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PARASITOLOGY

Natural infection and molecular detection of *Cytauxzoon felis* in a free-ranging *Puma concolor* in the state of Goiás, Brazil

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ABSTRACT: *The puma (Puma concolor Linnaeus, 1771), the most widely distributed felid species in the Americas, can be found in all Brazilian biomes. Nevertheless, few studies have focused on hemoparasites in this species. Cytauxzoon felis, a hemoparasite that can infect domestic cats, has also been described in wild felids in Brazil. To the best of our knowledge, this study is the first to diagnose the natural infection and molecular detection of C. felis in a P. concolor in the state of Goiás. This animal presented non-regenerative anemia and inclusion suggestive of piroplasmids within red blood cells. The amplified 551 bp fragment of partial Piroplasmida 18S rRNA gene sequence was 100% identical to corresponding sequences of C. felis available in GenBank. No specific treatment for cytauxzoonosis was administered, and after rehabilitation, the animal was reintroduced into the wild. This finding provides some evidence that P. concolor may act as a natural host of the parasite. The epidemiology, vector and pathogenicity of this hemoparasite in wild and domestic cats in Brazil deserves further investigation.*
Key words: *hemoparasites, Cerrado biome, wild felids, PCR.*

Infecção natural e detecção molecular de *Cytauxzoon felis* em *Puma concolor* de vida livre no estado de Goiás, Brasil

RESUMO: *O puma (Puma concolor Linnaeus, 1771) tem a maior distribuição entre os felídeos das Américas e é encontrado em todos os biomas do Brasil. No entanto, poucos estudos têm se concentrado nos hemoparasitos nesta espécie. Cytauxzoon felis, um hemoparasito que pode infectar gatos domésticos, também foi descrito em felídeos selvagens no Brasil. A saber, este estudo é o primeiro diagnóstico de infecção natural e detecção molecular de C. felis em um P. concolor do estado de Goiás. Este animal apresentou anemia arregenerativa e inclusão de piroplasmídeos nos glóbulos vermelhos. A amplificação do fragmento de 551 pb da sequência parcial do gene Piroplasmorida 18S rRNA foi 100% idêntica a sequências correspondentes de C. felis disponíveis no GenBank. Nenhum tratamento específico para citauxzoonose foi administrado e, após a reabilitação, o animal foi reintroduzido na natureza. Essa descoberta fornece algumas evidências de que P. concolor pode atuar como um hospedeiro natural do parasito. A epidemiologia, vetor e patogenicidade deste hemoparasito em gatos selvagens e domésticos no Brasil merecem uma investigação mais aprofundada.*
Palavras-chave: *hemoparasitos, bioma Cerrado, felídeos selvagens, PCR.*

Puma concolor Linnaeus, 1771 is a large felid distributed from southern Canada to the southern regions of South America. In Brazil, where its population is decreasing, the puma exhibits great adaptability and is present in all biomes of the country. However, information about the parasites that infect this wild felid is scanty (CUBAS et al., 2014).

The hemoparasite *Cytauxzoon felis* (Kier, 1979) belongs to the order Piroplasmida. After the first report of *C. felis* in North America, it is now considered an emerging worldwide tick-borne protozoan hemoparasite that parasitizes wild and domestic felids (MEINKOTH & KOCAN, 2005). Most of the infected wild felines remain asymptomatic; however, many cases of parasitized domestic cats (*Felis catus*) have been reported with severe clinical symptomatology, sometimes fatal (MEINKOTH & KOCAN, 2005; BROWN et al., 2010). The rapid course of the disease in domestic cats suggests they act as accidental and dead-end hosts to this parasite (KIER et al., 1987; BIRKENHEUER et al., 2008). However, studies have reported inconsistencies in the virulence of different isolates of *C. felis*, as some cats have been considered potential natural reservoirs

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after gone through a clinical course of the infection (RIZZI et al., 2015). Although, studies targeting detection of *C. felis* in wild felids have been carried out in Brazil (ANDRÉ et al., 2009; ANTUNES et al., 2018; FURTADO et al., 2017; SOUSA et al., 2018), data is still scarce. The current study aimed to detect *C. felis* in wild free-ranging *P. concolor* in the state of Goiás.

A puma (*P. concolor*) cub was rescued by the Goiânia Wild Animal Screening Center and taken for clinical treatment to the Wild Animal Clinic at the School of Veterinary Medicine and Animal Science of the Federal University of Goiás (HV/EVZ/UFG). The animal, which was parasitized by three *Rhipicephalus microplus* female ticks, presented an advanced state of cachexia, dehydration and first and second-degree burns on the palm and plantar cushions of both antimeres.

The animal was previously anesthetized with a mixture of ketamine (10 mg/kg) and midazolam (0.2 mg/kg) IM (intramuscular), and a blood sample was collected by cephalic venipuncture. Blood sample (5 mL) was sent to the Laboratory of Clinical Pathology of the Veterinary Hospital of EVZ/UFG. Red blood cell, leukocyte and platelet counts were performed using a hematological analyzer (Celltac Alpha/MEK-6550®, Nihon Kohden). Blood smear examination and hematocrit was determined by the microhematocrit method. Blood count revealed non-regenerative anemia, and stained blood smear (Panótico Rápido rapid hematology stain, Laborclin®, Brazil) showed inclusions suggestive of piroplasmids within red blood cells (Figure 1). The initial conjecture was *Cytauxzoon* sp., so polymerase chain reaction (PCR) was performed for confirmation. To this end, DNA was extracted from a blood sample using a DNeasy Blood & Tissue Kit (Qiagen®, Hilden, Germany), following the manufacturer's instructions, and the DNA was tested by a battery of PCR assays targeting bacteria of the Anaplasmataceae family (e.g. *Anaplasma* and *Ehrlichia*) and protozoa of the genera *Babesia*, *Cytauxzoon*, *Hepatozoon*, and *Theileria*. PCR protocols were performed using pairs of the oligonucleotides that amplify fragments of the following genes: 360 bp fragment of the Anaplasmataceae 16S rRNA gene, 551 bp fragment of the Piroplasmida 18S rRNA gene, and 670 bp fragment of the *Hepatozoon* spp 18S rRNA gene (ALMEIDA et al., 2012). Negative control (water) and an appropriate positive control sample (DNA of *Ehrlichia canis, Babesia vogeli*, and *Hepatozoon canis*) were run together with the *P. concolor* DNA sample.

The puma DNA sample was negative by the PCR protocols targeting organisms of the genus *Hepatozoon* and Anaplasmataceae family. PCR targeting a portion of the Piroplasmida 18S rRNA gene yielded amplicons of the expected size. Amplicons were treated with ExoSap (USB, Cleveland, OH, USA) and DNA sequenced in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyzer, Foster City, California, USA) with the same primers used for PCR. The obtained sequence (455 bp) was submitted to BLAST analyses (www. ncbi.nlm.nih. gov/blast) to infer the closest similarities available in GenBank. A BLAST analysis indicated that the 18S rRNA partial sequence was 100% identical to corresponding sequences of *C. felis* from several other wild felid species available in GenBank (MT458054, KY684003, MT904032, GU903911). The 18S rRNA partial sequence of *C. felis* generated in the present study from puma has been submitted to GenBank under the accession number MZ675364.

No specific medication was given for the treatment of cytauxzoonosis, and after a period of five months of recovery from clinical signs and rehabilitation, the puma animal was reintroduced to the place where it was rescued.

While the pathology and epidemiology of cytauxzoonosis has been studied in the USA (ZIEMAN et al., 2018), there have had isolated reports on the occurrence of this parasite in South America. In Brazil, cytauxzoonosis has been documented as fatal to exotic captive living lions (*Panthera leo*) (PEIXOTO et al., 2007), and subclinical infections have been documented in wild captive jaguars (*Panthera onca*), and free-ranging ocelots (*Leopardus pardalis*) and pumas (*Puma concolor*) (ANDRÉ et al., 2009; ANTUNES et al., 2018; SOUSA et al., 2018). FURTADO et al. (2017) reported high prevalence of *C. felis* in free-ranging jaguars (all 30 animals tested positive) from three different Brazilian biomes, Cerrado, Pantanal and Amazon. In view of available information about the pathology of this parasite in domestic cats and rare reports of cytauxzoonosis in other felids, it has been suggested that the number of deaths caused annually by this disease among wild felids in endemic areas may be undetected and underestimated (VERONESI et al., 2016).

While the natural wild reservoir of *C. felis* in North America is the bobcat (*Lynx rufus*) (SHOCK et al., 2011) and cytauxzoonosis is known to be transmitted by *Amblyomma americanum* and *Dermacentor variabilis* (REICHARD et al., 2010;

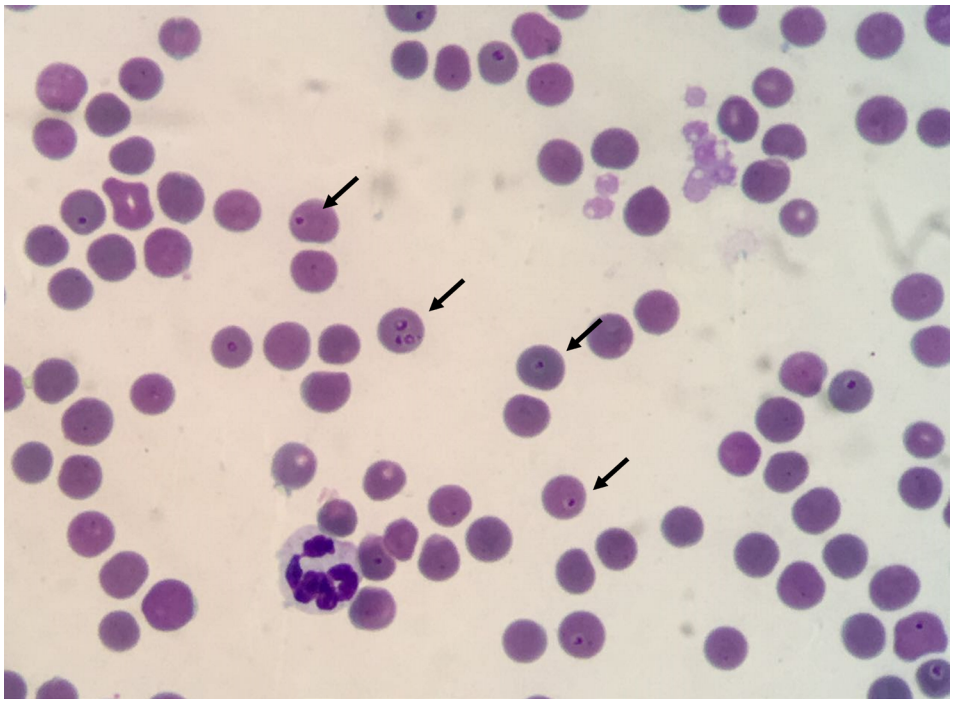

Figure 1 - Blood smear of *Puma concolor* cub presenting intraerythrocytic ovoid piroplasmids (black arrows) compatible with *Cytauxzoon felis* (Panótico Rápido® LB, 1000 x magnification).

SHOCK et al., 2014), its South American potential reservoir are believed to be wild Neotropical felids (ANDRÉ et al., 2009) and its vectors in the region remain unknown (FURTADO et al., 2017). Although, the present *C. felis*-infected puma was parasitized by *R. microplus*, there is no data on this tick species role in transmitting this protozoan. Therefore, further aspects of this hemoparasite need to be elucidated, such as exploring possible vectors in South America and other potential route of transmission; e.g., vertical transmission, which is reported to occur with other protozoan species (VERONESI et al., 2016).

ZIEMAN et al. (2018) reported wild bobcats chronically infected with *C. felis*, stating that a single host can propagate this parasite for years and that this single individual could be responsible for the introduction of *C. felis* in new regions. The home range of *P. concolor* in the Cerrado biome can cover 428 km^2 (SILVEIRA, 2004), and if this wild felid is a natural host, its large home range may enable it to disseminate *C. felis* to different regions and states.

Intraerythrocytic ovoid piroplasmids compatible with *C. felis,* similar to the morphological type described by JULIANO et al. (2004) and HARVEY et al. (2007), were found in the animal blood smear (Figure 1). Although, presenting anemia, cachexia and dehydration, the puma cub of this study did not present any other characteristic signs of cytauxzoonosis like those reported in other wild felids (PEIXOTO et al., 2007; ZIEMAN et al., 2018). Anemia, when present, is evident in the acute stage of cytauxzoonosis, and appears while piroplasms are detected in the bloodstream (NIETFELD & POLLOCK, 2002). In addition, due to rapid development, erythroid cell regeneration may not be initially noticeable in acute conditions (HARVEY et al., 2007). Although, the positive parasitological diagnosis by PCR and visual identification in blood smears, allied to the absence of clinical signs compatible with the disease, followed by recovery without specific treatment, may indicate effectiveness of the immune system (ANTUNES et al., 2018). Another hypothesis is that the *C. felis* strain in question may be less pathogenic and therefore unable to cause disease (pathogenesis) in susceptible feline species (JULIANO et al., 2004), as most cases reported in Brazil are nonfatal and asymptomatic (ANDRÉ et al., 2009).

The present finding highlighted the need for further investigations in Brazil to better comprehend *P. concolor* role as host of *C. felis*. To the best of our knowledge, this study is the first to diagnose natural infection of *C. felis* in *P. concolor* in the state of Goiás. Additional studies are needed in Brazil to shed light on the epidemiology and the real pathogenicity of this hemoparasite in wild and domestic cats in this country.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The study does not need bioethics approval as the sample comes from an animal treated at the EVZ/UFG Veterinary Hospital. Part of the extension project under registration PJ804-2018.

DECLARATION OF CONFLICT OF INTEREST

Authors declare they have no competing interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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