Short-Term Scientific Mission
A multinational field parasitological expedition on the track of the vector of *Onchocerca lupi*
14th- 19th April 2014
Scientific report

STSM applicant: ALESSIO GIANNELLI
Below, the report of the activities performed by the COST STSM applicant, Dr. Alessio Giannelli (ref code: COST-STSM-ECOST-STSM-TD1303-140414-044120), is provided. The scientific mission was carried out at the University of Èvora (Portugal), under the supervision of Prof. Helder Cortes, and according to the Work Plan previously provided.

Purpose of the STSM
The scientific mission aimed at identifying the vector of *Onchocerca lupi*, a neglected nematode infesting dogs with a concrete pathogenic potential, in order to elucidate the biology of this neglected filarioid of dogs. Accordingly, the applicant was trained on the diagnosis of filarioid infections in dogs, as well as on the collection of haematophagous insects, which have been suggested as putative vectors of *O. lupi*.
The research was framed into the TD1303 COST Action objectives of WG1 (One Health in the ecology of vector-borne diseases), as well as of WG (studies on rare and emerging vector-borne pathogens). In addition, this study allowed the applicant to exchange its experience in lab and in the field, learning new techniques and experiencing the specific environmental/epidemiological conditions.

Description of the activities carried out during the STSM
The working activities have been divided into three different main topics:

1) *Procedures on dogs*
The applicant was trained on the sampling procedures to diagnose canine filarioids in dogs. In particular, he collected blood samples from dogs, after owners consent, living in areas close to forests and rivers. All samples were examined for the presence of *Dirofilaria immitis*, *Dirofilaria repens* and *Acanthocheilonema reconditum* microfilariae. In addition, skin samples were collected using individual 3 mm 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 in the previous year (**Otranto et al., 2013**).
Owned and sheltered dogs living in the region of Algarve (southern Portugal) were sampled, pending the owners' consent, being a complete anamnesis and clinical history recorded for each dog. In addition, blood samples preserved in EDTA tubes and sera were collected, as well as ectoparasites (i.e., ticks and fleas). At the end of the fieldwork, a total of 10 dogs were sampled.

2) *Laboratory procedures*
A Knott-modified test was performed in order to detect microfilariae in the blood. In addition, skin samples were screened for the presence of skin-dwelling microfilariae, according to the procedures described in literature (**Otranto et al., 2013**). 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 (**Table 1**). In particular, microfilariae of *O. lupi*; *Cercopithifilaria bainae* and *Cercopithifilaria* sp. II sensu Otranto et al. 2012 were detected and identified.
In addition, each dog was screened for the presence of blood microfilariae, using the Knott modified method. Briefly, 1 ml EDTA blood was added to 9 ml of distilled water, and after centrifugation (3000 rpm×5 min), microfilariae were checked on the sediment and identified according to morphological keys (**Euzeby, 1981**). Microfilariae of *Dirofilaria immitis* were detected in a dog co-infested by *O. lupi*.

Finally, dogs were infested by ixodid ticks, which were morphologically identified as *Rhipicephalus* sp. II (**Dantas-Torres et al., 2013**)

3) *Collection of insects*
Haematophagus insects (i.e., simulids, biting midges, mosquitoes, and sand flies) were collected by using dry ice baited traps from the environment, in 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 and stored in individual eppendorf tubes, containing 70% ethanol to be molecularly screened for presence of helminths DNA. Female insects were allowed to fed on a *O. lupi* positive dog, 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.

Main results
During the STSM period, 10 dogs were sampled and those that scored positive for microfilariae are reported in the following. All species were morphologically and morphometrically identified.

Dog 1
Name: Old; **Age**: *circa* 10 years old; **Gender**: male; **Anamnesis:** The dog displayed weight loss, ocular discharge; alopecia; lymph node enlargement; pale mucous and was infested by ticks; **Result of the blood analysis**: negative for the presence of microfilariae; **Result of the skin snip**: 20 microfilariae *O. lupi*/20µl. **Collected ticks:** 6 engorged female, 12 un-engorged female and 8 male.

Dog 2
Name: Peluda; **Age**: *circa* 8 years old; **Gender**: female; **Anamnesis:** The dog displayed alopecia; lymph node enlargement; pale mucous and the presence of ticks; **Result of the blood analysis:** negative for the presence of microfilariae; **Result of the skin snip**: 16 microfilariae *Cercopithifilaria* sp. II /20µl. **Collected ticks:** 3 engorged female, 9 un-engorged female and 3 male. These specimens will be dissected 30 days post-collection (i.e., 17 May 2014) in order to describe, for the first time, developing larval stages of *Cercopithifilaria* sp. II.

Dog 3
Name: Nice; **Age**: *circa* 3 years old; **Gender**: Female; **Result of the blood analysis:** negative for the presence of microfilariae; **Result of the skin snip**: 8 and 4 microfilariae *O. lupi* and *C. bainae* in 20 µl, respectively. **Collected ticks:** 2 males. After tick dissection no developing larvae were detected.

Dog 4
Name: Cento; **Age**: *circa* 2 years old; **Gender**: Male; **Result of the blood analysis:** *D. immitis* microfilariae; **Result of the skin snip**: 10 microfilariae *O. lupi*/20µl.

Identification of insects

The participant was addressed on the sampling and identification procedures of blackflies and mosquitoes. During the STSM, he was able to identify the following insect species:

- Mosquitoes: s*Ochlerotatus caspius*; *Culex pipiens; Ochlerotatus detritus*
- Blackflies: *Simulium pseudoequinum; Simulium intermedium*.

The identification of immature stages of blackflies (i.e., larvae and pupae) is currently in progress at the University of Bari (Italy), while the detection of *O. lupi* developing larvae in fed blackflies is carried out at the University of Novi Sad (Serbia).

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-TD1303-140414-044120 in Portugal.

Évora, 16th of May 2014

Helder Carola Espiguinha Cortes