UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

DIPARTIMENTO DI MEDICINA VETERINARIA

COST Action Number TD1303 European Network for Neglected Vectors and Vector-Borne Diseases (EURNEGVEC)

STSM Report: Ivonne Pamela Llanes Acevedo

Subject: Short Term Scientific Mission

STMS Title: Diagnosis of canine vector-borne diseases in animals and detection in their ectoparasites from the Mediterranean area (WG4)

Beneficiary (Applicant): Ivonne Pamela Llanes Acevedo, Instituto de Salud Carlos III, Madrid- Spain
Reference: COST Action TD1303
Host institution: University of Bari- Italy, Department of Veterinary Medicine
Person responsible: Domenico Otranto (domenico.otranto@uniba.it)
Period: 10/10/2014 to 11/11/2014
Reference code: COST-STSM-ECOST-STSM-TD1303-011014-044378 Amount up to: EUR 2150

PURPOSE OF THE STMS

Short Term Scientific Missions (STSM) "Diagnosis of canine vector-borne diseases in animals and detection in their ectoparasites from the Mediterranean area" was aimed at strengthening the existing networks between laboratories and fostering collaborations by allowing scientists from other participating COST countries to visit University of Bari (Italy) that provided opportunities to get trained on entomology and parasitology education.

The research done contributed to the TD1303 COST Action objectives of "One Health" concept in the ecology of vector-borne diseases (WG1) and investigation of emerging vector-borne pathogens (WG4). In addition, the scientific mission allowed the participant to exchange experience in laboratory, research methods, learn new techniques about parasites and vectors and their epidemiological aspects. During the STMS, the researcher was trained to acquire relevant theoretical knowledge and practical training on European ticks' fauna and phlebotomine sand flies, especially on species of medical and veterinary importance.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSM

During the first week, the researcher attended lectures to furnish background information on biology and epidemiology for both ticks (Acarina: Ixodidae) and phlebotomine sand flies (Diptera: Psychodidae). From the second week until the end of the STSM, the attendant acquired and improved her practical skills of taxonomy, morphological identification, sampling and preservation procedures of these vectors:

1. <u>Acarina: Ixodidae</u>

a) Sampling of ticks

Thirty one ticks were collected from urban to rural areas (Table 1) from different regions of Spain, before starting the STMS at the University of Bari. This was the starting sample for future research work along the STSM.

b) Identification of immature and adult ticks

Once in the laboratory of the Department of Medicine Veterinary, University of Bari, all specimens collected from Spain (n=31) were separated according to their developmental stage. Furthermore, the applicant had free access to the tick collection stored at the host institution and at least 2 or 3 representative specimens (female, male, nymphs, larvae) of each species belonging to family *Ixodidae* were examined. Hence, the training in identification was extended to a higher number of species, including those that were not found in the sample.

<u>Morphological training-study</u>:

In order to achieve a more precise observation of morphological features some tick specimens (larva and nymph) were mounted using the procedure below:

1. Specimens were clarified in lacto-phenol for two hours.
2. Then were placed on a slide with a drop of Hoyer´s medium.
3. The specimen was placed on the dorsal view and a coverslip on its top.
4. Ticks were observed under a light stereomicroscope.

All ticks were morphological identified using keys and descriptions related to European ticks` fauna provided by Manilla et al. (1998) and Walker et al. (2000). The following characters were examined and/or measured: idiosoma (length and width); dorsal scutum (length and width, punctuation pattern, and shape of posterior margin in females); basis capituli (length and width); angles of basis capituli (position and shape); hypostomal dentition (number of rows); female porose areas (shape and distance between the two areas); female genital opening (shape); spiracular plates (shape), dorsal tail of spiracular plates (width); first festoon (width); lateral and postmediam grooves (shape); cervical pits (shape); cervical fields (shape); internal and external cervical grooves (shape and punctuation pattern); marginal lines (shape and punctuation pattern); male adanal plates (length, width at base, and presence/absence of median cusps on them); accessory plates (shape); male caudal process (presence/absence); spur on trochanter I (presence/absence); and body colour
(pattern).

Reference:

- Manilla G: *Fauna d'Italia. Acari-Ixodida*. Bologna: Ed. Calderini; 1998.
- Cringoli G, Iori A, Rinaldi L, Veneziano V, Genchi C. *Zecche Ixodidae*. Mappe Parassitologiche. Series editor. Università degli Studi di Napoli "Federico II". 2005.
- Walker JB, Keirans JE, Horak IG: Genus *Rhipicephalus (Acari, Ixodidae).* A guide to the brown ticks of the world. Cambridge: Cambridge University Press; 2000.

Table 1. Species of ticks (n = 31) included in this study, with data on hosts and geographical origin.

Collected Date	Collected Number	Locality	Province	Host	Species Identification	Gender	
						F	M
15/03/2014	2	Fuenlabrada	Madrid	Dog	*Rhipicephalus turanicus* *Rhipicephalus sanguineus*		2
17/04/2014	2	Alcala de Xivert Alcossebre	Castellón	Environment	*Rhipicephalus turanicus* *Hyalomma excavatum*	1	1
19/05/2014	1	Bochones	Guadalajara	Human	*Rhipicephalus sanguineus*		1
09/07/2014	2	Santa Maria- La Padilla	Madrid	Horse	*Rhipicephalus bursa* *Hyalomma marginatum*		2
24/05/2014	18	Navalagamella	Madrid	Cow	*Hyalomma excavatum* *Hyalomma marginatum*	11	7
20/05/2014	1	El Escorial	Madrid	Human	*Rhipicephalus* spp.	1	
09/07/2014	5	Santa Maria- La Padilla	Madrid	Horse	*Rhipicephalus* spp. *Rhipicephalus bursa* *Hyalomma marginatum*	1 1 1	2

2. <u>Diptera: Psychodidae</u>

a) Context and objective

Phlebotomine sand flies are small blood-feeding insects of medical and veterinary significance. Since the spread of leishmaniasis largely depends on the distribution of the vector species, the identification of circulating sand flies in endemic and areas is important for predictions of the risk and expansion of the disease. Sand flies are typically identified based on morphologic characteristics; mainly internal structures such as the cibarium, pharynx, spermatheca of females and terminal genitalia of males. Morphological classification requires considerable skill and taxonomic expertise, and has the potential risk of misidentification. During the STMS the priority of training was focus on identification the most prevalent phlebotomine species in the Mediterranean region as: *Phlebotomus perniciosus, Phlebotomus perfiliewi, Phlebotomus ariasi, Phlebotomus neglectus, Phlebotomus papatasi, Phlebotomus mascittii, Phlebotomus sergenti* and *Sergentomyia minuta*.

b) Mounting and Identification of phlebotomine sand flies

Phlebotomine sand flies examined for the morphological identification are preserved in the parasitological collection at the Department of Medicine Veterinary, University of Bari. Sand fly specimens have been collected during previous studies using sticky traps and light traps. For species identification, the external genitalia of males and head and posterior end of abdomen (last two tergites) of females were dissected using entomological needles and cleared with 10% potassium hydroxide solution at room temperature for 2 h. Then, the material was washed with water for 1–2 min, immersed in 10% aqueous solution of glacial acetic acid for 30 min, washed with water for 30 min and finally slide-mounted in Hoyer's solution as recommended by Lewis (1973). Identification was performed using the morphological keys provided by Killick-Kendrick et al. (1991) and Romi et al. (1994).

Reference

- Lewis, D.J., 1973. Phlebotomidae and Psychodidae (sand-flies and moth-flies). In: Smith, K.G.V. (Ed.), Insect and Other Arthropods of Medical Importance. British Museum (Natural History), London, pp. 155–179.
- Killick-Kendrick, R., Tang, Y., Killick-Kendrick, M., Sang, D.K., Sirdar, M.K., Ashford, .W., Schorscher, J., Johnson, R.H., 1991. The identification of female sandflies of the subgenus *Larroussius* by the morphology of the spermathecal ducts. Parassitologia 33, 335–347.
- Romi, R., Khoury, C., Bigliocchi, F., Maroli, M., 1994. Schede guida su acari e insetti di interesse sanitario. Rapporti Istisan 94/8, Roma.

c) Other laboratories procedures

The applicant was trained by other STSM participants, experts, and academic staff in veterinary medicine and parasitologists, on sampling procedures of many topics of the researches on-going at the Department. The applicant developed skills in molecular detection of other vector borne pathogens, identification of Metastroingiloydea of cats (*Troglostrongylus brevior*) and infestation of snails with L1 of these parasites, detection of *Cercopithifilaria* spp., etc.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Five tick species were identified by morphological analysis out of 31 samples collected: *R. bursa, R. turanicus, R. sanguineus, Hyalomma excavatum* and *Hyalomma marginatum.*

On the other hand, this training period has allowed the participant to develop expertise on the following scientific subjects as described on the STSM proposal:

- Identification of tick species infesting dogs in the Mediterranean area, acquired skills in sampling and identification procedure of variety of species in the collected material, including mainly genera of ticks: *Ixodes, Rhipicephalus, Dermacentor, Hyalomma, Haemaphysalis.*
- Identification of the most prevalent phlebotomine sand fly species living in southern Europe.
- Development of skills in molecular detection of other vector borne pathogens, identification of Metastroingiloidea of cats (*Troglostrongylus brevior*) and infestation of snails with L1 of this parasite, detection of *Cercopithifilaria* spp., etc.
- STSM has been an excellent opportunity to gain expertise to perform entomological studies in ticks and sandflies.
- Collaboration between laboratories is established.

FUTURE COLLABORATION WITH THE HOST INSTITUTION (IF APPLICABLE)

New project calls and opportunities will be searched to extend the collaboration between host and participant institutions. We are willing to extend the study areas to other Spanish regions and also perform more detailed analyses such as molecular identification for genetic comparison of vector populations between countries.

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

A further article will be prepared in collaboration; this will be based on the described and projected entomological study on ticks from Spain. We also expect to complete our sample to prepare the publication.

OTHER COMMENTS

I would like to thank COST for funding the STSM TD1303-011014-044378 that has allowed me to carry out this important and fruitful stay research.

My special gratitude to Mr. Prof. Domenico Otranto for welcoming me into the laboratory, and his team; particularly to PhD. Viviana Tarallo for availability and collaboration and his PhD Students Rafael Ramos and Alessio Giannelli who helped me in gaining many skills necessary in this research.

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

I herein confirm the present report regarding the COST-STSM-ECOST-STSM-TD1303011014-044378.

Valenzano, 10th of November 2014

Applicant and STSM participant: Ivonne Pamela Llanes Acevedo

Validated by: Prof. Domenico Otranto