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Case report

A fatal case of canine cutaneous leishmaniosis in a dog

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ABSTRACT. A 10-year-old intact female Chihuahua, 2.5 kg of weight and BCS 2 (range 1–5) was taken in for medical consultation due to the presence of four skin lesion, two in the ventral thoracic region and two in the dorsal region. The dog was receiving medication due to congestive heart failure. A cutaneous form of canine leishmaniosis was diagnosed using molecular tools from a 10-years-old Chihuahua dog living in the Caribbean region. The critical health condition of the geriatric patient may have evolved to a fatal renal failure. This report is the first of a fatal case of leishmaniosis in a dog from the endemic region in Mexico.

Key words: canine leishmaniosis, renal failure, *Leishmania mexicana*

Introduction

Leishmaniosis is a parasitic disease produced by different species of an intracellular protozoan from the genus *Leishmania*. In America, it is transmitted by a female hematophagous dipterous from the genus *Lutzomyia*. In dogs, three classical clinical forms of leishmaniosis may be present: cutaneous, mucocutaneous or visceral. However, not all dogs infected with *Leishmania* spp. will develop clinical signs of the disease. In Mexico, leishmaniosis is endemic in several states, particularly for the Southern area including the Yucatan Peninsula [1]. Several cases of cutaneous leishmaniosis in humans have been reported in this region of Mexico [2,3] but this condition has been rarely described in dogs. Only one cutaneous form of *Leishmania* in a dog has been described in the Caribbean region [4].

Here, we describe the clinical management and diagnosis using molecular tools of the first clinical case of canine leishmaniosis reported in the Peninsula of Yucatan.

Case presentation

A 10-year-old intact female Chihuahua, 2.5 kg of weight and BCS 2 (range 1–5) originally from Cozumel Q. Roo Mexico (20°31′00″ N 86°56′30″W) was taken in for medical consultation due to the presence of four skin lesion, two in the ventral thoracic region and two in the dorsal region. The lesions were ulcerated with a circular form without healing for three months. The dog was receiving Ramipril (0.625 mg/kg *q* 24hr) and furosemide (0.25 mg/kg *q* 24 hr) due to congestive heart failure. The dog was only fed commercial dog food and she was living indoors but with access to a backyard. Her vaccination program was complete and she had been dewormed six months ago; there were no other dogs or pets were living in the household. The owner reported that the dog had vomited at least twice every day for the last 3 days. During the physical examination, the dog's rectal temperature (36.6°C) and respiratory frequency (32 breaths/min) were below the normal range. A heart murmur, grade 4, was detected at the pulmonary valve. Teguments were icteric and none of the lymph

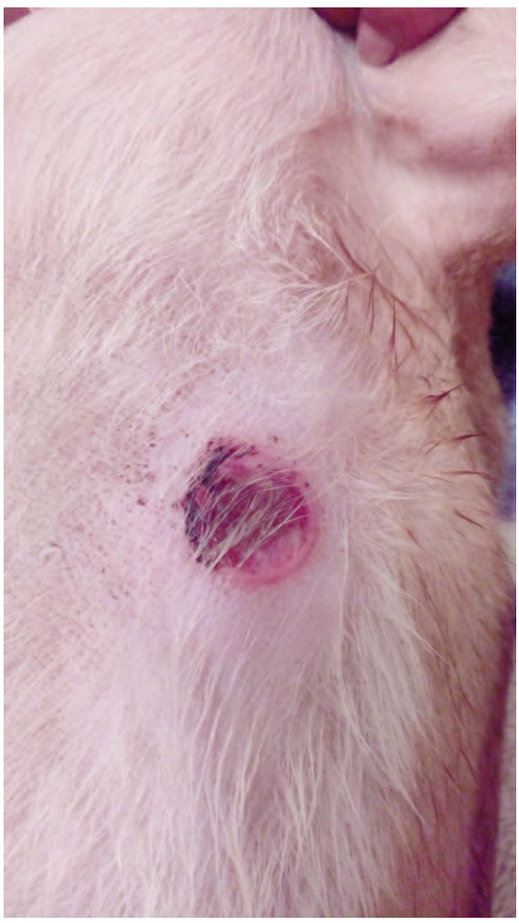

Fig. 1. Ulcerated skin lesion in the ventral region

nodes were reactive. Skin lesions were rounded, ulcerated and erythematous (Fig. 1). The pulmonary fields were clear. Skin samples were taken from the ulcerated lesions for histopathology and extraction of DNA for PCR studies. Blood samples were taken for a haematology and biochemical analysis. DNA was extracted from tissues using a phenol/chloroform/isoamyl alcohol extraction, and real-time PCR was performed. The primers amplified a 120-pb region of the kinetoplast of *Leishmania* spp. Once diagnosed positive, qPCR was performed to discriminate *Leishmania* species using a commercial Taq Universal SYBR Green Supermix (Biorad) for amplifications and primers.

The blood values are listed in Tables 1 and 2. From the red cells, PCV, MCV and platelets were below the normal range; a marked monocytocis was also present. Biochemical analysis revealed the presence of renal insufficiency and liver impairment.

Real-time PCR amplified for *Leishmania* spp., and later confirmed the species as *L. mexicana*. Cytology and histopathology revealed the presence of amastigotes in the tissue sampled. The histopathology was characterized by an ortho - keratotic hyperkeratosis in the epidermis, with moderate diffuse acanthosis and a severe zonal ulcer. In the dermis a diffuse, severe inflammatory infiltrate was found consisting of lymphocytes, plasma cells, neutrophils, monocytes and macrophages; global structures of 2–4 mm in length were found free in the cytoplasm of macrophages resembling amastigotes of *Leishmania* spp.

Due to the critical condition of the dog, it was recommended that she remain hospitalized for better monitoring and care. However, the owner refuses to leave the dog; one day later the dog died. No autopsy was allowed.

Cutaneous leishmaniosis has been described in the region where the present case was diagnosed; in this region *L. mexicana* appears to be related to the epidemiology of the disease in humans [1–2]. In this

Table 1. Complete blood count of dog

Parameters	Results	Range	Units
RBC	6.26	5.50-8.50	($\times 10^6$ μL^{-1})
HB	12.4	11.0-19-0	(gdL^{-1})
PCV	35.7*	39.0-56.	0 (%)
MCV	57.1*	62.0-72.0	(fl)
MCHC	34.7	30.0-38.0	(gdL^{-1})
Platelets	92.0*	200-460	
WBC	5.5*	6.0-17.0	($\times 10^6$ μL^{-1})
Lymphocytes	26.2	12.0-30.0	(%)
Monocytes	36.6*	2.0-9.0	(%)
Granulocytes	33.2*	60.0-83.0	(%)

*outside the normal range; RBC - red blood cells; HB - haemoglobin; PCV - packed cell volume; MCV - mean cell volume; MCHC - mean cell haemoglobin concentration; WBC - white blood cells

Table 2. Biochemical analysis results

Parameters	Results	Range	Units
Glucose	67.0	60-115	mg/dL
Urea	157.0*	10-26	mg/dL
Creatinine	4.01*	0.50-1.60	mg/dL
Uric acid	2.03*	0.10-1.50	mg/dL
Cholesterol	93.3	115.0-254.0	mg/dL
Triglycerides	196.7*	40.0-150.0	mg/dL
Total bilirubin	2.91*	0.0-0.60	mg/dL
Direct bilirubin	2.21*	0.0-0.10	mg/dL
Albumin	2.13*	2.30-4.0	g/dL
Total proteins	8.58*	5.5-7.5	g/dL
ALT	37.2	6.0-70.0	ul/L
AST	331.9*	8.0-49.0	ul/L
GGT	3.2	0.0-8.0	ul/L
P	15.69*	2.3-5.50	mg/dL
K	4.56	4.37-5.35	mm/dL
Na	107.4*	141.0-152.0	mmol/L
Ca	0.25*	2.25-2.83	mmol/L
Cl	108.5	105.0-115.0	mmol/L
Amylase	1344.0*	75.0-950.0	U/L
Lipase	91.3	25.0-250.0	U/L

*outside the normal range

case *L. mexicana* was diagnosed based on the clinical signs, epidemiological data from the region and histopathologic studies, later confirmed by PCR. Serological surveys in dogs from the Yucatan Peninsula have revealed the presence of *L. mexicana*, *L. braziliensis* and *L. infantum* [1]. Therefore, cutaneous and/or visceral presentations may be expected.

In humans cutaneous leishmaniosis is an inflammatory, localized process in which the parasites are restricted [5]. In dogs, however, immune complexes due to *L. infantum* are deposited in different organs such as liver and kidneys [6], similar to what is observed in *L. donovani* [7].

In the present case, blood abnormalities may have occurred due to the chronicity of the case, particularly the increase in plasma protein profile, which is considered one of the most reliable marker for monitoring canine leishmaniosis. Total serum protein levels are markedly increased in infected dogs and can reach more than 10g/dl probably due to high levels of β- and γ-globulins [8]. Following renal or hepatic damage, a drastic decrease in albumin levels can also be observed [6]. Renal damage may also be a clinical sign of canine leishmaniosis characterized by severe nephritis or glomerulonephritis with increased serum levels of urea and creatinine [9]. Immune complex deposits associated with renal failure could represent a model for better understanding the immunological aspects of leishmaniosis in dogs [10]. Chronic granulomatous hepatitis is found in about 5% of cases and causes vomiting, polyuria, polydipsia, poor appetite, and weight loss [6,11]

A normocytic, normochromic, poorly regenerative anaemia with medullar hypoplasia is also a common finding in canine leishmaniosis but not observed in the present case. However, thrombocytopenia was also likely in this case [6]. The monocytosis observed was probably the consequence of the chronic inflammatory and haemolytic process of the dog. Although the precise cause of death was not determined a severe renal failure was possibly a predisposing factor.

In conclusion, domestic dogs are important reservoirs of *Leishmania* spp. that develop clinical signs of the disease, particularly in endemic regions in which human cases are known. In this case, humans from the same household are at risk to be infected by the vector, especially because the dog

was kept indoor (i.e., the vector was in the household). Preventive measures by owners using repellents (e.g., impregnated collars, pour-on repellents) or fumigation of their backyards should be encouraged. Local health authorities should be informed of the cases for precise actions focused on the control of the vector. This investigation is the first molecular-confirmed report of a fatal clinical case of *L. mexicana* in a naturally infected dog from the endemic region of the Yucatan Peninsula, Mexico, where efforts to control the vector are non-existent.

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