

## ***Leishmania* infection dynamics in dogs in a confined environment in an endemic area**

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### **Background**

The aim of this study was to determine the circulation of *Leishmania infantum* between dogs living together in a confined environment in an endemic area and assess the efficiency of prevention against sand fly bites.

### **Materials and methods**

The study included 26 dogs living together in one country residence in Leros (an island in Greece, with a previously proven high *Leishmania* endemicity [1]). Blood and conjunctival swab samples were collected from each animal, at regular intervals for almost 3 years. Moreover, clinical examination of the animals, including body weight, were performed at the same time, and collars containing 10% w/w imidacloprid and 4.5% w/w flumethrin (Seresto<sup>®</sup>) were placed and replaced on all dogs every 7 months. Blood and conjunctival swab samples were analysed with a TaqMan-qPCR assay for the presence of *L. infantum* DNA [2], while serum samples were tested for the presence of *L. infantum* antibodies with a commercial ELISA assay (cutoff 1/100), according to the manufacturer's instructions (INGEZIM<sup>®</sup> *Leishmania*, Ingenasa, Madrid, Spain). Sand flies were collected using CDC miniature light traps and mechanical aspirators, speciated and molecularly tested for *L. infantum* throughout the three vector activity seasons.

### **Results**

Out of the 26 dogs included in the study, 4 tested positive during the inclusion phase. The health status of the infected animals was evaluated and treatment was administered if necessary. Among the four initially infected dogs, one exhibited clinical signs and had a high parasitic load (up to 14,980 copies/ml PBS on conjunctival swab) since the beginning of the trial. This dog remained positive for 21 months, when it died of leishmaniosis, despite receiving treatment. At the time of death, the dog had an extremely high parasitic load (391,500 copies/ml PBS on conjunctival swabs). Throughout the study, this dog was the only one to consistently show anti-*Leishmania* antibodies. Interestingly, 17 animals tested positive for *Leishmania* by qPCR analysis of conjunctival swabs in the sampling performed around the time of this dog's death, presenting, on average, a low parasitic load, ranging between 1 and 333.5 copies/ml PBS (Mdn: 9.5, M: 46, SD: 90.17). In all subsequent samplings and up to the end of the study all animals either reverted to or remained negative in all tests. Furthermore, sand flies captured during the study confirmed the presence of competent vectors of *L. infantum* in the environment, specifically *Phlebotomus neglectus* and *P. tobbi*, both recognized as competent vectors in the Mediterranean basin. All captured sand flies tested negative for *L. infantum*.

### **Conclusions**

The results of this study confirm that serology is not the optimal method to assess the early stage of infection, characterized by low parasitic loads. A long follow-up period is necessary to detect established infection. Moreover, prevention measures are capable of protecting healthy animals even when they are confined in the same environment as sick dogs by limiting infection challenges.

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**Conflict of interest:** None.

### **References**

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