



17.10.2014 Egypt, Murcia

To:

MC Chair

Prof. Dr. Andrei Mihalca

STSM coordinator

Prof. Dr. Dusan Petric

STSM scientific report

Title of the STSM: Habitat characterization and *Leishmania* spp. diagnosis in sandflies in periurban residential areas in southeast Spain.

Reference code: COST-STSM-ECOST-STSM-TD1303-010914-048433

Period: from 01/09/2014 to 10/10/2014

Host institution: Animal Health Department, Faculty of Veterinary Medicine, Murcia University, Murcia, Spain.

Host professor: Prof. Dr. Eduardo Berriatua, Animal Health Department, Murcia University, berriatu@um.es

Purpose of the STSM

My STSM has been performed at animal health department (parasitology section), faculty of veterinary medicine, Murcia University, Spain. The purpose of STSM was to develop a theoretical and practical training on (i) sampling and morphological speciation of sandfly species, (ii) *Leishmania* spp. infection PCR diagnosis in sandflies and mammal hosts, (iii) epidemiological and ecological data analysis.

Description of the work carried out during the STSM



My STSM allowed me to become involved in several aspects of *Leishmania* spp. and sandfly research including: (i) an investigation of sandfly abundance and distribution, (ii) *L. infantum* infection diagnosis in sandflies, dogs and rodents, (iii) isolation and culture of *L. infantum* from infected tissues, (iv) entomological and epidemiological data analysis.

During the first week of my stay I received formal lectures on basic epidemiological parameters and their applications in disease investigation and an introduction to the use of databases and statistical softwares EpiInfo and R. From the second week until the end of my STSM I participated on field trips to sample for sandflies and carried out morphological speciation of sandflies in the laboratory. On two occasions we visited Murcia's Zoonosis Control Centre to examine and collect samples from *Leishmania* infected dogs and learned to isolate and culture *Leishmania* strains from infected dogs. At the end of my STSM I carried out DNA purification and real-time PCR to diagnose infection in samples from dogs, sandflies and rodents.

Description of the main results obtained

I developed knowledge on the following issues:

- strategies to design ecoepidemiological studies.
- procedures to collect sandflies using sticky traps.
- identification of sandfly species using morphological parameters.
- DNA purification from vectors and hosts.
- Real time PCR analysis and principles of barcoding speciation methods.
- Clinical signs associated with *L. infantum* infection in dogs.
- Isolation and culture of *L. infantum* from infected tissues.
- Epidemiological and ecological data description and analysis using R and Epi-info programs.

Specific results obtained:



1. Entomological study of sandflies in periurban residential areas in Murcia

I participated in a longitudinal study of sandfly abundance, distribution and risk factors in the external environment of residential detached homes in periurban environments in Murcia, southeast Spain. We worked in 20 homes some with and other without dogs, and placed between 6 and 14 sticky traps in different places of the house plot including areas with dry and irrigated vegetation, log piles, window sills, dog and garden sheds, etc. Traps were changed weekly (starting 02/9/2014 till 09/10/2014) and during the 40 days we placed 875 traps averaging 175 traps per week. During my stay I was able to analyse the 300 traps collected during the first two weeks and we found 358 sandflies including 157 females (44%) and 201 males (56%) The species identified morphologically included *Sergentomyia minuta*, *Phlebotomus perniciosus* and *Phlebotomus papatasi*. The first is a vector of *Sauroleishmania* and *P. perniciosus* is the main vectors of *Leishmania infantum* in Spain. We found that sandfly abundance was greatest in covered areas, in the proximity of walls. Further analysis of the remaining traps need to be carried out for a more detailed description of ecological factors associated to sandfly abundance can be reached.

2. Diagnosis of *L. infantum* infection and culture isolation of the parasite

This part of the project focused on identifying clinical signs of *L. infantum* infection in dogs, developing real time PCR diagnosis on samples from clinical and subclinical infection from dogs and rats and vectors, and on isolation and culture of the parasite in laboratory culture media. I was able to examine dogs with typical clinical signs of *L. infantum* including weight loss, exfoliative dermatitis, onicogriphosis, conjunctivitis and lymph node enlargement. Three animals were euthanized on welfare grounds and I took spleen, bone marrow and lymph node tissue under sterile conditions. Samples were cultured in NNN (Novy-MacNeal-Nicolle) medium and promastigotes were observed 1 week later. In addition, we examined the role of rats as potential



reservoirs of *L. infantum* infection by analyzing infection in spleen samples from 48 individuals raised in a falcon farm. This was done by real-time PCR which involved DNA extraction and purification using a robot (Maxwell, Promega), measuring its concentration and purity using the nanodrop spectrophotometer and amplification of kinetoplast DNA fragment using a TaqMan probe test. As positive a control for DNA purification and PCR analysis we used laboratory *L. infantum* infected *P. perniciosus* (kindly provided by the research group at Prague University members of TD1303 Cost action). *L. infantum* DNA amplified from the sandflies and the dog samples but not from rats suggesting that they were not infected.

Future collaboration with the host institution (if applicable)

The STSM has been an excellent opportunity to develop basic knowledge on sandfly sampling and identification and ecological and epidemiological research of *L. infantum* infection. This is a stepping stone from which I will begin a survey of sandfly and Leishmania infection in Egypt with the support of Dr Berriatua and his team. A preliminary step will be to retrieve and publish the information available in Egypt on this aspects and the aim is to work together on a review paper on *Leishmania* infection in humans and animals in Egypt, where disease is considered sporadic.

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

In addition to the above review to describe available studies and reports on sandfly and Leishmaniosis in Egypt, we will coauthor an article on ecological and epidemiological analysis of sand flies habitat characterization in periurban residential area in southeast of Spain.

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



Sherif has been a hardworking and fast learning student and has very successfully executed the tasks previewed for his STSM, performing very well in both the field and the laboratory.

Other comments (if any)

From Murcia's perspective the STSM was very fruitful and our laboratories and expertise remain open to further STSM within COST action.

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Applicant
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