Epidemiological approach to the urbanisation of canine and feline *Leishmania* infection in Morocco

<u>Clara M. Lima^{1,2}*, Maria Bourquia³, Nada Haissen³, Abderrahmane Zahiri³, Nuno Santarém ¹, Luís Cardoso⁴, Anabela Cordeiro da Silva^{1,2}</u>

1. Department of Biological Sciences, Microbiology Laboratory, Faculty of Pharmacy, University of Porto, Porto, Porto, Portugal. 2. i3S, Institute for Research and Innovation in Health, University of Porto, Porto, Portugal. 3. Unité de Parasitologie et Maladies Parasitaires, Département de Pathologie et Santé Publique Vétérinaires, Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco. 4. Department of Veterinary Sciences, and Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal.

*clara.lima@ibmc.up.pt

Background

Leishmaniosis is endemic in Morocco, alongside other Mediterranean countries. Here, human leishmaniasis (cutaneous, CL; visceral, VL) is caused by *L. infantum* (VL; CL), *L. major* (CL) and *L. tropica* (CL) [1–3]. Although considered a rural and peri-urban health problem, affecting mostly children and those living in low socioeconomic conditions, human and canine *Leishmania* infection is being detected in urban settings [2,4]. Effective disease management relies on the understanding of its epidemiology, disease burden, and the level of preparedness of healthcare providers in implementing control measures. Since dogs are the main urban hosts of zoonotic *L. infantum*, and cats may play a role in transmission, we questioned the impact of leishmaniosis among companion and sheltered animals in urban centres of Morocco (Rabat and Fez). Furthermore, local veterinarians were surveyed on their understanding of animal leishmaniosis and current attitudes towards the management of this zoonotic disease.

Materials and methods

A serological and molecular survey is being conducted on canine (n=103) and feline (n=32) samples collected in Rabat and Fez between February and June 2023. Total anti-*Leishmania* IgG was detected by an ELISA assay, based on soluble *L. infantum* promastigote antigen (SPLA), as well as recombinant KDDR (rKDDR) and *Leishmania* cytosolic peroxiredoxin (CPX) antigens [5]. Molecular detection of *Leishmania* kDNA was performed by primers RV1 and RV2, further complemented with ITS-1 sequencing [6], whenever possible to amplify the latter. Additionally, a survey on veterinarians' knowledge, perceptions, and practices (KPP) regarding animal leishmaniosis in Morocco was conducted using Google Forms. The survey questionnaire was distributed online to potential respondents (n=220) via email and social media platforms, with a request for their participation.

Results

Twenty canine (20/101; 20%) and three feline samples (3/33; 9%) tested positive for *Leishmania* kDNA. ITS-1 sequencing confirmed *L. infantum* infection in two canids. Fifty veterinarians (22%) completed the questionnaire. The majority (45%) of them worked in Rabat and Casablanca, and 72% mentioned having diagnosed canine leishmaniosis (CanL) in the past. Skin lesions (92.5%) and lymphadenomegaly (80%) were associated with CanL. Importantly, 83% were unaware of international guidelines for leishmaniosis management. Regarding treatment options, 58.3% mentioned the use of allopurinol, 38.9% of meglumine antimoniate and 5.6% of miltefosine. Moreover, more than 30% used antibiotics for leishmaniosis treatment (doxycycline and marbofloxacin) and more than 64% did not recommend any prophylactic measures.

Conclusions

Our preliminary findings support the hypothesis that *L. infantum* is embedded in urban centers of Morocco, infecting dogs, causing illness, and posing a risk for zoonotic transmission. Besides, we report feline infection with *Leishmania* spp. in Morocco. Veterinarians require further education and support to increase their preparedness to control leishmaniosis.

Funding: This study is been funded by the Portuguese Foundation for Science and Technology (FCT) under the PhD scholarship 2020.07306.BD and in the framework of project PTDC/CVT-CVT/6798/2020.

Conflict of interest: The authors declare no conflict of interest.

References

- Laboudi M, Sahibi H, Elabandouni M, et al. A review of cutaneous leishmaniasis in Morocco: A vertical analysis to determine appropriate interventions for control and prevention. Acta Trop. 2018;187:275

 –83.
- 2. Boussaa S, Kasbari M, el Mzabi A, et al. Epidemiological investigation of canine leishmaniasis in southern Morocco. Adv Epidemiol. 2014;2014:1–8.
- 3. Kahime K, Boussaa S, Bounoua L, et al. Leishmaniasis in Morocco: diseases and vectors. Asian Pac J Trop Dis. 2014;4:S530–4. Kahime K, Boussaa S, Nhammi H, et al. Urbanization of human visceral leishmaniasis in Morocco. Parasite Epidemiol Control. 2017;2:1–6.
- Santarém N, Silvestre R, Cardoso L., et al. Application of an improved enzyme-linked immunosorbent assay method for serological diagnosis of canine leishmaniasis. J Clin Microbiol. 2010;48:1866–74.
- 5. Lachaud L, Marchergui-Hammami S, Chabbert E, et al. Comparison of six PCR methods using peripheral blood for detection of canine visceral leishmaniasis. J Clin Microbiol. 2002;40:210-215.