

STSM report Dušan Petrić

□ Purpose of the STSM

Short Term Scientific Missions (STSM) “A multinational field parasitological expedition on the track to the vector of *Onchocerca lupi*” (Host institution: Universidade de Évora; Period: 13/04/2014 to 18/04/2014; Reference code: COST-STSM-ECOST-STSM-TD1303-130414-044130; Approved amount: EUR 800) in collaboration with Prof. Helder Cortes and according to the Work Plan previously provided.

Short Term Scientific Missions was aimed at strengthening the existing networks and fostering collaborations by allowing scientists from other participating COST countries to visit University of Evora that provided opportunities to work on neglected parasite *O. lupi* and research aimed to discovery of its vector. Research done contributed 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). It also allowed participants to exchange experience in laboratory and field research methods, learn new techniques and gain access to specific environmental/epidemiological conditions concerning parasite and vectors, not available in their own countries. During the study, the applicant exchanged its experience in the field of insect vectors. In particular, he trained two PhD students on the study of vector sampling techniques and vector identification, as well as one ESRs.

□ Description of the work carried out during the STSM

a) Field sampling of haematophagous insects

The applicant sampled and trained other participants on the sampling procedures of haematophagous insects (i.e., simuliids, biting midges, mosquitoes and sand flies) from the urban and rural environment by using NS2 type dry ice baited traps, at specific sampling points, according to the insects’ behaviour. At the end of each sampling, insects were identified and stored in entomological cages, which were covered with a wetted towel, in order to maintain proper ventilation and humidity in the vivarium. In addition, pupae of black flies were collected every day directly from the water and freshly emerged insects were kept in individual boxes. Once in the laboratory, all specimens were separated according to their families and stored into *vivaria*. All specimens that died before the artificial feeding (see below) were morphologically identified to species level and stored in individual eppendorf tubes, containing 70% ethanol to be molecularly screened for presence of helminths DNA. Female insects were allowed to feed on a *O. lupi* positive dog (after owner consent), which was physically restrained into a confined tent. Plastic tubes containing the blood-sucking insects were placed on the dog shaved back. In addition, some

specimens were released in the tent and collected the day after and stored in plastic cages.

b) Rearing of the haematophagous insects

Rearing of separate individuals of blackflies is important method used for discrimination between species with similar morphology. Mature pupae and farate adult forms that may be observed inside the integument, were placed on wet filter paper put inside plastic tubes closed by cotton. This method of rearing allows observation of both adult and pupal exuvia morphological characteristics. In order to acquire as much as possible adult blackflies for feeding on the infected dog (under consent of the owner of the dog), beside application of dry ice baited traps, massive rearing of pupae to adult stage was conducted. Collected pupae of blackflies attached to the natural supporting material (aquatic plants) were kept in humid conditions in plastic cages to emerge. Part of the containers with pupae were also positioned outdoors, in the closed tent with a dog inside.

c) Identification of haematophagous insects

Identification of the adult stage of haematophagous insects was 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). Adults of blackflies reared from mature pupae were also identified. Detailed observation of the morphological characteristics was conducted by the use of stereo microscope. Applicant provided training in mosquito adults identification to other participants of STSM.

d) Procedures on dogs

The applicant was trained by the other participants on the sampling procedures to 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 inter-scapular region of dogs. All animals lived in endemic areas for canine onchocercosis, where an overall prevalence of up to 8.4% has been recorded.

e) Laboratory procedures of microfilariae detection

Applicant was also trained in a Knott-modified test in order to detect microfilariae in the blood. In addition, he participated screening of the skin samples for the presence of skin-dwelling microfilariae, according to the standard procedures. Briefly, 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 derma to the 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**

During the STSM, the following insect species were collected:

- Mosquitoes: *Ochlerotatus caspius*; *Culex pipiens pipiens*; *Ochlerotatus detritus*.
- Blackflies: *Simulium pseudequinum*; *Simulium intermedium*; *S. velutinum*

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 baina* and *Cercopithifilaria* sp. Il sensu Otranto et al. 2012.

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

□ **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-TD1303-130414-044130 in Portugal.

Évora, 16th of May 2014



Helder Carola Espiguiinha Cortes