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Case Report
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Leishmania enriettii in guinea pig (*Cavia porcellus*) in southern Brazil

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ABSTRACT.- De Souza ML, Fernandes FD, Hartmann G, Cassanego GR, Fighera RA, Vogel FSF, Sangioni LA. ***Leishmania enrietti* in guinea pig (*Cavia porcellus*) in southern Brazil**. *Pesquisa Veterinária Brasileira 45:e07576, 2025*. Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Av. Roraima 1000, Santa Maria, RS 97105-900, Brazil. E-mail: fagner.fernandes@acad.ufsm.br

Leishmaniasis is a parasitic disease with a worldwide distribution, transmitted to animals and humans through the blood meal of sand flies, caused by protozoa of the genus *Leishmania*. This report aims to describe the clinical, pathological, and molecular findings of mucocutaneous leishmaniasis caused by *Leishmania enriettii* in a guinea pig (*Cavia porcellus*). The animal presented ulcerated skin lesions in the right pinna. Samples from the lesion were evaluated by cytology, revealing the presence of amastigote forms of *Leishmania* spp. Histopathology showed a dermal inflammatory infiltrate consisting of macrophages, multinucleated giant cells, lymphocytes, plasma cells, and heterophils. The foam cells contained parasitophorous vacuoles filled with amastigotes. Molecular characterization identified the species as *L. enriettii*. The diagnosis of mucocutaneous leishmaniasis was based on morphological findings (macroscopic and microscopic) associated with molecular biology tests that allowed defining the etiological agent of the disease.

INDEX TERMS: Histopathology, leishmaniasis, rodent, *Leishmania enriettii*, protozoan.

RESUMO.- [***Leishmania enrietti* em cobaia (*Cavia porcellus*) no sul do Brasil.**] A leishmaniose é uma doença parasitária com distribuição mundial, transmitida a animais e humanos através do repasto sanguíneo de flebotomíneos, causada por protozoários do gênero *Leishmania*. Este relato tem como objetivo descrever os achados clínicos, patológicos e moleculares da leishmaniose mucocutânea causada por *Leishmania enriettii* em cobaias (*Cavia porcellus*). O animal apresentava lesões cutâneas ulceradas no pavilhão auricular direito. Amostras da lesão foram avaliadas por citologia, revelando a presença de formas amastigotas de *Leishmania* spp. A histopatologia mostrou um infiltrado inflamatório dérmico composto por macrófagos, células gigantes multinucleadas, linfócitos, plasmócitos e heterófilos. As células espumosas continham vacúolos parasitóforos cheios de amastigotas. A caracterização molecular identificou a espécie como *L. enriettii*. O diagnóstico da leishmaniose mucocutânea foi baseado em achados morfológicos (macroscópicos e microscópicos) associados a testes de biologia molecular que permitiram definir o agente etiológico da doença.

TERMOS DE INDEXAÇÃO: Histopatologia, leishmaniose, roedor, *Leishmania enriettii*, protozoário.

INTRODUCTION

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*, which are transmitted by sandflies. The disease is among the most neglected in the world and is expanding in Brazil, particularly in the southern region. Approximately 53 species of *Leishmania* have been described in different regions of the world, with 31 species parasitic in mammals and 20 pathogenic to humans (Akhoundi et al. 2016). *Leishmania enriettii* was described in 1940, infecting, among other rodents, the guinea pig (*Cavia porcellus*) (Muniz

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& Medina 1948), being the only vertebrate host reported for this species (Becvar et al. 2020). Until now, this species has been considered non-infectious to humans (Machado et al. 1994, Paranaíba et al. 2015) and exhibits rapid growth in the skin of the vertebrate host. *L. enriettii* causes severe ulcerated skin lesions in *C. porcellus*, which heal over time.

This study aimed to describe the clinical, anatomopathological, and molecular findings of mucocutaneous leishmaniasis caused by *L. enriettii* in *C. porcellus*.

CASE REPORT

Ethical approval. The present study was conducted using tissues from the animal treated at the Veterinaty Hospital of the "Universidade Federal de Santa Maria" (HVU-UFSM). Thus, evaluation by the Ethics Committee on the Use of Animals is not required.

Clinical and clinicopathological findings. A two-year-old male guinea pig (*Cavia porcellus*) was admitted to the HVU-UFSM due to a chronic skin lesion with approximately eight months of evolution on one of its ears. Upon physical examination, a multilobulated mass measuring 4 x 2 x 1.5 cm was observed on the right pinna. The affected surface was hairless, red, shiny, ulcerated, and exudative (Fig. 1). The mass bled easily upon palpation and had a soft consistency.

Cytologic and histopathologic findings. Some samples of the skin lesion were collected using fine-needle aspiration biopsy (FNAB) for cytological examination. The smears prepared from the obtained material showed a large number of foam cells with round to kidney-shaped nuclei, filled with parasitophorous vacuoles containing oval structures measuring approximately 4 x 3 µm. These structures had a round eccentric nucleus, a smaller rod-shaped structure (kinetoplast), and abundant cytoplasm, consistent with the morphology of *Leishmania* sp. amastigotes. The same structures were also observed freely throughout the background of the slide (Fig. 2). Plasma cells, lymphocytes, and some heterophils were also observed. Based on the cytological findings, a right unilateral conchectomy was recommended. The pinna was removed and sent for histopathology and molecular characterization. For histopathology, fragments of the pinna were collected, fixed in 10% formalin, processed routinely for histology, and stained with hematoxylin and eosin (HE). The lesion was characterized by a superficial and deep dermal inflammatory infiltrate consisting of macrophages, some multinucleated giant cells, and a smaller number of lymphocytes, plasma cells, and heterophils. The foam cells contained parasitophorous vacuoles filled with amastigotes (Fig. 3).

Molecular diagnosis. For molecular characterization, fragments of the auricular tissue were subjected to DNA extraction using commercial kits – Wizard® Genomic DNA Purification Kit (Promega®, Madison/WI, USA), according to the manufacturer's instructions. The concentration and purity of the extracted DNA from the samples were evaluated using a NanoDrop spectrophotometer (ThermoScientific®, USA). Polymerase chain reaction (PCR) amplification was targeted to the kinetoplast DNA (kDNA) minicircle region, which is a conserved region among all species of the genus *Leishmania*. The PCR primers used were leishmini-F (5'GGKAGGGGCGTTCTGC3') and leishmini-R (5'STATWTTACACCAACCCC3') according to Kocher et al. (2018), generating a 120 bp fragment. The generated amplicons were detected by electrophoresis on a 3% agarose gel using molecular weight markers (50 bp and 100 bp ladder, Ludwig Biotec®, Brazil) and a fluorescent dye (GelRed – Biotium®, Hayward/CA, USA). The amplified products were visualized under ultraviolet light on a transilluminator (Fig. 4). The amplified products were purified using the PureLink® Quick Gel Extraction and PCR Purification Combo

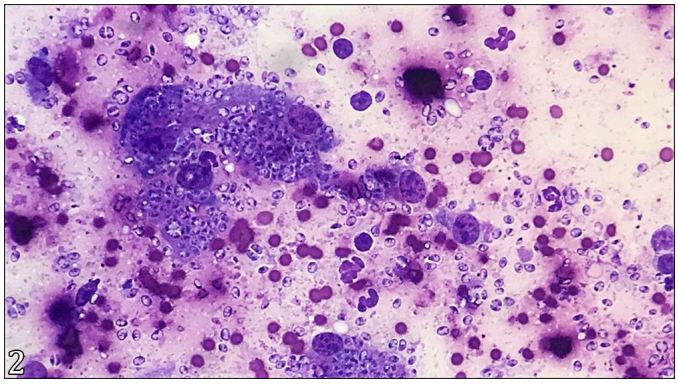

Fig. 2. A substrate of fine needle aspiration containing intra-histiocytic and free amastigote structures at the bottom of the slide. Morphology compatible with *Leishmania* sp. amastigotes. Rapid panoptic stain, obj. 20x. "Laboratório de Patologia Veterinária" (LPV-UFSM).

Fig. 1. Macroscopic aspect of the cutaneous lesion in *Cavia porcellus*. Multilobulated, alopecic, red, and shiny mass on the right pinna. "Laboratório de Patologia Veterinária" (LPV-UFSM).

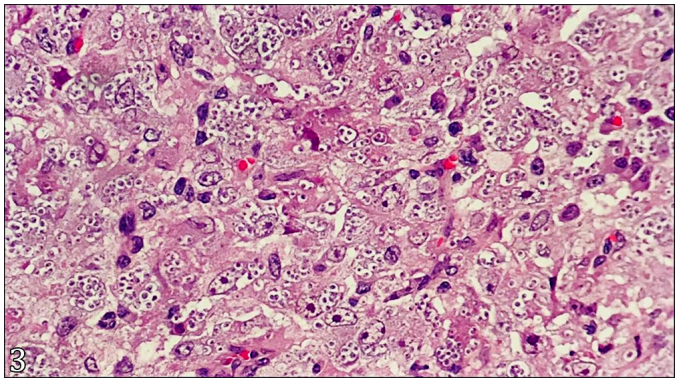

Fig. 3. The histological aspect of the cutaneous lesion, with foam cells containing parasitophorous vacuoles filled with amastigotes. HE, obj. 40x. "Laboratório de Patologia Veterinária" (LPV-UFSM).

Kit (Life Technologies, Carlsbad/CA, USA) according to the manufacturer's instructions. The samples were sequenced in duplicate in both directions using an ABI-PRISM 3100 Genetic Analyzer automatic sequencer (Applied Biosystems, Foster City/CA, USA) and aligned using the BioEdit Sequence Editor 7.0 program. The sample was sequenced, and the resulting data were deposited in GenBank (submission number 2936206). A multiplex PCR reaction was conducted aiming at identifying the *Leishmania* and *Viannia* subgenera, using the primers LSPF (5'GGGTAGGGGCGTTCTG3'), LVBR (5'GCGCGGCCCACTATA3'), LLAR (5'CCCCCAGTTGTGACCG3'), and LLCR (5'CCGATTTTTGAACGGGA3') (Conter et al. 2018). The obtained result was negative using the primers described by Conter et al. (2018), which are specific for the subgenera *Leishmania* and *Viannia*. This result is consistent with previous studies that classify *Leishmania enriettii* within the subgenus *Mundinia*, a genetically distinct group that does not amplify with primers targeting the other subgenera (Kuhls et al. 2011, Akhoundi et al. 2016). Therefore, the negative PCR result further supports the classification of the isolate as *L. enriettii*. The morphological findings, macroscopic and microscopic (cytology and histology), combined with molecular biology (PCR, multiplex PCR, and sequencing), allowed the identification of the etiological agent as *L. enriettii* in this animal.

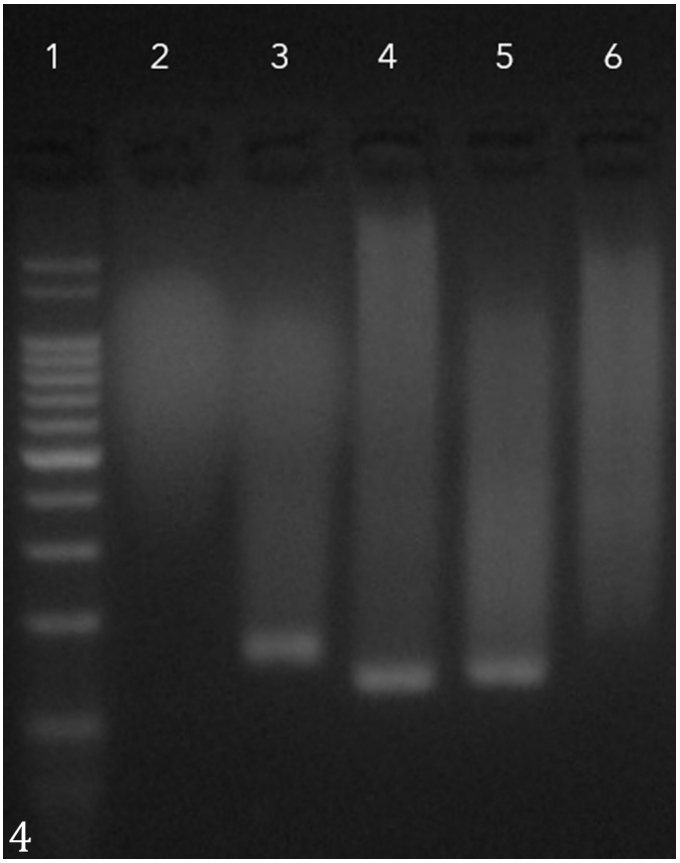

Fig. 4. Amplified products from leishmini primers on a 3% agarose gel. 1 = Molecular weight markers (100 bp); 2 = Negative control; 3 = Positive control; 4 = *Cavia porcellus* sample; 5 = *Cavia porcellus* sample; 6 = Negative DNA extraction control. "Laboratório de Doenças Parasitárias" (LADOPAR-UFSM).

DISCUSSION

Paranaíba et al. (2017) demonstrated that species of the *Leishmania enriettii* complex have a wide range of clinical manifestations and the ability to infect different vertebrate hosts and vectors. In a recent study, Soccol et al. (2021) isolated several strains of *L. enriettii* in guinea pigs in the state of Paraná. They did not detect the parasite in wild reservoirs, reinforcing the role of *Cavia porcellus* as a host for this species.

The lesions caused by *L. enriettii* infection in guinea pigs described in the literature are typically cutaneous and mucocutaneous and most often located on the pinna of the individuals (Paranaíba et al. 2015, Pinheiro et al. 2018, Ribeiro et al. 2023). In addition to ear lesions, it can also be observed in other areas of the body, such as the nostrils, limbs, upper lip, and genital region (Ecco et al. 2000, Fighera et al. 2003, Ribeiro et al. 2023).

The histopathological description of the lesion caused by *L. enriettii* in this study is characterized by an infiltrate of highly infected macrophages, a pattern very similar to other dermotropic species of the genus *Leishmania*. In the Ribeiro et al. (2023) study, histopathology of the skin lesions revealed histiocytic interstitial acanthotic dermatitis associated with a myriad of *Leishmania* organisms within macrophages cytoplasm. Furthermore, *L. enrietti* can cause a granulomatous lesion in the lung, which is characteristic of broncho-interstitial pneumonia with focal infiltrates of neutrophils, epithelioid macrophages, and multinucleated giant cells (Ribeiro et al. 2023). Experimentally, depending on the strain used, lesions can appear between the third and sixth weeks and dissipate between the tenth and fourteenth weeks post-inoculation (Paranaíba et al. 2015, Pinheiro et al. 2018).

Regarding the diagnosis, clinical suspicion began based on the clinical signs presented by the rodent. Similarly to what was demonstrated here, previously described cases of cutaneous leishmaniasis in guinea pigs attributed to *L. enriettii* presented as alopecic and ulcerated skin nodules or as alopecic and irregular thickening of the skin, attributed to the exuberant growth of amastigote forms in the skin (Muniz & Medina 1948, Machado et al. 1994, Ecco et al. 2000).

Currently, there is no standard diagnostic test for leishmaniasis caused by *L. enriettii* in rodents, and various methodologies are employed for diagnosis. Molecular biology has been widely used to characterize the species and provide a conclusive diagnosis based on clinical and histopathological findings (Paranaíba et al. 2017).

CONCLUSION

Leishmaniasis was diagnosed in a guinea pig naturally infected by *Leishmania enriettii* based on the association of clinical, histopathological, and molecular test findings. This study highlights the possibility that this pathogen is circulating in the central region of Rio Grande do Sul, Brazil.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

Credit author statement.- All authors contributed to the study conception and design and also to the final manuscript. Michelli L. de Souza, Fagner D. Fernandes, Fernanda S.F. Vogel and Luís Antonio Sangioni developed the methodology. Material preparation, data collection and analysis were performed by Michelli L. de Souza, Fagner D. Fernandes, Gabriela Hartmann, Guilherme R. Cassanego, Rafael A. Fighera, Fernanda S.F. Vogel and Luís Antonio Sangioni. Michelli L. de Souza, Fagner D. Fernandes performed the writing, reviewing and editing the manuscript.

Data availability statement.- The authors declare that all data used are available in this article.

REFERENCES

Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, Severo D. A historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. PLoS Negl Trop Dis 2016; https://doi.org/10.1371/journal.pntd.0004349

Becvar T, Siriyasatien P, Bates P, Volf P, Sádlová J. Development of *Leishmania* (*Mundinia*) in guinea pigs. Parasites Vectors 2020; https://doi.org/10.1186/s13071-020-04039-9

Conter CC, Lonardoni MVC, Aristides SMA, Cardoso RF, Silveira TGV. New primers for the detection *Leishmania* species by multiplex polymerase chain reaction. Parasitol Res 2018; https://doi.org/10.1007/s00436-017-5726-1

Ecco R, Langohr IM, Schossler JEW, Barros SS, Barros CSL. Leishmaniose cutânea em cobaias (*Cavia porcellus*). Ciência Rural 2000; https://doi.org/10.1590/S0103-84782000000300027

Fighera RA, Souza TM, Schmidt CA, Barro CSL. Leishmaniose mucocutânea em cobaios (*Cavia porcellus*). Medvep Rev Cient Med Vet Pequenos Anim Anim Estim 2003;1(3):177-182.

Kocher A, Valière S, Bañuls A-L, Murienne J. High-throughput sequencing of kDNA amplicons for the analysis of *Leishmania* minicircles and identification of Neotropical species. Parasitol 2018; https://doi.org/10.1017/S0031182017002013

Kuhls K, Alam MZ, Cupolillo E, Ferreira GEM, Mauricio IL, Oddone R, Feliciangeli MD, Wirth T, Miles MA, Schönian G. Comparative microsatellite typing of new world *leishmania infantum* reveals low heterogeneity among populations and its recent old world origin. PLoS Negl Trop Dis 2011; https://doi.org/10.1371/journal.pntd.0001155

Machado MI, Milder RV, Pacheco RS, Silva M, Braga RR, Lainson R. Naturally acquired infections with *Leishmania enriettii* Muniz and Medina 1948 in guinea-pigs from São Paulo, Brazil. Parasitol 1994; https://doi.org/10.1017/s0031182000076241

Muniz J, Medina HS. Leishmaniose tegumentar do cobaio (*Leishmania enriettii* n. sp.). Hospital (Rio J) 1948;33(1):7-25. PMid:18908199

Paranaíba LF, Assis RR, Nogueira PM, Torrecilhas AC, Campos JH, Silveira ACO, Martins-Filho OA, Pessoa NL, Campos MA, Parreiras PM, Melo MN, Gontijo NF, Soares RPP. *Leishmania enriettii*: biochemical characterisation of lipophosphoglycans (LPGs) and glycoinositolphospholipids (GIPLs) and infectivity to *Cavia porcellus*. Parasites Vectors 2015; https://doi.org/10.1186/s13071-015-0633-8

Paranaíba LF, Pinheiro LJ, Torrecilhas AC, Macedo DH, Menezes-Neto A, Tafuri WL, Soares RP. *Leishmania enriettii* (Muniz & Medina, 1948): a highly diverse parasite is here to stay. PLoS Pathog 2017; https://doi.org/10.1371/journal.ppat.1006303

Pinheiro LJ, Paranaíba LF, Alves AF, Parreiras PM, Gontijo NF, Soares RP, Tafuri WL. Salivary gland extract modulates the infection of two *Leishmania enriettii* strains by interfering with macrophage differentiation in the model of *Cavia porcellus*. Front. Microbiol 2018; https://doi.org/10.3389/fmicb.2018.00969

Ribeiro C, Koubiach KN, Faccini LS, Teixeira MC, Schüür FA, Thomaz-Soccol V, Barros CSL, Coelho ACB. Outbreak of leishmaniasis caused by *Leishmania enriettii* in guinea pigs (*Cavia porcellus*). Pesq Vet Bras 2023; https://doi.org/10.1590/1678-5150-PVB-7241

Soccol VT, Castro EA, Reifur L, Teixeira VN, Lange RR, Luz E. Guinea pigs naturally infected by *Leishmania enriettii*: clinical analyses, parasite isolation and identification. Braz Arch Biol Technol 2021; https://doi.org/10.1590/1678-4324-75years-2021210095