

Molecular unveiling of *Leishmania* spp. parasites in feline squamous cell carcinoma: preliminary results

Carolina Oliveira¹, Joaquim Henriques^{2,3,4}, Joana Santos^{2,5}, Andreia Valença^{2,6,7,8}, José Catarino^{2,3}, David Ramilo^{2,9}, Margarida Alves^{2,9,10}, Carla Maia¹¹, Pedro Faísca^{2,5,9}, André Pereira^{2,6,9,11}

¹Faculty of Veterinary Medicine, Lusófona University, Lisbon University Center, Lisbon, Portugal.

²Research in Veterinary Medicine (I-MVET), Faculty of Veterinary Medicine, Lusófona University, Lisbon University Centre, Portugal.

³AniCura Atlântico Hospital Veterinário, Mafra, Portugal.

⁴NOVA4Health, NOVA Medical School (NMS), Universidade Nova de Lisboa (UNL), Lisbon, Portugal.

⁵DNAtech, Laboratório Veterinário, Lisbon, Portugal.

⁶Superior School of Health, Protection and Animal Welfare, Polytechnic Institute of Lusophony, Lisbon, Portugal.

⁷Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal.

⁸Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), Centre, Lisbon, Portugal.

⁹Veterinary and Animal Research Centre (CECAV), Faculty of Veterinary Medicine, Lusófona University, Lisbon University Centre, Portugal.

¹⁰CBIOS - Research Center for Biosciences and Health Technologies, Lusófona University, Lisbon, Portugal.

¹¹Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health (LA-REAL), Instituto de Higiene e Medicina Tropical (IHMT), UNL, Lisbon, Portugal.

Objectives: Feline leishmaniosis due to *Leishmania infantum* is an emerging vector-borne disease, often coexisting with other morbidities, including neoplasia. This study aims to explore the possible association between *Leishmania* spp. infection and squamous cell carcinoma (SCC) in cats, through the employment of advanced molecular techniques.

Material and Methods: Genomic DNA was extracted from 86 paraffin-embedded blocks containing feline biopsy lesions with a histopathological pattern compatible with SCC diagnosis. The presence of *Leishmania* DNA in SCC samples was screened using a nested-PCR protocol with primers targeting the parasites' small ribosomal DNA (SSU-rDNA).

Results: *Leishmania* SSU-rDNA was identified in a sample (1.2%, 1/86) of an SCC located on the nasal planum of a 10-year-old female European Shorthair cat.

Conclusion: The molecular evidence of *Leishmania* parasites in a feline SCC reinforces the hypothesis that *Leishmania* infection may be associated with SCC. This finding highlights the need for further investigation into the role of *Leishmania* in the pathogenesis of SCC, as well as the importance of considering *Leishmania* infection in the differential diagnosis and treatment of this malignancy.

Keywords: Squamous cell carcinoma, *Leishmania*, DNA, Nested-PCR.

Funding: This study was supported by the Instituto Lusófono de Investigação e Desenvolvimento under the exploratory project "FELIS - Feline Exploration of *Leishmania* in Squamous Cell Carcinoma" (<https://imvet.ulusofona.pt/investigacao>).