

***Hepatozoon* spp. in a hoary fox (*Lycalopex vetulus*) from Uberlândia, Minas Gerais State, Brazil**

Hepatozoon spp. na raposinha-do-campo (*Lycalopex vetulus*) em Uberlândia (MG)

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Abstract

Canine hepatozoonosis, which has been diagnosed in several regions of Brazil, can also affect wild canids. A male hoary fox (*Lycalopex vetulus*) was treated in August 2008 at the Center for Wildlife Studies of the Veterinary Hospital, Federal University of Uberlândia, in the municipality of Uberlândia, Minas Gerais, Brazil. After a preliminary clinical assessment, the fox was subjected to hematological and hemoparasite exams, which revealed *Hepatozoon* spp. gametocytes in the blood smear. This is the first report of the presence of *Hepatozoon* spp. in a hoary fox (*Lycalopex vetulus*) in the Triângulo Mineiro region.

Keywords: Wild canids. Hemoparasitosis. Hepatozoonosis.

Resumo

A hepatozoonose canina, que tem sido diagnosticada em diversas regiões do Brasil e pode acometer também canídeos silvestres. Um macho de raposinha-do-campo (*Lycalopex vetulus*) foi atendido no Núcleo de Estudos em Animais Selvagens do Hospital Veterinário da Faculdade de Medicina Veterinária da Universidade Federal de Uberlândia em agosto de 2008, no município de Uberlândia (MG). Após prévia avaliação clínica, realizou-se exame hematológico e a pesquisa de hemoparasitas e gametócitos do *Hepatozoon* spp. foram detectados



no esfregaço sanguíneo. Este é o primeiro relato da presença de *Hepatozoon* spp. na raposinha-do-campo (*Lycalopex vetulus*), na região do Triângulo Mineiro.

Palavras-chave: *Canídeos selvagens. Hemoparasitoses. Hepatozoonose.*

Introduction

The hoary fox (*Lycalopex vetulus*) is a native Brazilian canid that lives in the fields and savannahs of the states of Mato Grosso, Goiás, Minas Gerais and São Paulo, and was first identified as *Lycalopex vetulus*, by Lund (1842), according to Gomes (2006). It is a carnivorous species whose diet includes birds, small rodents and insects (GOMES, 2006).

Two species of the genus *Hepatozoon* have been described in domestic dogs: *H. canis*, reported in Europe, Asia, Africa, South America and the United States, and *H. americanum*, which, so far, has only been diagnosed in the United States. In Brazil, the only species found to infect dogs is *H. canis*.

Canine hepatozoonosis is a disease that has been diagnosed in several regions of Brazil. Various published articles have revealed that, although *H. canis* is present throughout this country, its prevalence is highly variable, depending on state, origin of the animals (i.e., dogs from rural or urban areas) and diagnosis methodology (i.e., blood smears or PCR) (O'DWYER, 2011).

H. canis is frequently imputed as the infectious agent of hepatozoonosis (FORLANO et al., 2007). In Brazil, *H. canis* infection was diagnosed in a crab-eating fox (*Cerdocyon thous*) in the region of Botucatu, state of São Paulo (ALENCAR; KOHAYAGAWA; SANTARÉM, 1997). Recent studies have involved the molecular characterization of *Hepatozoon* sp. in wild canids from Brazil (ANDRÉ et al., 2010; CRIADO-FORNELIO et al., 2006).

Criado-Fornelio et al. (2006) detected *Hepatozoon* sp. exhibiting similarity to *H. canis* genotype "Spain-1" (a common genotype among wild canids, but not among domestic dogs) and *Hepatozoon* sp. showing similarity to *H. americanum*. Recently, André et al. (2010) found samples of *Hepatozoon* sp., phylogenetically distinct from *H. canis*, based on a phylogenetic analysis of the 18S rRNA gene. André et al. (2010) also detected *Hepatozoon* sp.,

phylogenetically related to *H. americanum* in bush dogs in the wild and in zoos of Cuiabá, Mato Grosso State, Brazil.

Fishman et al. (2004) asserted that infections caused by *Ehrlichia* sp. and *Hepatozoon* spp. are endemic in red foxes (*Vulpes vulpes*) from Israel – considered natural reservoirs of the infection in domestic dogs. They observed that the distribution of canine hepatozoonosis among wild carnivores in Texas coincided with the geographical distribution of the infection in domestic dogs, indicating the importance of wild animals in the epidemiological chain of this disease (MERCER et al., 1988).

The purpose of this communication is to report, for the first time, a case of natural infection caused by *Hepatozoon* spp. in a hoary fox (*Lycalopex vetulus*) from the Triângulo Mineiro region, Uberlândia, Minas Gerais State, Brazil.

Materials and methods

A male hoary fox (*Lycalopex vetulus*), showing a good body mass index score and weighing 4.3 kg (Figure 1), was brought to the Center for Wildlife Studies by the Environmental Police of Uberlândia in August 2008 and was treated at the Veterinary Hospital of the Faculty of Veterinary Medicine (Famev/UFU).

The animal displayed calm behavior, despite being physically restrained with a snare and a polyethylene basket muzzle. Then, biological material was collected, without requiring general anesthesia. Clinical examination was performed according to the recommendations of Feitosa (2004). The animal's physiological parameters, such as the rectal temperature (38 °C), cardiac frequency (120 bpm) and respiratory frequency (36 mpm) were considered normal for the species. The presence of ticks was not observed during clinical examination.



Figure 1 - Hoary Fox (*Lycalopex vetulus*) captured in the municipality of Uberlândia, Minas Gerais State, Brazil

Source: Research data.

After preliminary clinical assessment, 3 mL of blood was drawn by cephalic venipuncture. The blood sample was then transferred to a vacutainer tube containing 0.1 mL of EDTA-K₂ (dipotassium ethylenediaminetetraacetic acid). The blood was analyzed at the Clinical Analysis Laboratory of the Veterinary Hospital of the Faculty of Veterinary Medicine (Famev/UFU), using an ABC VET[®] automated cell counter.

Hemoparasite detection was performed by examining a peripheral blood smear from the marginal ear vein. The sample were stained by the May-Grünwald-Giemsa method (FERREIRA NETO et al., 1982) and used for hemoparasite diagnosis and differential white cell count.

Results

The hepatozoonosis was diagnosed based on the identification of *Hepatozoon* spp. gametocytes in the blood smear (Figure 2).

After the animal's adaptation to the site, it showed a normal appetite, water intake and bowel function. The hepatozoonosis occurred with absence of symptoms during the 40-day period of the

animal's stay at the Veterinary Hospital, after which, the animal was transferred to a zoo in Sabiá Park in the municipality of Uberlândia.

The therapy consisted of two doses of imidocarb (5 mg.kg⁻¹ IM, 14-day interval) 15 minutes after atropine (0.044 mg.kg⁻¹, SC) was applied, in order to reduce the side effects of imidocarb. Simultaneous therapy was established with oxytetracycline (four applications of 10 mg.kg⁻¹ IM at 48-hour intervals).

Discussion

Baneth, Harmelin e Presentey (1995) claimed that *H. canis* infection can be diagnosed through the detection of gametocytes contained in neutrophils and monocytes in peripheral blood smears, as well as by molecular techniques such as indirect immunofluorescence, PCR and Western blot (O'DWYER et al., 2001). The 18S rRNA gene-based PCR is the technique of choice in the diagnosis of *Hepatozoon* parasites (ANDRÉ et al., 2010; CRIADO-FORNELIO et al. 2006; MIRANDA et al., 2011; RUBINI et al., 2005).

The hepatozoonosis occurred in the absence of symptoms during the 40-day period when the animal was under observation. A study of domestic

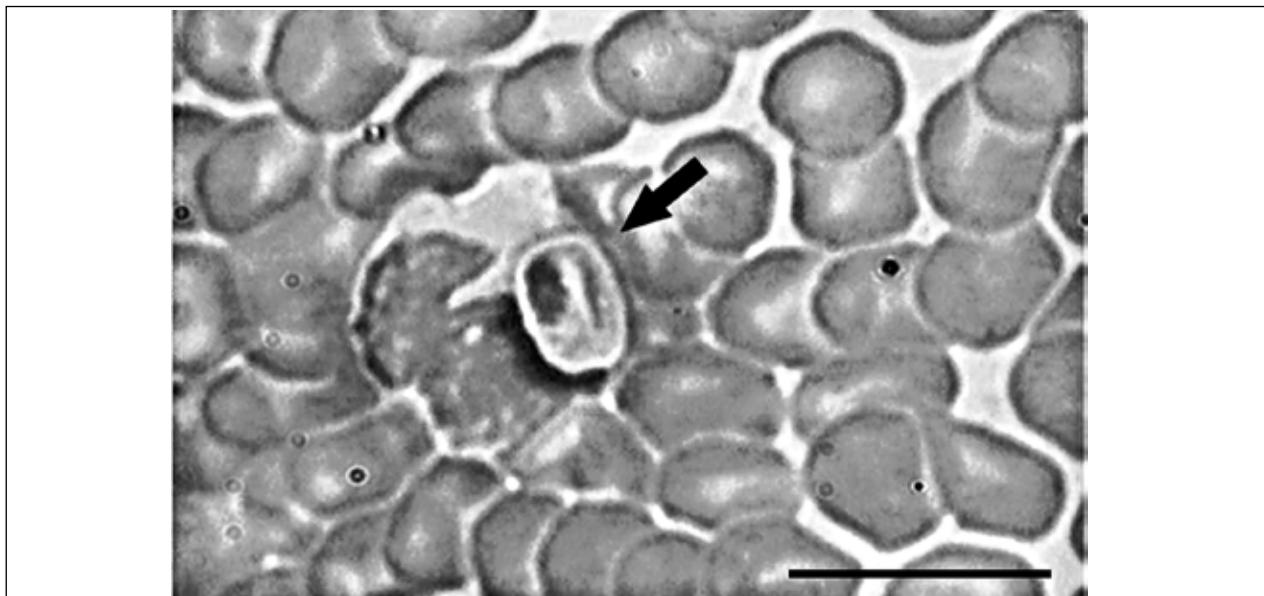


Figure 2 - *Hepatozoon* spp. gametocyte in peripheral blood smear from the hoary fox (indicated by the arrow)

Legend: Optical microscopy with 100× magnification, May-Grünwald-Giemsa coloration (bar = 100 µm).

Source: Research data.

dogs in the same region found that 33.92% of the animals were naturally infected by *Hepatozoon* sp. and remained asymptomatic (MUNDIM et al., 2008). In fact, Gomes (2006) reported that protozoan infections in wild canids are often described as asymptomatic.

A study involving coyotes (*Canis latrans*) in Oklahoma showed that 56.25% (9/16) of the animals presented natural infection by *H. americanum*. This was confirmed by the presence of the agent in skeletal and cardiac muscles. The coyotes presented several species of ticks, including adult *Amblyomma maculatum* (KOCAN et al., 1999).

Reports of canine hepatozoonosis in Brazil have revealed a higher occurrence in rural dogs than in urban animals. This is attributed to the proximity of rural dogs to the wild populations considered natural reservoirs of the agent. Moreover, the wide variety of tick species present in rural areas increases the transmission rates of the pathogen (O'DWYER et al., 2001).

According to O'Dwyer et al. (2001), *H. canis* is a protozoan transmitted by ticks, which infects dogs worldwide. Its clinical form differs from the subclinical form, ranging from apparently healthy dogs to animals presenting severe clinical symptoms. The common vector of *H. canis* transmission is the tick *Rhipicephalus sanguineus*. However, other tick species

related to canine hepatozoonosis, such as *Amblyomma ovale*, have been identified in rural areas (FORLANO et al., 2005; RUBINI et al., 2009). In Brazil, *R. microplus* was recently reported as the possible vector of *H. canis* to an infected domestic dog from a rural area (MIRANDA et al., 2011). In the United States, *Amblyomma maculatum* is the vector of canine hepatozoonosis caused by *H. americanum* (EWING; MATHEW; PANCIERA, 2002).

In Italy, Gabrielli et al. (2010) reported the presence of *Hepatozoon canis* in red fox (*Vulpes vulpes*). Based on molecular diagnostic methods, the authors found that 13.4% of the foxes (16/119) and 2.1% of the ticks (6/290) in their study were positive for *H. canis*. This confirms the importance of *R. sanguineus* as a vector, and suggests that *Ixodes ricinus* may also be implicated in parasite transmission. The peridomestic habits of *V. vulpes* and global warming are expected to magnify the impact of this vector-borne disease and the transmission of *Hepatozoon* to domestic animals.

André et al. (2010) evaluated the presence of *Hepatozoon* spp. in 165 captive wild felids and 100 captive wild canids using molecular techniques. The authors found five canids (two bush dogs, one fox, one crab-eating fox, and one maned wolf) positive for *Hepatozoon* spp. This agent may be a potential pathogen and an opportunistic parasite in concomitant infections or immunocompromised animals.

Domestic dogs with high parasite levels presented acute symptoms such as fever, anorexia, oculonasal discharge, paralysis, weakness, lethargy, lung alterations, diarrhea, and general signs of weakness during the chronic phase of the disease (MUNDIM et al., 1994, 2008). According to Garret et al. (2005), the factors that influence morbidity and mortality of hepatozoonosis are the host's age, exposure time and the quantity of ingested oocysts, as well as its immunological status.

Hematological alterations found in the crab-eating fox *Cerdocyon thous* caused by *H. canis* were neutrophilia, eosinophilia, monocytosis and anemia (ALENCAR; KOHAYAGAWA; SANTARÉM, 1997). Domestic dogs with hepatozoonosis usually present normochromic normocytic anemia, leukocytosis, neutrophilia and nuclear deviation of neutrophils to the left (MUNDIM et al., 2008). Nevertheless, the animal showed hematological values within reference parameters for domestic (MEINKOTH; CLINKENBEARD, 2000) and wild canines (GOMES, 2006).

The therapeutic protocol employed in this study was efficient and comparable to a study using wolves (GOMES, 2006). To date, no therapeutic protocol has proved to be effective in eliminating *H. canis* or *H. americanum* from tissues (LAPPIN, 2010). The animal's health was monitored on weekly basis through blood smears, always showing negative results, which allowed the transfer and integration of the animal to the zoo mentioned previously.

Conclusion

This is the first report of *Hepatozoon* spp. infecting a hoary fox (*Lycalopex vetulus*) in the Triângulo Mineiro region, Minas Gerais State, Brazil.

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