

STSM Report Yusuf Özbel

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-TD1303-220614-044127; Host institution: University of Novi Sad; Period: 22/06/2014 to 29/06/2014; Approved amount: EUR 800) in collaboration with Prof. Dusan Petric and according to the Work Plan previously provided.

There is an important gap of knowledge on the distribution of the sand flies and mosquitoes and the pathogens carrying by these important haematophagous insects in Balkan area. Therefore, Short Term Scientific Missions was aimed (i) to exchange the knowledge and tools related to the collection methods between applicants and host researcher, (ii) to evaluate state-of-the-art and new techniques of adult sampling; (iii) to collect sand fly and mosquitoes in the locations where no information is available in Serbia and Kosovo; (iv) to clarify and standardize storage techniques which are necessary for future parasitic or viral studies and identification methods for sand fly and mosquitoes; (v) 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. During the field work, the applicants exchanged their experience in the field of insect vectors. In particular, three PhD students were trained during the study of vector sampling techniques and identification of sand fly and mosquito species.

2. Description of the work carried out during the STSM

a. Sampling of sand flies and mosquitoes 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 and sticky paper traps coated with castor oil, at specific sampling points, according to the insects’ behavior. All traps were placed 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 Niš located in Southern Serbia and sampling was done between 23 and 25 June. In Kosovo, the villages located in middle and southern Kosovo in Prizren (villages of Zhur, Vermiče, Landoviçe and Krusha e Madhe), Gyilan (villages of Livoç and Cerniçe) and Junik (villages of Isniq and Turkaje) were selected according to previous visceral leishmaniasis case history. The published papers, mainly in local language, were also used for choosing the locations in Serbia and Kosovo. The applicants were trained by the other participants on the sampling methods of sand flies and mosquitoes.

b. Storage procedure and identification of insects

At the end of each sampling night, the samples were checked individually on dry ice; mosquitoes were identified in genus or species level by stereo microscope and stored in dry-ice especially for future viral studies while sand flies were separated from other flies and kept on dry-ice. Additionally, 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.

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 lab. Detailed observation of the morphological characteristics was conducted by the use of stereo microscope. Host provided training in mosquito adult identification to other participants of STSM.
- Regarding sand flies, dry-ice was placed into the petri dish and slide on it, under the stereo microscope only genitalia was cut, put to the alcohol for later identification and kept the 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.

3. Description of the main results obtained

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 different traps and around 500 sticky papers were set and the following insect species were collected:

- Mosquitoes (around 900 specimens): *Culex* spp., *Culiseta* spp., *Aedes* spp., *Anopheles* spp. The species identification will be finalized later and certain number of each species will be determined.
- 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.

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 are willing to extend the study areas to other Balkan countries and also perform more detail analyses such as morphometry and molecular identification for phenotypic and genetic comparison of vector populations between countries.

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.



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

I herein confirm the report of Prof. Dr. Yusuf Özbel regarding the COST - STSM-ECOST-STSM TD1303 - 220614-044127 in Serbia.

Novi Sad, 4th July 2014

Yours sincerely

Prof. Dr. Dušan Petrić