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Case Report

First description of parasite load and clinicopathological and anatomopathological changes in a dog naturally coinfected with *Dioctophyme renale* and *Leishmania infantum* in Brazil

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ABSTRACT

This article reports the case of a domestic dog naturally coinfected with the nematode *Dioctophyme renale* and with the protozoan *Leishmania infantum*. The dog exhibited no clinical signs but had normocytic hypochromic anemia, hyperproteinemia, hyperglobulinemia, hypoalbuminemia, and hematuria. Necropsy revealed eight *D. renale* specimens in the abdominal cavity and in right kidney whose parenchyma was atrophied. Histopathological analysis showed glomerular atrophy, fibrosis and a marked diffuse pyogranulomatous inflammatory infiltrate in the right kidney. Moderate multifocal granulomatous peritonitis was observed in the greater omentum. Several *Dioctophyme renale* eggs were present amidst the inflammatory infiltrate of the right kidney and greater omentum. *Leishmania infantum* parasites were detected in perirenal adipose tissue of the right kidney, greater omentum, spleen, bone marrow, and popliteal lymph node. The high *D. renale* load and the severe and uncommon histological alterations associated with the eggs of this parasite may have been influenced by coinfection with *L. infantum*.

1. Introduction

Zoonotic visceral leishmaniasis (ZVL) is caused by the protozoan *Leishmania infantum* in Latin America, southern Europe, the Middle East, and Central Asia (Burza et al., 2018). Transmission of this protozoan to humans and other mammals occurs mainly through the bite of infected female sandflies. Domestic dogs are the main reservoir in urban areas (Brasil, 2006). In dogs, *L. infantum* infects macrophages in different tissues where it is associated with granulomatous inflammation (Koutinas and Koutinas, 2014).

Dioctophymatosis is caused by the nematode *Dioctophyme renale*, which has a worldwide distribution. This helminth is commonly found in the right kidney or abdominal cavity of the definitive host, which includes domestic animals, especially dogs (Kommers et al., 1999), wild animals (Rocha et al., 1965; Kumar et al., 1972; Mace and Anderson, 1975; Milanelo et al., 2009; Ishizaki et al., 2010; Echenique et al., 2018; Trindade et al., 2018), and humans (Chauhan et al., 2016). These hosts are infected by ingestion of aquatic oligochaetes, fish or frogs (Mace and Anderson, 1975). In the kidneys of dogs, *D. renale* causes primarily atrophy or complete destruction of the parenchyma (Sapin et al., 2017).

In dogs, coinfection of *L. infantum* with other pathogens can increase the susceptibility to ZVL (Toepp et al., 2019); however, little is known about coinfections of *L. infantum* with helminths. Therefore, the objective of this study was to describe the parasite load and clinicopathological and anatomopathological changes in a domestic dog naturally coinfected with *D. renale* and *L. infantum*.

2. Materials and methods

2.1. Animal

A 4-year-old male mongrel dog had a positive serology result for

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anti-*L. infantum* antibodies by a rapid immunochromatography test (Bio-Manguinhos, Rio de Janeiro, Brazil) and enzyme immunoassay (Bio-Manguinhos). This dog was privately owned and was from the city of Barra Mansa (22°32′25.19″S and 44°10′35.33″W), Rio de Janeiro State, Brazil, a ZVL-endemic area. The animal was fed commercial chow and home-made food, had unrestricted access to a street near a river, and had never traveled.

2.2. Clinical examination and sample collection

The dog was submitted to inspection of the skin and mucosae and palpation of the superficial lymph nodes and organs. Peripheral blood samples were collected from the cephalic vein. The animal was then euthanized by intravenous injection of an overdose of sodium thiopental and potassium chloride as a control measure of ZVL (Brasil, 2006).

During necropsy, fragments of the two kidneys, greater omentum, spleen, skin, popliteal lymph node and bone marrow aspirate were collected, fixed in 10% neutral buffered formalin, and processed for embedding in paraffin (FFPE) for histopathology and immunohistochemistry. Additionally, fragments of the skin, popliteal lymph node and bone marrow were immersed in sterile saline for isolation of *Leishmania* spp. by culture. A spleen fragment was also collected and frozen for singleplex quantitative real-time PCR (qPCR). Urine was collected by cystocentesis for urinalysis.

2.3. Analysis

Complete blood count, serum biochemistry and urinalysis were performed. The reference values described by Thrall et al. (2012), Kaneko et al. (2008), Rizzi et al. (2010), and Meuten (2012) were used. The nematodes were identified according to Vicente et al. (1997). For histopathology, 5-µm FFPE tissue sections were stained with hematoxylin-eosin (Carson and Cappellano, 2015). For the detection of amastigote forms of *Leishmania* by immunohistochemistry, 5-µm FFPE tissue sections were processed according to Oliveira et al. (2017). For culture, the collected tissues were seeded onto biphasic NNN/Schneider's Insect Medium and incubated at 26–28 °C (Madeira et al., 2009). The isolates were identified by multilocus enzyme electrophoresis (Cupolillo et al., 1994). Quantification of *L. infantum* load in the spleen by singleplex qPCR was performed according to Oliveira et al. (2017).

3. Results

No clinical sign was observed. The animal had normocytic hypochromic anemia, with 4.48×10^6 red blood cells/µl and hematocrit of 28.0%, hyperproteinemia (total protein 9.3 mg/dl), hyperglobulinemia (globulin 6.78 mg/dl), and hypoalbuminemia (albumin 2.52 mg/dl). Urea, creatinine and other hematological and biochemical parameters were normal (Supplementary Table 1). Urinalysis showed turbidity, traces of protein, hematuria, and non-larvated *D. renale* eggs (Supplementary Table 1).

Necropsy revealed the presence of eight nematodes of red color: two free males in the greater omentum and six (four females and two males) inside the right kidney (Fig. 1). Male nematodes measured 18.5 to 25.5 cm in length and 0.4 cm in width and females measured 43.2 to 52.0 cm and 0.8 cm, respectively. These parasites were identified as *D. renale*. The parasitized kidney was enlarged, had a pale color and an irregular surface, and exhibited marked atrophy of the parenchyma (Fig. 1; Supplementary Fig. 1). No macroscopic alterations were observed in the left kidney.

Microscopically, the right kidney showed a pyogranulomatous inflammatory infiltrate in the cortex (Fig. 2), medulla and capsule, as well as in perirenal adipose tissue. Lymphocytes, plasma cells, and multinucleated giant cells were also present. Several larvated and non-larvated eggs of *D. renale* were detected in the cited histological regions

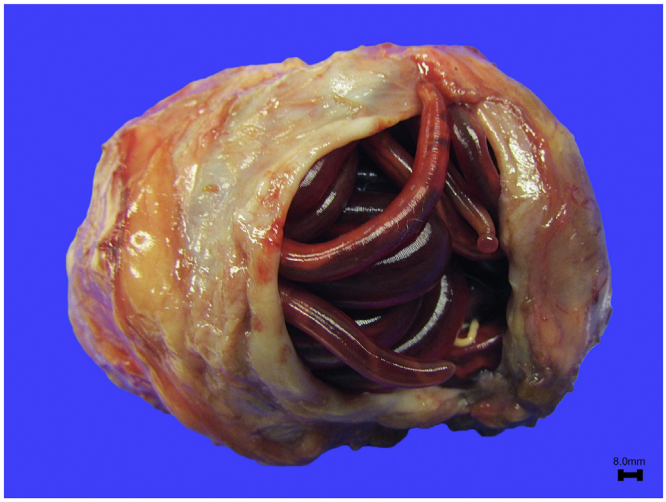

Fig. 1. Right kidney of a dog coinfected with *Dioctophyme renale* and *Leishmania infantum*. Note the enlarged organ with a pale color and irregular surface. Sectioning of the kidney revealed the presence of red *D. renale* specimens in the parenchyma. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 2, Supplementary Fig. 2). Additionally, parenchymal fibrosis and glomerular atrophy and destruction were observed (Fig. 2), as well as dilated collecting ducts and renal pelvis. Hyaline cylinders were found inside the convoluted tubules and collecting ducts (Fig. 2). The greater omentum exhibited moderate multifocal granulomatous peritonitis with multinucleated giant cells engulfing multiple non-larvated *D. renale* eggs (Fig. 3). A diffuse lymphoplasmacytic inflammatory infiltrate containing a small number of macrophages was observed in the spleen and popliteal lymph node. No microscopic alterations were found in the left kidney, skin or bone marrow.

Using immunohistochemistry, *Leishmania* amastigote forms were detected in perirenal adipose tissue of the right kidney (Fig. 4), greater omentum (Supplementary Fig. 3), spleen, and popliteal lymph node. Additionally, isolates of *L. infantum* were obtained from bone marrow and popliteal lymph nodes. The spleen was positive for *L. infantum* DNA and the parasite load expressed as the natural logarithm of the number of parasite genome equivalents (gEq)/ng was 6.175.

4. Discussion

Mistieri et al. (2014) observed coinfection with *D. renale* in 5 of 9 dogs seropositive for anti-*L. infantum* antibodies from the state of Rio Grande do Sul, Brazil. However, these authors did not investigate the load of this protozoan or the clinicopathological and histological alterations associated with coinfection as done in the present study. The fact that the present dog lived in a ZVL-endemic area and could have ingested aquatic oligochaetes, fish or frogs, since it had access to a river and these hosts, may explain coinfection with the two parasites. Mascarenhas et al. (2019) found low numbers of *D. renale* larvae in fish from a dioctophymatosis-endemic area in Brazil and suggested that the main source of infection for dogs in urban areas are oligochaetes ingested together with water.

Anemia, hyperproteinemia due to hyperglobulinemia, as well as the absence of clinical signs and azotemia in the dog studied here has also been reported for dogs infected only with *D. renale* or *L. infantum* (Nakagawa et al., 2007; Mesquita et al., 2014; Paltrinieri et al., 2016). The hypoalbuminemia observed was probably caused by proteinuric nephropathy and inhibition of albumin production in the liver by cytokines due to *L. infantum* infection (Paltrinieri et al., 2016). The absence of azotemia might be explained by the fact that the non-parasitized left kidney plays a compensatory role (Kommers et al., 1999;

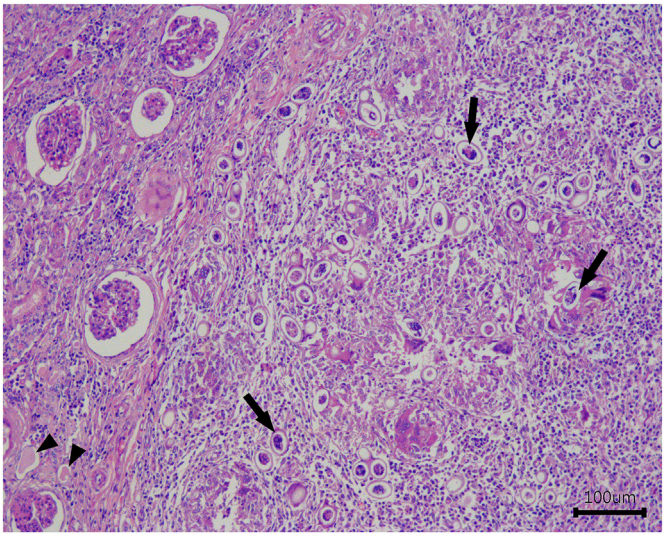

Fig. 2. Histological findings in a dog coinfected with *Dioctophyme renale* and *Leishmania infantum*. Right kidney. Pyogranulomatous nephritis, glomerular destruction and atrophy, and non-larvated *D. renale* eggs (arrows) in the cortical region amidst an inflammatory infiltrate. Hyaline cylinders are present in the lumen of convoluted tubules (arrowheads). HE.

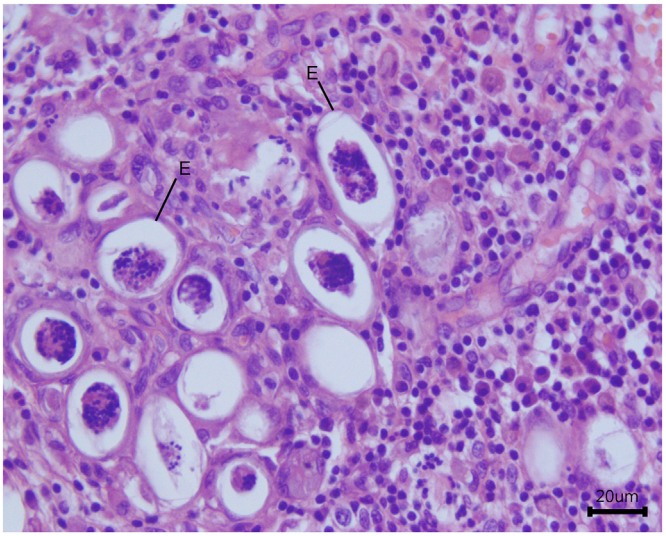

Fig. 3. Histological findings in a dog coinfected with *Dioctophyme renale* and *Leishmania infantum*. Greater omentum. Granulomatous peritonitis mainly composed of macrophages and, to a lesser extent, of lymphocytes, plasma cells, neutrophils and multinucleated giant cells engulfing multiple non-larvated *D. renale* eggs (E). HE.

Mesquita et al., 2014).

The anatomopathological changes observed in the right kidney were compatible with the severe chronic nephropathy described in canine dioctophymatosis (Kommers et al., 1999; Silveira et al., 2015; Sapin et al., 2017). Few studies have reported pyogranulomatous nephritis and granulomatous peritonitis with multinucleated giant cells, associated with *D. renale* eggs, as observed in the present study (Silveira et al., 2015; Sapin et al., 2017). The nephritis, fibrosis and glomerular atrophy observed in the right kidney have also been described in infections of dogs with *L. infantum* and are caused mainly by deposition of immunocomplexes (Koutinas and Koutinas, 2014; Silva et al., 2019). Furthermore, the detection of amastigote forms of *Leishmania* spp. in the greater omentum indicates that, together with the eggs of *D. renale*, this protozoan contributed to granulomatous peritonitis.

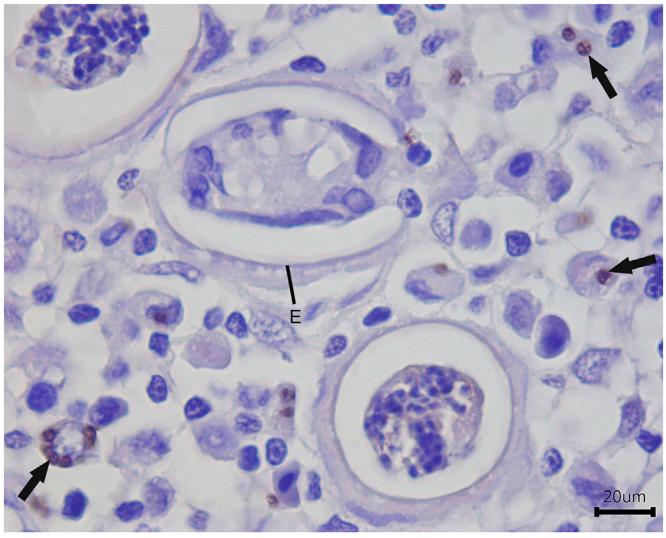

Fig. 4. Histological findings in a dog coinfected with *Dioctophyme renale* and *Leishmania infantum*. Right kidney. Eggs of *D. renale* (E) and brown-stained amastigote forms of *Leishmania* sp. (arrows) inside macrophages in perirenal adipose tissue. Immunohistochemistry. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The observation of larvated *D. renale* eggs in the kidney parenchyma in this study is an uncommon finding, which has only been reported by Kumar et al. (1972) in a maned wolf (*Chrysocyon brachyurus*). During its typical life cycle, non-larvated eggs of *D. renale* containing one or two cells are eliminated in the urine of definitive hosts. In water, first-stage larvae develop in eggs at a temperature of 14 to 30 °C (Mace and Anderson, 1975). The detection of first-stage larvae in *D. renale* eggs present in the kidney parenchyma in this study demonstrates that these larvae are able to develop at temperatures of 38 to 39.2 °C and under other conditions in the kidney tissue such as oxygen concentration, moisture, pH, and presence of an inflammatory reaction. No larvated eggs were found in the urine of the dogs studied here, probably because they were trapped in the kidney parenchyma.

The *D. renale* load was high in the right kidney considering that one to three parasites are usually found in this organ (Kommers et al., 1999). However, the splenic *L. infantum* load was lower than the median parasite load of 6.961 observed in the spleen of dogs from the same region and infected only with *L. infantum* (Oliveira et al., 2017). Overinfection of mice with *L. infantum* aggravated immunosuppression, which resulted in higher parasite loads, increased elimination of eggs, and longevity of the nematode *Heligmosomoides polygyrus* (González-Sánchez et al., 2018). Thus, in the present study, the high *D. renale* load and the severe and uncommon histological alterations associated with the eggs of this parasite may have been influenced by coinfection with *L. infantum*. However, this coinfection does not seem to have increased susceptibility to ZVL.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vprsr.2019.100351.

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publication.

Ethical statement

The study was approved by the Ethics Committee on Animal Use of the Oswaldo Cruz Foundation (permit number: LW-54/13).

Declaration of Competing Interest

The authors declare no conflict of interest regarding the publication of this manuscript.

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