



25.7.2014 Prague, Murcia

STSM scientific report

Title of the STSM: Epidemiology and diagnosis of *Leishmania infantum* infection and ecology of vector sandfly species in Spain.

Reference:ECOST-STSM-TD1303-280414-044115

STSM dates: from 28-04-2014 to 26-06-2014

Location: Animal Health Department, Murcia University, Murcia, Spain

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

Purpose of the STSM

My STSM took place at Faculty of Veterinary Medicine, University of Murcia in Spain (from 28.4. to 26.6.). Purpose of the STSM was received theoretical and practical training on (i) general epidemiological concepts, (ii) data description and their analysis using free software R (R-project), (iii) sampling and morphological speciation of sand fly species and (iv) molecular diagnosis of *Leishmania* infection in sand flies.

Description of the work carried out during the STSM

During first weeks of my stay I became acquainted with the objectives of epidemiological research and attended lectures on basic epidemiological parameters and their application to disease investigation. From the third week of May until the end of my STSM I carried out field and laboratory work on an on-going entomological study of sandfly distribution and *Leishmania* infection in Murcia. In addition, I was able to complete the epidemiological analysis and write scientific article on a study of the dynamics and diagnostic potential of antibodies against sandfly salivary gland antigens in dogs from a *Leishmania infantum* endemic area.

Description of the main results obtained

This work has allowed me to develop expertise on the following scientific subjects as described on the STSM proposal:



1. Objectives of epidemiological and ecological research.
2. Epidemiological study types.
3. Sample size calculations in epidemiological studies.
4. Epidemiological and ecological data description using the R program (*R Development Core Team*).
5. Analysis of causal relationships including the use of multivariable regression models using R.
6. Validation of diagnostic tests using ROC (Receiver Operating Characteristic) analysis
6. Sandfly abundance and distribution study design.
7. Morphological speciation of sandflies.
8. Real time PCR diagnosis of *Leishmania* spp. infection in sandflies.

1. Entomological study of sandfly distribution and Leishmania infection in Murcia

The study involved investigating sandfly distribution in residential detached houses in the outskirts of Murcia where *L. infantum* incidence is particularly high. During six weeks between May and July we placed between 6-14 sticky traps in each of 9 study houses and traps were changed weekly. We isolated 877 sandflies of the following species: *Phlebotomus perniciosus*, *Phlebotomus papatasi*, *Phlebotomus sergenti* and *Sergentomyia minuta*. The most abundant species was *S. minuta* and although the ratio of females and males was almost 1:1 the great majority of females belong to species *S. minuta*. This species is a recognised vector for *Sauroleishmania* but not of *L. infantum*. PCR tests were carried out in 70 *S. minuta* females and in 10 laboratory *L. infantum* infected *P. perniciosus* females. *Leishmania* amplification was detected only in laboratory infected *P. perniciosus* and not in field sandflies, suggesting infection rate in *S. minuta* is low. Further PCR tests in *P. perniciosus* are required to estimate *L. infantum* infection in *P. perniciosus*. In addition I collaborated in a preliminary analysis of environmental factors associated with sandfly distribution in study houses which indicated that sandfly abundance was highest in areas close to walls and protected from direct sun light and with abundant irrigated vegetation.



*II. Dynamics and diagnostic potential of antibodies against sandfly salivary gland antigen in dogs in an *L. infantum* endemic area*

The results from this study were analysed using advanced statistical techniques including multilevel regression models, and written up for publication in a scientific journal. This was a two year longitudinal study in experimental dogs kept in an *L. infantum* endemic area in Italy. Dogs were tested for *L. infantum* by indirect immunofluorescence antibodies (IFI), PCR and culture and sandfly antibodies were measured by recombinant enzyme-linked immunosorbent assay (ELISA). The study shows that it is possible to use recombinant ELISA tests to detect antibodies against sandfly salivary gland antigens in dogs with a good sensitivity and specificity. Dogs develop antibodies shortly after exposure but their concentration increased very significantly after the second year of exposure possibly associated to hypersensitivity inflammatory reactions. Moreover, antibody concentration was associated to clinical *L. infantum* infection. This could indicate that *L. infantum* infection in dogs could be related to constant reinfections or alternatively, that dogs developing leishmaniasis, which are known to be prone to developing non-effective antibody responses to the parasite, also develop strong humoral responses to sandfly bites, more so than dogs not developing leishmaniasis.

Future collaboration with the host institution (if applicable)

The STSM has been an excellent opportunity to develop an ongoing collaboration on sandfly ecology and epidemiology of *L. infantum* infection. Infected sandflies provided by the Czech group have allowed setting up *L. infantum* PCR to diagnosis infection in sandflies in Murcia. On the other hand the Czech student has benefitted from epidemiological data analysis and gained experience on performing entomological studies in *L. infantum* endemic areas.

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

We have collaborated in the writing up of the results from the above mention study on dog's antibody response to sandfly salivary gland antigens. A further article will be prepared in collaboration on the results of the previously described entomological study. We also expect to complete a study comparing several methods of DNA extraction from *L. infantum* infected sandflies.



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

Tatiana has been an outstanding student and has very successfully executed the tasks previewed for her STSM. Her learning of basic and advanced epidemiological analysis and the use of the “R” program has been remarkable. In addition, she performed very well in the laboratory when carrying out the identification and morphological speciation of sandflies and in the diagnosis of *L. infantum* infection in sandflies using real-time PCR methods.

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 TD 1303.

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Profesor of Animal Health.

Mgr. Tatiana Košťálová