and collected after sunrise, around 08:00 AM.

The sampling locations, mainly rural villages, were selected in the towns of Aleksinac and Nis located in Southern Serbia and sampling was done between 23th and 25th June 2014. In Kosovo, the villages located in middle and southern Kosovo in Prizren (villages of Zhur, Vermiče, Landoviče and Krusha e Madhe), Gyilan (villages of Livoç and Cerniče) and Pece / Junik (villages of Isniq and Turkaje) were selected according to previous visceral leishmaniasis case history. The previously published papers, mainly in local language, were also used for selecting the locations in Serbia and Kosovo. The other participants trained on the sampling methods of sand flies and mosquitoes by the applicants. Sampling was done between 26th and 28th June 2014 in Kosovo.

b. Storage and identification

After each sampling night, the samples were checked individually on dry ice; mosquitoes were identified to genus or species level by stereomicroscope, and pooled according to sex and physiological condition and stored in dry ice especially for future viral studies. Larvae and pupae of mosquitoes were collected when they found in the sampling locations and they kept in individual plastic bags in their natural water for later

identification. Sand fly specimens were separated from other flies and kept on dry ice. They also pooled in terms of sex and physiological conditions.

A dry-ice procedure was applied to keep the insects frozen for viral studies;

- During the collection of the traps, the sampling bags were separated from the traps and directly put to the box containing dry-ice,
- After arrival to the field lab, we put some dry ice into the thick nylon bag and sand flies, mosquitoes and other flies were separated on this nylon bag. Mosquitoes could identify according to their morphological features in the field laboratory. Detailed observation of the morphological characteristics was conducted by the use of stereomicroscope. Host provided training in mosquito adult identification to other participants of STSM.
- Regarding sand flies, dry ice was put into the petri dish and slide on it, under the stereomicroscope only genitalia was cut, put to the alcohol (70%) for later morphological and molecular identification and kept the rest of body on dry ice for virus study. For virus isolation, salivary glands of sand flies are more important because viruses are mainly concentrating into salivary glands. For this reason, only genitalia kept in the alcohol will be used for identification. In this way, the insects were always kept in cold from the field to the lab. Later identification will be also done by DNA barcoding techniques.

3. General results

The most of the catching nights, the climatic conditions were not suitable especially for sand fly catching. The strong rain and storms were happened. In any case, during the STSM period, a total of 120 traps and around 500 sticky papers were set and the following insect species were collected:

- Mosquitoes (around 900 specimens): *Culex* spp., *Aedes* spp., *Anopheles* spp., *Culiseta* spp. The number of each species will be determined after final identification.
- Sand flies (around 350 specimens): The identification will be done after the mounting of the genitalia of individual specimens. DNA-barcoding was also planned for vector identification.
- Pathogens: The screening of the wild-caught specimens for the presence of possible pathogens such as *Leishmania*, phleboviruses, West Nile viruses,

Japanese Encephalitis virus, etc., will be performed by molecular techniques in due time.

A dry-ice procedure was developed for keeping the specimens in the field conditions and suitable storage of different insects in good condition for molecular and viral studies.

In addition, we obtained useful knowledge on standardization of sampling methods, strength and weakness of current using collection methods, differences between classic and state-of-art collection and identification methods, sampling protocols, how to collect specimens and how to process specimens if we want to make entomological, ecological studies and virus/parasite isolation studies from same specimens in same time period, what is the best protocol to transfer the collected specimens to laboratories. We believe that on one side, we shared all these obtained information with young scientists, local authorities and local people, and we also established sustainable network between the laboratories in Balkan countries.

4. Future collaboration with the host institution

Applicant will provide new training for hosts from the University of Novi Sad, to acquire additional knowledge in entomology, vector sampling and artificial feeding techniques of sand flies and mosquitoes. New project calls and opportunities will also be searched for extending the sampling area in other Balkan countries. We also decided to search possible opportunities to be articulated to new European project such as Vectornet and to be shared our knowledge into these projects. We want to extend the study areas to other Balkan countries and also perform morphometric and molecular analyses for phenotypic and genetic comparison of vector populations between countries for phylogenetic studies. In this way, we believe that, the screening of wild-caught samples by PCR and/or DNA barcoding techniques and relevant bioassays to assess the diversity and prevalence of pathogens will contribute the possible risk analyses on vector and related pathogens for Europe.

5. Foreseen publications/articles resulting from the STSM (if applicable)

The results of this STSM will be published in 1 or 2 peer-reviewed journals.

6. Confirmation by the host institution of the successful execution of the STSM

This will be written by Prof. Dušan Petrić-Novi Sad University.



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

I herein confirm the report of Prof. Bulent Alten regarding the COST - STSM-ECOST-STSM TD1303 -220614-044123 in Serbia.

Novi Sad, 4th July 2014

Yours sincerely

Prof. Dr. Dušan Petrić