

First report of *Hepatozoon sp.* In a dog in the Western Amazon, Brazil

Canine hepatozoonosis is a worldwide disease, caused by protozoa of the genus *Hepatozoon*, whose transmission occurs when the dog ingests ticks or part of them containing sporulated oocysts. The objective was to report the identification of subclinical *Hepatozoon sp.* in a dog in the state of Rondônia, in the Western Amazon, Brazil. A female dog, two years old, mixed breed, resident in the urban area of the municipality of Rolim de Moura (Rondônia) was taken to a veterinary clinic due to lameness of the right hind limb. In the anamnesis, the animal did not present pain on palpation of the affected limb. There were ectoparasites in the animal's body (fleas and ticks). Blood was collected in a tube with EDTA to perform the complete blood count and blood smears. The blood count showed normocytic and normochromic anemia and thrombocytopenia. In blood smears, gametes were observed in neutrophils. Given the report, we concluded that *Hepatozoon sp.* circulates in dogs from the Western Amazon, emphasizing the importance of including the investigation of hemoparasites in the veterinary clinical routine, even when the animal is asymptomatic.

Keywords: Apicomplexa; *Hepatozoon canis*; Hepatozoonosis; Hemoparasites; Rondônia.

Primeiro relato de *Hepatozoon sp.* em cão na Amazônia Ocidental, Brasil

A hepatozoonose canina é uma doença de distribuição mundial, causada por protozoários do gênero *Hepatozoon*, cuja a transmissão ocorre quando o cãoingere carrapatos ou parte deles contendo oocistos esporulados. Objetivou-se relatar a identificação de *Hepatozoon sp.* sob a forma subclínica em um cão no estado Rondônia, na Amazônia Ocidental, Brasil. Um cão fêmea, de dois anos de idade, sem raça definida, residente na zona urbana do município de Rolim de Moura (Rondônia) foi levada a uma clínica veterinária devido a claudicação do membro posterior direito. Na anamnese, o animal não apresentou dor à palpação do membro afetado. Havia ectoparasitos no corpo do animal (pulgas e carrapatos). Foi coletado sangue em tubo com EDTA para a realização do hemograma e esfregaços sanguíneos. O hemograma revelou anemia normocítica e normocrômica e trombocitopenia. Nos esfregaços sanguíneos foram observados gamontes em neutrófilos. Diante do relato, concluímos que *Hepatozoon sp.* circula em cães da Amazônia Ocidental, enfatizando a importância de incluir a pesquisa de hemoparasitos na rotina clínica veterinária, mesmo quando o animal se encontra assintomático.

Palavras-chave: Apicomplexa; *Hepatozoon canis*; Hepatozoonose; Hemoparasitos; Rondônia.

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INTRODUCTION

Hepatozoonosis is a worldwide disease, caused by protozoa of the genus *Hepatozoon* (Apicomplexa; Hepatozoidae), which parasite neutrophils and monocytes of vertebrates, which are intermediate hosts (BORGES et al., 2015). It performs a heteroxene biological cycle, having blood-sucking invertebrates as its definitive hosts (BASSO et al., 2019). In dogs, the life cycle of *Hepatozoon* spp. happens asexually: merogony, in various tissues and organs, followed by gametogony in leukocytes. In the tick, considered the main definitive host, a sexual cycle occurs with the formation of sporulated oocysts. *Hepatozoon* transmission occurs when the dog ingests ticks or part of them containing sporulated oocysts (DEMONER et al., 2013; DUARTE et al., 2016). Thus, the sporozoites are released and reach the blood circulation and various organs, such as bone marrow, spleen, liver, kidneys, intestines, lungs and lymph nodes, where the merontes will be formed. Merozoites are released from merontes, they invade neutrophils and monocytes forming gametes, where ticks become infected during the blood meal (DEMONER et al., 2013).

In Brazil, the first report of hepatozoonosis was described in dogs domiciled in rural areas where these animals had contact with wild fauna, considered reservoirs of the pathogen. Ticks and fleas have been referred to as vectors (O'DWYER et al., 2001; RUBINI et al., 2008).

Domestic dogs can be infected with *Hepatozoon americanum*, hitherto restricted to the United States, and *Hepatozoon canis*, described in several countries on all continents, including Brazilian states (RUBINI et al., 2008; PALUDO et al., 2005; DUARTE et al., 2016).

The clinical findings of hepatozoonosis are nonspecific, becoming a problem in the diagnosis of the disease. However; anemia, intermittent fever, anorexia, depression, muscle hyperesthesia, paraparesis, bloody diarrhea, lymphadenopathy, ocular and nasal discharge are the most common clinical signs of the disease (BANETH, 2006; IVANOV et al., 2008; O'DWYER, 2011).

The parasite is identified frequently by light microscopy, during the blood smear. The identification of gametes within neutrophils and monocytes is widely used, however, this technique may have low sensitivity due to the low parasitic load (RUBINI et al., 2008; O'DWYER, 2011; BRAZ et al., 2015).

The objective was to report the identification of subclinical *Hepatozoon* sp. in a dog in the state of Rondônia, in the Western Amazon, Brazil.

A female dog, two years old, mixed breed, weighing 9.2 kg, residing in the urban area of the municipality of Rolim de Moura (Rondônia) was taken to a veterinary clinic due to lameness of the right hind limb. In the anamnesis, the animal did not present pain on palpation of the affected limb. Mucosal staining parameters, capillary filling time, temperature, heart and respiratory rate were normal. There were ectoparasites in the animal's body (fleas and ticks). The owner reported there was no lack of appetite and no strange behavior was observed in the animal. Blood was collected in a tube with EDTA (ethylene diamine tetra-acetic acid) for the performance of the complete blood count and blood smears, stained with rapid panotic Laborclin®.

The red blood cell series showed normocytic and normochromic anemia and thrombocytopenia

(Table 1). In the blood smear the presence of gametes of *Hepatozoon* sp. was identified. (Figure 1). In this study, a 9% parasitemia was detected based on the count of parasitized cells in the observation of 100 leukocytes. Doxycycline (5 mg/Kg/VO/BID for 28 days), Sufa + Trimetropim (15 mg/Kg/VO/BID for 10 days), Ranitidine (2 mg/Kg/VO/BID for 10 days), Imidocarb (5 mg/Kg/VO/BID two applications with an interval of 15 days) and Meloxicam (0.2 mg/Kg/VO/SID for 7 days). The animal's owner did not return for clinical follow-up and preparation of the healing slide.

Table 1: Blood count of a two-year-old female positive for *Hepatozoon* sp. in the Western Amazon, Brazil.

SÉRIE VERMELHA	Valores de Referência		Resultados	
Hemácias ($10^3/\mu\text{L}$)	5,5 – 8		4,21	
Hemoglobina (Hb)	12 – 18		9,7	
Hematócrito (Ht)	37 – 55		28,1	
V.C.M (fentonlitros)	60 – 77		66,7	
H.C.M (picogramas)	19 – 23		23,0	
C.H.C.M (%)	31 – 36		34,5	
Proteínas Totais (g/dL)	5,5 – 8,0		7,0	

SÉRIE BRANCA	Valores de Referência		Resultados	
	Relativos (%)	Absolutos ($/\mu\text{L}$)	Relativos (%)	Absolutos ($/\mu\text{L}$)
Leucócitos totais	----	6.000 – 17.000	----	8.300
Bastonetes	0 – 3	0 – 300	00	00
Neutrófilos	60 – 77	3000 – 11.500	69	5.727
Eosinófilos	2 – 10	100 – 1.250	05	415
Linfócitos	12 – 30	1.000 – 4.800	17	1.411
Basófilos	Raros	Raros	00	00
Monócitos	3 – 10	150 – 1.350	09	747

PLAQUETAS:	200.000 – 500.000/ mm^3	97.000/ mm^3
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μL = microliters; MCV = Mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; g/dL = grams per deciliter; mm^3 = cubic millimeters. Canine CBC showing normocytic and normochromic anemia (Ht = 28.1%; Hb = 9.7) and thrombocytopenia (97,000/ mm^3 of platelets).

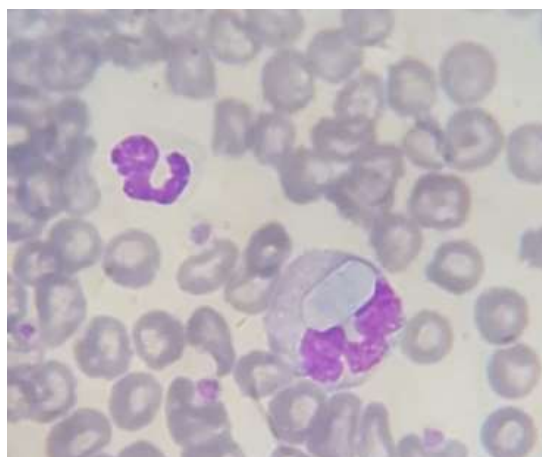


Figure 1: Gamete of *Hepatozoon* spp. in neutrophil (arrow) of dog. Optical light microscopy. 1000x magnification.

Although thrombocytopenia is not a common finding of hepatozoonosis (O'DWYER, 2001; PALUDO et al., 2003; IVANOV et al., 2008; O'DWYER, 2011; BORGES et al., 2015), it was reported in a study in the Midwest region (RAMOS et al., 2010). However, low hemoglobin and PCV values, thrombocytopenia, leukocytosis and neutrophilia associated with a high liver enzyme count were suggestive of acute inflammation in response to *H. canis* (SARMA et al., 2012).

The hematological variations present in hepatozoonosis are proportionally related to the degree of parasitemia, immune response and presence of co-infections by other pathogens (IVANOV; TSACHEV, 2008;

O'DWYER, 2011. Generally, the absence of symptoms reveals a degree of parasitemia below 5%. More severe symptoms with a lethal evolution were found in dogs with high levels of parasitemia (BANETH, 2006). Anemia is the most common finding in the diagnosis of the disease (MUNDIM et al., 1994; O'DWYER et al., 2001; BANETH, 2006; IVANOV et al., 2008; O'DWYER, 2011; DEMONER et al., 2013; ANTUNES et al., 2015; BORGES et al., 2015), as was observed in this case, however, associated with fever, lethargy and weight loss (ROOPALI et al., 2017). Ataxia and lameness have also been reported in dogs infected with *Hepatozoon*, due to the development of osteomyelitis (SAKUMA et al., 2009).

Although molecular tests are among the most sensitive methods for diagnosing hepatozoonosis, they are not available in the veterinary laboratory routine (OTRANTO et al., 2011). Therefore, light microscopy is the most used technique, being simple and fast for the visualization of the parasite gametes, a fact that occurred in this study.

In Brazil, hepatozoonosis has been reported in states in the South (LASTA et al., 2009), Southeast (RUBINI et al., 2005; SPOLIDORIO et al., 2009; PEREIRA et al., 2011), Midwest (MUNDIM et al., 1994; PALUDO, 2011; SPOLIDORIO et al., 2011; ANTUNES et al., 2015), Northeast (RAMOS et al., 2010), North (GOMES et al., 2016) and so far without records in the state of Rondônia. Therefore, we are reporting the first case of canine hepatozoonosis in the Western Amazon.

This case of hepatozoonosis can be classified as a subclinical accidental finding, as the animal did not present any of the most common clinical signs described in the various reports (PALUDO et al., 2005; BANETH, 2006; IVANOV et al., 2008; O'DWYER, 2011). Ticks, in particular *Rhipicephalus sanguineus*, are considered the main biological vector of hepatozoonosis (DEMONER et al., 2013; BANETH, 2011; DUARTE et al., 2016) which probably explains the finding, since the owner reported not controlling ectoparasites in the animal, which was infested.

Given the report, we concluded that *Hepatozoon* sp. circulates in dogs from the Western Amazon, emphasizing the importance of including the investigation of hemoparasites in the veterinary clinical routine, even when the animal is asymptomatic. Alerting the local population regarding the control of ectoparasites is necessary, because in addition to hepatozoonosis, they transmit other pathogens such as *Babesia* spp. and *Ehrlichia* sp., which when associated can lead the animal to an acute and fatal condition.

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