

**Short Term Scientific Mission Report**  
**Prevalence and geographic distribution of vector-borne diseases and its co-infection in domestic dogs and wild reservoirs from the inland region of Portugal - Serological and morphological approaches**  
**15<sup>th</sup> September – 3<sup>rd</sup> October 2014**

**STSM applicant:** Ana Margarida Pignateli Vasconcelos de Assunção Alho

**Host Institution:** Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland

**WG where the application's topic belongs:** WG1 - The “One Health” concept in the ecology of vector-borne diseases

**Purpose of the STSM:** Canine Vector-Borne Diseases (CVBDs) are an emerging problem in Veterinary Medicine and their zoonotic potential poses a global threat to human health (Otranto et al, 2009; Otranto et al, 2009b). As a consequence of environmental and climatic changes, increased drug resistance among both vectors and pathogens, enhanced international mobility of companion animals along with social and cultural human changes, CVBDs are spreading worldwide, becoming highly prevalent and distributed in a short space of time (Knols and Takken, 2007; Beugnet and Marie, 2009).

The successful diagnosis, treatment and prevention of these infections are extremely dependent upon firm awareness by owners and practitioners. Despite the advances that have been made in molecular and serology fields related to these infections, there are still some areas that had not followed the trend. Portugal is an endemic country for several CVBDs (Cardoso et al., 2012), mainly due to its mild Mediterranean climate that promotes favourable conditions for vectors development and survival. Co-infection is also a frequent event in dogs, since several arthropods are competent vectors of more than one pathogen and share the same environment. Co-infections represent a major issue considering the synergistic and complex disease expression in sick dogs, impairing the achievement of a definitive diagnosis and selection of proper therapeutic agents.

However, accurate data concerning national prevalence and geographic distribution of different CVBDs and co-infections is scarce, and the existing studies are merely punctual and fragmentary. Particularly, at the inland regions, data on the distribution of arthropods and CVBDs is anecdotal and outdated, probably as a result of poorly implemented veterinary diagnostic services and a lack of surveillance at local or regional levels. Besides these factors, the impact of the recent socio-economic crisis in Portugal has resulted in a reduction of the number of prophylactic treatments, namely those directed against vectors (Madeira de Carvalho et al., 2013).

For the aforementioned reasons, a large-scale epidemiological survey was designed in order to address the actual problem, characterizing the risk of many CVBDs and overcoming the missing data. Therefore, a multicenter study was carried out, in stray dogs maintained in public shelters from the Northern, Central and Southern inland regions of Portugal, screening the prevalence and geographical distribution of CVBDs that affect these neglected regions.

The present project proposal will allow a better understanding of CVBDs currently present in Portugal, bringing new insights concerning the complex interactions between pathogens. Accurate and updated data will be important in establishing the best strategies for effective treatment and control approach to these challenging diseases, promoting higher health standards and overcoming general unawareness of owners and practitioners.

### **Description of the work carried out during the STSM**

---

**Task 1) Sample collection:** A multicentre study was carried out in stray dogs maintained in public shelters from the Northern, Central and Southern inland regions of Portugal (Bragança Guarda, Castelo Branco, Portalegre, Évora, Beja and Faro), totalling 248 shelter dogs from seven National districts. Animals were clinically examined and a complete record was kept for each dog. Blood collection was performed (2-5 ml from the cephalic vein) and serum and plasma were separated.

Samples obtained from wild reservoirs were very few since hunting season was already finished when the STSM grant was attributed. For that reason, the analysis of the samples from wild reservoirs was not included in this report.

**Task 2) Prevalence assessment of *Dirofilaria immitis*** circulating antigen using an antigen commercial kit. In order to clarify *Dirofilaria* species currently circulating in the country, the Modified Knott's technique was performed to concentrate and distinguish microfilariae of *D. immitis*, *D. repens*, *A. dracunculoides* and *A. reconditum*, based on morphometric criteria published by Magnis et al. 2013. (Task performed at the Laboratory of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine, Lisbon University, Portugal)

**Task 3) Seroprevalence assessment of *Leishmania infantum* using an enzyme-linked immunosorbent assays (ELISA).** Sera was tested using an ELISA based on soluble antigens derived from promastigote-derived antigens of *L. infantum*, as described by Mettler et al., 2005. (Task performed at the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland)

**Task 5) Seroprevalence assessment of *Angiostrongylus vasorum* using combined detection of circulating *A. vasorum* antigen and parasite-specific antibodies, using enzyme-linked immunosorbent assay (ELISA).** Sera was tested for circulating *A. vasorum* antigens using mono and polyclonal antibodies in a sandwich-ELISA (sensitivity 95.7%, specificity 94.0%), as described by Schnyder et al., 2011. Concurrently, serum was tested for specific antibodies against purified *A. vasorum* adult stage antigen (sensitivity 85.7%, specificity 98.8%), as described by Schucan et al., 2012. (Task performed at the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland)

**Task 6) Data analysis, manuscript writing and submission.** All data and results were gathered and statistical analysis are being performed, describing and comparing prevalence in the surveyed regions, using the programs SPSS (Statistical Package for the Social Sciences) IBM v.21.0 and SAS (Statistical Analysis Software) v. 9.3.

### **Description of the main results obtained**

---

A total of 248-dog samples were collected in shelters of the inland region of Portugal, 112 females and 136 males, with an average age of 4.1 years old.

-Regarding the assessment of *D. immitis*, an average prevalence of 2.8% was found in the dogs belonging to the seven districts surveyed, using a female *D. immitis* antigen kit and the Knott test. The district with the highest prevalence was Beja (9.1%), followed by Guarda (6.7%), Faro (2.7%) and Castelo Branco (2.5%). No dirofilariosis was found in Bragança, Portalegre and Évora. Using the Knott test, it was also possible to observe *Acanthocheilonema* spp. microfilariae, in the samples collected in Beja and Faro districts, with a prevalence of 4.5% and 2.7%, respectively. No *D. repens* was found in any of the seven districts surveyed.

-Considering the assessment of *L. infantum*, an average prevalence of 18.4% was found in the dogs belonging to the districts surveyed, using the ELISA technique and considering a cut-off value higher than 0%. The district with the highest prevalence was Guarda (33.3%), followed by Faro (27.0%), Beja (24.4%), Bragança (19.2%), Portalegre (15.2%) and Castelo Branco and Évora, both with 10.0%. Positive samples do *L. infantum* were found in all the seven districts surveyed, confirming the endemicity of this disease and its wide distribution all over Portugal.

-Confirmation of the positive samples to *A. vasorum* antibody and antigen using a sandwich ELISA is still in progress at the Institute of Parasitology, University of Zurich, Switzerland. Results will be available soon.

After gathering all the results, a complete statistical analysis will be performed to assess CVBDs co-infection and risk factors in domestic dogs from the inland regions of Portugal.

### **Foreseen publications/articles resulting from the STSM**

---

The results of this STSM will be published in 2 international peer-reviewed journals and presented in international conferences.

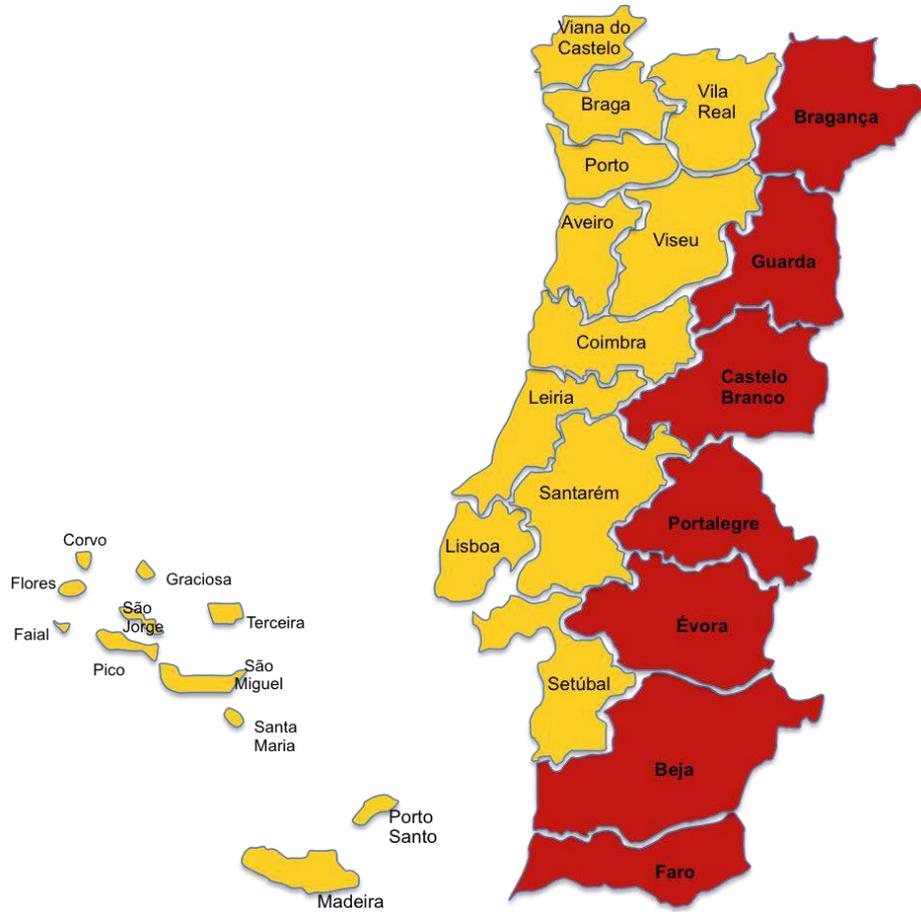
### **References**

---

- Beugnet, F., Marie, J. (2009). Emerging arthropod-borne diseases of companion animals in Europe. *Veterinary Parasitology*, 163, 298–305.
- Cardoso, L., Mendão, C., Madeira de Carvalho, L. (2012). Prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, *Borrelia burgdorferi* sensu lato, *Anaplasma* spp. and *Leishmania infantum* in apparently healthy and CVBD-suspect dogs in Portugal - a national serological study. *Parasites & Vectors*, 5, 62.
- Knols, B.G.J., Takken, W. (2007) Alarm bells ringing: more of the same, and new and novel diseases and pests. In *Emerging Pests and Vector-Borne Diseases in Europe* (Takken, W., Knols, B.G.J., eds), pp. 13–19, Wageningen Academic Publishers.
- Madeira de Carvalho, L.M., Alho, A.M., Matos, M., Sousa, S., Miranda, L.M., Anastácio, S., Otero, D., Gomes, L., Nunes, T., Otranto D., Belo, S., Deplazes, P. (2013). Some emerging canine vector borne diseases and antiparasitic control measures in companion animals in Portugal – Recent updates. XVIII Congreso de la Sociedad Española de Parasitología, Las Palmas de Gran Canaria, Spain, Keynote - CL.3, Proceedings pp. 100.
- Magnis, J., Lorentz, S., Guardone, L., Grimm, F., Magi, M., Naucke, T.J., Deplazes, P. (2013). Morphometric analyses of canine blood microfilariae isolated by the Knott's test enables *Dirofilaria immitis* and *D. repens* species-specific and *Acanthocheilonema* (syn. *Dipetalonema*) genus-specific diagnosis. *Parasite & Vectors*; 6, 48.
- Mettler, M., Grimm, F., Capelli, G., Camp, H., Deplazes, P. (2005). Evaluation of Enzyme-Linked Immunosorbent Assays, an Immunofluorescent-Antibody Test, and Two Rapid Tests (Immunochromatographic-Dipstick and Gel Tests) for Serological Diagnosis of Symptomatic and Asymptomatic *Leishmania* Infections in Dogs. *Journal Clinical Microbiology*, 43, 5515-5519.
- Otranto, D., Dantas-Torres, F., Breitschwerdt, E.B. (2009). Managing canine vector-borne diseases of zoonotic concern: Part one. *Trends in Parasitology*, 25(4), 157-163.
- Otranto, D., Dantas-Torres, F., Breitschwerdt, E.B. (2009b). Managing canine vector-borne diseases of zoonotic concern: Part two. *Trends in Parasitology*, 25(5), 228-235.
- Schnyder, M., Maurelli, M.P, Morgoglione, M.E., Kohler, L., Deplazes, P., Torgerson, P., Cringoli, G., Rinaldi, L. (2011). Comparison of faecal techniques including FLOTAC for copromicroscopic detection of first stage larvae of *Angiostrongylus vasorum*. *Parasitology Research*, 109 (1), 63-69.
- Schucan A., Schnyder M., Tanner I., Barutzki D., Traversa D. and Deplazes P. (2012). Detection of specific antibodies in dogs infected with *Angiostrongylus vasorum*. *Veterinary Parasitology* 185, 216–224.

**Attachment files**

Fig. 1 - Map of Portugal highlighting the seven inland areas that were surveyed (red colour)





UZH, Institute of Parasitology,  
Winterthurerstrasse 266a, CH-8057 Zurich

President of the Portuguese Foundation for  
Science and Technology

**Prof. Dr. Peter Deplazes**  
Director  
Phone +41 44 635 85 02  
Fax +41 44 635 89 07  
deplazes@access.uzh.ch

Zurich, 3<sup>rd</sup> October 2014

### Confirmation by the host institution of the successful execution of the Short Term Scientific Mission

I, Peter Deplazes, Professor of Parasitology and Director of the Institute of Parasitology, University of Zurich, confirm that Ana Margarida Pignateli Vasconcelos de Assunção Alho, stayed from 15<sup>th</sup> September to 3<sup>rd</sup> October in Zurich, at the Institute of Parasitology, on the behalf of a Short Term Scientific Mission Grant (EurNegVec TD1303 COST Action) which activity report is in attachment.

Sincerely,

University of Zurich  
Institute of Parasitology

Prof. Dr. Peter Deplazes  
Director

APPROVED BY EAEVE/FVE IN 2008