

***Leishmania infantum* DNA detection and sand flies blood feeding on wild captive animals from Madrid zoos: preliminary results**

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Background

In Spain, leishmaniosis is an endemic zoonotic disease caused by the protozoan *Leishmania infantum* and transmitted mainly by *Phlebotomus perniciosus*, which can affect humans, dogs, and other hosts [1]. Previous studies have demonstrated the presence of *L. infantum* infection in captive animals in zoos, such as Madrid Zoo Aquarium [2] and Faunia [3]. This makes entomological investigations necessary to determine the presence of sand flies, as well as the detection of the parasite and feeding source and thus obtain information on the risk of *L. infantum* infection in the wild animals housed in these zoos.

Materials and methods

An entomological survey was carried out from June to October from 2019 to 2020 at the Madrid Zoo Aquarium and from 2020 to 2021 at Faunia during the sand fly activity period. Sticky traps were placed for three to four days every fortnight in different locations of the zoos. Sand flies were collected and identified in the laboratory with identification keys. Finally, duplex real-time PCR (qPCR) was carried out to detect *Leishmania* and mammalian DNA. The analysis of blood meal preferences was done by amplification of 359 bp fragment of cytochrome b gene followed by sequence analysis.

Results

A total of 142 *P. perniciosus* females were captured, with preliminary results indicating that 10.6% (15) of them had fed on mammalian blood. Of the 15 females tested, 9 had fed on humans (*Homo sapiens*), 5 on rabbits (*Oryctolagus cuniculus*) and 1 on ring-tailed lemur (*Lemur catta*).

Out of all the females tested for *L. infantum*, the 96 females corresponding to the Madrid Zoo Aquarium were negative. In contrast, out of the 46 *P. perniciosus* females from Faunia, 36 were tested and 23 were found to be positive (63,8%). Notably, 3 females previously identified as positive for *L. infantum* had fed on human blood. Additionally, the sand fly that had fed on lemur blood also had *L. infantum* DNA.

Conclusions

The study findings suggest a risk of *L. infantum* infection transmission due to the presence of *Phlebotomus* in Madrid zoos. Further research is needed to explore the roles of humans and animals in maintaining the biological cycle.

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