STSM report Aleksandra Ignjatovi

Ćupina

Short Term Scientific Missions (STSM): “A multinational field parasitological expedition on the track to the vector of *Onchocerca lupi”*
Host institution: Universidade de Èvora, Portugal; person in charge: Helder Cortes
heldercortes@gmail.com
Period: 13/04/2014 to 18/04/2014
Applicant: Aleksandra Ignjatović Ćupina, PhD
Reference code: COST-STSM-ECOST-STSM-TD1303-130414-044119
Approved amount: EUR 800

PURPOSE OF THE STSM

Short Term Scientific Missions was conducted according the previously provided working plan. STSM was aimed to strengthening the collaboration and networking between scientists from other participating COST countries. The visit to the University of Evora provided opportunities to work on neglected parasite *O. lupi* and to conduct the research on its possible vector.

This mission allowed participants to exchange experience in field and laboratory research methods and techniques not available in the own country and to acquire additional knowledge on specific environmental and epidemiological conditions concerning parasite and vectors. During the study, the applicant exchanged its experience in the field of insect vectors. In particular, she trained two PhD students in identification of haematophagous insects, techniques of rearing blackflies, as well as techniques of preparing and feeding of insect vectors on the host.

The performed research activities were related to the TD1303 COST Action objectives of “One Health” concept in the ecology of vector-borne diseases (WG1) and investigating rare and emerging vector-borne pathogens (WG5).

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

1. IDENTIFICATION OF HAEMATOPHAGOUS INSECTS

Identification of the adult stage of haematophagous insects was mainly focused on the material captured by dry ice baited traps (type NS-2) positioned in the studied area where cases of dogs infected with *O. lupi* were confirmed. The trapping results were positive for three insect families of medical importance: mosquitoes (Diptera, Culicidae), blackflies (Diptera, Simuliidae) and sand flies (Diptera, Psychodidae, Phlebotominae). Identification of immature stages of blackflies collected in the highly productive breeding site within the affected area was also conducted.

Moreover, adults of blackflies reared from mature pupae were identified. Detailed observation of the morphological characteristics was conducted by the use of binocular microscope with adequate magnification. The identification was done according few identification keys for blackflies (Knoz, 1965; Rivosecchi, 1978; Bass, 1998; Jedli

ka et al., 2004; Rivosecchi et al., 2007) and recent identification key for mosquitoes (Becker et al, 2010).

The applicant provided training in blackfly identification to other participants of STSM.

2. REARING BLACKFLIES

Rearing of separate individuals of blackflies is important method used for confident identification of species with similar morphology. Mature pupae, recognized by dark color and the formed farate adult that may be observed inside the integument, were placed on wet filter paper put inside plastic tubes closed by cotton. This method of rearing allowed observation of both adult and pupal exuvia morphological characteristics.

In order to acquire as much as possible blackfly females ready for feeding on the infected dog (under consent of the owner of the dog), beside application of dry ice baited traps, massive rearing of adults was conducted. Collected mature pupae of blackflies attached to the natural supporting material (acquatic plants) were kept in humid conditions in plastic cages after until their emergence. Part of the rearing material was also positioned outdoors, in the closed tent with a dog inside.

3. PREPARING AND FEEDING THE INSECTS ON THE HOST

Collected individuals from traps (mosquitoes, blackflies and few individuals of sand flies) were fed on the infected dog placed in the closed tent (3m x 3 m x 2 m). The feeding of insects was conducted by:

a) Releasing of insects (mosquitoes and blackflies) inside the tent, where the dog was continually exposed to bites.

   Releasing of the insects collected by traps was performed in the evening hours. Recollecting of fed individuals, recognized by enlarged abdomen was conducted regularly each day in the morning hours by application of the insect aspirator. Unfed individuals were left in the tent until the next recollection. After the end of the experiment all of the visually unfed mosquitoes and blackflies were collect and preserved in ethanol 70% for further analysis.

b) Application of glass tubes, with 5-7 blackflies per tube for stimulated feeding of blackflies on the dog on the selected body region.

   The tubes with females were placed and hold tight on skin of the forehead or inter-scapular region of the dog, where highest number of *O. lupi* microfilaria was previously detected.

All of the fed individuals, mosquitoes and blackflies, were placed in plastic insect cages (BugDorm-1, MegaView Science Co., Ltd, Taiwan), 30 cm x30 cm x30 cm, supplied by the source for additional feeding (gauze soaked with 10% sugar solution) and covered by wet dark towel in order to maintain adequate humidity of about 80% and semi dark conditions to inhibit the excessive activity that would lead to energy overconsumption and affect the lifespan. All specimens that died before the feeding on the dog were morphologically identified to species level and stored in individual eppendorf tubes, containing 70% ethanol for further molecular analyses on the presence of helminths DNA.

The period of duration of the STSM was not long enough to observe the final results of the research, the development of microfilaria to the infective L_3 stage. In fact, at the moment when this report was wrote many of insects fed on the dog were still alive. Therefore, the last phases of the research started within the STSM action is still in process. Soon after the death of each fed insect individual, they are identified, dissected in the saline solution in order to check visually the presence of infective L_3 microfilaria *O. lupi* and then placed in 70% ethanol for further molecular analysis on parasite detection.

4. LEARNING ABOUT PARASITE DETECTION IN BLOOD AND SKIN SAMPLES AND DISSECTED INSECT TISSUES

The STSM was the excellent opportunity to learn and acquire the knowledge and practical skills on parasite detection in the host and vector. Participants of the STSM, experts in veterinary medicine trained the entomologists, also participants of STSM how to recognize the symptoms of infections of dogs with *O. lupi* and demonstrated the procedure of blood and skin snips sampling. However, the most interesting and applicable parts of the STSM for the applicant, as being entomologist, were the trainings given by both veterinarians and parasitologists on the techniques of processing the samples for detection of the microfilarial parasites in the blood smear and skin tissue of the definite host, techniques of dissection and detection of microfilaria in the intermediate host (i.e. insect vector), methodology for permanent preparations of filarial larvae on slides and finally, taxonomical identification of the infective stages of filarial worms according identification key of Bain & Chabaud, (1986).

a) Procedures on dogs

The applicant was trained by other STSM participants, experts in veterinary medicine and parasitologists, on the sampling procedures of diagnose canine filarioids in dogs: a) How to collect blood and skin samples from dogs, after owners consent, to assess the presence of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema reconditum* microfilariae, as well as *O. lupi* and *Cercopithifilaria* spp. Skin samples were collected using individual 3mm diameter biopsy punches, performed on the head and inter-scapular region a dog.

b) Laboratory procedures of microfilariae detection

The applicant was also trained in a Knott-modified test in order to detect microfilariae in the blood and in screening of the skin samples for the presence of skin-dwelling microfilariae, according to the standard procedures. Skin snips were soaked in saline solution (NaCl 0.9%) and left overnight at room temperature, in order to allow the migration of microfilariae from the skin sample to the saline solution. Sediments were individually observed under a light microscope (i.e., two fields of 18mm×18mm coverslip each) and microfilariae found were identified, according to their morphological and morphometrical features.

- **DESCRIPTION OF THE MAIN RESULTS OBTAINED**

Majority of the collected blood sucking insects during the STSM were mosquitoes and blackflies. In the mosquito fauna the dominant species was Culex Pipiens Complex followed by *Ochlerotatus caspius*. Few specimens of *Oc. detritus* were also recorded. Blackfly fauna was represented by three species: *Simulium pseudequinum* as the dominant species, *Simulium intermedium* and *S. velutinum*. All of the blackfly species identified during this mission have been recorded in Portugal in the past and listed in the last edition of the revised taxonomic and geographical inventory of world blackflies (Adler & Crosskey, 2014).

Ten dogs were sampled for microfilariae and all isolated species of microfilaria were morphologically and morphometrically identified. In particular, microfilariae of the following filarioid species infesting dogs were detected: *O. lupi*; *Cercopithifilaria bainae* and *Cercopithifilaria* sp. II sensu Otranto et al. 2012.

The detection of *O. lupi* developing larvae is currently in still progress in Laboratory of Medical Entomology, University of Novi Sad (Serbia) where the applicant is affiliated.

- **FUTURE COLLABORATION WITH THE HOST INSTITUTION**

Applicant will provide new training for hosts from the University of Evora, to acquire additional knowledge in entomology, vector sampling and artificial feeding techniques.

FORESEEN PUBLICATIONS/ARTICLES RESULTING FROM THE STSM (IF APPLICABLE)

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

CONFIRMATION BY THE HOST INSTITUTION OF THE SUCCESSFUL EXECUTION OF THE STSM

I herein confirm the present report regarding the COST-STSM-ECOST-STSM-TD1303-130414-044119 in Portugal.

Évora, 16th of May 2014

Helder Carola Espiguinha Cortes

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The Applicant is grateful to the COST action for the essential support to the STSM, to Prof. Helder Cortes, the Host Coordinator from the University of Evora for hospitability and excellently organized mission and to all of the participants for collaboration and sharing their knowledge and experiences in their field of expertise.

Report done on May, 13th, 2014

Applicant and STSM participant:

Dr Aleksandra Ignjatović Ćupina