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Fatal infection caused by *Cytauxzoon felis* in a captive-reared jaguar (*Panthera* onca)

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ABSTRACT

Fatal infections with *Cytauxzoon felis* are common in domestic cats, and jaguars (*Panthera onca*) are believed carriers of this protozoan. Fatal *C. felis* infections have never been described in jaguars before. Thus, this study describes such an infection in a 5-month-old captive-reared jaguar that presented hyporexia and died at 26 days after the first clinical signs. During necropsy, samples were taken from several tissues, some were fixed in 10% formalin and process for histopathological diagnosis, and some samples were used for DNA extraction, amplification via PCR and sequencing of the potential pathogens. Grossly, marked splenomegaly and icterus were observed. Histologically, numerous structures that are morphologically compatible with *Cytauxzoon* spp. schizonts obliterated multiple blood vessels in the brain, leptomeninges, spinal cord, lungs, heart, skeletal muscle, adrenal gland, kidneys, spleen, small intestine and pancreas. *C. felis* was identified by PCR in many organs. Thus, *C. felis* infection in jaguars can be fatal, and the clinicopathological findings are similar to those of cytauxzoonosis in other wild and domestic felid species.

1. Introduction

Cytauxzoon felis (Piroplasmorida: Theileriidae) is a protozoan parasite that infects wild and domestic felids (Cohn and Birkenheuer, 2011). In North America, bobcats (*Lynx rufus*) are the most common natural hosts and reservoirs of this protozoan, acting as source of infection for other felid species (Garner et al., 1996; Zieman et al., 2018; Wang et al., 2017). In Brazil, including the state of Mato Grosso do Sul (Antunes et al., 2018; Juliano et al., 2004), asymptomatic carriers of *C. felis* have been identified among domestic, wild, exotic free-ranging, and exotic captive-reared felids (bobcats, pumas, jaguars, and ocelots) (André et al., 2009, 2015; Filoni et al., 2012; Furtado et al., 2017b; Wang et al., 2017; Raimundo et al., 2021). Despite the detection of this protozoan predominating in wild felids, cases of infections in domestic felids are increasing, including in Brazil. (André et al., 2017; Maia et al., 2013). However, little is known about the epidemiology of cytauxzoonosis (André et al., 2015).

Jaguars (*Panthera onca*) are considered the biggest predators in tropical America, and the great majority are located in Brazil (Sanderson et al., 2002; Sollmann et al., 2008). They have great importance in their biomes as apex predators and play a role as asymptomatic carriers of *C. felis* (André et al., 2015; Furtado et al., 2017b), yet this characterization is based on sporadic reports.

Domestic cats are more susceptible to the clinical and fatal forms of this disease than wild felids (Cohn and Birkenheuer, 2011; Wang et al., 2017). However, fatal cytauxzoonosis has been described in North American bobcats (Nietfeld and Pollock, 2002) and captive tigers (Garner et al., 1996) and in South American captive-reared lions (Peixoto et al., 2007). Fatal infection with *C. felis* in jaguars has not been described previously. Thus, this study describes such a case in a captive-reared young Brazilian jaguar (*Panthera onca*), including its clinical, anatomopathological, and molecular aspects of this condition.

2. Material and methods

A jaguar (*Panthera onca*) was subjected to a necropsy and

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histopathological exams at the Anatomic Pathology Laboratory (LAP), Faculty of Veterinary Medicine and Husbandry, Federal University of Mato Grosso do Sul, Campo Grande, Brazil. Its clinical history was obtained from the veterinaries responsible for the case. During the necropsy, fragments of many organs were collected, fixed in 10% formalin, processed routinely, stained with hematoxylin and eosin (HE) and examined microscopically. Additional fragments of the liver, spleen, heart, central nervous system, lungs, lymph nodes, and kidney were collected for PCR exam.

PCR reactions were performed after DNA extraction of the tissues collected based on Araújo et al. (2009) adapted methodology (100 mg of tissue incubated in 20 mg/ml of proteinase K, for 12 hours at 56 °C). The reactions were carried out in a final volume of 25 µL containing 10 mM of Tris–HCl (pH 8.3), 50 µM of KCl, 1.5 mM of MgCl2, 0.2 mM of each desoxynucleoside triphosphate, 1.5 U of Taq DNA polymerase (Invitrogen), 11 pmol of each primer, and approximately 100 ηg of genomic DNA. To amplify a 651 bp fragment from the ITS1 and 18S rRNA gene were used the primer set 5′ CGATCGAGTGATCCGGTGAATTA 3′ and 5′ GCTGCGTCCTTCATCGATGTG 3’ (forward and reverse, respectively). The following parameters were used in the thermocycling program: 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 sec, 60 °C for 30 sec and 72 °C for 90 sec. A final extension step at 72 °C for 10 minutes was performed (Brown et al., 2010). The amplification products were viewed under an ultraviolet light after electrophoresis on 1,5% agarose gel stained with GelRed® (Biotium Inc., Fremont, CA) according to the manufacturer’s instructions.

The amplicons obtained were pooled together, purified with ExoSAP-IT® PCR Product Cleanup (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in both directions using an automatic sequencer (ABI 3130, Applied Biosystems, Waltham, MA, USA). The electropherograms were analyzed in a consensus sequence obtained with the program GeneStudio 2.2.0.0 (GeneStudio, Inc.). The consensus sequence was used to search for homologies with DNA sequences available in Genbank using the program Blastn (Altschul et al., 1990), and then the resulting sequence was deposited in the same data bank.

Additionally, RT-PCRs were performed for FeLV and FIV diagnosis, from RNA extracted with Trizol Reagent (Thermo Fisher scientific), according to the manufacturer’s instructions, from a pool sample containing liver, spleen, heart, central nervous system, lungs, lymph nodes, and kidney fragments. Transcriptions and PCRs were performed as described by Furtado et al. (2017a). All PCR products were analyzed by electrophoresis on 1.5% agarose gel as described above. A FeLV and FIV-positive sample of a domestic cat was used as a positive control.

3. Results

A male captive-born 5-month-old jaguar presented hyporexia. Antibiotic therapy and fluid therapy were administered, but the jaguar died 26 days after the initial signs. About 20 days before the animal became ill, a 2-month-old jaguar that was highly infested with ticks, weak, and in poor body condition was introduced to the other animal’s environment. A few weeks later, the introduced animal recovered completely.

The necropsied jaguar had regular body condition with slightly pale ocular mucosa. Slight to moderate jaundice was seen in the oral and ocular mucosa, and in subcutaneous tissue (Fig. 1). Gross findings consisted of marked splenomegaly (Fig. 2), with rounded edges and meaty appearance on the cut surface; enlarged liver with an evident lobular pattern and diffusely yellowish color; and heavy, non-collapsed lungs with smooth and bright pleura surfaces.

Microscopically, macrophages partially or fully obstructed multiple small and medium blood vessels in the brain, leptomeninges, spinal cord, lungs, heart, skeletal muscle, adrenal gland, kidneys, spleen, small intestine and pancreas. The macrophages had expanded cytoplasm with 7-µm diameter, round to oval shape, and pleomorphic and basophilic structures compatible with *Cytauxzoon* sp. schizonts (Figs. 3 and 5). These structures occasionally adhered to the endothelial surface. Mature schizonts containing 2-µm-diameter, oval or spindle-shaped, basophilic merozoites were observed occasionally (Fig. 4), as fibrin thrombi that partially filled blood vessels of the kidneys and heart. The lungs showed marked alveolar edema. The spleen showed hemosiderosis, red pulp, and lymphoid depletion. Hypercellularity was observed in the bone marrow by erythroid precursors, indicating regeneration process.

Fig. 1. Gross findings of *Cytauxzoon felis* fatal infection in a jaguar. Moderate icterus in the ocular mucosa. The enophthalmos indicates severe dehydration.

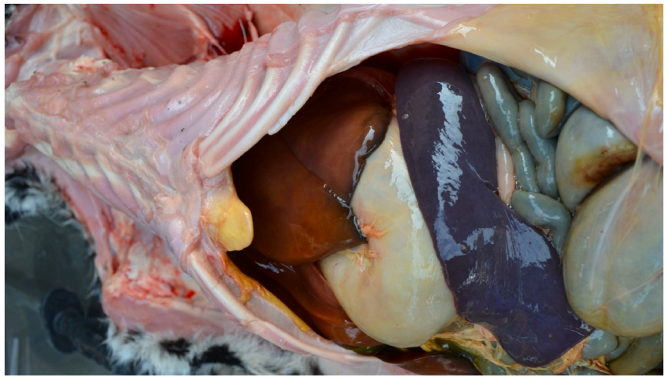

Fig. 2. Gross findings of *Cytauxzoon felis* fatal infection in a jaguar. The spleen is severely enlarged.

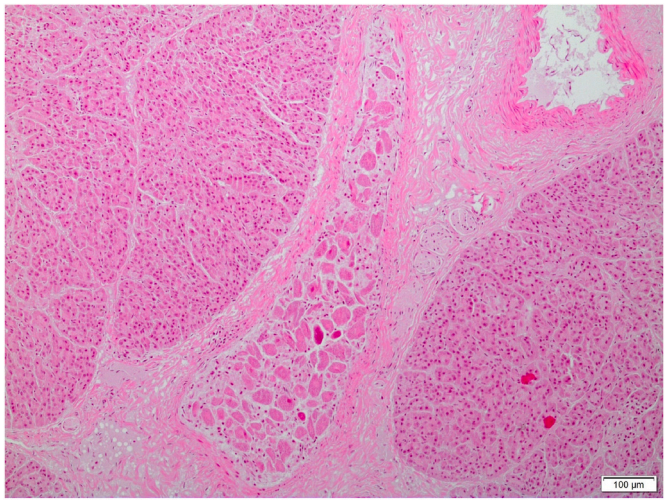

Fig. 3. Micrograph of the pancreas on infected jaguar. The central blood vessel is partially obstructed by macrophages containing high numbers of *C. felis* schizonts. HE.

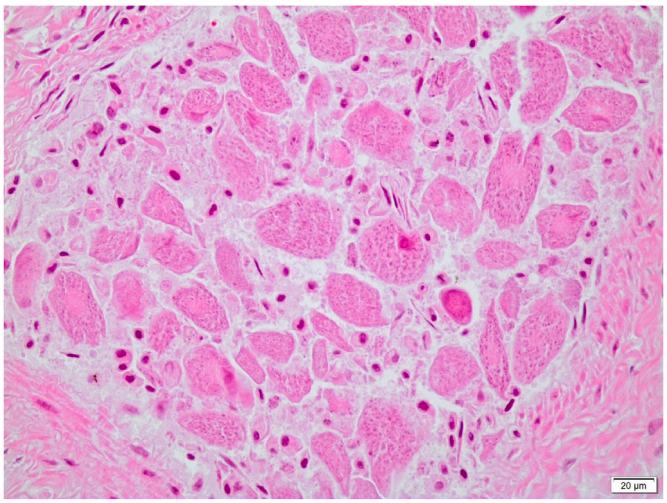

Fig. 4. Micrograph of the pancreas on infected jaguar. The macrophages are enlarged up to twice normal size and contained cytoplasmic schizonts. Note eccentric and pyknotic nuclei of macrophages. HE.

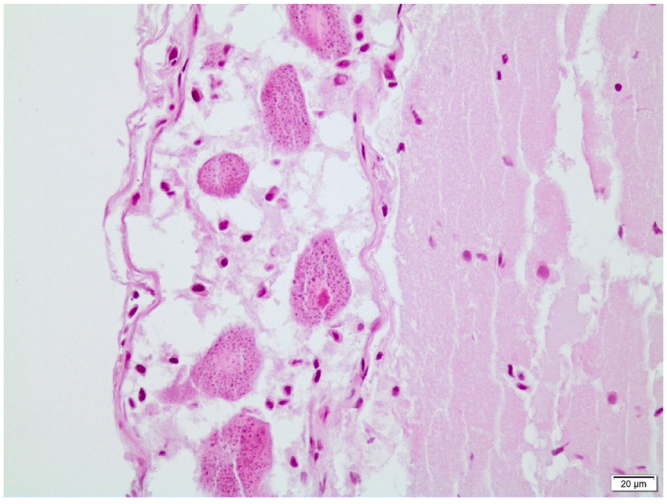

Fig. 5. Micrograph of the brain on infected jaguar. Schizonts inside macrophages contain numerous round to oval 1–2 µm diameter basophilic organisms (merozoites). HE.

C. felis was detected via genomic screening in the liver, spleen, heart, brain, lungs, and kidneys. The sequenced genomic fragment (525 pb) had 96.75% similarity to DNA sequences of *C. felis* available in Genbank. The DNA sequence obtained in this study was deposited in Genbank as MW591997. RT-PCR exam for FIV and FeLV were negative.

4. Discussion

A diagnosis of cytauxzoonosis was made based on the anatomopathological aspects, histological visualization of schizonts and merozoites in blood vessels, and the detection of *C. felis* in tissues with PCR. Serological tests performed in the Cerrado, Pantanal, and Amazon biomes of Brazil (Furtado et al., 2017b) identified *C. felis* in 75–100% of the jaguars investigated, and all of them were clinically healthy, suggesting that this species is a carrier of the protozoan in Brazil. This study adds to this observation in showing the possibility of young jaguars developing fatal infection by *C. felis*.

Anorexia and the necropsy and histopathological findings corroborate previous descriptions of cytauxzoonosis in other felid species (Aschenbroich et al., 2012; Garner et al., 1996; Maia et al., 2013; Nietfeld and Pollock, 2002; Peixoto et al., 2007), which detected schizonts in the cytoplasm of intravascular macrophages. This finding characterizes the tissue phase of cytauxzoonosis, which is essential for the establishment of a fatal infection (Nietfeld and Pollock, 2002).

In this study, it was not possible identify the source of infection. The necropsied jaguar did not have ticks, which is important in the transmission of *Cytauxzoon* spp. (Cohn and Birkenheuer, 2011). In North America, the vectors of this protozoan are well known (Cohn and Birkenheuer, 2011; Garner et al., 1996; Zieman et al., 2017), but in South America and Italy (Veronesi et al., 2016), the importance of ticks in the transmission of cytauxzoonosis is still unknown (Furtado et al., 2017b; Peixoto et al., 2007). Nevertheless, it is possible that they could also be an important source of infection in these countries. In this study, the absence of ticks on the jaguar does not rule them out as potential vectors of *C. felis*, similar to what was seen in a previous study (André et al., 2009).

The introduction of new felids in captivity is considered a risk for transmission (Millán et al., 2007), and jaguars can act as carriers even when they recover from the clinical disease (Furtado et al., 2017b). Thus, it is not possible to exclude the young tick-infested jaguar that was introduced before the necropsied jaguar became ill as a source of infection. However, as clinicopathological and molecular tests were not performed on the other jaguar, infection by *C. felis* could not be confirmed.

5. Conclusions

C. felis infection is fatal to young jaguars, similar to domestic and wild felid species. Cytauxzoonosis should be investigated in young jaguars with icterus.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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