

Report a case of cutaneous and visceral canine leishmaniasis from Queretaro, Mexico

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RESUMEN

Reporte de un caso de leishmaniasis canina cutánea y visceral en Querétaro, México.

Leishmania infantum es el agente causal de la leishmaniasis canina cutánea y visceral, una enfermedad ampliamente descrita en varios países de Europa y América. En el presente trabajo se describe el caso de un canino raza Presa Canario nacido en México que fue remitido a consulta por una lesión alopecica en plano nasal y linfadenomegalia generalizada. Se confirma un caso de leishmaniasis con las improntas, encontrando macrófagos con amastigotes de *Leishmania*. Debido al deterioro del animal se realizó la eutanasia y la necropsia. Se tomaron muestras de piel, ganglios linfáticos, hígado, riñón y bazo para realizar los análisis histológicos y moleculares. En muestras de piel, bazo y riñón se observaron lesiones granulomatosas con amastigotes y se confirma con el análisis molecular que la especie es *L. infantum*. El registro de este caso sugiere un riesgo potencial de esta enfermedad en una región no endémica del país.

ABSTRACT

Leishmania infantum is the causal agent of cutaneous and visceral canine leishmaniasis, a disease widely described in Europe and Latin America. This study describes a case of a canine Canary mastiff breed, born in Mexico that was referred to consult by an alopecic lesion and generalized lymph adenomegaly. Diagnosis of leishmaniasis was

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established by impression smears of the lesion that showed amastigotes within macrophages. Due to the poor body condition of the animal, euthanasia and necropsy were conducted and samples taken from skin, lymph nodes, liver, kidney, and spleen to perform histological and molecular analysis. In skin, spleen and kidney granulomatous lesions were observed showing amastigotes of *Leishmania*. The diagnosis of *L. infantum* was confirmed by using molecular tools. The report of this case suggests the potential risk of this disease in a non-endemic region from Mexico.

INTRODUCTION

In the Americas, *Leishmania infantum* was introduced with the European settlement (1). Currently, distribution extends from the southern United States to northern Argentina (2). Visceral leishmaniasis is considered a zoonosis, where domestic dogs are presumed to be the most important reservoir (1). Some infected dogs may eventually control the parasite and not develop the disease; however, others may develop progressive leishmaniasis showing a wide spectrum of manifestations, from benign skin lesions to fatal forms of visceral leishmaniasis (3).

To date, studies of canine leishmaniasis in Mexico have been scant and composed of records scattered across different geographical sites. There are two records of canine visceral leishmaniasis imported from Spain, such cases were in two Bull Terriers (5), both infected with *Leishmania infantum*. Furthermore, cases of canine leishmaniasis have been reported in endemic regions in Mexico. In Tabasco and Quintana Roo, three dogs and their tutors were shown to be infected with *L. mexicana* (4). Canine populations from Yucatan and Quintana Roo demonstrated the presence of antibodies against *L. braziliensis*, *L. infantum* and *L. mexicana* as well as co-infections (6, 7). Other studies of canine leishmaniasis have been reported, although the parasite species was not determined in Chiapas (8) and Guerrero (9).

We report the case of canine leishmaniasis from a non-endemic region in the State of Queretaro, Mexico. The dog, born in Queretaro, Mexico, had never been in an endemic region, yet it's father grew up in Spain and the mother was born in Mexico. The clinical situation of both parents is unknown.

CASE PRESENTATION

In December 2017, a 3.5-year-old male Presa Canario breed was referred to the private veterinary clinic in San Juan del Rio, Queretaro, Mexico. The tutor had observed continuous bleeding of the footpads and onychogryphosis. During clinical examination generalized lymphadenopathy, and a nodule in the nasal planum of approximately 2 cm we observed, as well as alopecia on the snout and ocular periphery (Figure 1).

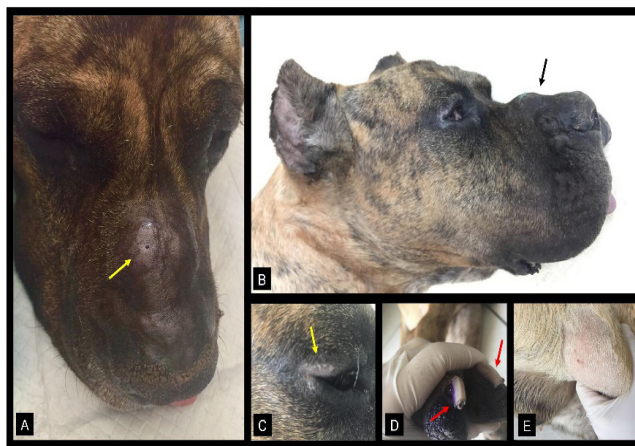


Figure 1. Clinical manifestations of the dog with leishmaniasis from Querétaro, México. Dermatitis and alopecic lesions A-B. Nodular dermatitis and alopecic lesion in the nasal plane (arrows). C. Periocular alopecia (yellow arrow). D. Onychogryphosis (red arrows). E. Prescapular lymph adenomegaly.

For diagnosis, smears were taken from the nodule and lymph nodes using fine needle puncture and then fixed with methanol and stained with Giemsa. Biopsies from skin nasal planum and a lymph node were taken and stained with Hematoxylin-Eosin (H&E). The diagnosis of leishmaniasis was confirmed by direct observation of amastigotes from smears and biopsies showing large amounts of *Leishmania* amastigotes (Figure 2).

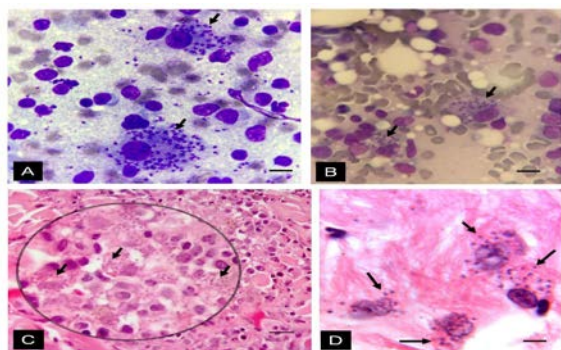


Figure 2. Amastigotes (arrows). A-B. Macrophages from a lymph node smears (Giemsa stain). C. Granulomatous dermatitis with intracytoplasmic amastigotes. D. Skin. H&E stain (C and D). Scale bar: 5 μ m (A, B, D); 20 μ m (C).

Given the clinical deterioration and poor long-term prognosis, the dog was euthanized, and a necropsy was performed with the tutor's consent. The main findings to the macroscopical study were lymph adenomegaly and splenomegaly (**Figure 3A and 3B**). Samples from skin, lymph nodes, spleen, kidney, liver, and heart were taken and fixed in 10% buffered formalin for subsequent paraffin inclusion and processed by routine H&E staining for histopathological study. Granulomatous lesions in skin, lymph nodes and spleen associated to *Leishmania* amastigotes were observed (**Figure 3C and 3D**) as well as focal interstitial nephritis with scarce amastigotes within macrophages.

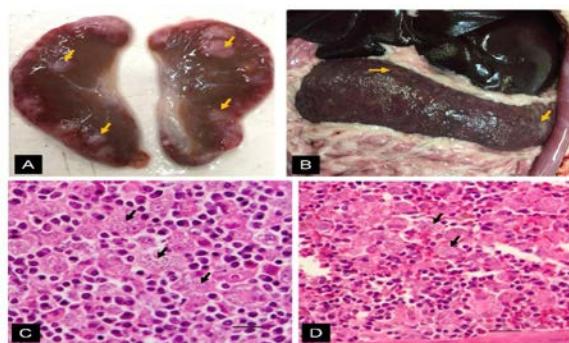


Figure 3. Macroscopic and microscopic analysis. A. Longitudinal section from a Lymph node showing nodular changes to granulomatous lesions (yellow arrows). B. Splenomegaly with multinodular lesions on capsule (yellow arrows). C. Lymph node with amastigotes (black arrows) in granulomatous lesions. D. Granulomatous lesion in spleen with amastigotes in macrophages (black arrows). H&E stain. Scale bar: 20 μ m (C and D).

For molecular studies samples from skin, kidney, spleen, heart, and lymph node were taken for molecular studies and were frozen (-80°C) until DNA extraction. DNA was extracted from approximately 25 mg of tissues (skin, kidney, spleen, heart, and lymph node), using a commercial DNA extraction kit (DNeasy Blood and Tissue kit, Qiagen, Hilden, Germany), following the manufacturer's instructions. To determine the presence of *Leishmania*, oligonucleotides based on the *Leishmania* mini-circle kinetoplast DNA, L.MC-1S, and L.MC-1R were used as described previously (10). Amplification reactions were done in 25 μ l of reaction mixture: Taq PCR Master Mix (Qiagen, Hilden, Germany), 100 ng of the corresponding oligonucleotides, and 1 μ l of tissue extract corresponding to 100 ng of DNA. As a positive control for *Leishmania* DNA and *Leishmania mexicana* (SOLIS) were used. PCR products were analyzed in 1.5% agarose gel electrophoresis in Tris-acetate EDTA (TAE) buffer at 80 V, stained with 0.5 $\mu\text{g}/\text{mL}$ ethidium bromide, and visualized under ultraviolet light. PCR products were purified and sequenced at the Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud, Instituto de Biología, UNAM. The sequences obtained were analyzed using the software BioEdit version 7.0.5. Edited DNA sequences were compared to those available in the National Center for Biotechnology Information (NCBI), US National Library of Medicine, Basic Local Alignment Search Tool (BLAST). Topology were developed with the program Molecular Evolutionary Genetics Analysis (MEGA) version 11.0, using the ClustalW algorithm and with Maximum Likelihood (ML) with Bootstrap of 1000 repetitions, with the probabilistic model of GTR+G+I (General Reversible Time + Gamma Distribution with invariant sites). The estimation of paired genetic distances was obtained with Bootstrap of 1000 repetitions, with the Maximum Composite Likelihood probabilistic model.

The tissues were tested by PCR to detect evidence of infection. The results were positive to genus *Leishmania* parasites (**Figure 4A**). The use of species-specific primers IR1/LM17 revealed that the

infecting parasites were negative *Leishmania* (*L.*) *mexicana* (Figure 4B). For additional confirmation that the infecting parasites were *Leishmania*, the PCR amplifications obtained with the primers IR1/LM17 were sequenced. With validation of the obtained sequence through a BLAST tool, the platform yielded an identity percentage of 96.5% and expected value about 6×10^{-15} of match, corresponding to *Leishmania infantum*. The analysis involved 12 nucleotide sequences, with a total of 78 positions in the final dataset. The isolate is placed in a different phylogenetic subgroup, together with the sequence M93416.1 of *L. infantum*, with genetic distance of 0.44% (Figure 4C).

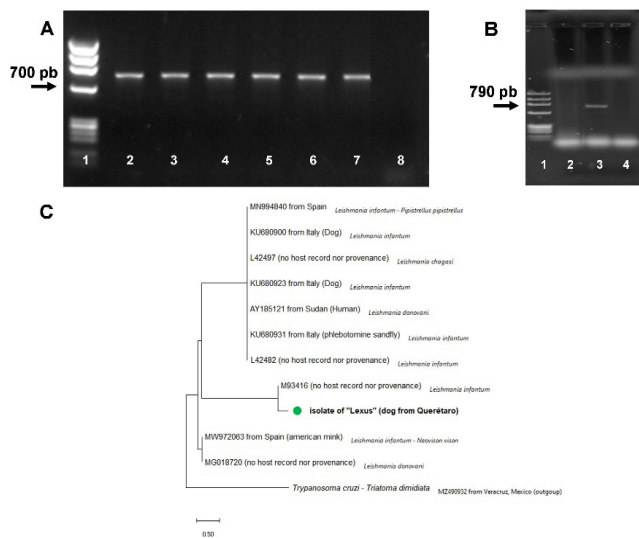


Figure 4. Gel electrophoresis of PCR-based products of *Leishmania*. A. DNA ladder (Lane 1). Tissues analyzed: skin (Lane 2), kidney (Lane 3), spleen (Lane 4), heart (Lane 5), lymph node (Lane 6) were positive for genus *Leishmania* (Lanes 2-6), positive control with reference strain of *Leishmania* (Lane 7) and negative control (without DNA: Lane 8). B. DNA ladder (Lane 1), DNA from skin (Lane 2), positive control of *Leishmania mexicana mexicana* (Lane 3), negative control (without DNA: Lane 4). C. Phylogenetic tree for the DNA sequence obtained from the infected dog, using maximum likelihood (ML) analysis. *Trypanosoma cruzi* was used as an outgroup to root the tree. Symbol in green represents the sequences of this study.

DISCUSSION

Our results represent the first documented *Leishmania infantum* infection in a dog from a

non-endemic region of Mexico. The diagnosis of leishmaniasis was established by smears and samples taken from organs to perform parasitological and histological examinations, as well as by molecular confirmation.

We observed *Leishmania* amastigotes in all tissue samples and the infecting parasite was identified as *L. infantum* by PCR and sequencing. Some species causing Canine leishmaniasis (CanL) in the Americas are *L. infantum*, *L. amazonensis*, *L. panamensis*, *L. braziliensis* (11), *L. guyanensis* (12) and *L. mexicana* (4).

In Mexico, the magnitude of the epidemiological risk to the inhabitants from CanL is unknown. However, a study showed that there can be co-infection by more than one species and *L. mexicana* is the most prevalent species (7). Dogs may develop a dissemination of the parasite in the skin and internal organs, which is lethal if not appropriately treated (3). In the current case, the animal had progressive clinical signs of skin lesions and visceral involvement. Onychogryphosis, alopecia, lymphadenopathy, and splenomegaly were clinical signs the dog infected with *Leishmania infantum*. This finding is consistent with those published by other authors (9, 13).

Transmission of *Leishmania infantum* has been reported for *Lutzomyia longipalpis* and *Lu. evansi*. Furthermore, there are other non-vectorial transmissions such as vertical and venereal, transfused blood products from infected blood donor and there is the possibility of direct dog-to-dog transmission by bite wounds (14). Additionally, traveling with dogs or the importing them from endemic regions are other ways to acquire CanL (2). In this case, the infection was probably acquired through vertical transmission. This may explain why a dog without a history of travel outside of its birthplace has acquired *Leishmania infantum* in Queretaro, where no cases of leishmaniasis have been registered.

To obtain a final sensitive and specific diagnosis it is important to use more than one method (15). In addition to skilled personnel are required, due to the wide spectrum of clinical symptoms that

occur in CanL. Therefore, it is necessary to develop accessible, non-expensive tests for its correct identification, and adequate treatment. On the other hand, to reduce the risk of *Leishmania* infection, it is important to promote prevention of sandfly bites in noninfected dogs and spread in infected dogs.

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